

PS.01.001 CAN PLANT RESIDUE QUALITY INFLUENCE SOIL ENZYMATIC FUNCTIONS DURING THE DECOMPOSITION PROCESS IN SOIL?

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Intrinsic characteristics of plant residues which correspond to the substrate for microbial growth and enzyme activities is a key factor determining the kinetics of decomposition in soils (Bertrand et al., 2006; 2009). These processes are partly mediated by extra-cellular enzymes involved in degradation, transformation and mineralization of plant residues (Sinsabaugh et al., 2009). Thereby, exoenzyme assays can provide functional information to further understand the relationships between soil microbial activity and the nature of carbon entering the soil (Sinsabaugh et al., 1991).

The main objective of this study was to analyse from a dynamic point of view the relationships between contrasted carbon mineralization kinetics due to different residue quality and enzyme activity profiles. Enzyme activities in soil and on the decomposing residues were compared to achieve functional interpretation of enzyme action.

To do so, incubations of aerial parts (leaf) and underground parts (root) of maize plants were performed in an agricultural loamy soil. These incubations were performed under controlled conditions for 43 days at 15°C and a water potential of -80kP with mineral nitrogen added. A dynamic characterization of plant residue quality (polysaccharide and lignin contents), of specific-enzymes activity (Xylanase, Cellulase, CBH-1 and Laccase), of microbial biomass C and of residue C mineralization was performed.

Our results showed that, after 43 days, maize leaves mineralized significantly more C than maize root. Indeed this contrasted C kinetics was related to different chemical composition of the residues, with leaves having higher cyto-soluble content and lower Klason Lignin content than roots. Enzymes activities determined directly on plant residue were systematically higher in leaves than roots. High xylanase and cellulase activities measured at the start of incubation while laccase activity was low which is more pronounced in case of roots.

Abstract Category

01 Biogeochemistry

PS.01.002 EXTREME BIOGEOCHEMICAL HETEROGENEITY: MODELLING ANTARCTIC AQUATIC MICROBIAL ECOLOGY THROUGH THE MELTWATER PONDS OF BRATINA ISLAND, ANTARCTICA

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On the rock- and sediment-covered undulating surface of the McMurdo Ice Shelf surrounding Bratina Island (Victoria Land, Antarctica) lays a unique collection of aquatic communities (meltwater ponds) confined to a very small area. Many of the ponds are naturally isolated from one another and show striking physiochemical heterogeneity ranging from fresh water to hypersaline conditions, coupled with steep vertical stratifications of oxygen and salinity. These characteristics make the Bratina ponds the perfect model for investigating biogeochemistry at an extremely localized level by removing uncontrollable variables such as temperature and light

availability, and the simple food webs in the system (higher trophic levels are relatively scarce) enable comprehensive descriptions of biotas. This study aims to characterise physicochemical parameters controlling microbial community structure in relation to well-established intense geochemical gradients. Using a specially designed micromanipulator sampling system, sets of water samples were taken precisely across the geochemical gradient of seven selected ponds during '09-'10 mid-austral summer. This research employs a suite of genetic approaches (DNA fingerprinting, 454 pyrosequencing, and qPCR) matched with geochemistry (ICP/MS, nutrients, etc) to identify how the communities are structured in relation to their environment. Based on our preliminary data, DNA fingerprints from four selected ponds showed that geochemistry and physical attributes are statistically correlated with microbial community structure, and that oxygen concentration and conductivity explain most of the variation in the microbial populations; the results validate our initial hypothesis of geochemically-driven diversity in these pond communities. In addition to ongoing data analysis, an additional temporal study utilizing quantitative methods will provide specific information on how populations respond to changes in physicochemical conditions.

Abstract Category
01 Biogeochemistry

PS.01.003 CLIMATE AND NITROGEN AMENDMENT EFFECTS ON THE CH₄-CYCLING MICROBIAL COMMUNITY OF A PINE FOREST

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Methane (CH₄) consumption in upland soil systems is primarily performed at atmospheric [CH₄] by uncultured methanotrophs. The growth rate of the atmospheric [CH₄] has become erratic in the past 20 years after several decades of monotonic increase. This could indicate a shift in the net methane flux of well-drained ecosystems that consume methane and/or poorly-drained that release methane. A study site was chosen in the Pinelands of NJ, USA, with a continuous canopy cover over well-drained and poorly-drained plots, with similar inclines, not more than 40 m apart. This site was chosen to investigate the effect that differences in drainage, variation in climate, and addition of nitrogen would have on the methane-cycling community size, diversity and function. Sites were established in 2007 and were monitored year-round with thermocouples and moisture probes. Chamber bases were implanted into the soil in the center of each plot. Methane flux was quantified by closing the chambers, collecting air within tedlar bags at set intervals, and running those samples on a gas chromatograph-flame ionization detector. Soil cores were extracted and frozen on each day of gas measurement. In order to investigate the diversity and relative abundance of the microorganisms associated with observed variation in methane flux, PCR of bacterial and archaeal 16S rRNA genes was coupled with DNA microarray analysis using the Phylochip. Three years of research has shown massive and variable methane flux into and out of the soil. Both the well- and poorly-drained sites are capable of consuming and releasing large quantities. Clear correlations have emerged between soil methane consumption and lower soil moisture, precipitation and inorganic nitrogen. Varied responses to nitrogen addition hint at the role of ammonia oxidizing bacteria in the methane cycle. The soil

selected for Phylochip has shown the most diverse methane flux profiles across years, seasons, and nitrogen addition levels.

Abstract Category
01 Biogeochemistry

**PS.01.004 TOXICITY OF AMENDMENT FOAM TECHNOLOGY ON
SHEWANELLA ONEIDENSIS MR-1**

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In situ remediation strategies involving water-based delivery of remedial amendments such as calcium polysulfide (CPS) to the vadose zone or groundwater have the potential to mobilize contaminant plumes in the environment. In addition, preferential flow paths observed in the vadose zone can limit the distribution of the remedial amendment, often bypassing low permeability zones in which majority of contaminants might be located. Foam-based delivery of remedial amendments can be used to evenly distribute the remedial amendment throughout the vadose zone and potentially saturate low permeability zones. However, little is known about the effects of this technology on the indigenous microorganisms at contaminated sites. While it is possible that CPS and/or foam-based delivery technology may be inadvertently used as an electron donor or acceptor during remediation strategies, these amendments may, in fact, be toxic to native microbial communities. A series of bench-scale experiments were designed to determine the effects of foam amendment technology on bacterial cells. *Shewanella oneidensis* MR-1 was chosen as a candidate organism for these studies because of its ability to respire metals and radionuclides under anoxic conditions as well as its ability to inhabit an oxic environment like the vadose zone targeted in this study. *S. oneidensis* MR-1 cultures were exposed to different concentrations of the remedial amendment CPS and sodium lauryl sulfate (SLS) surfactant (i.e., foam). Experiments focused on the toxicity of CPS and SLS on *S. oneidensis* MR-1 as well as the ability of the bacteria to use CPS as a fortuitous electron acceptor. Data from these experiments provide insight into the interactions between the indigenous microorganisms of the vadose zone, surfactant and remedial amendment.

Abstract Category
01 Biogeochemistry

**PS.01.005 SULFATE-REDUCING BACTERIA ATTENUATE ACID MINE
DRAINAGE**

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Acid mine drainage persists as mining worldwide continues. It is an acidic, iron-rich leachate that causes the dissolution of metals. At our study site, pyritic Davis mine in Rowe, Massachusetts, the acid mine drainage is naturally attenuated. We hypothesize that the attenuation of acid mine drainage is chiefly facilitated by sulfate-reducing bacteria found in anoxic sediment at our site because sulfate reduction decreases acidity leading to the

precipitation of dissolved metals. To quantify the mechanisms by which sulfate-reducing bacteria attenuate acid mine drainage, a suite of molecular and geochemical techniques were applied to a series of microcosms representing the site. The microcosms revealed that the sulfate-reducing bacteria can be stimulated to attenuate acid mine drainage which was demonstrated by the increase in pH, decrease in oxidation-reduction potential, and production of sulfides and ferrous ions. Ferrous sulfide precipitates forming in the microcosms were detected by x-ray diffraction and electron microprobe analysis. The rate of sulfate reduction in the microcosms stimulated with glycerol and micronutrients was higher than the rate in unamended microcosms and killed controls as measured from distilled radiolabeled sulfides. This increased rate of sulfate reduction coincided with a greater number of sulfate-reducing bacteria determined by QPCR of the dissimilatory sulfite reductase gene. In addition, the production of sulfide increased both the pH and ferrous ion concentration. Similarly, inhibition of sulfate reducing bacteria produced the opposite trends for all mentioned parameters. Molybdate as a specific inhibitor of sulfate reduction as well as nitrate as a non-enzymatic inhibitor of sulfate and iron reduction both resulted in no increase of pH, no decrease of oxidation-reduction potential, nor production of ferrous ions. The findings of this study demonstrate that sulfate-reducing bacteria attenuate acid mine drainage.

Abstract Category
01 Biogeochemistry

PS.01.006 THE ROLE OF PLANTS IN REGULATING GROSS NITROGEN TURNOVER RATES

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The role of plants in regulating N dynamics is ambiguous. On one hand, by exuding easily available carbon compounds plants create an environment with a high microbial biomass and activity in the proximity to the roots, and consequently, high N mineralization rates. On the other hand, a high microbial activity results in a high microbial N demand and there are some evidence suggesting that plants and soil microorganisms compete for N.

In order to resolve this ambiguity and determine the role of plants in regulating N dynamics we designed an experiment where Ponderosa Pine, Sitka Spruce and Western Hemlock seedlings were grown in separate boxes. Each box was divided into one compartment containing the plant and its roots, separated from a second compartment by a membrane that excluded roots and mycorrhiza but still allowed for free movement of the soil solution. The boxes were incubated in a ¹³C₂ enriched atmosphere and soil samples collected from the two compartments 6 days after the pulse labelling. The rate of root exudation was then estimated by the amount of ¹³C recovered in the respective compartment.

The gross N immobilization and mineralization was directly related to the rate of root exudation, suggesting top-down regulation of N turnover in the proximity of roots. At low exudation rates and the absence of roots, the N mineralization was sufficient to meet the N demand. On the other hand, the combination of high root exudation rates and the presence of plant roots seemed to

cause a situation where the N demand exceeded the rate of mineralization. The findings suggest that plants may create a situation where they compete with microorganisms for N. However, the mineralization rate, i.e. the production of plant available N per time unit, was always higher in the presence of roots. Thus, given the order of magnitudes longer lifespan of trees compared to soil microorganisms, creating a seemingly competitive situation may still be an evolutionary sound strategy.

Abstract Category
01 Biogeochemistry

PS.01.007 GLOBAL PATTERNS IN BACTERIAL AND ARCHAEAL COMMUNITIES ASSOCIATED WITH MARINE HOT SPOTS OF THE ANAEROBIC OXIDATION OF METHANE

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Cold seeps, sulfate-methane transition zones and some hot vents have been identified as submarine habitats characterized by the anaerobic oxidation of methane (AOM) as the dominating biogeochemical process. To search for specific signatures of bacterial and archaeal communities associated with AOM hot spots, environmental samples from the Gulf of Mexico, Guaymas Basin, the Haakon Mosby Mud Volcano, Aarhus Bay, New Zealand, Costa Rica, Japan, Kongo, North Sea, Black Sea, Antarctica, Hydrate Ridge, North Carolina and the Storegga Seep off Norway were included in a comparative biodiversity survey. The V6 region of the 16S rRNA was PCR amplified from DNA extracted from these environments and subjected to massively parallel pyrosequencing. A total of 478,594 bacterial and 472,320 archaeal sequences were examined. Generally, the bacterial and archaeal communities show a high similarity on the phylum level, indicating that AOM zones select for distinct microbial associations globally. However, for the archaeal communities, the estuarine sediments from North Carolina, the Black Sea microbial mats and the Guaymas hot vent sediments appear distinct from the other environments. This may be due to a combination of the presence of ANME1 and Crenarchaeota in these environments, phyla which are not largely abundant in the other environments. For the bacterial communities, the Weddell Sea extinct seep in Antarctica, the Aarhus Bay and hot Guaymas samples are distinct from the other sites, potentially due to the presence of JS1 bacteria at the Weddell seep and Guaymas and the dominance of Firmicutes in Aarhus Bay. Further statistical analyses of the relationships between abundant and rare microbial types from similar geochemical regimes across the globe will be presented.

Abstract Category
01 Biogeochemistry

PS.01.008 MICROBIAL DIVERSITY AS A CONTROLLING FACTOR OF AEROBIC METHANE CONSUMPTION

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Aerobic methane oxidizing bacteria (MOB) play a vital role in the global climate by degrading the greenhouse gas CH₄. The process of CH₄ consumption is sensitive to disturbance leading to strong variability in CH₄ emission from ecosystems. Mechanistic explanations for variability in CH₄ emission from soil and sediment ecosystems may be found in the diversity and ecology of these microbes. The objective of the presented work is to link the community composition and ecology of these microbes to the environmental variability observed in CH₄ consumption.

Methane consumption was investigated in a river floodplain along the river Rhine. Methane oxidation kinetics were determined in vitro in slurry incubations as well as on intact cores. Methanotrophic community composition was assessed using pmoA-based micro array and QPCR on mRNA as well as DNA. Stable isotope probing (SIP) of lipids and mRNA was applied to detect the active methanotrophic species.

The flooding regime in the Rhine floodplain established a distinct CH₄ consumption pattern with a maximum exactly in the part of the floodplain between permanently and irregularly flooded sites. This pattern was mirrored by the MOB community composition. Diversity index as assessed by micro array and activity components (initial consumption, V_{max}, V_{max}/K_m) were positively correlated. These analyses as well as SIP showed that γ -proteobacterial MOB were responsible for the observed kinetics with a distinct optimum in the gradient whereas α -proteobacterial MOB increased with decreasing flooding intensity.

In general it can be concluded that the environmental disturbances shaped the CH₄-consuming microbial community leading to differential eco-distribution of MOB. The relative abundance of specific subgroups controlled CH₄ consuming activity which makes it evident that knowledge on the microbial community composition is necessary to predict effects of environmental change on methane cycling.

Abstract Category
01 Biogeochemistry

PS.01.009 WHAT IS THE PHYSIOLOGICAL RESPONSE OF MICROBES TO DROUGHT?

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Microbes cope with environmental changes by physiologically adapting to cues such as temperature and moisture. These responses happen at the molecular scale, but ultimately serve as controls on ecosystem-level biogeochemical cycles. One example of this phenomenon is the buildup of summer-time microbial biomass in a dry California grassland. Our aim was to chemically characterize the increase in biomass with the hypothesis that it was due to the accumulation of osmolytes, cellular compounds used to prevent desiccation. Beginning in summer 2007, we made monthly measurements of soil moisture, total dissolved organic C and N, microbial biomass C and N and individual amino acids in total soil and in microbial biomass. We incubated samples in the lab to quantify C mineralization on rewetting. Over the summer, microbial biomass carbon went up by 62%. We expected the added biomass was due to the buildup of glutamate and proline, known osmotic compounds. However, proline was rarely

present, and while glutamate concentration did increase (by 3.4 $\mu\text{g C/g}$ dry soil over summer) it did not account for the rise in biomass carbon. The laboratory incubation showed that as summer soils dry the amount of bioavailable carbon increased. While there may be summer-time microbial osmoregulation, the chemical nature of that material is not amino acids, as culture studies suggested it should be. Work is underway to quantify other putative osmolytes, e.g. trimethylglycine, as well as to profile the other constituents of the microbial metabolome. By chemically characterizing the physiological response of microbes to drought, we gain insight on the energetic costs of adapting to drought, and can extrapolate how this may translate into ecosystem-level C and N cycling patterns. With the predicted increase of drought frequency and severity in the future, it is critical to understand the microbial controls on these processes.

Abstract Category
01 Biogeochemistry

PS.01.010 HYDROGEN ECOLOGY AND CYANOBACTERIAL-BACTERIAL INTERACTIONS IN BENTHIC MARINE MICROBIAL MATS

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Microbial mats are compact, stratified microbial communities that are exposed to immense light and chemical variations over a day-night cycle. In mats from Elkhorn Slough, CA amounts of hydrogen (H_2) emitted to the atmosphere under dark, anoxic conditions are orders of magnitude higher than under daylight conditions. The vast majority of H_2 originates from the upper phototrophic strata. Our goal is to understand the ecophysiological determinants on H_2 production and identify microorganisms that produce and consume H_2 in these systems. Depriving the mat of light or inhibiting oxygenic photosynthesis with DCMU during the day, resulted in reduced H_2 production during the following night (~20-fold and ~4-fold respectively). This suggests that oxygenic phototrophs are key H_2 -producers. Small subunit rDNA and rRNA pyrosequencing of ~3K OTUs in nighttime samples revealed the presence of a diversity of *Bacteria* and *Eukarya* (no *Archaea*). However, *Microcoleus chthonoplastes* seemed to be the dominant active microorganism. The H_2 -evolving H_2 -ase of *M. chthonoplastes* is up-regulated 3-fold at night compared to the day as determined using a tiled H_2 -ase microarray (~44K probes targeting ~1.2K H_2 -ases). Spatial localization of H_2 -producers/consumers is an important determinant of net H_2 production, as homogenization of the mat (physical disruption of mat architecture) increased H_2 production ~2.5-fold. Addition of molybdate to inhibit sulfate-reducing bacteria (SRB) also increased net H_2 production ~2.5-fold, implying that SRBs are important H_2 -consumers. PCR-based studies of expressed H_2 -evolving H_2 -ases and biomarker genes of putative H_2 -consumers will complement efforts to identify microorganisms that modulate H_2 emission. Research into the importance of cyanobacterial-bacterial interactions in H_2 ecology is progressing via nanoscale-imaging using a combination of stable isotope probing and *in situ* hybridization with 16S rRNA-targeted probes.

Abstract Category
01 Biogeochemistry

PS.01.011 COMPARATIVE GENOMICS AND PHYSIOLOGY OF FOUR NEUTROPHILIC NITRATE-DEPENDENT FE(II) OXIDIZERS

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Nitrate-dependent Fe(II)-oxidizing bacteria are ubiquitous and diverse. Completed or draft genome sequences are now available for *Acidovorax ebreus* strain TPSY, *Azospira suillum* strain PS, *Dechloromonas aromatica* strain RCB, and *Pseudogulbenkiania ferrooxidans* strain 2002. All members of the Betaproteobacteria, strains PS and RCB belonging to the order of Rhodocyclales, strain TPSY is a member of the Comamonaceae and strain 2002 the Neisseriales. All four strains have been demonstrated to grow mixotrophically with Fe(II), nitrate and acetate, but only strain 2002 grew lithoautotrophically with Fe(II), CO₂, and nitrate. Strain 2002 and TPSY oxidized U(IV) to soluble U(VI) coupled to nitrate reduction. Little to nothing is known about the biochemistry and genetics which govern neutrophilic nitrate-dependent Fe(II)-oxidation and it is hoped a comparative approach to this metabolism will aid the determination of the pathway(s) in these strains. A comparison of cytochrome content revealed strain 2002 had approximately 40 cytochrome domain-containing proteins, not differing significantly in numbers from the majority of known iron metabolizing organisms, but appeared to have a more extensive suite of cytochromes including B5 and Ni-Fe hydrogenase b-type cytochrome which may have a role in U(IV) oxidation observed for *Thiobacillus denitrificans*. Previous studies have indicated that c-type cytochromes play a significant role in microbial Fe(III) reduction and in phototrophic/aerobic acidophilic Fe(II) oxidation. A number of genes encoding candidate proteins with similarity to PioABC and MtrABC were present in these strains. These proteins, particularly MtrABC, are involved in Fe(III) respiration by *Shewanella* spp. Interestingly, none of the strains were capable of Fe(III) reduction suggesting that the MtrABC-like proteins may be involved in Fe(II) oxidation in a reverse pathway.

Abstract Category
01 Biogeochemistry

PS.01.012 CHEMICAL AND MOLECULAR CHARACTERISATION OF COASTAL SAND DUNE PROFILES IN SOUTHEAST QUEENSLAND, AUSTRALIA

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Sand dunes are widespread along over 30,000 km long coast in Australia and are important habitats for the coastal fauna and vegetations. The objective of this study was to examine the chemical characteristics through the soil profile and their associations with microbial community composition to improve the understanding of soil forming processes. Two sand dune soil profiles (Podzols in Australia, or Spodosols, USDA) were selected in the Cooloola area of southeast Queensland for this study. A total of eleven soil samples were collected from the two profiles

and subject to analysis of total soil C and N, delta 13C and delta 15N (by mass spectrometer), P, S, Fe, Al, Mg, K, Ca, Mn, Cu, B, Mo, Na, Zn (by ICP-AES) and pH (water). The soil DNA was extracted using Powersoil DNA Isolation Kit (Mo Bio) and 16 rRNA gene was amplified by PCR (nested) using 9bfm and 1512uR PCR primers. DGGE was performed using a DCode Universal Mutation Detection System (Bio-Rad). Data on the DGGE band pattern and Intensity and on the chemical parameters were analysed by principle component analysis and redundancy analysis. Results have shown clear downward translocation processes in organic compounds, Fe, Al, Ca, K and Mg through the soil profile. The soil profile was characterised by the distinct B horizons (dark brown /light brown sand with patches of organic and Al/Fe compounds). The typical B horizons were characterised with high concentrations of C (2.82-5.06%), S (742-1072 mg kg⁻¹) and Al (912-2147 mg kg⁻¹), high C/N (69-85) and C/P (900-1658) ratios and low P/S ratio (0.03-0.04) and pH (3.73-4.92). The PCA analysis on DGGE data showed clear separation of the two profiles. The redundancy analysis indicated the C/N, C/P, C/S ratios and pH were predominant factors governing the DGGE patterns. It is concluded that the leaching of organic/Al compounds are the key process in determining the microbial community composition during the formation of the sand dune profile.

Abstract Category
01 Biogeochemistry

PS.01.013 ECOLOGICAL STOICHIOMETRY PREDICTS COMPLEX NUTRIENT RECYCLING IN MICROBIAL FOOD WEBS: FLAGELLATES SHIFT FROM N TO P RECYCLING AS GROWTH SLOWS

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Ecological stoichiometry focuses on the balance between multiple nutrient elements in resources and in the consumers of those resources. The major consumers of bacteria in aquatic food webs are the heterotrophic and mixotrophic flagellate protozoa. Despite the importance of this consumer-resource pair to nutrient dynamics in aquatic food webs, little data are available addressing those factors that may control nutrient regeneration by flagellate predators of bacteria. *Ochromonas danica*, a mixotrophic bacterivore, was used as a model consumer to study the relationships among temperature, growth rate, and element stoichiometry while grazing on the bacterium *Pseudomonas fluorescens*. Both organisms were grown independently in chemostats at ecologically relevant growth rates (doubling times between ~5 h and 24 h) and temperatures (ranging between 15 and 28°C). The element composition of each organism was determined (as C, N, and P). A matrix was developed that predicts nutrient regeneration by *Ochromonas* growing at a given rate but grazing upon *Pseudomonas* growing over a range of rates. Nutrient regeneration matrices were generated for temperatures between 15 and 28°C. At 15°C, *Ochromonas* growing with doubling times between 12 and 24 h will regenerate P when grazing upon bacteria having doubling times ranging between 5 and 24 h. *Ochromonas* will shift to N regeneration when growing more rapidly (doubling times < 9 h). These relationships change as temperature rises, and ultimately, at 28°C, *Ochromonas* shifts to exclusively N regeneration. At low temperatures, nutrient regeneration was more a function of flagellate growth rate than bacterial growth rate.

Abstract Category
01 Biogeochemistry

PS.01.014 A NOVEL STABLE ISOTOPE LABELLING AND PROBING TECHNIQUE SUCCESSFULLY TRACES CARBON BELOWGROUND IN 22-YEAR OLD SITKA SPRUCE (*Picea sitchensis*)

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Plants provide nutrients to the soil microbial community through root exudates and litter; many soil microbes are highly dependent on this plant carbon (C) for growth and metabolism. In turn, plants are dependent on certain rhizosphere microbes for the breakdown of soil organic matter into plant-available nutrients, and for protection from soil pathogens. This research assesses the ability of a novel stable-isotope-labeling and probing technique to trace the flow of photosynthates from large trees into the roots and soil, and forms part of a study assessing the importance of retention trees in maintenance of soil organisms after harvest. This technique will enable us to observe flow of C belowground by measuring "spikes" in ¹³C levels in CO₂ efflux from soil, as well as, in future, into the microbial and faunal biomass and soil organic matter. Double-labeled aspartic acid (¹³C/¹⁵N) was injected directly into the phloem of three 22-year old Sitka spruce trees located at the edge of a plantation in Gisburn forest, Lancashire, UK. Soil ¹³CO₂ efflux was measured along a transect 0-50 meters from the base of each tree over the course of a month. Soil CO₂ was significantly enriched in ¹³C, relative to the baseline, with observable peaks close to the base of the tree on days 13-23 after labeling. These results suggest that stem injection is a viable option for measuring the flow of carbon belowground from mature trees. Previous research assessing rhizosphere C flow has been limited to smaller plants and seedlings, with the exception of FACE experiments; however, seedlings will likely interact with their environment differently than established trees. Mature trees have the potential to greatly influence above and belowground forest communities due to their developed root system, large canopy, and larger volume of photosynthates. Therefore, it is important to develop techniques that will allow us to explore C dynamics at a greater scale.

Abstract Category
01 Biogeochemistry

PS.01.015 SPATIAL AND TEMPORAL VARIABILITY OF MICROBIAL COMMUNITIES FROM PRE- AND POST-ERUPTION MICROBIAL MATS COLLECTED FROM LOIHI SEAMOUNT, HAWAII

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Loihi Seamount is an active submarine volcano that marks the southernmost extent of the Hawaiian hotspot. The volcano was discovered to be active in 1987, and diffuse hydrothermal vents (T_{max} 37°C) with high Fe(II) concentrations were discovered near the summit of the volcano with thick (2-10 cm) iron-oxide encrusted microbial mats growing at the vent orifices.

The Volcano erupted in 1996 forming a 250m deep caldera with hydrothermal venting up to 200°C. Loihi now contains multiple hydrothermal vents with fluids ranging from 8-65°C almost completely devoid of sulfide. In this study, we show how the microbial mat communities at these vents changed during the eruptive event and how they have transitioned as the vent fluids have cooled.

Community fingerprints from over 70 discrete microbial mat samples from 1993 to 2009 were analyzed using terminal restriction length polymorphisms coupled with clone library and group-specific quantitative PCR. The samples form two distinct clusters designated Loihi Cluster 1 and Loihi Cluster 2. Loihi Cluster 1 is the largest group and the communities are generally dominated (15-73%) by phylotypes belonging to the ζ -Proteobacteria indicating primary production fueled by iron oxidation. All pre-eruption and post-eruption communities with $T_{max} < 50^\circ\text{C}$ cluster within Loihi Cluster 1. Loihi Cluster 2 is comprised of only post-eruption samples with $T_{max} > 50^\circ\text{C}$ and are generally dominated (20-100%) by ϵ -Proteobacteria. The presence of ϵ -Proteobacteria in this group suggest these microbial mats are dominated by sulfur- or hydrogen-oxidizing primary production. These results indicate that microbial mats at Loihi Seamount transitioned from iron-oxidizing to sulfur- or hydrogen-oxidizing primary production shortly after the eruptive event transitioning back to iron-oxidation as the vents cooled and sulfide decreased. This represents an example of true community succession in chemoautotrophic communities at an active deep-sea volcano.

Abstract Category
01 Biogeochemistry

PS.01.016 INVESTIGATING BENTHIC NITROGEN FIXATION AT SITES OF NATURAL CARBON LOADING ALONG THE EAST PACIFIC MARGIN

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Nitrogen fixation, the biological conversion of N_2 to NH_3 , is mediated by select microorganisms to obtain nitrogen for growth. This process supplies a source of N to other organisms unable to assimilate N_2 directly, as well as closes the global N cycle by consuming N_2 produced by denitrification. Traditionally, N_2 fixation is thought to dominate in oligotrophic regions such as marine surface waters, while denitrification dominates in organic rich locations, such as heterotrophic sediments. Recently, several findings have suggested that N_2 fixation occurs in previously unexpected locations, such as marine sediments. In order to explore N_2 fixation further, we collected samples from four locations along the East Pacific Margin: Costa Rica (CR); Eel River Basin, California, USA (ERB); Monterey Bay Canyon, California, USA (MB); and Hydrate Ridge, Oregon, USA (HR). The CR, ERB, and HR sites are active methane seeps. Methane seeps have previously been suggested as sites of N_2 fixation, potentially caused by N limitation due to the unusually high carbon input unaccompanied by nitrogen. The MB samples were taken from three whalefall locations; sites where both C and N would be at least initially elevated due to the degrading whale biomass. Anaerobic incubations of bulk sediment from these sites with $^{15}\text{N}_2$ or $^{15}\text{NH}_4^+$ were conducted under a variety of conditions. Bulk Elemental Analysis as well as cell-specific Fluorescence *in situ* Hybridization coupled to nanoscale Secondary Ion

Mass Spectrometry (FISH-NanoSIMS) were employed to track ^{15}N into biomass as a proxy for N_2 fixation and growth. Results indicate variable N_2 fixation rates between and within sites, a strong increase in N_2 fixation in the presence of methane (>10 fold), an inhibition of N_2 fixation by high levels (2 mM) of ammonium, and variable growth between the ANME-1 and ANME-2 subgroups in the same incubation conditions.

Abstract Category
01 Biogeochemistry

PS.01.017 ANAEROBIC OXIDATION OF METHANE IN LAKE CONSTANCE SEDIMENTS

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Freshwater lakes contribute with 2-10% to the total emissions of the potent greenhouse gas methane. In Lake Constance aerobic oxidation of methane has been described extensively but anaerobic oxidation of methane remained cryptic. Anaerobic oxidation of methane with sulfate as terminal electron acceptor has been reported in various environments including freshwater habitats. Recently also nitrate and nitrite were shown to act as electron acceptors for methane oxidation in eutrophic freshwater habitats, and bacteria belonging to the NC10 phylum are most likely accountable for this process. The aim of this study was to check for anaerobic oxidation of methane in Lake Constance and to reveal which electron acceptors are used.

We performed tracer experiments to follow $^{14}\text{CO}_2$ formation from $^{14}\text{CH}_4$ in sediment incubations in the presence of different electron acceptors, namely nitrate, nitrite, and sulfate. The diversity of NC10 bacteria was assessed via clone libraries, and T-RFLP profiles were used to compare the community composition between different sediments. No evidence for sulfate-dependent methane oxidation was found, but addition of nitrate significantly increased $^{14}\text{CO}_2$ formation in incubations of profundal sediment. In addition, bacteria of the NC10 phylum were detected in Lake Constance sediments and the community structure differed between profundal and littoral sediments.

These results clearly indicate that Lake Constance sediments have the potential for anaerobic methane oxidation coupled to denitrification. This process seems to be more important in profundal sediments than in the littoral zone, and the differences in the community structure of the NC10 bacteria may reflect this disparity. Anaerobic oxidation of methane *in-situ* is possibly often masked by aerobic methane oxidation in oligotrophic habitats due to the close spatial proximity of the reactant transition zones but may still play a significant role in mitigating methane emissions.

Abstract Category
01 Biogeochemistry

PS.01.018 DNA, rRNA AND mRNA-BASED STABLE ISOTOPE PROBING OF METHANOTROPHS IN LAKE SEDIMENT

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Methane oxidizing bacteria (methanotrophs) mitigate the release of methane from numerous environments, including lakes. In this study we investigated the diversity and activity of methanotrophs in oligotrophic Lake Stechlin (Germany) sediment using stable isotope probing (SIP) approaches. In addition to DNA-SIP and rRNA-SIP, which are well established techniques, we show that mRNA-SIP is also a viable approach to examine active methanotrophs. 16S rRNA and *pmoA*, which encodes a subunit of the methane monooxygenase enzyme, were analysed following the incubation of sediment with ¹³C-methane and the separation of ¹³C-labelled DNA and RNA from unlabelled nucleic acids. The incubation with ¹³C-methane was performed over a 4 day time course and the *pmoA* genes and transcripts became progressively labelled over the incubation such that approximately 60% of the *pmoA* genes and 80% of the transcripts were labelled at 96 hours. The labelling of *pmoA* mRNA was quicker than *pmoA* genes, demonstrating that mRNA-SIP is more sensitive than DNA-SIP. The presence of unlabelled *pmoA* transcripts at 96 hours suggests that some methanotrophs were active but not incorporating ¹³C. Although type II methanotrophs were found in low abundance at the start of the incubation, they were labelled during the incubation indicating that this population is present in the sediment and can be rapidly stimulated. The correlation between the abundance of labelled *pmoA* genes and transcripts suggested that type Ia methanotrophs in Lake Stechlin sediment possess higher cellular *pmoA* transcript abundances than do the type Ib and type II methanotrophs; this suggests that the relative abundance of *pmoA* transcripts between methanotroph genera may not reflect their relative activity. In general, we show that DNA, rRNA and mRNA-SIP are all valuable tools for examining the activity of methanotrophs in the environment and that each can provide useful complementary information.

Abstract Category
01 Biogeochemistry

**PS.01.019 MERCURY METHYLATING MICROBIOTA ASSOCIATED WITH
MERCURY CONTAMINATED STREAM SEDIMENTS**

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High concentrations of Hg(II) and methyl mercury (MeHg) in streams at the Department of Energy (DOE) reservation in Oak Ridge, TN, are an ongoing concern. Sulfate-reducing bacteria are the primary methylators, but other methylators and the mechanism of MeHg formation are unknown. Sixty sediment samples were collected from five contaminated sites over two years and analyzed for bacterial community composition and geochemistry, respectively. Community characterization via GS 454 FLX pyrosequencing resulted in 588,699 sequences targeting the 16S rDNA V4 region. The community was represented by 24 phyla and unclassified Bacteria including *Proteobacteria* (22.9-58.5%), *Cyanobacteria* (0.2-32.0%), *Verrucomicrobia* (3.4-31.0%), *Acidobacteria* (1.6-30.6%), *Bacteroidetes* (0.03-14.4%), *Chloroflexi* (0.3-6.3%), *Firmicutes* (0.03-5.2%), *Planctomycetes* (0.03-3.9%), *Gemmatimonadetes* (0.05-1.3%), WS3 (0.01-1.1%), OD1 (0.04-0.8%), *Actinobacteria* (0.01-0.8%), and TM7 (0.01-0.2%). Redundancy analysis indicated significant correlations between community structure and both seasonal and geochemical variation. Of the known methylators, only 3064 sequences (0.52 % of the total)

were identified as *Deltaproteobacteria* by the RDP classifier at the 99% confidence threshold. Clustering at a 97% similarity cut off indicated that the most prevalent *Deltaproteobacteria* 16S sequences (a cluster of 497 sequences) were similar to *Desulfobulbus propionicus*. The next largest group (197) was most similar to an uncultured *Desulfobulbus*. Constrained ordination techniques indicated significant positive correlations between *Desulfobulbus* spp. and methyl mercury concentrations in the contaminated sites. Isolation of *Desulfobulbus*-like microorganisms has thus far yielded 5 species that are currently being tested for Hg methylation and demethylation capabilities. This is the first study to demonstrate a positive relationship between MeHg at a field site and specific members of the *in-situ* microbial community.

Abstract Category
01 Biogeochemistry

PS.01.020 AN INTEGRATED STUDY OF THE INFLUENCE OF NITROGEN ADDITIONS ON SOIL BACTERIAL COMMUNITY STRUCTURE AND FUNCTION

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Ecosystems worldwide are receiving increasing amounts of reactive nitrogen (N) through anthropogenic activities. Although the effects of increased N inputs on plant communities have been reasonably well studied, few comparable studies have examined impacts on whole soil bacterial communities, though they play critical roles in ecosystem functioning. We sampled soils from two experimental N gradients in contrasting ecosystem types, a grassland and an agricultural field. By examining shifts in bacterial communities across these contrasting ecosystem types using a variety of approaches (barcoded 16S rRNA pyrosequencing, catabolic profiling, and comparative metagenomics), we could test competing hypotheses about the direct and indirect factors that might drive bacterial responses to elevated N inputs. Both the phylogenetic and functional characteristics of soil bacterial communities were highly responsive to N additions. We observed predictable and consistent changes in the phylogenetic structure of the bacterial communities across both ecosystem types, changes that were closely associated with shifts in bacterial carbon dynamics and metagenomic profiles. These results suggest that bacterial community composition and function are more structured by the indirect effects of N additions on soil carbon availability (via an increase in plant productivity), than by the direct effects of N additions, a pattern that was confirmed with a microcosm study conducted with a broader array of soil types.

Abstract Category
01 Biogeochemistry

PS.01.021 VARIATIONS IN THE ACTIVE BACTERIAL COMMUNITY WITH GROUNDWATER CHEMISTRY IN A PRISTINE AQUIFER

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Bacteria in anoxic aquifers catalyze many critical biogeochemical reactions, yet little is known about how the composition of the active community correlates with groundwater chemistry. In this study, we compare bacterial communities in the Mahomet aquifer of east-central Illinois using 16S rDNA-based methods. Using terminal restriction fragment length polymorphism (T-RFLP) and clone libraries, we examine how the distribution of active bacteria attached to in situ sediment samplers varies with the chemistry of groundwater. Although ferrous iron concentrations in Mahomet groundwater are, on average, one hundred times greater than sulfide concentrations, we observe no correlation between the concentration of either species and community structure. Instead of being composed predominantly of iron-reducing bacteria as might be expected from the iron-rich groundwater, both iron reducers and sulfate reducers comprise a significant proportion of the 2554 16S rDNA sequences we analyzed from 12 wells across the aquifer. By quantifying differences in T-RFLP profiles between wells using multivariate statistics, we find that bacterial communities from areas of the Mahomet with high sulfate concentrations are more similar to each other than to communities from areas with low sulfate concentrations. Clones related to *Desulfobacter* and *Desulfobulbus* spp., known sulfate reducers, are more predominant at high sulfate concentrations (21%) than low (7-9%). Conversely, populations closely related to iron-reducing *Geobacter*, *Geothrix*, and *Desulfuromonas* spp. are more prevalent in areas of low sulfate concentrations (41-51% of the total clones) than high (27%). Iron reducers are more abundant when sulfate is limited, but sulfate reducers comprise an equivalent proportion of the microbial community in high sulfate areas. This suggests that rather than being excluded by iron reducers, sulfate reducers can be abundant in aquifers with iron-rich groundwater.

Abstract Category
01 Biogeochemistry

PS.01.022 TEMPERATURE SENSITIVITY OF MICROBIAL EFFICIENCY AND IMPLICATIONS FOR SOIL CARBON STORAGE

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The objectives of this study were to examine the temperature sensitivity of microbial growth efficiency in soils subjected to chronic warming and to evaluate the potential impact of altered efficiencies for soil C storage using the DAYCENT soil organic matter model. Soil samples were collected from the Soil Warming x Nitrogen Addition Study at Harvard Forest in Petersham, MA, USA. Microbial efficiency was determined in control and heated soils by amending samples with ¹³C-labeled glucose or phenol, incubating at three temperatures (5, 15 and 25°C) and tracing the ¹³C into microbial biomass, DOC, and respired CO₂. Microbial efficiency of glucose-amended soil was temperature insensitive. In contrast, the efficiency of phenol utilization exhibited a strong temperature response. Phenol-amended soils had much lower efficiencies compared to glucose (12-32%) and showed nearly a 60% drop in efficiency when incubated at 25 versus 5°C. The effect of temperature on efficiency was not altered by chronic soil warming. Reducing the efficiency parameter in DAYCENT by a comparable degree as observed in our experiment (for phenol) resulted in a loss of about 400 g C m⁻² over a six year period. The decrease in efficiency led to a decreased microbial biomass due to more C lost as

CO₂ during organic matter decomposition. When soil warming was applied in the model, more soil C was lost for a comparable decrease in efficiency. Our results suggest that the degree of compound lability determines the temperature sensitivity of microbial efficiency. Regardless of compound lability, soil warming did not directly affect the temperature sensitivity of microbial efficiency. However, long-term warming may alter compound lability in the soil and thus determine the direction of warming effects on both microbial communities and soil C storage.

Abstract Category
01 Biogeochemistry

PS.01.023 MOLECULAR, GENETIC AND PHYSIOLOGICAL CONTROL OF MANGANESE (II) OXIDATION IN PROTEOBACTERIA

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Due to their high sorptive capacity, manganese (III, IV) oxides have a profound impact on the geochemical cycling of metals. By catalyzing both oxidation and reduction of Mn, bacteria influence the levels of Mn(III, IV) oxides in the environment. In this work, Mn(II) oxidation by the proteobacteria *Aurantimonas manganoxydans* SI85-9A1 and *Pseudomonas putida* GB-1 was studied using both molecular genetic techniques and in vitro activity assays. In *P. putida* GB-1 a two-component regulatory pathway is essential for Mn(II) oxidation, while the flagellar regulatory pathway and a small, RNA-binding protein implicated in cell density sensing in *Pseudomonas aeruginosa* PAO1 have been shown to also regulate oxidation. A mutant strain of *P. putida* GB-1 has been isolated that fails to oxidize Mn(II) except when grown in close proximity to wild-type bacteria. This activation of oxidation was shown to be due to a secreted protein whose identity has yet to be determined. For both *P. putida* GB-1 and *A. manganoxydans* SI85-9A1, the Mn(II)-oxidase activity has been localized to the outer membrane fraction and loosely-bound outer membrane fraction, respectively, using cellular fractionation and separation of membrane components. In vitro assays examining oxidation in the partially purified fractions are being developed to probe the molecular requirements of Mn(II) oxidation. In *A. manganoxydans* SI85-9A1, the addition of antioxidant enzymes such as superoxide dismutase and catalase disrupt manganese oxidation, while the addition of hydrogen peroxide and calcium enhance it. In *P. putida* GB-1, hydrogen peroxide, NADH and NAD⁺ stimulate oxidation while copper, superoxide dismutase, and catalase inhibit it. Calcium does not appear to affect oxidation in *P. putida* GB-1 extracts. These results suggest that reactive oxygen species may be involved in Mn(II) oxidation and that, at least in *P. putida* GB-1, oxidation is tightly regulated.

Abstract Category
01 Biogeochemistry

PS.01.024 MICROBIAL COMMUNITY STRUCTURE AND ACTIVITY DURING AMENDMENT WITH LONG-TERM ELECTRON DONOR SOURCES FOR BIOREDUCTION OF GROUNDWATER CONTAMINANTS

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Longer-term maintenance of reduced zones is considered a primary impediment to a biostimulation approach to uranium-contaminated groundwater remediation. Here we present an experiment exploring the use of slow-release substrates (SRS) to achieve longer periods of sustained U bioimmobilization. Objectives included tracking microbial populations as the subsurface manipulation zone transitioned through reduction and re-oxidation phases, correlating microbial and geochemical observations, and thereby improving predictions of the long-term effectiveness of remediation activities. SRS, consisting mainly of emulsified vegetable oil, was injected once into a contaminant plume which was sampled via monitoring wells for over 12 months. Prokaryotic communities were characterized using 16S rRNA gene pyrosequencing and functional gene analyses. The subsurface amendment of SRS resulted in sequential reduction of nitrate, Mn(IV), Fe(III), and sulfate over the first 20 days; soluble [U] decreased by >80% for up to 4 months and remained at <50% of original levels after >1 year. Groundwater bacterial community richness and diversity decreased sharply after SRS amendment, then slowly increased as SRS was consumed and re-oxidation occurred. Members of the *Veillonellaceae* and *Desulfobacteraceae* families were identified as key taxa for initial oil degradation (triglyceride hydrolysis and long-chain fatty acid-oxidation) and providing a sustained source of labile electron donors critical to maintaining reduced conditions. Sulfate (*Desulforegula*, *Desulfovibrio*) and iron (*Geobacter*) reducers predominated and presumably acted syntrophically to achieve long-term U reduction. H₂-oxidizing, methanogenic archaea flourished after other electron donors and acceptors were largely depleted. Although diversity rebounded, community compositions remained distinct from the initial, pre-amendment samples, indicative of a lasting effect on bacterial and archaeal community structures for > 9 months.

Abstract Category
01 Biogeochemistry

PS.01.025 THE DIVERSITY AND ACTIVITY OF METHANOGENS IN A WET, TROPICAL FOREST DURING PLANT DECOMPOSITION

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Methanogens in tropical soils play an important role in methane-based climate forcing. Methanogens in the wet, tropical Luquillo Experimental Forest in Puerto Rico produce methane during persistent oxic-anoxic fluctuations in soil, leading to methane efflux. Our goals were to assess potential rates of soil methane production over an elevation transect spanning three forest types and to assess methane production dynamics during plant decomposition in this soil. To investigate the diversity and activity of methanogens in Luquillo, anoxic microcosms were set up with soil from 0-10 and 10-20cm from the Lower, Middle and Upper sites and methane was monitored over time. Following consistent methane production, RNA was extracted from each microcosm to be used to construct a clone library of mcrA. Difluoromethane and ¹³C-labeled

plant material were added to additional microcosms from the Middle site under three environmental conditions; consistent oxic, consistent anoxic and four day oxic-anoxic flux. Methane was monitored every four days and RNA was extracted from microcosms destructively sampled throughout the experiment. The abundance of *mcrA* and bacterial and archaeal 16S in both sets of microcosms were measured using qPCR. Methanogens in the Upper and Middle sites produced more methane than those in the Lower site. The methanogens at 0-10cm from the Upper site had higher rates of methane production than those at 10-20cm. Methanogens in the plant-amended anoxic condition microcosms produced more methane than in the oxic and fluctuating microcosms. Methane production in the plant-amended oxic and anoxic portions of the fluctuating condition mirrored the methane production in the consistent oxic and anoxic conditions, respectively. Based on these data, more methane is produced at the higher elevation sites in Luquillo and the persistent fluctuating condition does not lead to more methane production.

Abstract Category
01 Biogeochemistry

**PS.01.026 DISSECTING THE CARBON CYCLE BENEATH OUR FEET:
QUANTIFYING THE ROLE OF MICROBIAL DECOMPOSERS OF PLANT LITTER
INPUTS TO SOIL**

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The most important zone in the soil in terms of the soil carbon cycle is that influenced by the presence and activity of plant roots, known as rhizosphere. Plant root litter in the soil results in greater microbial activities and numbers than in non-planted soil with important consequences for plant nutrition and health. Microbial respiration of plant litter is also an important soil-to-atmosphere flux of carbon dioxide. However, our current knowledge of the carbon cycle beneath our feet and of the bacteria involved, is poor. Knowing how much rhizosphere carbon flow a particular microbial group is responsible for, and under which range of soil conditions, would enhance our ability to predict system resilience or how the system may respond to environmental change.

The overall aim of this project was to develop a new methodological approach to allocate C utilization to key bacterial groups in soil. The method involves the combination of the well-established techniques of the radioactive substrate labelling (^{14}C), Fluorescence In Situ Hybridisation and Flow Cytometry with the application of Fluorescence Activated Cell Sorting.

The new method revealed that the bacterial group pseudomonad represents 3.0-4.5% of the total bacteria extracted from intact soil systems planted with ryegrass. The pseudomonads are known to be important in terms of plant health. Cloning of the sorted pseudomonad fraction revealed >97% specificity which validated the new methodology.

This research has strategic relevance in that it develops a technique that has promise for broader future application. This is done namely by contributing to knowledge which will allow

landowners to reduce damaging emissions (CO₂) and non-renewable inputs (pesticides, fertilizers) and can therefore help satisfy environmental policy and consumer demands for a more sustainable agriculture.

Abstract Category
01 Biogeochemistry

PS.01.027 DENITRIFIER COMMUNITIES, DENITRIFICATION AND NITROUS OXIDE EMISSIONS IN AGRICULTURAL AND RIPARIAN ZONES

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Denitrification is a major contributor to the greenhouse gas nitrous oxide (N₂O). Riparian and agricultural zones differ in soil properties, soil water content, plant species, and nutrients that can influence denitrifier community dynamics, and therefore denitrification and N₂O emissions. Denitrifier community abundance, diversity and denitrification gene mRNA levels were quantified on three different dates in a corn field and riparian zone adjacent to the Thomas Brook in Nova Scotia, Canada. Sampling was done at eight positions along four transects spanning agricultural and riparian zones. The abundance of *nirK* denitrifiers was significantly higher in the riparian compared with the agricultural zone whereas *nirS* denitrifier abundance was lower in the riparian zone compared to the agricultural zone. The *nosZ* denitrifier abundance and *nosZ* gene mRNA levels did not show significant differences between zones. Diversity of *nirS* community structure showed significant differences between agricultural and riparian zones only in June (R = 0.22). In contrast, *nirK* community structure changed significantly between the riparian and agricultural zones at all dates (R = 0.25-0.31). N₂O emissions were higher from the agricultural zone compared to the riparian zone (3.7 vs 0.3 g N₂O-N ha⁻¹). Relationships between denitrifier community structure and environmental factors were explored using averages over transects. *nirK* community structure was most strongly related to soil temperature, soil nitrate concentration and N₂O emissions ($\rho = 0.445$). *nirS* denitrifiers structure was not closely related to environmental factors. The results indicate that the denitrifier communities targeted in this study responded differently to land use zones and that the denitrifier community abundance and diversity showed few changes despite differences in environmental and edaphic factors between the agricultural and riparian zones.

Abstract Category
01 Biogeochemistry

PS.01.028 CURRENT MOLECULAR METHODS THAT TARGET DENITRIFICATION GENES DO NOT DETECT KEY DENITRIFYING TAXA

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Our research indicates that PCR primer sets targeting denitrification genes systematically underestimate the diversity and abundance of denitrifying bacteria, and that geochemical, cultivation, genomic and metagenomic mining approaches are necessary to characterize denitrifying microbial communities. Our objective is the in situ characterization of denitrifiers in the contaminated terrestrial subsurface. Groundwater and sediment were recovered from the Oak Ridge Integrated Field Research Challenge (OR-IFRC) site and used to isolate denitrifying bacteria and genomic DNA. DNA extracts were analyzed using PCR, TRFLP, sequencing, and quantitative PCR targeting rRNA and nitrite- and nitrous-oxide reductase genes. Bacteria from 5 genera (*Afipia*, *Bacillus*, *Hyphomicrobium*, *Intrasporangium* and *Rhodanobacter*) within 3 phyla were isolated and subjected to genetic and phenotypic analyses. All of the isolates reduce nitrate to gaseous end products (N₂O, N₂) and show high rRNA gene sequence identity to gene sequences retrieved from the site. Denitrification genes were identified in draft genomes sequenced from 2 of the isolates (*Rhodanobacter*, *Intrasporangium*). Although all isolates were denitrifiers, we were limited in our ability to amplify their denitrification genes (*nirS*, *nirK*, *nosZ*) with commonly used primers. Comparison of genome sequences from known denitrifiers to primer sequences revealed a remarkable sequence divergence in *nirK* and *nosZ* targets, indicating that key denitrifying taxa would not be detected in environmental samples using current methods. Using newly designed primers that take advantage of our genome and metagenome data-mining efforts, quantitative molecular results indicated that members of the Gammaproteobacterial genus *Rhodanobacter* were abundant and broadly distributed in acidic samples from the OR-IFRC site. Our results indicate that current molecular methods used for determination of the abundance and diversity of denitrifiers should be re-evaluated.

Abstract Category
01 Biogeochemistry

PS.01.029 DIFFERENCES IN NITRATE USAGE BY COEXISTING SULFATE-REDUCING BACTERIA ASSOCIATED WITH ANAEROBIC METHANE-OXIDIZING ARCHAEA

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The anaerobic oxidation of methane serves as the primary biological sink for oceanic methane. Syntrophic associations between anaerobic methane oxidizing archaea (ANME) and sulfate-reducing bacteria (SRB) appear to carry out this process. In addition to the commonly observed *Desulfosarcina* (DSS), a SRB partner affiliated with the *Desulfobulbaceae* (DSB) has also been found in association with ANME. We used FISH to reveal that this association between ANME and DSB is robust across diverse methane seep habitats in the Eel River Basin in which it consistently co-occurs with ANME/DSS aggregates. We observed a trend towards higher DSB/ANME abundance in shallower sediment horizons where nitrate would most likely be available. Members of DSB group are capable of using nitrate as a terminal electron acceptor in

addition to sulfate while DSS are restricted to sulfate, introducing the possibility that ANME/DSB aggregates may be able to couple methane oxidation to the reduction of nitrate, defining a separate niche from that of ANME/DSS. In order to explore this possibility, we used nano-scale secondary ion mass spectrometry (nanoSIMS) to measure ¹⁵N incorporation by ANME/DSB versus ANME/DSS aggregates from the same ¹⁵N-labeled nitrate incubation. In addition to direct assimilation, the incorporation of nitrogen from nitrate can be explained by the fact that dissimilatory nitrate reduction by SRB produces ammonium, which provides a nitrogen source. We also measured ¹⁵N incorporation from ¹⁵N-labeled ammonium incubations. Preliminary results suggest a trend towards higher levels of ¹⁵N incorporation by ANME/DSB than by ANME/DSS aggregates from the ¹⁵N-labeled nitrate incubation. No trend was observed in ¹⁵N incorporation by ANME/DSB versus ANME/DSS aggregates from the ¹⁵N-labeled ammonium incubation. Results indicate ANME/DSB and ANME/DSS differ in their ability to either assimilate nitrate directly or use it a terminal electron acceptor.

Abstract Category
01 Biogeochemistry

PS.01.030 MICROBIOLOGY OF A MASSIVE IRON HYDROXIDE DEPOSIT ASSOCIATED WITH CIRCUMNEUTRAL COAL MINE DRAINAGE

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Bituminous coal mining in the Appalachian region during the 19th and 20th centuries has resulted in many abandoned mines. In southwest Pennsylvania, a large mineral deposit composed largely of iron hydroxides has formed at the drainage portal of the abandoned and flooded Ocean No. 2 bituminous coal mine. The mineral deposit forms as carbon dioxide degasses from the mine waters as they flow over a 10 m-high waterfall. Vertical curtains and laminations reminiscent of those observed in speleothem dripstones and flowstones are formed. The goal of our study is to characterize the mineralogy, geochemistry, and microbiology of the mine water and its associated mineral deposit. Here, we present the results of preliminary studies on the microbial community. Liquid, solid and semi-solid media enrichment methods demonstrated the presence of aerobic and microaerophilic iron-oxidizers as well as anaerobic sulfate-reducers associated with the deposit. The rapid burial of organic matter (leaves, etc.), analogous to that observed in calcareous tufa deposits, is believed to provide a source of subsurface labile carbon used by anaerobic bacteria. The distribution of microbes within the deposit is being analyzed by the sectioning of deposit material and direct cell visualization using fluorescent DNA stains and SEM. The community composition has also been studied using fluorescence in situ hybridization (FISH) and phospholipid fatty acids (PLFA). Procedural difficulties associated with the application of these methods to this particular environment will be presented. Preliminary results suggest an active and diverse microbial community associated with the mine drainage deposit.

Abstract Category
01 Biogeochemistry

PS.01.031 DISTRIBUTION AND DIVERSITY OF ARCHAEOAL COMMUNITIES IN SELECTED CHINESE SOILS

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Genomic studies for environments showed Archaea are global distributed not only in extreme environments, but also in moderate habitats, which implies that they may make a significant contribution to microbe-mediated processes in ecosystems. In order to monitor the distribution and diversity of Archaea in Chinese agricultural soils, a total of 26 topsoil samples (classified into four types, i.e. cinnamon soil, brown soil, fluvo-aquic soil and red soil) and 6 soil profile samples (0-100 cm depth) along Northern to Southern China were studied using molecular techniques including real-time PCR and terminal restriction fragment length polymorphism combined with sequencing. Archaeal 16S rRNA gene copy numbers ranged from 4.96×10^6 to 5.62×10^8 copies g^{-1} dry soil, which showed significant positive correlation with soil pH, soil organic carbon, total nitrogen and NH_4^+ -N in the topsoils, while only significantly correlated with pH and NH_4^+ -N content along soil profiles. The percentage of archaeal 16S rRNA gene copy numbers in prokaryotes (sum of bacteria and archaea) were from 0.34% to 8.55 % in the topsoils, while the percentage increased with soil depth ranged from 0.20 to 9.26 %.

Phylogenetic analysis revealed that Group 1.1a, Group 1.1b and *Thermoplasmatales* dominated in cinnamon soils, brown soils, and fluvo-aquic soils, but Group 1.1c, Group 1.3 and *Thermoplasmatales* in red soils. The canonical correspondence analysis showed that soil archaeal community diversity was divided along soil pH. These results implied that a stable component of archaeal community represented in soil microbial communities, while community structure changed largely with different soil types. An increment of Archaea in the soils with depth showed that archaea may be particularly adapted to unfavorable environmental situation and chronic energy stress.

Abstract Category

01 Biogeochemistry

PS.01.032 MERCURY REDUCTION BY BACTERIA ISOLATED FROM INFORMAL MINING ZONES

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Peru is a mining country and there are many places polluted by informal mining using mercury. The aim of this research was the selection of bacterial strains resistant to mercury, as well as to demonstrate their capacity to reduce mercury in solution when they are inoculated in pure and mixed cultures in order to be used for bioremediation.

Samples of soil from informal mining gold sites in Peru were collected and fifteen mercury resistant bacteria were isolated and tested for reduction of mercury. Six selected strains were studied in their growth characteristics and mercury reduction as well as the identification of the *mer A* gene by PCR

Strains RM6, RM7, RM9, RM11, RM12 and RM13 were selected for their capacity to reduce mercury in solution. The six bacterial strains belong to the genus *Pseudomonas*. Inoculated in pure cultures, these strains reduce mercury in solution although in different percentages: RM9, RM11 and RM12 reduce 93% to 97% of the mercury, while strains RM6, RM7 and RM13 reduce 80% to 85%. The consortium of all six bacterial strains showed a mercury reduction of 84%. Approximately 91% of mercury in solution was reduced in 1 hour and this reaction was not associated to bacterial growth. Using specific primers, the *merA* gene was amplified from genomic DNA of the bacterial strains, which would suggest the activity of the mer operon as a mechanism of mercury resistance.

Due to their ability to reduce mercury in solution, it is advisable to carry out more research on the selected strains since they could be useful in future bioremediation processes.

Abstract Category
01 Biogeochemistry

PS.01.033 MICROBIAL DIVERSITY IN ULTRA-BASIC SERPENTINIZING CEDARS SPRINGS

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The springs and aquifers of the actively serpentinizing Cedars peridotite in coastal Northern California are ultra-basic, highly-reducing, and present extreme challenges to life. We report a culture independent survey of the multi-domain microbial diversity in the Cedars springs. The Cedars springs are characterized by some of the most extreme environmental and nutritional conditions on the planet; very high pH (11.50 - 11.85), extremely low E_h (< -550 mV), low ionic concentrations (≤ 2 mM Na^+ , K^+ , Cl^-), no detectable phosphate, nitrate, nitrite, or ammonium, low amounts of trace metals, and relatively high concentrations of Ca^{2+} (1-5 mM). In addition, the water contains H_2 and CH_4 , representing a very significant energy source, however these waters contain neither oxygen, dissolved inorganic carbon (DIC - CO_3^{2-} , HCO_3^- , CO_2), or significant concentrations of any other potential electron acceptor. Two springs named Grotto Pool Spring 1 (GPS1) and Barnes Spring 5 (BS5) within the Cedars area were chosen as sampling sites within the area. GPS1 is a rare spring in that it allows for the collection of pristine ultra-basic and highly-reducing water directly from the spring source. The waters from the BS5 spring, however, enter a small pool from below leading to a highly stratified water column with the bottom waters remaining highly reducing and oxygen free. The results show that in anoxic water *Chloroflexi* spp, belonging to a new clade, dominated the Bacteria, whilst a new group likely belonging to the Euryarchaeota were the major Archaea. Eukaryotes were absent. The dominant species in oxic waters were unidentified *Comamonadaceae* spp similar to clones from other alkaline, non-saline environments. This preliminary microbial diversity analysis of these springs shows that a specialized community has adapted to life in some of the harshest environments on earth. We thank O.J. Johnson for his earlier contributions. The work was supported by the J. Craig Venter Institute.

Abstract Category
01 Biogeochemistry

PS.01.034 SEASONAL TRANSITION OF BACTERIA, ARCHAEA, AND EUKARYOTE COMMUNITIES ALONG REDOX POTENTIAL CHANGES IN RICE PADDY SOIL

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Rice paddy soil is a unique environment where redox potential drastically changes during rice growing season. As redox potential decreases, various anaerobic biochemical processes can occur, including denitrification, metal and sulfate reductions, and methanogenesis. However, response of microbial communities to redox potential changes has not been investigated in detail. In this study, we investigated the transition of bacteria, archaea, and eukaryote communities along changes in soil redox potential during one rice growing season.

Soil samples (n=20) were collected weekly or biweekly from April to October in 2009 from a rice paddy soil in Niigata, Japan. The soil chemical and biological properties, such as soil redox potential and CH₄ fluxes, were measured for each sample. From each soil sample, DNA and RNA were extracted, and cDNA was synthesized from RNA. Amount of small subunit ribosomal RNA from bacteria, archaea, and eukaryote was measured by using domain-specific quantitative PCR. Community structures were analyzed by PCR-denaturing gradient gel electrophoresis targeting small subunit ribosomal RNA. Bands specific to some samples were excised from the gel and sequenced.

The occurrence of various biogeochemical processes, as well as the increase in the amount of ribosomal RNA, was observed as soil redox potential decreased. DNA-based denaturing gradient gel electrophoresis revealed the stability of the bacterial and archaeal community structures regardless of soil redox potential changing; however, RNA-based analysis detected some populations as redox potential decreased. In contrast to bacteria and archaea, eukaryotic community structure was greatly influenced by redox potential changes.

Our results suggest that eukaryotic community was more sensitive than bacteria and archaea communities. Only minor fraction of the bacteria and archaea communities responded to the redox potential changes, and majority of bacteria and archaea were stable in rice paddy soil.

Abstract Category
01 Biogeochemistry

PS.01.035 THE EFFECTS OF HYDROGRAPHY AND ANTHROPOGENIC PRESSURE ON BENTHIC NITROGEN CYCLING IN THE GULF OF FINLAND (BALTIC SEA)

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Baltic Sea is one of the largest brackish water bodies in the world characterized by shallow water depth and stagnated water column. The stagnation of the water column is sporadically broken up by inflow of North Sea water that ventilates the main basins of the Baltic Proper. During inflow event the old saline and oxygen depleted bottom water from the main basins is pushed over to

Gulf of Finland, strengthening the local stagnation and consequently worsening the oxygen conditions. The last major inflow occurred in 2003 and since then the Gulf of Finland has been suffering from extremely poor bottom water oxygen conditions. In addition to poor oxygen conditions causing phosphorus release from the sediments, the anthropogenic nutrient loading to Gulf of Finland is very high and the total nitrogen load per volume of water is the highest among Baltic Sea sub-basins. Anoxia drastically affects the benthic nitrogen cycle by shutting down the coupled nitrification-denitrification pathway that naturally removes bio-available nitrogen from the water ecosystem. We measured sediment nitrification, denitrification and dissimilatory nitrate reduction to ammonium (DNRA) rates in 2008 at the central Gulf of Finland. The nitrification and denitrification rates were extremely low and nitrate was reduced by DNRA rather than denitrification. DNRA intensifies the vicious cycle of eutrophication because instead of being removed, nitrogen remains available for primary producers causing intense algal blooms, which deplete the bottom water oxygen even more when decomposed. Overall the results highlight the low resilience of the natural nitrogen purification system in the Gulf of Finland, and that the eutrophication of the area is driven by anthropogenic nutrient loading as well as the unique hydrography of the Baltic Sea.

Abstract Category
01 Biogeochemistry

PS.01.036 SULFUR-REDUCING BACTERIA MAY LOWER CORROSION RISK IN OIL FIELDS BY COUPLING OXIDATION OF OIL ORGANICS TO REDUCTION OF SULFUR-POLYSULFIDE TO SULFIDE

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Sulfate-reducing bacteria are generally considered to have a profound impact on the petroleum industry as their sulfide production activity contributes to reservoir souring and pipeline corrosion. SRB can be controlled by injecting biocides into pipelines and above-ground facilities. A recent "green" alternative for controlling reservoir souring is to inject nitrate, as nitrate is relatively harmless and is ultimately reduced to nitrogen gas. Resident nitrate-reducing bacteria (NRB) reduce nitrate to nitrite, which is a strong inhibitor of SRB, thereby inhibiting sulfide production. However, NRB-mediated oxidation of sulfide with nitrate and/or chemical reaction between nitrite and sulfide can generate sulfur-polysulfide (S-PS), which can expedite corrosion. S-PS is also rapidly formed by chemical reactions, when sour produced waters, containing substantial sulfide concentrations, are exposed to air. Once formed, S-PS can be removed by either of two alternative routes, which may thus reduce corrosion risk. In the presence of (i) excess electron acceptor (e.g. nitrate), NRB may further oxidize the S-PS to sulfate, whereas in the presence of (ii) excess electron donor (oil organics, e.g. acetate) the S-PS may be reduced back to sulfide. A specialized group of sulfur-reducing bacteria catalyzes this reaction. A representative of this group, *Desulfuromonas acetoxidans*, derives energy for growth from the reaction: $4 \text{ sulfur} + \text{acetate} = 4 \text{ sulfide} + 2 \text{ CO}_2$. Because oil field waters tend to be electron donor rich and electron acceptor poor, one would expect S-PS to be removed by the second route. A survey of the microbial community in produced waters from an oil field with low bottomhole temperatures indicated *Desulfuromonas* species to be common. Hence, once S-PS is formed by reaction of excess sulfide with a limiting concentration of nitrate or oxygen, it may

subsequently be effectively removed through the activity of sulfur-reducing bacteria such as *Desulfuromonas*.

Abstract Category
01 Biogeochemistry

PS.01.037 TRANSCRIPTOMIC ANALYSIS OF SULFATE REDUCTION AND ITS INHIBITION BY NITRITE IN *DESULFOTOMACULUM REDUCENS* MI-1

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Sulfate-reducing bacteria (SRB) are a diverse group of microorganisms that play a key role in the biogeochemical cycle of sulfur. The mechanism of sulfate reduction has been studied extensively on Gram-negative bacteria *Desulfovibrio* spp. In those organisms, the periplasm is crucial for the establishment of a proton motive force as protons are released via the oxidation of H₂ and electrons shuttled back intracellularly for sulfate reduction. In contrast, very little is known about the mechanism in Gram-positive bacteria that lack a periplasm. *Desulfotomaculum reducens* is a representative of that group with a complete and annotated genome sequence. In this study, we carry out a transcriptomic analysis of sulfate reduction to unravel its mechanism and to evaluate the effect of nitrite - a known inhibitor of sulfate reduction- on the process. Data from customized whole-genome microarrays were obtained under sulfate respiration with pyruvate, lactate and butyrate, and compared to those from fermentation of pyruvate. Surprisingly, key genes for sulfate reduction - adenylylsulfate reductase and the dissimilatory sulfide reductase- were constitutively expressed. Genes up-regulated during sulfate reduction included those encoding heterodisulfate reductases, NADH-oxidoreductase and the nitrite reductase (*nrfHA*). These results suggest their involvement in electron transfer during sulfate respiration. Nitrite concentrations inhibiting sulfate reduction were considerably lower for *D. reducens* than those reported for *Dv. vulgaris*. Genes encoding the annotated nitrite reductase were not upregulated in presence of nitrite suggesting that it may not be the nitrite reductase. Expression of another gene encoding a hydroxylamine oxidoreductase, was correlated with nitrite reduction pointing to its potential role in that process. The results allowed the development of a model for sulfate-reduction adapted for Gram-positive bacteria and that differs from that for Gram-negative SRB.

Abstract Category
01 Biogeochemistry

PS.01.038 EFFECT OF FERTILIZATION AND PLANT DIVERSITY ON DENITRIFIER AND BACTERIAL COMMUNITIES IN AGRICULTURAL SOIL

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Denitrifying microbes release nitrogen to the atmosphere, contributing to emissions of nitrous oxide, a potent greenhouse gas, and a loss of nitrogen in agricultural soils. Recent work has identified a cluster of denitrifiers that is dominant in conventionally fertilized agricultural soil but absent in forest soil. The cluster is phylogenetically associated with *Nitrosospira*, which are ammonia oxidizers capable of denitrification and may benefit from nitrogen fertilizers. Our aim was to test if fertilization of agricultural soils is a key factor for bacterial communities and denitrifiers in particular, taking also into account the effect of plant cover. We sampled soils within the Kellogg Biological Station Long-Term Ecological Research site with similar agricultural history and crop species but with or without fertilizer input. To separate the effects of fertilization and plants, we also included plots with different crops and native successional vegetation. Denitrifier communities were compared using clone libraries for the copper-containing nitrite reductase gene *nirK*. Total bacterial communities were evaluated by 454-pyrosequenced 16S rRNA gene tags. Fertilized and unfertilized crop plots and soil under native vegetation all showed distinct denitrifier and overall bacterial communities. No marked difference in denitrifier diversity could be detected among plots with corn, soybean or a corn-soybean-wheat rotation. The denitrifier cluster dominant in fertilized soil was rare or absent in all unfertilized soils based on *nirK* data. Similarly, bacterial 16S tags showed lower abundance of *Nitrosospira* in unfertilized soils. The results suggest that nitrogen fertilization of agricultural soils drives denitrifier communities and may regulate the occurrence of denitrifying ammonia oxidizers. However, vegetation type (crops versus native vegetation) was also an important factor for denitrifier and overall bacterial community composition.

Abstract Category
01 Biogeochemistry

PS.01.039 LINKING BACTERIAL COMMUNITY PATTERNS TO AMINO SUGAR AND AMINO ACIDS PROFILES IN TWO SWISS LAKES OF DIFFERENT TROPHIC STATUS

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Studies on the transformation of organic nitrogen compounds such as amino-sugars and -acids in lacustrine ecosystems are rare, although they are quantitatively important components of the dissolved and particulate organic matter and of biopolymers like peptidoglycane and chitin. In this study, amino-sugar and -acid concentration profiles and isotope ratios were linked to a qualitative and quantitative analysis of the bacterial community in two Swiss lakes. The lakes distinguish themselves by their nutrient and redox conditions: Dimictic Lake Brienz is oligotrophic and fully oxic while meromictic Lake Zug is eutrophic and it is permanently anoxic below 160m at the sampling site. The lakes were sampled in spring and fall 2009. We collected water from 10 different depths, sediment samples from sediment traps installed between the two sampling campaigns and from the first centimeters of a sediment core recovered at the sampling site. The vertical changes in microbial assemblages were analyzed by Denaturing Gradient Gel Electrophoresis and Automated Ribosomal Intergenic Spacer Analysis. A moving window similarity analysis applied to the community fingerprints was used to provide information on the species turnover with depth as a proxy for the recycling of bacterial biomass and related to

changes in organic nitrogen chemistry. Sequencing of 16S rRNA gene fragments was used to identify the main bacterial taxa allocated to shifts in biogeochemical patterns. To reveal how microbes themselves contributed as a source of amino-acids and -sugars, we determined total bacterial counts by flow cytometry and the bacterial biomarkers muramic acid and D-amino acids.

This study provides new information on the significant role of bacteria in the formation and turnover of organic nitrogen compounds during sedimentation in lacustrine systems.

Abstract Category
01 Biogeochemistry

PS.01.040 THE ROLE OF ARCHAEA IN THE TURNOVER OF ROOT AND MYCORRHIZAL EXUDATES IN SOIL

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The understanding of what regulates the turnover of dissolved organic carbon (DOC) in soil is an important part of the overall understanding of the flow of carbon between soil and atmosphere. Even if certain aspects of DOC turnover has been thoroughly studied, only a little is known about the microorganisms involved. For example, archaea has been shown to be dominant in the turnover of small organic acids in marine environments, but the importance of archaea in the turnover of DOC in soil is unknown. Furthermore, plant roots exudate DOC and studies have shown that the abundance and diversity of archaea in the rhizosphere and mycosphere differs from that of the bulk soil. Based on the findings above, it is reasonable to suspect that easily available DOC in the form of root and mycorrhizal exudates may be a carbon source for archaea in soil as well. We therefore designed an experiment with the aim to investigate the role of archaea in the turnover of these compounds. Our hypothesis was that archaeal abundance would be higher in soil with high concentration of root exudates. To test the hypothesis three different tree species were grown in separate boxes. Each box was divided by membranes into four compartments. The first compartment contained the mycorrhized plant. The mycorrhizal hyphae, but not the plant root, could proliferate through the membrane into the second compartment. These two compartments were separated from two additional compartments by a membrane that only allowed free movement of the soil solution containing the exudates. The boxes were incubated in a $^{13}\text{CO}_2$ enriched atmosphere for 4 hours and soil samples were collected from all compartments 3 and 6 days after the pulse labelling. Root exudation rates are estimated by the amount ^{13}C recovered from the different compartments, measured by IRMS. The abundance of archaea is estimated by quantitative PCR and will then be related to the rate of root exudation. Preliminary results will be discussed.

Abstract Category
01 Biogeochemistry

PS.01.041 LINKING BEECH LITTER STOICHIOMETRY TO MICROBIAL DECOMPOSER COMMUNITY STRUCTURE AND FUNCTION

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Microbes exert a major control on biogeochemical cycles by decomposing natural compounds, affecting climate and biosphere. However little is known about how the stoichiometry of leaf litter in terms of carbon (C) nitrogen (N) phosphorus (P) ratios drive the decomposition process. To elucidate how stoichiometry and biochemistry affect the decomposition process of beech (*Fagus sylvatica*) litter a terrestrial microcosm experiment was conducted. Our aim was to follow changes of beech litter stoichiometry and biogeochemical processes in response to stress treatments. In addition to gaseous element losses (CO₂), we examined the release of nutrients prone to leaching. A semi-quantitative metaproteomics approach (1D-SDS PAGE combined with liquid chromatography and tandem mass spectrometry; unique spectral counting) was employed to investigate the impact of litter stoichiometry on structure and function of the decomposing community. Beech litter from different Austrian sites covering C:N ratios from 45 to 66 and C:P ratios from 652 to 1467 were incubated at 15°C and 60% moisture for six months. The microcosms were subject to changes in temperature (+30°C and -15°C) and moisture to monitor the influence of environmental stress. Litter stoichiometries (C:N, C:P) turned out to be strong predictors for respiration, N and P losses, and fungal and plant spectral counts. (i) Faster decomposition occurred in beech leaf litter with narrow C:nutrient ratios; which therefore showed higher respiration rates, higher fungal and lower plant spectral counts. (ii) Increased nutrient losses as leachates were observed from this litter. (iii) A strong functional response of the microbial community to environmental extremes was found. The impact of stress on microbes was most pronounced in sites with narrow C:N ratios. Our results indicate a similar stoichiometric demand of microbes, with temporal changes which result in differences in nutrient cycling on substrates with different C:N:P ratio.

Abstract Category
01 Biogeochemistry

PS.01.042 BIOGENIC FORMATION AND LOCALIZATION OF TELLURIUM NANOCRYSTALS BY *SHEWANELLA* SPECIES

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Certain toxic elements support the metabolism of diverse prokaryotes by serving as respiratory electron acceptors for growth. Tellurium (Te) demonstrates properties similar to those of elements known to be toxic to human, and has applications in industrial processes, which are rapidly growing in importance and scale. The perceived importance of tellurium in biological systems has lagged behind selenium (Se), because of tellurium's lower crystal abundance, lower oxyanion solubility and biospheric mobility. This chalcogen is rarely found in the nontoxic, elemental state (Te⁰), but its soluble oxyanions, tellurite (TeO₃²⁻) and tellurate (TeO₄²⁻), are toxic for most forms of life even at very low concentrations. *Shewanella* species are gram-negative

facultative anaerobes capable of utilizing a broad range of electron acceptors, including several solid substrates such as Fe(III) and Mn(IV) oxides. *Shewanella* species can reduce tellurite [TeO₃²⁻] to elemental tellurium [Te(0)] under anaerobic conditions. This reduction results in the formation of unique crystalline Te(0) nanoarchitectures as end products. The needle-like shaped granules of element tellurium are localized in the cytoplasm or near the cytoplasmic membrane, and each species forms a similar structures. Those formed by *Shewanella* species are needle-like (50 ~ 200 nm length), which cluster together, forming larger rosettes composed of numerous individual shards. EDX and SADE indicate that nanostructures are composed entirely of Te and are crystalline. Several different *S. oneidensis* MR-1 deletion mutants which are related with electron transfer cytochrome c genes, produced tellurium nanostructures. However, they are show different reduction rates. These results indicate that deleted genes play a significant role and different mechanisms are related in tellurite reduction. Additional work is needed to describe more details about molecular mechanisms of the tellurite reduction by *Shewanella* species.

Abstract Category
01 Biogeochemistry

PS.01.043 FATTY ACIDS AND ETHANOL AFFECT METHANOTROPH ACTIVITY IN AN ACIDIC FEN CAPABLE OF UTILIZING ATMOSPHERIC METHANE

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Sphagnum-covered mires harbor mono- and polycarbotrophic aerobic methanotrophs. *Methylocystis* species are restricted to one carbon compounds, one *Methylocapsa* species grows on acetate, and *Methylocella* species prefer fatty acids and ethanol as substrate instead of methane. Soluble organic compounds are produced by fermentation and plant root exudation in mires. It is likely but has not been demonstrated that organic compounds interfere with in situ methane oxidation. This study assessed (a) *pmoA* gene diversity in a fen (Schlößpnerbrunnen, Germany), (b) the capability of fen methanotrophs to consume atmospheric methane, and (c) the effect of alternative carbon sources on methane oxidation and *pmoA* expression. Frequent genotypes were closely related to *pmoA* of *Methylocystis* strains capable of atmospheric methane utilization. *pmoA2* was retrieved from fen RNA indicating the occurrence of enzymes capable of atmospheric methane utilization. Indeed, soil consumed atmospheric methane. *pmoA* of uncultured methanotrophs that presumably consume atmospheric methane in soils were absent. Thus, the detected *Methylocystis* species may have been capable of atmospheric methane utilization. Supplemental propionate, acetate, or ethanol lowered methane (0.1%) oxidation rates. Methane oxidation occurred subsequent to the consumption of supplemental organic compounds. The expression of *Methylocystis pmoA* genes was down regulated at acetate concentrations above 1 mM, whereas glucose did not reduce *pmoA* expression. Hence, acetate, propionate, and ethanol may impair the capacity of fen methanotrophs to oxidize methane. The mechanism remains unresolved since *pmoA* expression could have been reduced by either the toxicity of supplemental organic compounds or by a metabolic switch from methane to alternative substrate utilization. The findings suggest that fermentation and plant-derived organic compounds might be overlooked determinants of the net methane flux in mires.

Abstract Category
01 Biogeochemistry

PS.01.044 USING HYDROGEN ISOTOPES TO ELUCIDATE BIOLOGICAL HYDROGEN PRODUCTION PATHWAYS

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Biological H₂ production by H₂ase enzymes plays an important role in anaerobic microbial metabolism and has enormous potential as an environmentally sustainable energy source. Despite considerable progress in elucidating H₂ metabolism, the regulation of and flux through key H₂ production pathways remain largely undefined. Our goal is to improve understanding of biological H₂ production by using H isotope ratios to dissect proton fluxes through different H₂ase enzymes and from different substrates. We hypothesized that the isotope ratio of H₂ produced by various H₂ases would differ, and that the H isotope ratios would allow us to define the contribution of different enzymes when more than one is present in vivo. We chose *Shewanella oneidensis* (*S.o.*) MR-1, a facultative anaerobe capable of transferring electrons to a variety of terminal acceptors, including protons, as a model system for in vivo studies. *S. o.* encodes one [FeFe]- and one [NiFe]-H₂ase.

We purified three [FeFe]-H₂ases (*S.o.*, *Clostridium pasteurianum*, and *Chlamydomonas reinhardtii*) and two [NiFe]-H₂ases (*S. o.* and *Desulfovibrio fructosovorans*) and determined the isotope ratio of the H₂ produced by each. For in vivo analysis, wild-type, electron transfer-deficient, and H₂ase-deficient strains of *S. o.* cultures were grown in serum bottles in medium containing lactate as an electron donor, with the O₂ in the initial headspace as the initial electron acceptor.

All five H₂ases produced H₂ with a unique isotopic signature. In vivo measurements of H₂ production showed that its isotopic content was consistent with in vitro measurements and that electron transport deficiency impacts H₂ase activity, and suggests that the [NiFe]- and [FeFe]-H₂ases became active at different times.

Different H₂ases express different fractionation factors in vitro, and H isotope ratios can be exploited to dissect pathways of H₂ production in vivo.

Abstract Category
01 Biogeochemistry

PS.01.045 THE ROLE OF SAR11 BACTERIA IN CONTROLLING THE MOLECULAR-LEVEL COMPOSITION OF MARINE DISSOLVED ORGANIC MATTER

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The SAR11 clade of the α -proteobacteria is numerically dominant within the heterotrophic bacterioplankton in a wide variety of marine environments, particularly within oligotrophic regimes. Genomic, proteomic and culture-based studies all point to an efficient carbon metabolism that enables these organisms to thrive in a dilute heterogeneous medium. However, the impact of this metabolism on the dissolved organic matter (DOM) pool has not yet been directly measured. Here, we examine the changes in molecular-level composition of DOM in the presence of *Pelagibacter ubique* (HTCC1062), a member of the SAR11 clade, when grown under light or dark conditions. We utilized ultrahigh resolution mass spectrometry (ESI FT-ICR MS) to detect and resolve thousands of compounds within DOM isolated from *P. ubique* cultures, in comparison to abiotic controls. The abiotic controls had identical DOM composition, regardless of light exposure. However, unique compounds were observed in *P. ubique* cultures; some were common to all culture conditions and some were unique to either light or dark. Compounds common to all cultures frequently had elemental formulae similar to carbohydrates, consistent with recent findings that SAR11 genomes have a limited metabolic repertoire for carbohydrate oxidation. The unique pools of DOM compounds observed in light vs. dark conditions suggest an as-yet unknown role for light-driven metabolism in carbon processing. We anticipate that the pursuit of these experimental approaches will further elucidate the role of SAR11 in altering the DOM composition of the ocean.

Abstract Category
01 Biogeochemistry

PS.01.046 ELECTRON DONOR DEPENDENT IRON AND SULFATE REDUCTION AND MICROBIAL COMMUNITY DEVELOPMENT

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Subsurface contamination by heavy metals or organic contaminants is a big concern for human health and environmental protection. In situ bioremediation is one option for reducing levels of contaminants. Introducing suitable substrates into the subsurface can stimulate the growth of iron or sulfate reducing microorganisms which often reduce contaminants either directly or by the production of reactive Fe(II) and sulfur species. We investigated the effects of electron donors on the enrichment of specific communities capable of iron/sulfate reduction. We created bicarbonate-buffered systems with acetate, lactate, or glucose as the electron donor and with Fe(III) oxides and sulfate as the electron acceptors. Sediment collected from the Old-Rifle-ERSP-IFRC site was used as the microbial seeding material. Terminal Restriction Fragment Length Polymorphism (T-RFLP) and 454-based sequencing technology/metagenomic analysis were used to monitor changes in the microbial communities. Mineral transformations were monitored using x-ray diffraction (XRD) and x-ray absorption fine-structure (XAFS) analysis. All electron donors promoted ferrihydrite reduction, but with different rates and extents: glucose>lactate>acetate. Sulfate reduction occurred only with lactate and acetate. X-ray analysis suggested that siderite was formed with glucose and iron sulfide and vivianite were formed with lactate or acetate. T-RFLP yielded unique community profiles depending upon the availability of specific electron donors. The communities with glucose shifted rapidly and remained constant,

consistent with the rapid reduction of iron. Incubations with acetate and lactate also showed major community shifts over time. These community profiles were different from each other and from the community profiles in glucose system. Incubations with lepidocrocite or goethite also showed both iron and sulfate reduction with lactate or acetate. These results will be correlated to microbial community analyses.

Abstract Category
01 Biogeochemistry

PS.01.047 BIOGEOCHEMICAL REDOX ACTIVITIES AND LINKED MICROBIAL COMMUNITY OF HANFORD 300 AREA SUBSURFACE SEDIMENTS

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Vadose zone and groundwater has been contaminated with uranium from the wastes process ponds in 300 Area, U.S. Department of Energy (DOE)'s Hanford site's southern extremity in central Washington State, where fuel fabrication had occurred. As part of site characterization efforts and installation of the experimental well field at Hanford Integrated Field Challenge (IFRC), a series of subsurface core sediment samples were collected to probe and characterize potential biogeochemical processes impacting contaminant behavior in Hanford 300 Area subsurface sediments. Hanford formation sediment was incubated either with or without sulfate (0.63 mM). Microbial reduction of Fe(III) in the Hanford sediments was faster with sulfate than without. The heat-treated sediments did not exhibit Fe reduction either with or without sulfate. Sulfate was reduced in Hanford sediment and the sediment-associated sulfide accounted for 63% of the initial sulfate. Nitrate was also reduced in all three sediments, albeit at different rates. Microbial U(VI) reduction was observed in the Hanford formation sediments both with and without sulfate, while Ringold oxidized and Ringold reduced sediments showed adsorption of U(VI), but not obvious microbial reduction. Microbial community analysis was performed by constructing 16S rRNA gene libraries from the bioreduced Hanford formation sediments both in the presence and absence of sulfate. *Clostridia* and *β-proteobacteria* were commonly detected phylotypes while sulfate-reducing and sulfur-disproportionating were also relatively abundant in the sediment incubated with sulfate. *Symbiobacterium* was common in the sediment incubated without sulfate. *δ-Proteobacteria* were also more common in microcosms without sulfate; *Geobacter* and *Desulfuromonas* were both detected. Dissimilatory sulfite reductase (*dsr*) genes were detected by PCR of DNA extracted from both Hanford formation sediments bioreduced with and without sulfate (104 d).

Abstract Category
01 Biogeochemistry

PS.01.048 BACTERIAL GROWTH EFFICIENCY IN SOILS ACROSS A GRADIENT OF LAND USE

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Soil is rich in organic carbon (SOC) that is vulnerable to shifts in land management and climate. A major portion of the SOC loss into the atmosphere as CO₂ is mediated by microbial activity. Transformation of SOC, including plant litter and root exudates, by bacterial communities in soil can be quantified through measurement of bacterial growth efficiency (BGE). BGE is defined as the capacity of bacteria to incorporate carbon substrates consumed into bacterial biomass. It is defined as $BP/(BP+BR)$, where BP is bacterial production and BR is bacterial respiration. BGE is commonly assumed to be in the narrow range of 0.35-0.45 in SOC models for various soil types and land use, including cropland soils. This project explores the range of BGE for bacterial communities from soil across a gradient of land uses at the Kellogg Biological Station Long Term Ecological Research site. BP was measured as the amount of ¹⁴C-substrate or ³H-leucine incorporated into biomass material, while BR was measured as the amount of CO₂ respired from the ¹⁴C-substrate or total oxygen consumed, respectively. Preliminary results suggest that BGE differs for soil under different land management and can be as low as 0.16, as found in the forest site. BGE for bacterial communities in cropland soils is more variable and is hypothesized to correlate positively with primary productivity. BGE is also highly dependent on the type of substrate that is assimilated: BGE measurements using a single readily oxidizable carbon source are typically higher than BGE measured using native SOC. Replacing a single, generalized BGE value with site-specific values for BGE should improve the accuracy and predictive capacity of local and global models of the carbon cycle.

Abstract Category
01 Biogeochemistry

PS.01.049 THE EFFECTS OF EXOGENOUS PYOVERDINES ON PYOVERDINE SYNTHESIS AND MANGANESE OXIDATION BY *PSEUDOMONAS PUTIDA* GB-1

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In aerobic and circumneutral environments, microorganisms produce siderophores to facilitate iron uptake. Fluorescent pseudomonads, including the Mn(II) oxidizing bacterium *Pseudomonas putida* GB-1, produce pyoverdines, fluorescent siderophores that can be taken up via specific cognate receptors. Previously, however, it was shown that pyoverdines can inhibit Mn-oxide formation and as Mn-oxides control the fate of various metals and organic compounds, it is important to understand how various pyoverdines affect iron metabolism and Mn-oxide formation. Here, various pyoverdines were siderotyped and their effects on *P.putida* GB-1 were investigated. Using cross-streaking and cell culture techniques, it was determined that homologous pyoverdines, identical to that of *P.putida* GB-1, inhibit Mn-oxide formation and increase pyoverdine concentration. Of the heterologous pyoverdines tested, that from *P.putida* KT2440 inhibited Mn-oxide formation and also showed significant pyoverdine production probably due to iron-limitation. Interestingly, another heterologous siderotype had no effect on either Mn-oxide formation or pyoverdine production. A non-pyoverdine producing mutant of *P.*

putida GB-1 exhibited growth inhibition in the presence of pyoverdine from *P. putida* KT2440, but not by any other pyoverdine tested. Based on these observations, there are three classes of pyoverdines which have three different effects on *P. putida* GB-1. First, homologous pyoverdines that can be utilized, increase pyoverdine concentration, have no effect on growth test, and inhibit Mn-oxide formation. Second, heterologous pyoverdines that cannot be utilized increase pyoverdine concentration and inhibit growth and Mn-oxide formation. Third, heterologous pyoverdines that can be utilized do not increase pyoverdine concentration and do not affect Mn-oxide formation. Therefore, the type of siderophores present and its usability can growth and Mn-oxide formation in complex microbial systems.

Abstract Category
01 Biogeochemistry

PS.01.050 RESOURCE ADDITIONS DRIVE SHIFTS IN SOIL MICROBIAL COMMUNITY STRUCTURE AND FUNCTION IN A TROPICAL RAIN FOREST

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Recent methodological advances have greatly improved our ability to describe soil microbial composition and diversity, yet we lack an understanding of the relationships between soil microbial community composition and fundamental ecosystem processes. In this study, we addressed two related questions: First, do changes in soil resource availability (in this case soil organic carbon [C]) drive changes in soil microbial community composition? Second, are phylogenetic changes in soil microbial community composition accompanied by shifts in functional traits that could influence C cycling? To address these questions, we conducted a leaf litter manipulation experiment (with treatments consisting of 0X and 2X normal litter fall) in a tropical rain forest in Costa Rica. We measured soil microbial community composition shifts resulting from those varied C inputs using quantitative PCR, and we conducted laboratory experiments to investigate the functional significance of those observed community changes. Our results showed changes in leaf litter C inputs drove significant shifts in microbial community composition at the phylum level, and those phylogenetic shifts corresponded to different soil respiration rates between treatments. For instance, low leaf litter inputs corresponded to proportional increases in the oligotrophic members of the soil community (e.g., Acidobacteria), and the low C community soil showed a decreased ability to respire labile C. In addition, when we inoculated sterilized soil with microbial communities from the leaf litter treatments, communities from soil exposed to low leaf litter inputs respired significantly less CO₂ relative to soil communities from control plots. These results suggest changes in C inputs to soils could drive significant shifts in the both the phylogenetic and functional attributes of the soil microbial biota, changes that could ultimately affect soil CO₂ losses to the atmosphere.

Abstract Category
01 Biogeochemistry

PS.01.051 ACCUMULATION AND SPECIATION OF IODIDE-125 BY SUBSURFACE AEROBIC BACTERIA AT A U.S. DOE NUCLEAR WASTE STORAGE FACILITY

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Iodine-129 is an important risk driver for the disposal of nuclear waste because of its perceived mobility in the environment, excessive inventory, toxicity, and long half-life. Iodine can be strongly bound to macromolecular organic matter which can significantly decrease or increase its transport, bioavailability, and transfer to man. Microbial activity (most likely oxidases, perhydrolases, and/or peroxidases) has been implicated in the halogenation of soil organic matter, but very little is known about the proposed process, especially in the subsurface environment. We are thus investigating the role of bacteria in the speciation and mobility of iodine-129 in subsurface aquifers. Approximately 350 microbes were isolated from sediments collected at the Savannah River Site (Aiken, SC). A total of 7 phyla (54 genera) are represented, mainly dominated by *Betaproteobacteria* (29%), *Firmicutes* (15%), and *Actinobacteria* (12%). None of the isolates exhibited iodide oxidizing activity when grown on agar plates containing iodide and starch. However, yellow discoloration was noted in 2/24 enrichment cultures containing slurries of SRS sediment and iodide (1 mM) after 22 weeks, indicating that iodide was oxidized to iodine. FISH analysis revealed that the microbial community in this positive enrichment culture was primarily composed of eubacteria (~53%), particularly high GC content gram (+) bacteria (~77%). Isolates (n=138) were screened for iodide accumulating ability using iodide-125 as a tracer. Three strains were identified as iodide accumulators, but specific uptake was low ranging from 0.10 to 0.20% and was not correlated with iodide concentration. H₂O₂ stimulated iodide accumulation by these strains suggesting that peroxidases may be involved. Efforts are underway to identify the organisms responsible for iodide oxidation in the enrichment cultures and to determine if these strains, iodide accumulators, or other strains are capable of iodinating soil organic matter.

Abstract Category

01 Biogeochemistry

PS.01.052 LITTER QUALITY REGULATES SOIL STABLE CARBON DYNAMICS BY DRIVING MICROBIAL COMMUNITY SHIFTS: A NOVEL CONCEPTUAL MODEL

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Understanding if/how microbial species act as a valve between C sequestered in soils versus released into the atmosphere in natural ecosystems is important but challenging. Small changes in the equilibrium between inputs and outputs to the soil stable C pool could have a significant impact on atmospheric CO₂ concentrations, which may either reduce or exacerbate the

consequence of burning of fossil fuel. However, the heterogeneity of the soil C pool makes detecting changes in the soil's pool size difficult.

In our conceptual model, the dynamics of the soil stable C pool were assessed by comparing the contributions of between microbial stimulating action (soil priming effect) and microbial anabolic activities (microbial necromass effect). We hypothesized that fungal dominance, driving by high-C/N ratio-litter inputs, leads to not only higher rate of CO₂ release by decomposing soil stable C, but also higher accumulation of microbial-derived C by incorporating microbial residues into the stable C pool. Both channels will determine the change of soil stable C pool size at different temporal scale. Mathematical simulations based on the new model are conducted under different plausible scenerios.

In general, although these pathways can be clearly differentiated at a conceptual level it is often extremely difficult at the experimental level to discriminate between them in both space and time. Consequently, while individual studies have shown that these can all occur, probably simultaneously in the same soil system, we are currently unable to rank the relative importance of each process. Finally, in order to elucidate the mechanism and improve our ability to predict C cycling, we call for more studies that investigate these processes particularly regarding the microbial necromass effect.

Abstract Category
01 Biogeochemistry

PS.01.053 HIGH N₂O EMISSION RATES FROM WETLAND SEDIMENTS IN PANTANAL (BRAZIL) ARE DRIVEN BY THE HYDROLOGICAL CYCLE AND RAIN INCIDENTS.

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High emission rates of the potent greenhouse gas and ozone depleter N₂O is often linked to nitrogen-rich environments that undergo sudden changes from oxic to anoxic conditions due to increasing water saturation. Such emissions are poorly investigated in tropical ecosystems. The aim of this study was to evaluate N₂O emission in relation to spatial and temporal oxic-anoxic transitions in a tropical wetland.

Soil porewater O₂ and N₂O concentration profiles were measured to a depth of up to 10 cm with microsensors in flooded soil cores, and the *in situ* efflux of N₂O from wetland soil was estimated with static chambers along a transect at two different lakes.

Upon flooding of soil cores O₂ was quickly depleted rendering the soil anoxic after a few hours. Anoxia was followed by an immediate and large increase in the concentration of N₂O concentrations within the flooded soil resulting in diffusion to the overlying oxic water phase with a peak emission of ~4500 µg N m⁻² hr⁻¹.

The *in situ* efflux of N₂O was very dynamic (100 - 3050 μg N m⁻² hr⁻¹) with peak emission rates occurring immediately after rain events. These peak emissions were of similar size and duration as found in the core flooding experiments and thus supported our hypothesis that incomplete denitrification in the soil due to sudden natural wetting events cause a significant increase in N₂O emission from this tropical wetland soil (~3-10 kg N ha⁻¹ over ~40 days).

Porewater NO_x and soil pH was found to vary in both space and time with highest NO_x concentrations (~30 mM) and lowest pH (4.5-5) in drying soil while no detectable NO_x and highest pH (6-6.5) was found in wet soil near rivers or lakes. The fluctuating soil NO_x suggests that the hydrological cycle drives a decoupled nitrification and denitrification activity.

Furthermore, low pH in the nitrate-rich, dry soil may inhibit the N₂O reductase enzyme and hence explain the high emission rates of N₂O.

Abstract Category
01 Biogeochemistry

PS.01.054 NEUTROPHILIC FE(II)-OXIDIZING AND FE(III)-REDUCING BACTERIA COEXIST IN ESTUARINE GROUNDWATER OF AN AUSTRALIAN SUBTROPICAL COASTAL CATCHMENT

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The highly-weathered Poona catchment (SE QLD, Australia) is dominated by *Pinus* plantation forestry, with native vegetation along waterways adjacent poorly-drained soils. Subsurface groundwater near the catchment estuary represents a stratified system with potential for mixing of fresh- and marine-waters. We hypothesize this groundwater system is dynamic, with land surface organic matter, minerals and nutrients infiltrating and rapidly processed through biogeochemical reactions. Tidal recharge may affect local flow systems seasonally and transport electron acceptors into the aquifer, increasing microbial activities related to local biogeochemistry.

In a survey of groundwater from eight boreholes across the estuarine transect, laboratory cultivation techniques combined with microscopy and 16S rRNA-based molecular analysis were used to estimate presence and approximate abundance of potential Fe cycling bacteria. Results showed neutrophilic Fe(II)-oxidizing bacteria were ubiquitous, with levels in groundwater up to 10⁴-10⁵ cells ml⁻¹. TGGE analysis indicated the most common laboratory-culture-enriched member was related to *Sideroxydans lithotrophicus* (96% sequence identity). Other common members included *Dyella*-, and *Burkholderia*-related bacteria. Neutrophilic Fe(III)-reducing bacteria were also detected in all boreholes, with highest level (10⁸-10⁹ cells ml⁻¹) corresponding with the highest level of Fe(II)-oxidizing bacteria. In addition, lower levels of sulfate-reducing bacteria were found in all boreholes (10-10³ cells ml⁻¹).

Coexistence of neutrophilic Fe(II)-oxidizing and Fe(III)-reducing bacteria with low levels of sulfate-reducing bacteria indicates microbial Fe cycling may be the biogeochemically predominant driver of electron flow in the estuarine groundwater system. Such Fe cycling has

important biogeochemical implications in aquatic habitats, e.g. stimulation of carbon mineralization, as well as suppression of sulfate reduction and methanogenesis.

Abstract Category
01 Biogeochemistry

**PS.01.055 DENITRIFICATION AND NITROUS OXIDE PRODUCTION
POTENTIAL OF THE DAIRY CATTLE MANURE COMPOST SURFACE LAYER**

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Background and aims Nitrous oxide, a greenhouse gas, is emitted through the cattle manure composting process and needs to be mitigated. Our previous work revealed that the source of this gas is the denitrification that occurs just after the compost is turned, and the accumulated NO_x-N on the surfaces of compost piles was suggested to be the main cause of nitrous oxide production. The aim of this study is to understand the mechanisms underlying nitrous oxide emission by using stable isotope techniques and measuring the denitrification potential of the compost surface in vitro. Methods Dairy cattle manure was mixed with dried grass and formed into a pile (4 metric tons). A sample taken from the surface (to a depth of 30 cm) was used to test the denitrification potential by the acetylene block method. The nitrous oxide concentration was measured by a gas chromatography-electron capture detector, and then the ratio of both stable isotope bulk ¹⁵N and site preference values were measured by gas chromatography-isotope ratio mass spectrometry. Results Nitrous oxide was actively emitted immediately after the surface samples were taken. However, the NO₂-N-added core sample produced relatively little gas; the production rate was significantly lower than those of the surface samples. As the composting process progressed, the nitrous oxide production rate and nitrous oxide reduction rate slowed down. The observed nitrous oxide samples with the acetylene block method showed 0-10‰ site preference values. The reduced nitrous oxide samples showed up to 10‰ increases in site preference values. Conclusions Nitrous oxide emission results mainly from the compost surface zone, and the core zone has relatively poor denitrification potential. The contribution of bacterial denitrification to nitrous oxide emission was confirmed. The results suggested that the nitrous oxide reduction process contributes to the elevation of site preference values of nitrous oxide emitted between turnings.

Abstract Category
01 Biogeochemistry

**PS.01.056 THE EFFECTS OF SODIUM BISULFATE ON THE BACTERIAL
POPULATION STRUCTURE OF DAIRY COW WASTE**

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Background and Aims: Sodium bisulfate (SBS) is commonly used in the poultry and dairy industries to acidify waste for the reduction of volatile organic compound emissions. Although commonly used, little is known about how SBS affects the bacterial population structure of waste. **Methods:** To determine the effect of SBS on the microbial population structure of fresh waste we applied SBS three times a week, at four different levels (0, 50, 100, 150 g/m²) to the waste that accumulated on the dirt floors of four chambers (22 m x 11 m), housing eight lactating dairy cattle, located at the University of California, Davis, CA. Waste samples were taken from the chamber floors on days 1, 4, and 7 two-hours post SBS application and analyzed for aerobic, anaerobic and coliform bacteria. We also extracted DNA from waste samples and constructed 16S rRNA gene sequence libraries containing 1,728 sequences from each treatment. **Results:** Bacterial culture analysis revealed significant reductions in coliform, aerobic and anaerobic bacterial numbers after SBS treatment; however, over time the bacterial populations recovered to levels roughly equivalent to those of the untreated controls. The 16S rRNA gene sequences analysis revealed that the microbial population structure of the waste was altered by the addition of SBS, with significant reductions in 16S rRNA sequences associated with the phyla *Bacteroidetes* and *Firmicutes* and significant increases in those associated with the phyla *Proteobacteria* and *Spirochaetes* ($P < 0.01$). However, over time these sequences reverted to levels that resembled those observed in the untreated waste. Interestingly the percentage of sequences associated with *E. coli* increased by several orders of magnitude after the initial application; however, they too reverted to normal over time. **Conclusions:** Initially, SBS application resulted in alterations to the microbial population structure of fresh waste; however, over time these alterations revert to normal.

Abstract Category
01 Biogeochemistry

PS.01.057 SPATIALLY RESOLVED STABLE ISOTOPE ANALYSIS AT THE MICROBIAL COMMUNITY LEVEL

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Stable isotope analysis of microbial substrates, metabolites, biomarkers, whole microbial cells, and environmental constituents provide valuable information on substrate flow, biogeochemical cycling, microbial activity and physiological connections between microbial communities. Yet, spatial arrangement is typically sacrificed during sample preparation for isotope analysis. We present a laser ablation technique which, we hypothesize, when it is coupled to an isotope ratio mass spectrometer (IRMS) will permit spatially prescribed isotope analysis at microbial relevant scales. We are using a commercially available (Cetac) Nd:YAG laser (incident $\lambda = 266\text{nm}$) with a movable stage, camera, and focusing and targeting system. When trained on a sample the laser ablates the surface, sending the generated particulates along a helium stream and into a capillary combustion reactor. The resulting CO₂ is cryogenically concentrated then released into a Thermo Delta V plus for isotope ratio measurement. We used comparison of $\delta^{13}\text{C}$ measurements from the laser ablation and traditional elemental analyzer IRMS approach to

measure the system accuracy and precision, both typically better than 0.5%. The system sensitivity permits measurement on 60ng carbon and the optics support spatial resolution to 25µm. We measured δ¹³C content of cells and extracted RNA dried onto a slide. We are exploring the isotopic boundaries associated with a microbial mat isolated from a MgSo₄-rich meromictic lake. This laser ablation IRMS system uses almost exclusively commercially available components. When combined with a suitable IRMS the system permits spatially resolved isotopic analysis with minimal sample preparation and short analysis times. Given its spatial sensitivity, laser ablation IRMS can help distinguish isotopic boundaries at the microbial level as well as permit detailed substrate tracking useful in isotope labeling experiments.

Abstract Category
01 Biogeochemistry

**PS.01.058 LACTATE ENRICHMENT OF URANIUM AND CHROMIUM
CONTAMINATED HANFORD GROUNDWATER SAMPLES**

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The Department of Energy site at Hanford, WA, has been impacted by U and Cr. In an attempt to stimulate microbial remediation, in-situ lactate enrichment experiments are ongoing. To bridge the laboratory to field gap, we inoculated triplicate anaerobic, continuous-flow reactors with Hanford groundwater. The reactors were fed from a single carboy containing a defined medium with 30 mM lactate at a rate of 0.22 ml/min under continuous nitrogen flow at 9 ml/min for twelve weeks. Cell counts, organic acids, gDNA (for qPCR and pyrosequencing) and gases were monitored biweekly. Cell counts remained low (less than 1x10⁷ cells/ml) during the first two weeks, but by day 20 had increased to greater than 1x10⁸ cells/ml and 1x10⁹ cells/ml by day 37. Metabolite analysis showed a steady decrease in lactate concentrations over time with complete consumption, and therefore lactate limitation, by day 37. Pyruvate and formate concentrations ranged from 20-40 µM and 1.48-1.65 mM, respectively during the first week, then were undetectable by day 15. Acetate was present in low amounts during the first week, but reached 10 mM by day 20 and 40 mM by day 22. Carbon dioxide and methane were also present in low concentrations early on and increased to 0.65-0.75 mM and 10-20 µM, respectively. At the conclusion of the experiment, samples from each reactor were serially diluted for isolation of individual organisms with particular metabolic functions; specifically methanogens, iron-reducing and sulfate-reducing bacteria. From these results and pyrosequencing analysis, we show that microbial community dynamics shifted over time to form a stable enriched microbial community. Comprehensive investigations such as these allow for the examination of not only which nutrient source will accelerate site remediation, but also provide insight to evaluate remediation strategies and identify which enriched community members may be important for bioremediation.

Abstract Category
01 Biogeochemistry

PS.01.059 WHAT THE MUCK? IDENTIFYING PRESERVED MICROBIAL PROTEINS FROM BERING SEA SEDIMENTS

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The oceanographic community focuses much of its attention on understanding the recycling and net fluxes of organic carbon and nutrients in order to better forecast environmental parameters controlling the global 'health' of our oceans. The long-term burial of organic carbon ultimately preserves atmospheric oxygen, and understanding the mechanisms that control the fate of this large, reactive pool is required to evaluate the global carbon cycle and the role of microbial catalysis. We have identified proteins from Bering Sea sediments in order to evaluate these mechanisms leading to the long-term preservation of marine-derived protein. The Bering Sea is a highly productive system dominated by the seasonal production of diatoms. Our previous work has tracked the loss and selective preservation of proteins from diatom blooms as they descend to the ocean floor and undergo the early stages of degradation in surface sediments. Here we examine sediment samples from deeper sediment horizons to evaluate long-term preservation over geological time scales. Methods were developed for extracting proteins from sediments using denaturing buffers and gel electrophoresis prior to bottom-up proteomic analyses. Peptides were analyzed on a LTQ-Orbitrap mass spectrometer, which provided high mass accuracy on precursor ions and peptide fragment-ion mass maps. Mass spectra were searched against a concatenated database that included proteomes from two diatoms and the Global Ocean Sampling (GOS) survey (>6 million bacterial proteins). More than 100 proteins were confidently identified from Bering Sea sediments. Over 80% of these proteins were of diatom origin, suggesting that novel protective mechanisms exist to preserve diatom proteins as they are transported and buried in the sediment for hundreds of years. This is the first study to demonstrate the isolation and identification of proteins from 500+ year old oceanic sediments.

Abstract Category
01 Biogeochemistry

PS.01.060 ELECTRON SHUTTLE EFFECTS ON MICROBIAL COMMUNITY DEVELOPMENT UNDER IRON- AND SULFATE-REDUCING CONDITIONS

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The projected performance of subsurface remediation techniques is often based on insufficient knowledge of the mechanisms of biogeochemical transformation of contaminants in the

subsurface, particularly as they relate to the structure and activity of subsurface microbial communities. We hypothesize that the addition of an electron shuttling compound (2,6-anthraquinone disulfonate (AQDS)) to iron-rich material that is inoculated with sediments collected from the US DOE ERSF's Old Rifle IFC site will result in changes to microbial community structure and function that is dependent upon the presence/absence of the electron shuttling compound. We characterized mixed batch experiments over a 60-day period by microbial community analysis (using 454 Titanium-based tag sequencing of the V3-V4 region of the 16S rRNA-encoding gene) and geochemical measurements (wet chemical and synchrotron-based analyses) to elucidate natural biogeochemical processes that can be augmented to foster stabilization of subsurface contaminants. Specifically, we investigated evolution of natural microbial community structure, the production of Fe(II), the loss of sulfate, and the consumption of acetate within material inoculated with sediment collected from the Old Rifle ERSF IFC site, after it was biostimulated via the addition of acetate and augmented with AQDS. Geochemical characterization of the material identified an increase in the concentration of reduced iron and a decrease in the concentration of sulfate with time. The microbial community analysis indicated an expansion of the Firmicutes over time in both mixed batch experiments. An increase in the relative abundance of the Deltaproteobacteria was observed in the first half of the experiment when augmented with AQDS compared to experiments absent of AQDS. Conversely, a greater increase in the relative abundance of Bacteroidetes was observed in the first half of the experiment for the batch not containing AQDS.

Abstract Category
01 Biogeochemistry

PS.01.061 ANALYSIS OF PUBLISHED *NIFH* PRIMERS- WHO ARE WE MISSING?

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Determining the diversity of diazotrophic bacteria within an environment plays a fundamental role in understanding the nitrogen cycle on global to organismal scales. With our current knowledge of diazotrophic prokaryotes present in terrestrial, aquatic, marine, and symbiotic systems, are we able to capture the full diversity of diazotrophs within any given ecosystem? PCR-based methods targeting the *nifH* gene are the most commonly used approach to studying diazotroph diversity. However, this approach has inherent limitations. One such limitation is the inability of primer sets to anneal to all *nifH* genes within an environmental sample. We addressed this issue using Perl scripts to compare 19 previously published primer sets, ostensibly designed to be universal in nature, to 200 full length *nifH* sequences of known taxonomy. Individual forward and reverse primer sequences were compared to each of the 200 *nifH* sequences. For each primer we determined the number of mismatches between the primer and sequence, taking into account 0, 1, or 2 mismatches. From this analysis we were able to theoretically determine the universality of a primer set. Our results identified some of the most commonly used primer sets as very universal (matching over 90% of *nifH* sequences in the data set without mismatches), while others were not nearly as universal (matching less than 50%, taking mismatches into consideration). We also identified which taxonomic groups a specific set would potentially miss in a diversity study. These results will provide important information on the strengths and

weaknesses of these primer sets and serve as an invaluable tool for analyzing sequence data obtained using the primer sets analyzed in this study, allowing researchers to better understand the taxonomic limitations of their primers when studying the diversity of diazotrophs within specific systems.

Abstract Category
01 Biogeochemistry

PS.01.062 RESPONSE OF N-PROCESSES AND MICROBIAL TRANSCRIPTS TO SOIL WET-UP

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The first rainfall following the hot, dry summer in Mediterranean annual grasslands causes large and rapid carbon dioxide and nitrous oxide emissions. The goal of this study was to understand microbial mediation of nitrogen cycling following wet-up and how specific microbial functional groups responded to the wet-up event in terms of time since the disturbance and relation to nitrous oxide emissions. Using laboratory simulations of rainfall, we monitored pools of ammonium and nitrate; rates of gross nitrogen mineralization and gross nitrification; effluxes of carbon dioxide and nitrous oxide; and gene transcripts for ammonia oxidation, nitrite oxidation, nitrite reduction, and nitrous oxide reduction at timepoints from 15 minutes to 72 hours following the water pulse. Results from two California annual grassland soils show significant increases in the soil ammonium pool within three hours of water addition followed by significant increases in soil nitrate within nine hours of water addition. Nitrogen mineralization was very high ($16 \mu\text{g N-NH}_4^+ \cdot \text{g dry soil}^{-1} \cdot \text{d}^{-1}$) within five hours of water addition. Gross nitrification was also very high, with the rate being highest (and most variable) at nine hours after wet-up ($83 \mu\text{g N-NO}_3^- \cdot \text{g dry soil}^{-1} \cdot \text{d}^{-1}$). Nitrous oxide efflux followed a pattern similar to that of gross nitrification, peaking at nine hours. Transcripts for ammonia oxidation (β -Proteobacterial *amoA*) were elevated earlier than those of nitrite oxidation (*nxrA* of *Nitrobacter* spp.). Transcript response was similar in both soils, but the response continued longer in one soil. These data build on previous studies showing rapid microbial response to wet-up and high nitrogen mineralization by demonstrating the responses of ammonia oxidation, nitrite oxidation, and denitrification during this dynamic event.

Abstract Category
01 Biogeochemistry

PS.01.063 CHARACTERIZATION OF IN-SITU MICROBIAL COMMUNITIES WITHIN A DEEP COLUMBIA RIVER BASALT FLOWTOP PRIOR TO CO₂ INJECTION

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Deep geologic sequestration of supercritical CO₂ (scCO₂) is one solution for the removal of excess CO₂ from the atmosphere. A pilot well located at Wallula Gap in eastern Washington

State was drilled and characterized to examine the potential for CO₂ sequestration in the Columbia River Basalt Group (CRBG). Microbial communities in groundwater sampled from selected CRBG depth intervals were characterized during borehole advancement as a baseline for assessing community response to the CO₂ injection. Groundwater from five depth intervals was collected onto 0.2 µm filters and frozen in the field, then stored at -80°C until analysis. Cell concentrations on the filters were estimated by quantifying extracted microbial DNA. Bacterial community diversity was determined by terminal-restriction fragment length polymorphism (T-RFLP) and clone library construction based on the 16S rRNA gene. Samples contained 9 to 1830 pg of DNA mL⁻¹, which corresponds to approximately 5 x 10³ to 9 x 10⁵ cells mL⁻¹. The deepest depth interval contained the greatest amount of biomass based on extracted DNA and also harbored the most diverse bacterial community (by T-RFLP), while the three middle depth intervals contained similar but less diverse communities. The shallowest depth interval also hosted a unique bacterial community. Clone library results supported the findings from T-RFLP. Clones representing the *Proteobacteria* composed the majority of the library, with some clones related to putative hydrogen utilizers. The microbial characterization conducted within the Wallula Gap pilot well indicates that the CRBG hosts a diverse bacterial community. Future work will examine how microbial communities change in response to the formation of a strong chemical gradient associated with the injection of scCO₂ into the deep basalt flowtop

Abstract Category
01 Biogeochemistry

PS.01.064 INVESTIGATING THE ACTIVITY OF AMMONIA-OXIDIZING ARCHAEA AND BACTERIA IN AN AGRICULTURAL SOIL BY STABLE ISOTOPE PROBING

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Ammonia oxidation, the first step in nitrification, is a crucial part of the global nitrogen cycle. The key step of the conversion of ammonia to nitrite by ammonia-oxidizing organisms is catalyzed via the ammonia monooxygenase (AMO) enzyme. While for a long time bacteria were believed to be solely responsible for this process, recent findings revealed another microbial group presumably involved in ammonia oxidation: the ammonia-oxidizing archaea, members of the Crenarchaeota. Ammonia-oxidizing archaea are found in a wide variety of environments including marine systems, hot springs, and soils. Due to the widespread linkage between ammonia oxidation and autotrophic CO₂ fixation in ammonia-oxidizing prokaryotes, they might also play an important role in the global carbon cycle and provide an additional sink for the greenhouse gas CO₂. But although ammonia-oxidizing archaea clearly outnumber their bacterial counterparts, especially in terrestrial habitats, still little is known about their actual activity and contribution to ammonia oxidation in soils.

In this study we used RNA-stable isotope probing (RNA-SIP) as an approach to unravel the activity of ammonia-oxidizing archaea in an agricultural soil. Soil microcosms were incubated with ammonia and CO₂ and general ammonia oxidation activity was monitored using chemical gas and soil analyses. Specific activity of ammonia-oxidizing archaea and bacteria was investigated using DNA- and RNA-SIP on the basis of assimilation of labeled ¹³CO₂, followed

by quantitative PCR (qPCR) of the 16S rRNA and *amoA* genes. RNA-SIP, in contrast to DNA-SIP, revealed activity of ammonia-oxidizing archaea in this soil. This may indicate that archaea play an important role in ammonia oxidation in this environment, but grow more slowly than the ammonia-oxidizing bacteria.

Abstract Category
01 Biogeochemistry

PS.01.065 MID-INFRARED SPECTROSCOPY CAN PREDICT GENETIC AND ENZYMATIC CHARACTERISTICS OF SOIL MICROBIAL COMMUNITIES IN TEMPERATE GRASSLANDS AND PASTURES

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The determination of genetic and enzymatic characteristics of soil microbial communities of large numbers of soils is generally time and cost consuming. In order to overcome such restrictions, we tested the applicability of diffuse reflectance Fourier-transform mid-infrared spectroscopy (DRIFT-MIRS) combined with partial least-squares regressions (PLSR) which has been previously acknowledged to be a fast and reliable technique to predict physicochemical soil properties. However, DRIFT-MIRS/PLSR has not been applied for predicting soil microbial properties. In this study, this chemometric approach was tested on 162 soil samples derived from temperate, differently managed grasslands and pastures that have been analyzed by microbiological, enzymatic and genetic methods. DRIFT-MIRS/PLSR resulted in good predictions of soil microbial biomass carbon (MBC), microbial biomass nitrogen (MBN) and genomic soil DNA (DNA) which corresponded accurately to the actual measurements as confirmed by their coefficients of determination (R²) and residual prediction deviations (RPD): i.e. MBC, R² = 0.94, RPD = 5.35; MBN, R² = 0.90, RPD = 3.09; DNA, R² = 0.84, RPD = 2.49. Sound prediction models were further obtained for selected enzyme activities (e.g. β -glucosidase, R² = 0.94, RPD = 4.01; urease, R² = 0.93, RPD = 3.72; xylosidase, R² = 0.86, RPD = 2.72) and substantiated the potential for predicting genetic parameters such as the abundance of functional prokaryotic genes (nitrite reductase, *nirS* gene (R² = 0.86, RPD = 2.73); *nirK* gene (R² = 0.72, RPD = 1.90)). In conclusion, DRIFT-MIRS/PLSR demonstrated to be an excellent tool to predict soil microbiological properties of grasslands and pastures differing in their land use intensity. Further studies are required to strengthen the performance of chemometric models for predicting genetic data and to confirm their applicability in other terrestrial environments.

Abstract Category
01 Biogeochemistry

PS.01.066 USE OF 15N-DNA STABLE ISOTOPE PROBING TO DETERMINE ACTIVE SOIL BACTERIA AND FUNGI INVOLVED IN DECOMPOSING LEAF RESIDUES OF DIFFERENT CHEMICAL QUALITY

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Plant residues play an important role in maintenance of soil organic matter and nutrient cycling; especially in tropical conditions where microbial decomposition is proceeding fast. As limited information has been obtained to date on the genetic identity of active soil microbial communities and their specific interactions and key roles in organic residue decomposition in tropical soils, a ¹⁵N-DNA stable isotope probing (¹⁵N-DNA-SIP) approach was used to identify particularly bacteria and fungi actively involved in the decomposition of plant residues of different biochemical quality in a topical soil. ¹⁵N-labelled (90 atom-%) and unlabelled (control) maize (C-to-N ratio: 32; cellulose content: 24.9%) and soybean (15; 15.5%) leaf residues were mixed with a tropical Vertisol and incubated for 15 days under controlled conditions. Genomic soil DNA was isolated and subjected to ¹⁵N-DNA-SIP. Density-resolved DNA fractions obtained from ¹⁵N-DNA-SIP were further analyzed by 16S rRNA and 18S rRNA gene-based DGGE analysis combined with sequencing of selected DGGE bands to identify bacteria and fungi able to assimilate litter derived nitrogen. Residue addition induced new bands and modified relative intensity of already existing bands in ¹⁵N-enriched SIP fractions. Phylogenetic analysis of heavy fractions (highly ¹⁵N enriched (72 atom-%)) revealed that in the maize treatment, the active bacterial community was dominated by *Bacillus* spp., *Saccharopolyspora* spp. and *Pseudonocardia* spp., whereas soybean residues promoted *Bacillus* spp., *Saccharopolyspora* spp., *Streptomyces* spp. and *Arthrobacter* spp, all known as key players involved in the decomposition of organic matter. For fungi, Ascomycota was found to be particularly involved in early residue decomposition. In conclusion, our results indicated that ¹⁵N-DNA-SIP was suited, if supplemented with an unlabelled residue control, to identify active decomposing bacteria and fungi in both ¹⁵N plant residues evaluated.

Abstract Category
01 Biogeochemistry

PS.01.067 MULTIPLE AND SIMULTANEOUS RESPIRATION MEASUREMENTS OF COMPLEX MICROBIAL COMMUNITIES

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Respiration is an important emergent feature of microbial communities, integrating the aerobic activities of all microbes present. Most methods for measuring respiration, including electrodes and colorimetric assays, limit the capacity for obtaining replicate measurements while manipulating environmental conditions. The availability of an oxygen optode in each well of a 24-well microtiter plate coupled with the Sensor Dish Reader, a plate reader that excites and reads the optode, offers the potential for multiple, simultaneous measurements of oxygen concentration. Our aim was to optimize this system for measuring rates of oxygen consumption in environmental samples, including soil slurries which are particularly challenging due to the complex physical matrix of soil. The optode microtiter plate was modified by isolating wells from the atmosphere with an adhesive foil, preventing oxygen influx during respiration

measurements over the duration of 120 minute experiments. To produce a homogenous solution, glass beads were added to each well of the plate, and the plate was placed on the plate reader while fixed to a rotary shaker. These modifications removed all effects of settling particles seen in samples without glass beads. Respiration was measured in both pure cultures of bacteria and soil slurries using the modified optode system and compared to microrespirometry measurements from an oxygen electrode. Respiration rates and variability between replicate measurements were similar for both systems. The modifications made to the optode system allow for bacterial respiration measurements of complex communities and pure cultures that are accurate and exhibit little variation within a sample. The modified optode system allows for the simultaneous manipulation of multiple variables, where a large capacity for replication is necessary.

Abstract Category
01 Biogeochemistry

PS.01.068 SOIL pH DRIVES THE COMPOSITION AND GROWTH OF BACTERIA, WHILE COMPETITIVE EXCLUSION DETERMINES FUNGAL GROWTH

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One of the most influential factors that regulates the structure of microbial communities in soil is pH, however, it is often hard to isolate the role of pH from other factors also likely to vary between different soils. A unique long term (>100 yr) experiment, Hoosfield acid strip, Rothamsted Research, UK, presents a pH gradient (pH 4.5 to 8.3), within 180 m in the same arable soil. We have studied how pH influences the size (PLFA, qPCR, ergosterol, SIR-biomass) and composition (pyrosequencing, PLFA) of the fungal and bacterial communities. In addition, we have tested the influence of pH on the growth of fungi (acetate in ergosterol incorporation) and bacteria (leucine incorporation), and on overall decomposition (respiration) in this soil.

Soil pH had a marginal influence on estimates of total microbial biomass as well as bacterial and fungal biomass. Respiration, for example, was only decreased by 30% toward low pH. The composition of the microbial community was highly influenced by pH (explaining 40% of the variation in PLFA composition, with an $r^2=0.97$ with pH), and the composition of the bacterial community influenced more by pH (29% of the variation in bacterial sequences with a $r^2=0.994$ with pH; driven in part by changes in relative abundance of acidobacteria subgroups 1, 3 and 6 as well as gamma-proteobacteria) compared with fungi (17% of the variation with an $r^2=0.55$ with pH). Fungal growth increased more than 5-fold toward low pH, while bacterial growth decreased in the same interval, yielding a fungal:bacterial growth ratio that increased about 30-fold toward low pH. Using an inhibitor (bronopol) to specifically suppress bacterial growth resulted in increased fungal growth at all pH demonstrating that competition by bacteria is important in determining fungal growth patterns with regard to soil pH.

Abstract Category
01 Biogeochemistry

PS.01.069 RELATIVE GENE EXPRESSION OF DISSIMILATORY SULFITE REDUCTASE IN DESULFOVIBRIO VULGARIS CORRELATES TO EXTREMELY LOW SULFATE REDUCTION RATES

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We tested whether the relative gene expression of dissimilatory sulfite reductase (*dsr*), a critical enzyme in the sulfate reduction pathway, decreases with rates of sulfate reduction that might be found in natural systems. We grew *Desulfovibrio vulgaris* with formate as an electron donor, acetate as a carbon source and sulfate in fed-batch reactors under formate-limiting conditions. To simulate low reaction rate conditions characteristic of oligotrophic environments such as anoxic aquifers, we constrained the rates of sulfate reduction from 0.1 $\mu\text{M h}^{-1}$ to 21 $\mu\text{M h}^{-1}$ by controlling the rate of formate addition into each fed batch reactor using a syringe pump. Over a typical 144 hour experiment, steady state formate concentrations were maintained near zero while only incrementally increasing the volume of the 100 ml reactors. We used quantitative-PCR to measure the number of *dsr* mRNA transcripts and the number of *dsr* genes present in nucleic acids extracted from cells sampled at different time points. Independent of any extraction efficiency, the ratio of mRNA transcripts to the gene copy number provided a relative metric of gene expression. We observed a well-defined relationship between the rate of sulfate reduction and the number of *dsr* mRNA transcripts per cell. Cultures with the highest sulfate reduction rate had a relative *dsr* gene expression level 36 times greater than in cultures with the lowest reduction rate. These results suggest even lower rates may be tested and that rates of sulfate reduction in the field may be inferred by measuring the relative *dsr* mRNA in sulfate reducing populations found in the environment. Such rate measurements are difficult to make directly because the microbial mediated substrate consumption cannot be observed readily in many environments, such as in aquifers.

Abstract Category
01 Biogeochemistry

PS.01.070 IMPACT OF OXYGEN AND HERBICIDES ON CELLULOLYTIC AND SACCHAROLYTIC AGRICULTURAL SOIL BACTERIA

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Cellulose is the most abundant biopolymer in agricultural ecosystems and is degraded by soil microbes. Cellulolytic microbial communities in aerated agricultural soils are confronted with fluctuations of oxygen availability and the potential toxic effects of anthropogenic pesticides. The phylogenetic identities of prokaryotes that participate in cellulose degradation in soils are poorly resolved, as are factors that impact on their activity. Aerobic and anaerobic cellulolytic and saccharolytic prokaryotes were identified by 16S rRNA stable isotope probing in an aerated agricultural soil. The conversion of cellulose, cellobiose, and glucose to fermentation products under anoxic conditions was concomitant to the reduction of nitrate and ferric iron. Under oxic conditions, these substrates were mineralized to carbon dioxide. [¹³C-U]cellulose was mainly degraded under anoxic conditions by the *Kineosporiaceae*, cluster III *Clostridiaceae*, and novel

taxa of *Bacteroidetes*. Under oxic conditions, novel taxa of *Bacteroidetes* and *Chloroflexi*, and a family-level taxon of *Planctomycetes* were involved. Independent of oxygen availability, active sub sets of community members in [¹³C]cellobiose and [¹³C]glucose treatments differed from those in [¹³C]cellulose treatments. Cluster I *Clostridiaceae* were detected under anoxic conditions, whereas *Intrasporangiaceae* and *Micrococcaceae* were prevalent under oxygen-enriched conditions. The herbicides MCPA (4-chloro-2-methylpenoxyacetic acid) and Bentazon impaired cellulose-linked microbial processes. Impairment was most pronounced under anoxic conditions. These findings suggest that (a) a large uncultured diversity of *Bacteria* was involved in the overall degradation of cellulose, (b) oxygen selectively activated sub sets of the microbial community, (c) saccharolytic taxa were dissimilar to cellulolytic taxa, and (d) MCPA and Bentazon impaired the microbial metabolism of cellulose.

Abstract Category
01 Biogeochemistry

PS.01.071 SULFATE REDUCING MICROBES: BIOGEOCHEMISTRY OF MICROCOSM NUTRIENT SUPPLEMENTATION

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Microcosms were constructed to investigate the role additions of molasses, sulfate, both or neither had on the quantity of sulfate reducing microorganisms, and the subsequent effects this known route of biogenic sulfide production would have on soluble minerals, including sulfide, iron and arsenic. The results indicate that addition of any nutrient increases the copies of DsrB, a marker for sulfate reduction, per gram of sediment as compared to the negative control. This increase occurs before 14 days, and appears stable to at least 56 days. Soluble iron is freed during the first 14 days, but is decreased to near 0 ppm over 56 days, in sulfate amended microcosms. Concentration of arsenic was variable over time and did not correlate to any other measured factor. Biomass increased with the addition of molasses, as measured by 16S copy number, but was not linked to increases in DsrB copies. Finally, phosphorus levels became elevated in samples amended with sulfate.

Abstract Category
01 Biogeochemistry

PS.01.072 THE EFFECT OF VIRUSES ON PHYTOPLANKTON AND PROKARYOTIC NUTRIENT ASSIMILATION: A SINGLE CELL APPROACH

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Viruses are the most abundant entities in the world's oceans. Viral infection leads to rapid cellular lysis, thereby influencing the global carbon and nutrient cycles, acting as a driving force in microbial community composition and succession. However, the effect of viruses on the physiology and inorganic nutrient assimilation of the host cells is still poorly understood. Here we present results of incubation experiments with ^{13}C - and ^{15}N -labeled substrates in order (1) to quantify the effects of virus (PgV-07T) on carbon and nitrogen assimilation of host algae (*Phaeocystis globosa*) during infection, and (2) to examine how viral mediated algal lysis influences bacterial substrate assimilation and community composition. We determined abundance of algae, bacteria and viruses by flow cytometry; measured bulk assimilation responses by Isotope Ratio Mass Spectrometer (IRMS), quantified carbon and nitrogen assimilation by single algae cells by nano-scale secondary-ion mass spectrometer (nanoSIMS), and identified the main bacterial groups associated with infected and non-infected algae by using fluorescence in situ hybridization. Bulk assimilation responses revealed no differences in carbon and nitrogen assimilation until the point of lysis (18 h post infection). However, single cell quantification of carbon and nitrogen assimilation indicates that inorganic carbon and nitrogen assimilation by infected algal cells during infection was only about half of the total carbon and nitrogen assimilated by non-infected algae. Viral mediated algal lysis resulted in a shift in bacterial community, with *Alteromonas* sp. dominating soon after algal lysis. In conclusion, our preliminary results indicate that viral infection changes carbon and nitrogen assimilation by algae, which may have a substantial but so far poorly understood impact on oceanic carbon and nutrient cycles.

Abstract Category
01 Biogeochemistry

PS.01.073 DEPTH DISTRIBUTION OF DISSOLVED B-VITAMINS AND PROKARYOTES IN THE EASTERN TROPICAL SOUTH PACIFIC

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B-vitamins are required coenzymes for many metabolic processes and are suspected to be important growth factors in the environment. Recent studies have highlighted the importance of prokaryotes for the production of B-vitamins in the marine environment for auxotrophic eukaryotic phytoplankton. To determine the availability of these metabolites for auxotrophic phytoplankton, we completed the first depth-profile measurements of a suite of B-vitamins (B1, B2, B5, B7, B9, and B12) in the Eastern Tropical South Pacific (ETSP) (March 2010) using a newly developed LC-MS method. Dissolved B-vitamin concentrations were measured at 15 depths at 6 locations ranging from coastal upwelling to open ETSP waters and correlated to prokaryote and eukaryotic phytoplankton abundance as well as inorganic nutrient and dissolved metal (e.g., Co and Fe) concentrations. The field data was complemented with shipboard incubations experiments to establish the impact of those two trace elements on B-vitamin synthesis as well as the impact of B-vitamins on the phytoplankton community composition of the ETSP. Although growth limitation has primarily been observed for vitamin B12, our preliminary data suggest that the addition of vitamin B7 (biotin) resulted in an increase in

chlorophyll in some of our stations. The relevance of this new B-vitamin data set on the biogeochemical cycles of the ETSP will be discussed.

Abstract Category
01 Biogeochemistry

PS.01.074 MICROBIAL GROWTH AND DIVERSITY IN A HUMIC-FREE ENVIRONMENT ON THE COTTON GLACIER, ANTARCTICA.

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A supraglacial stream forms annually on the Cotton Glacier, Antarctica. Analysis of dissolved organic matter (DOM) from this stream in 2004-05 and again in 2009-10 showed that the concentration was low (44-48 $\mu\text{M C}$), and lacked humic signatures, unlike typical DOM of microbially-based ecosystems. Our results indicate that DOM in this system is seasonally formed from soluble microbial products and that a reservoir of recalcitrant humified DOM does not pre-exist. In most aquatic ecosystems, humic DOM acts as a natural sunscreen and the absence of humics may thus represent an additional stressor influencing the microbial community. Nonetheless, the stream contained an active microbial assemblage with bacterial cell abundances from 2.94×10^4 — 4.97×10^5 cells ml^{-1} , and bacterial production ranging from 58.8-293.2 $\text{ng C l}^{-1} \text{d}^{-1}$. Chlorophyll-a concentrations ranged from 0.3 to 0.53 $\mu\text{g l}^{-1}$ indicating that algal phototrophs were the probable source of the DOM. Microbial isolates produced a rainbow of pigment colors, suggesting adaptation to UV stress, and were similar to those from other cryogenic systems (*Cytophagales* and *β -Proteobacteria* lineages). Clone library analysis of the microbial assemblages from the stream water, ice, sediments and aeolian communities were significantly different, but still related to organisms from other cold temperature environments. Taken together, these results suggest that the occurrence of related phylotypes from diverse glacial environs is due to similar survival strategies and that UV stress due to the absence of humics is important in supraglacial streams. Supraglacial streams provide an example of contemporary microbial processes on the glacier surface and a natural laboratory for studying the microbial adaptation to the absence of humics, as well as chemical processes controlling the eventual genesis of humic DOM.

Abstract Category
01 Biogeochemistry

PS.01.075 FUNCTIONAL GENE DIVERSITY AND GEOCHEMISTRY STUDIES IN URBAN AQUATIC AND SEDIMENT SYSTEM IN SINGAPORE

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Singapore with its population density of 7000/km² is the third most densely populated country in the world. Current domestic water consumption, of 155 liters/capita/day is still higher than many European countries. High population density and scarce renewable water resources have placed Singapore low in water poverty index. Further increase in population will put extra pressure on its already limited water resources. Given these urban pressures, it is critical to understand and manage the water resources more efficiently combined with lower energy regimes for circulation and reuse.

Towards this, we have adopted a holistic and integrated approach combining microbial ecogenomics, chemistry, hydrology and modeling. GeoChip analysis of soil, sediment and water samples from 22 locations of a model catchment covering land use patterns such as residential, industrial, commercial and vegetation cover showed a total abundance of 13,747 genes. Genes involved in organic remediation(35%), metal resistance(18%), carbon and nitrogen cycling(11%) and antibiotic resistance (6%) were highly abundant in microbial communities across the catchment. Genes from carbon fixation(1%), energy processes(1.6%) and phosphorus cycling(2%) categories were least abundant. Carbon degradation(3.5%) and sulfur cycling(4.5%) were comparable with antibiotic resistance. While there were 4 fold differences in their total abundance between sites, the relative abundance had little variation. Shannon's diversity index from 6.8 to 7.3 also showed little variation between sites. Such a trend of low variation clearly shows effects of urban anthropological pressure on microbial communities. Due to the small size of catchment (50km²) in urban environment the expected difference in microbial diversity, based on different land use patterns are not seen. Hence, small changes in urban activities can affect the narrow spectrum of microbial diversity, which underpins strong efforts in better management practices.

Abstract Category
01 Biogeochemistry

PS.01.076 MICROBIAL DIVERSITY IN TROPICAL PEATLANDS FROM INDONESIA

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Peatlands cover nearly 4% of earth surface of which, more than 50% (22 million hectares) of the tropical peatlands are in Indonesia. Deforestation and drainage of peatlands for agriculture and rehabilitation purposes, has caused rapid oxidation of peat biomass leading to carbon emissions of nearly 2 Gigatons/year which constitutes nearly 3-8% of global emissions due to fossil fuels. Our 11 study transects are located near Jambi, Sumatra island covering 48 sq km and differing in peat depth, land cover, water table, states of degradation and management systems. Data is gathered for land-use pattern from remote sensing and on-site measurements for gas emissions, microbial diversity and geochemical parameters at various depths during dry and wet seasons.

Here we report, microbial diversity data based on 360 rDNA clones from samples taken from 2 different sites and at 2 depths within one site. Of these clones, only 12.5% were identified species, of which 23% were common between the 2 sites, whereas 40% and 37% were unique to each site. When compared within the site, 23% species were common to both the depths, whereas 32% and 45% were unique to above and below water table respectively. Hence, even with limited clone coverage, very high level of microbial diversity is seen in peatlands. High degree of variation between sites as well as at the two depths is evident. Dominant species belonged to alpha- and gamma-proteobacteria, of which *Stenotrophomonas maltophilia* and *Brevundimonas sp.* were most abundant. Wood degradation studies were done to indicate peat biomass decomposition potential of microbes. Significant wood decomposition (recorded as weight loss) above (27-94%) and below (8-18%) the water table was observed in less than 4 months. In future, we hope to increase coverage of clones and include geochemical parameters to allow us to develop associations between microbial species and geochemical characteristics of peatland.

Abstract Category
01 Biogeochemistry

PS.01.077 BIOGEOCHEMICAL CYCLING AND MICROBIAL COMPOSITION IN HYDROTHERMAL PLUMES FROM LOIHI SEAMOUNT, HAWAII

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Loihi Seamount is a mid-plate hotspot volcano located ~15 kilometers off the southeast corner of the Big Island of Hawai'i. The hydrothermal fluids at Loihi are enriched in carbon dioxide, methane, ammonia, phosphate, iron and manganese, and have very little hydrogen sulfide. As fluids mix with seawater, redox disequilibria set up an ideal environment for chemoautotrophy to occur. We studied biogeochemistry and microbial community composition in the hydrothermal fluids and non-buoyant hydrothermal plumes at Loihi during two cruises in Fall 2008 and 2009. We measured dissolved organic carbon (DOC), nitrate + nitrite, and dissolved ammonia in samples collected with either ROV Jason II (hydrothermal fluids) or a CTD rosette (hydrothermal plumes). Elevated concentrations of DOC are detected in the non-buoyant plumes, and DOC increased with distance from the seamount, suggesting *in-situ* autotrophic production in the plumes. High ammonia is measured in fluids, ranging from 0.7-4.2 micromolar. A strong, negative correlation between nitrate+nitrite and ammonia is determined in the hydrothermal fluids, but no samples contained 0 micromolar nitrate+nitrite, indicating that subsurface ammonia oxidation is likely occurring. Ammonia in the non-buoyant plume was detected up to 4 kilometers from the Loihi summit. Concentrations of ammonia in the non-buoyant plume distal from the summit were 0-0.375 micromolar during the two cruises, while background concentrations at depths above and below the plume were below detection. This indicates that Loihi is a significant source of ammonia to deep waters up to several kilometers away. This possibly supports autotrophic ammonia oxidation within these plumes, a potential source of the elevated DOC measured in these samples. Preliminary analysis of microbial community composition within these samples will be discussed.

Abstract Category
01 Biogeochemistry

**PS.01.078 EFFECT OF CONTRASTING SOIL TREATMENTS ON DENITRIFIERS ,
DENITRIFICATION AND NITROUS OXIDE EMISSIONS USING OXIC SOIL
MICROCOSMS**

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In agriculture, compost and mineral amendments can influence soil microorganisms involved in denitrification, providing organic carbon and readily-utilizable N sources which are major drivers of the production of the greenhouse gas nitrous oxide (N₂O). In a vineyard soil located in Tuscany, Italy, mineral fertilization or compost amendment were applied annually since 2001. The long term effects of mineral fertilizer (conventional management system, CS), and compost (organic management system, OS) was investigated in oxic soil microcosms for 21 days using three treatments, no fertilizer, ammonium nitrate (NH₄NO₃) or compost amendment. Denitrifier abundance, denitrification gene mRNA levels, N₂O emissions and denitrification (N₂O+N₂) rate were quantified. Abundance of *nirS*, *nirK* and *nosZ* denitrifiers was significantly higher in OS compared to CS soil for all treatments. Compost addition caused a significant increase in *nirK* abundance for soils under OS and CS over time, while *nirS* and *nosZ* denitrifiers increased significantly only in OS soil following compost treatment. In the remainder of the treatments, the denitrifier abundance was relatively stable overtime. In OS soil, *nosZ* mRNA levels were higher at any time point for all treatments compared with CS soil. Furthermore, *nosZ* mRNAs significantly increased over time after compost treatment for both CS and OS soils, while for the no fertilizer and NH₄NO₃ treatments, *nosZ* transcripts were relatively stable overtime. N₂O emissions were higher in both soils after NH₄NO₃ addition compared with no fertilizer and compost treatments whereas denitrification was significantly higher following NH₄NO₃ treatment in OS soil compared with CS soil. Contrasting soil managements changed soil properties, leading to clear effects on soil denitrifier communities and nitrous oxide emissions, thereby influencing the ecological impact of anthropogenic activities in this soil ecosystem.

Abstract Category
01 Biogeochemistry

**PS.01.079 MICROBIAL COMMUNITY DIVERSITY EFFECTS ON THE
MAGNITUDE AND STABILITY OF GREENHOUSE GAS FLUXES ACROSS LAND
MANAGEMENT CLINES**

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Land use has a dramatic impact on ecosystem processes, with soil microbial communities in standard row crop agriculture increasing rather than reducing greenhouse gas fluxes including

methane and carbon dioxide. Ecological models suggest that the magnitude and stability of an ecosystem process are correlated with species richness when the process is associated with a specialized metabolism. Conversely, when many organisms in a given environment contribute to the same ecosystem function, the process is stable, but not affected by species richness. While the production of carbon dioxide is a highly redundant type of metabolism, characteristic of all heterotrophic microbes, methane oxidation is a very specialized process that only a limited number of microbes can carry out. To ascertain this relationship between ecosystem function and richness, the diversity of methanotrophs was determined using molecular surveys of the *pmoA* gene, overall bacterial diversity was determined from 454-pyrosequencing 16S 'tag' data, and in situ measurements of methane and carbon dioxide were made across a range of land uses at the Kellogg Biological Station Long Term Ecological Research site. We established that, consistent with the model, the rates of methane consumption were lowest and the variability highest in sites that harbored the least methanotroph diversity. Conversely, estimates of total bacterial diversity in soil were not related to the rate or stability of carbon dioxide emission. Additionally, the composition and diversity of the methanotroph community varies in soils of crops considered for biofuels at the Great Lakes Bioenergy Research Center sites. Our results suggest that it may be possible to manipulate the diversity of organisms with highly specialized metabolisms to influence the magnitude and stability of the ecosystem processes they catalyze.

Abstract Category
01 Biogeochemistry

PS.01.080 MICROBES PRODUCE UNNEGLIGIBLE THERMOGENESIS-LIKE METHANE IN DEEP SUBSURFACE

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The origin of gaseous hydrocarbons is essential to assess the migratory pathway and reserve capacity during hydrocarbon exploration, and to delineate the extent of carbon cycling in terrestrial gas-oil-water systems. Conventional approaches use specific ranges of isotopic compositions and relative abundances of hydrocarbons to distinguish various source mechanisms (including thermal maturation, methanogenesis, and abiotic process). These fingerprints have been found to be microbially obscured in several gas and petroleum reservoirs, rendering the quantification of the contributions from source and post-formation processes challenging. In this study, we conducted laboratory microcosm incubations to demonstrate that acetate-consuming methanogenic populations produced methane with carbon isotopic compositions more enriched in ¹³C than hydrogenotrophic methanogens did, and highly resembling those in amendment-free incubations, in previous field studies, and of thermogenic methane. The majority of these methanogens were affiliated with *Methanosaeta thermophila*, a thermophilic archaeon solely relying on acetate for energy conservation. Such microbial methane constituted a significant proportion (>50%) of the observed methane in the investigated site. When extrapolating the incubation results to a hypothetical rock column extending to a depth of ~1.8 km, methanogenesis could feasibly account for a prospective hydrocarbon accumulation in deep subsurface.

Abstract Category
01 Biogeochemistry

PS.01.081 MEASUREMENT OF ASSIMILABLE ORGANIC CARBON (AOC) IN OLIGOTROPHIC GROUNDWATER AQUIFERS

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AOC determination is often avoided due to the tedious nature of the conventional bioassay, although it potentially controls bacterial growth. A new method was proposed some years ago, in which plating was replaced by fluorescence staining of the natural community combined with flow cytometry as a rapid and straightforward method. We adapted this method, mainly used as a quality parameter in water distribution systems, to pristine oligotrophic groundwater aquifers to quantify the pool of natural bioavailable carbon compounds. Samples were obtained from different alpine karst springs (average DOC of 0.48 mg L⁻¹), in order to estimate the detection limit of the approach. The amount of AOC in alpine groundwater varied between 10 and 130 µgC L⁻¹, representing between 2 and 35 % of the DOC fraction. Although there was no correlation with DOC, a highly significant correlation between the low heterotrophic prokaryotic production (avg. 38 pmol C L⁻¹h⁻¹) and AOC was observed. The fraction of AOC available for the autochthonous community was not necessarily identical with the overall AOC pool present in the environment but depended on factors such as incubation temperature and limitation by trace elements. Thus, the AOC pool might be divided into a potentially usable AOC and an actually used AOC fraction. Currently, we are trying to apply the method to the respective aquifer sediments. We also perform further tests to estimate the possible effect of viral lysis during the incubation time, since it is known that viral lysis influences prokaryotic production and growth efficiency. The method provide valuable information even in environments where the concentration of available carbon is extremely low and showed its potential for, i.e. more detailed information on AOC along trophic gradients or during changes of nutrient availability.

Abstract Category
01 Biogeochemistry

PS.01.082 EFFECT OF MUTIL-FUNCTIONAL THERMO-TOLERANT PHOSPHATE- AND POTASSIUM-SOLUBILIZING BACTRIA ON BIOFERTILIZER PREPARATION

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Multi-functional thermo-tolerant potassium- and phosphate-solubilizing isolates: *Bacillus licheniformis* A3 and *Bacillus subtilis* H8 were selected from animal and agricultural waste

composts and had diverse enzymatic and potassium- and phosphate-solubilizing activities. Both of them possessed phosphate-solubilizing activities of aluminum phosphate, calcium phosphate, ferric phosphate, hydroxyapatite, and rock phosphate, potassium-solubilizing activities of feldspar, illite, and kaolinite, and enzyme activities of α -amylase, chitinase, CMCase, keratinase, lipase, protease, pectinase, and xylanase. Inoculating these microbes in biofertilizer preparation could shorten maturity period, improve quality, increase soluble potassium and phosphorus contents, and promote populations of bacteria, phosphate-solubilizing bacteria, and potassium-solubilizing microbes. Inoculation of *B. subtilis* H8 in biofertilizers preparation had the highest soluble potassium content (1.84 ± 0.03 g kg⁻¹); while inoculation of *B. licheniformis* A3 presented the highest phosphate content (1.55 ± 0.02 g kg⁻¹). Therefore, it is prospective to prepare biofertilizers with high soluble potassium and phosphate contents by the inoculation of mixed multi-functional thermo-tolerant potassium- and phosphate-solubilizing microbes for sustainable agriculture.

Abstract Category
01 Biogeochemistry

PS.01.083 DIVERSITY OF ARSENIC(V) REDUCTASE (*arrA*) GENES ACROSS REDOX GRADIENTS WITHIN DELTAIC SOILS OF SOUTHEAST ASIA

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Arsenic cycling within soils and sediments of the Mekong Delta is strongly affected by drastic redox fluctuations caused by seasonal monsoons. Repeated oxidation and reduction of soils, which contain 10-40 mg kg⁻¹As, may lead to the eventual downward movement of arsenic to the underlying aquifer. Extensive flooding during monsoon seasons creates anoxic soil conditions that favor anaerobic microbial metabolic processes, including arsenate [As(V)] respiration-a process contributing to the mobilization of arsenic. Amplification of a highly conserved functional gene encoding As(V) reductase, *arrA*, can be used as a marker to detect the genetic potential for As(V) respiration in environmental samples. In the present study, we examine the presence and diversity of *arrA* genes amplified from multiple study sites within the Mekong Delta at various soil depths (10, 50, 100, 200, and 400 cm). Geochemical analysis of soil pore water, collected using lysimeters and passive samplers (peepers) at the corresponding depths, show that successful amplification of *arrA* genes using degenerate primers corresponds with an increase dissolved arsenic at 50, 100, 200 cm depths. Our findings help illustrate the potential for using functional gene expression to understand operative geochemical processes and, in particular, conditions promoting the mobilization of arsenic within flooded, tropical soils-systems where arsenic is having a devastating impact on human health by contaminating regional aquifers.

Abstract Category
01 Biogeochemistry

PS.01.084 EVIDENCE FOR AUTOTROPHIC GROWTH OF ARCHAEAL AMMONIA OXIDISERS IN SOIL

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Metagenomic and cultivation-based studies have revealed the existence of ammonia-oxidizing archaea (AOA) containing ammonia monooxygenase subunits encoded by *amoA*, *amoB* and *amoC* genes. Archaeal *amoA* genes are more abundant than *amoA* genes of ammonia oxidizing bacteria (AOB) in a range of environments and archaea appear to contribute significantly to nitrogen cycling in oceans and in many soils. However, no autotrophic archaeal ammonia oxidizer has yet been isolated from soil, our understanding of the relative contributions of AOA and AOB to soil nitrification is limited and it is not known whether ammonia oxidation by AOA is linked to autotrophy. The aim of this study was to determine the relative roles of AOA and AOB in soil ammonia oxidation and whether ammonia oxidation was linked to autotrophy. Stable isotope probing (SIP) was used to assess CO₂ fixation by AOA and AOB during nitrification in soil microcosms. Combined nitrite + nitrate concentration increased in microcosms incubated with ¹³CO₂ or ¹²CO₂ for 14 and 28 days and was coupled with a significant increase in archaeal, but not bacterial *amoA* gene abundance. Nitrification was also accompanied by significant increases in the relative intensities of two bands in DGGE profile of archaeal *amoA* genes, but no change in bacterial *amoA* DGGE profiles was detected. DGGE and quantitative PCR analysis of ¹³C-labelled DNA demonstrated significant incorporation of ¹³CO₂ into archaeal *amoA* genes during active nitrification, but not into bacterial *amoA* genes. The results therefore provide strong evidence for an association between ammonia oxidation and autotrophic growth by soil archaea and for a dominant role of archaea in ammonia oxidation in this soil.

Abstract Category
01 Biogeochemistry

PS.01.085 IMPROVING SOIL MICROBIAL COMMUNITY STRUCTURE AND FERTILITY BY COTTON STRAW AMENDMENTS

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Maintaining the soil fertility and controlling pollution from the fertilizer application are important for keeping the high soil productivity of sustained agriculture. Crop straw amendments are available for improving soil conditions by increasing organic matter and effective microorganisms in soil. However, little is known about the cotton straw amendments on soil microbial ecology till now. The objective of this study was to use plate count, Biolog, phospholipid fatty acid (PLFA) analysis and 16S rDNA clone library to determine the changes of microbial community that occur in the soil of an organic orchard by cotton straw amendment. The results showed that the cotton straw application could improve the bacterial community metabolic activity, and markedly changed the structure of microbial community, such as the members of the *Gammaproteobacteria* phylum were increased while the members of the *Chloroflexi* phylum were decreased markedly; the concentration of cotton straw in soil was positive correlation with the number of the culturable microorganisms and biomass. In

conclusion, the cotton straw amendments could promote the cucumber seedling growth by improving soil microbial community structure and increasing the soil organic matter (OM), available nitrogen (AN), available phosphorus (AP) and available potassium (AK) significantly.

Abstract Category
01 Biogeochemistry

**PS.02.001 METHANOGENS IN THE CANADIAN HIGH-ARCTIC:
CONTRIBUTION TO THE CARBON-CYCLE FEEDBACK EFFECT ON CLIMATE
CHANGE**

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Permafrost thaw due to climate change is predicted to form anaerobic wetlands and provide previously frozen carbon stores as substrates for microbial decomposition. This is expected to favour methanogenesis and the release of methane, a greenhouse gas 23 times more potent than carbon dioxide. This project aims to analyse samples from Canadian High-Arctic permafrost for the diversity, abundance, and activity of methanogens, as well as the rate of methane release in response to increased temperatures. This will help predict the contribution of methanogens to the positive carbon-cycle feedback effect of permafrost thaw on climate change. The diversity of active methanogens was assessed by DNA stable isotope probing (DNA-SIP) and sequencing of 16S rRNA and *mcrA* genes from microcosms supplemented with ¹³CO₂ and ¹³C-labeled acetate. The abundance of methanogens was determined through quantitative PCR (qPCR) targeting the *mcrA* gene. The rate of methane release was determined by supplementing microcosms with ¹⁴CO₂ and ¹⁴C-labeled acetate and monitored by gas chromatography with a flame ionization detector. To assess the response of methanogens to the temperature increases associated with climate change, microcosms were incubated at temperatures 5°C higher than *in situ*. DNA-SIP and qPCR showed that both the diversity and abundance of active methanogens in Canadian high Arctic permafrost is quite low. The abundance varied according to various factors, including water content, depth, and extractable organic content. The increase in temperature of 5°C caused higher rates of methane production in isolated controls, and is predicted to occur in Canadian High-Arctic samples. An increase in permafrost temperature promotes methanogen activity and thus may lead to a significant release of methane into the atmosphere, contributing to the positive feedback effect on climate change in the coming century.

Abstract Category

02 Climate Change

**PS.02.002 METHANOGENS ARE GLOBALLY UBIQUITOUS IN AERATED SOILS
AND ARE ACTIVE UNDER SIMULATED NATURAL CONDITIONS — THE RARE
BIOSPHERE IN ACTION**

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Methanogenic archaea are traditionally thought to be active in strictly anoxic environments. Consequently, the major known sources of biogenic methane are highly reduced environments with a constant influx of organic material such as: marine and lake sediments, gastrointestinal tract of ruminants and termites, natural wetlands and rice paddies. In contrast to this textbook notion, we found methanogens of the types *Methanosarcina* and *Methanocella* to be present in many types of upland soils sampled globally, including desert soil crusts. These methanogens could be readily activated by incubating the soil as slurry under anaerobic conditions, as seen by

rapid methane production within a few days without any additional carbon source. Analysis of 16S rRNA gene copy numbers, transcript copy numbers and the isotopic analysis of the methane all indicated a dominance of (up to 90%) *Methanosarcina* mediated acetoclastic methanogenesis. Using a specifically designed terminal restriction length polymorphism (TRFLP) assay, we were able to detect the presence of these methanogens in many of our pre-incubated soil samples and in similar proportions to the post-incubated samples indicating no significant enrichment bias. In a follow-up factorial experiment, we incubated desert crust soil in microcosms under oxic, anoxic, full light, darkness, flooded and drained conditions, in all possible combinations, for 42 days. Methane production and transcripts of the key enzyme, methyl coenzyme M reductase (*mcrA*), were observed in all microcosms, from 7 days onwards. Isotopic data shows that under oxic conditions the primary methanogenic route is hydrogenotrophic rather than acetoclastic. Our results show that methanogens are apparently active in anoxic niches of aerated soils when water content is sufficiently high, such as after rain events. This hints to a possible, and formerly unknown, contribution of such ecosystems to the global methane budget.

Abstract Category
02 Climate Change

PS.02.003 CAPACITY AND MECHANISMS OF ADAPTATION OF ACTINOBACTERIA OF VARYING CLIMATE-HISTORIES TO ALTERED PRECIPITATION

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Recent studies of bacterial communities across contrasting terrestrial environments have recorded soil moisture as an edaphic variable strongly regulating community structure in a seemingly predictable manner. Phylogenetic analysis across broad scales found preferential distribution of microbial groups into 'wet' and 'dry' environments. While a large number of taxa were associated with 'wet' conditions, gram-positive bacteria, in particular *Actinobacteria* consistently demonstrated a strong positive response to decreasing soil moisture. Such grouping by moisture preference suggests a functional basis selected for on evolutionary timescales. This study examines the mechanistic rationale for such distribution and the genetic capacity for adaptation to decreasing precipitation. *Actinobacteria* were isolated on a complex carbon medium (humic acid vitamin agar) from samples taken from rainfall exclusion and control plots in a Puerto Rican wet tropical forest. Isolates were exposed to a range of water potentials (from -0.00086 to -5.0 MPa) in minimal media. RNA was extracted, sequenced and mapped against reference genomes. Metabolic adaptation was assessed by growing isolates in media containing either ¹³C- or ¹⁵N-labeled substrates and monitoring transfer of labeled compounds through the metabolic pathway by LC-MS-MS. Exposure to a range of water potentials demonstrated a low adaptive capacity of tropical soil *Actinobacteria*. Isolates exhibited stress under small decreases in water potential, with growth inhibited below -0.3 MPa. The result of transcript and metabolic analyses demonstrated a number of pathways by which *Actinobacteria* alleviate osmotic stress. Key within the metabolome is the production of compatible solutes protecting against

desiccation at higher salt concentrations. Results suggest that at a local scale recent climate history may constrain osmoregulatory adaptive capacity in *Actinobacteria* despite an evolutionary history of drought tolerance.

Abstract Category
02 Climate Change

PS.02.004 THE IMPACT OF CLIMATE CHANGE ON SOIL BACTERIAL COMMUNITY COMPOSITION IN COASTAL REDWOOD FORESTS

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This project examined the potential impacts of climate change on the composition of soil microbial communities in coastal redwood forests. Understanding the response of soil communities to climate change is important for predicting changes in ecosystem services and of interest to ecosystem stewards. A 3-way reciprocal transplant experiment was conducted across a latitudinal gradient of coastal redwood forests. Samples were collected 1 year and 3 years after transplanting. Bacterial community composition was analyzed using a high-density 16S rDNA microarray (PhyloChip). Climatic variables and soil variables (rainfall, soil moisture, soil temperature, soil C and N availability, pH, soil texture) were measured. Changes in community composition were assessed with non-metric multidimensional scaling (for the entire community) and ANOVA (for individual taxa). The relationships between bacterial community composition and climatic and edaphic variables were examined with Mantel tests. The change in climate had a significant influence on bacterial community composition. The amount of rainfall and its impact on soil moisture were the strongest and most significant correlates with community composition. In addition, the number of bacterial species that responded to the change in climate increased from year 1 to year 3. The results indicate that changing patterns of precipitation may influence bacterial community composition at a regional scale. While many environmental factors may also influence changes in community composition over time, it appears that the number of species that respond to the impact of climate change increases with time; three years may not be long enough to assess the long-term impact of climate change on microbial community composition.

Abstract Category
02 Climate Change

PS.02.005 THE HOMOMOLOGY MODELING AND TvAPI TRANSCRIPTION FACTOR GENE EXPRESSION IN *Trichoderma virens* RESPONSE TO TEMPERATURE STRESS

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The climate change, as one of the most important environmental conditions, has critical influence on microbial survival and ecology. It has been reported to be associated with directly effects on microbial metabolism and mechanism, but there are very few studies in literature, which have reported in beneficial fungi. The aims of this study, we investigated the impact of culture temperature stress on the TvAP1, involved in the transport of secondary metabolites, gene expression of *Trichoderma virens*. The changes in ADP-Glo kinase and defense/cell wall enzymes activities including catalase, peroxidase and glucanase were also determined. The TvAP1 protein secondary and tertiary structure was predicted. One mL spore suspension (1×10^8) were added to 100 mL VMS media and cultured at difference temperature on a rotary shaker. The fungal tissue were directly sampled for microscopy or collected by centrifugation and used for future experiments. Our results indicate that CAT, POX, GLU activities and TvAP1 gene expression generally decreased with culture temperature, whereas ADP-Glo kinase increase-changed as temperature stress, indicating that all five indicators played a major role probably due to enhanced activity of the fungal respiratory and energy metabolism pathway. The phylogenetic tree showed that TvAP1 closest neighbour is AP1 from *Neurospora crassa*. The secondary structure of TvAP1 using PSIPRED revealed helices, strands and coils in the protein structure. The tertiary structure prediction of TvAP1 by Pymol showed β/α fold. The program VERIFY 3D and the Ramachandran plot using RAMPAGE software assessed the quality of the predicted structure with acceptable scores. Our findings indicate that temperature stress caused increase biomass and ADP-Glo kinase while decrease in the defense/cell wall enzyme activities and transcription factor gene expression that might be these key gene and enzymes help *T. virens* adapt for survival to climate change as temperature stress.

Abstract Category
02 Climate Change

PS.02.006 IMPACT OF TEMPERATURE ON SOIL CARBON MINERALIZATION AND MICROBIAL COMMUNITY STRUCTURE

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In tropical country such as India, as a result of increased temperature, the release of CO₂ from soil organic carbon may be governed by the temperature sensitivity of decomposing organic matter in soil and the amount of precipitation. However the magnitude of this effect is uncertain and critically dependent on the response of soil organic carbon to changes in climate. In the present study, the carbon mineralization rate and temperature sensitivity of various contrasting soils (representing five soil orders and grouped as low, medium and high carbon status) of India having different carbon contents was determined. The soils were incubated aerobically at five temperature gradients i.e., 20, 25, 30, 35 and 40°C under laboratory conditions. Soil respiration increased linearly with increase in temperature from 20 to 40°C at different rates in all the three soils having different carbon content. Low carbon soils showed higher carbon mineralization (14-24% C) and high water soluble carbon as compared to medium and high carbon soils as the temperature increased. Furthermore, the availability of more nutrients because of greater mineralization of soil organic matter, led to increase in abundance of gram positive bacteria,

which exhibits the broadest capability to decompose organic polymer. However, the recalcitrant pool in this soil is less vulnerable to decomposition as being evidenced by not much change in fungi: bacterial ratio. In high and medium carbon soil, the increases of temperature led to the significant decline in fungi:bacterial ratio thereby resulting in decline in recalcitrant pool of the carbon. Whereas in low carbon soil, there is not much change in fungi: bacterial ratio with the increase in temperature. Thus this study indicates that small incremental increase in temperature in low carbon soil results in high carbon dioxide emissions as compared to medium and high carbon soil, thereby making low carbon soil more vulnerable to warming.

Abstract Category
02 Climate Change

PS.02.007 MOLECULAR ECOLOGICAL NETWORKS AND THEIR CHANGES IN RESPONSE TO ELEVATED CO₂

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Understanding the interactions among different species and their responses to environmental changes is a central goal in ecology. Although the network interactions and their responses to environmental changes have been intensively studied in macroecology, very little is known in microbial ecology. Here, using large-scale metagenomics sequencing and hybridization data, we describe a novel random matrix theory-based conceptual framework for identifying network interactions in microbial communities, termed as molecular ecological networks (MENs). Both phylogenetic and functional MENs of the 16 microbial communities from various habitats possessed general characteristics of many complex systems such as scale-free, small-world, modular and hierarchical. Our results also indicated that elevated CO₂ had significant impacts on the overall architecture of microbial community interaction networks and dramatically altered the interactions among different microbial populations and functional groups. As an example, the interactions of the populations involved in nitrogen fixation became much more complex under elevated CO₂ than ambient CO₂. In addition, the changes in network structure were significantly correlated with soil carbon and nitrogen dynamics, and plant productivity, indicating the importance of network interactions in ecosystem functioning. Elucidating network interactions in microbial communities is critical to studying biodiversity, global change biology, ecology, and systems microbiology.

Abstract Category
02 Climate Change

PS.02.008 AMMONIA OXIDIZING BACTERIAL AND ARCHAEAL COMMUNITIES RESPOND DIFFERENTLY TO NITROGEN DEPOSITION

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The Domains Archaea and Bacteria contain the vast majority of Earth's biodiversity and biomass, and their members play critical, often exclusive, roles in many biogeochemical cycles

and ecosystem services. Human-induced global change, particularly with respect to increased nitrogen deposition, has the potential to drastically alter how soil nitrifying communities perform their biogeochemical function. Additionally, global change can alter how microbial communities interact with each other and with the associated plant communities. This study, performed in the context of the long-term Jasper Ridge Global Change Experiment (JRGCE) in a California grassland ecosystem, examines how ammonia-oxidizing Archaea and Bacteria (AOA and AOB, respectively) respond to nitrogen deposition. We have compared how DNA-based versus RNA-based ammonia-oxidizing communities respond to global change manipulations over several seasons. We have employed ultra-deep 454-pyrosequencing techniques to examine these communities using the ammonia mono-oxygenase (*amoA*) functional gene marker. Effects of nitrogen deposition have been examined at several phylogenetic levels and linked this community information to gross rates of nitrification using ¹⁵N stable isotopic methods. Ammonia-oxidizer and plant communities have been compared using multivariate statistical methods. Our results show that both AOB communities are highly influenced by nitrogen deposition in both their abundance and community structure, while few changes are seen in the AOA community. These changes are linked to increased nitrification rate in the elevated nitrogen deposition plots. Our results further show that the relationship between the AOB and plant communities fundamentally changes under long-term nitrogen deposition manipulation. This positive feedback loop may enhance the rate of change in ammonia-oxidizer communities, which may further elevate nitrification rates.

Abstract Category
02 Climate Change

PS.02.009 CLIMATE CHANGE AND DROUGHT IMPACT ON CARBON SEQUESTRATION IN AUSTRALIAN FORESTS: THE ROLE OF SOIL MICROBIAL DIVERSITY

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The resilience of Australian forest ecosystems is likely to be exceeded by an unprecedented combination of climate change and drought. Forest survival and adaptation to future climate change conditions are dependent on soil microbial communities, which are the main agents responsible for litter decomposition and nutrient cycling. Here we describe how elevated atmospheric CO₂ concentrations and drought affect belowground microbial communities associated with Australian eucalypt forests. *Eucalyptus saligna* trees were grown for three years in the field under controlled temperature conditions and exposed to ambient (*ca.* 380 ppm) or elevated (*ca.* 640 ppm) atmospheric CO₂ concentrations and different watering regimes in 12 whole tree chambers. We assessed bacterial and fungal community structure and biomass using a combination of RNA- and DNA-based molecular fingerprinting, phospholipid fatty acid and quantitative PCR approaches in addition to pyrosequencing. Multivariate analysis of community profiles showed that elevated CO₂ intensified the effect of drought stress by significantly altering the bacterial and fungal community composition associated with *E. saligna* rhizosphere and bulk

soil. As opposed to simply increasing the biomass of soil-borne microbes at ambient CO₂ under drought conditions, elevated atmospheric CO₂ strongly selected for opportunistic plant-associated microbial communities and effected shifts in microbial community composition. Collectively, our data demonstrate that alterations to atmospheric CO₂ concentrations combined with drought conditions drastically modify the structure of soil bacterial and fungal communities. These responses of soil-borne microbial communities are expected to impact biodiversity and soil food-web interactions, and may influence the direction and magnitude of terrestrial ecosystem/atmosphere feedbacks that regulate global change in Australian forest ecosystems.

Abstract Category
02 Climate Change

PS.02.010 CYANOBACTERIAL EXUDATES — LAND OF COCKAIGNE FOR HETEROTROPHIC BACTERIA?

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Cyanobacterial exudates mostly consist of carbohydrates but also of amino acids and potentially of toxins. Increasing temperatures may lead to changes in quantity and also in quality of exudates. If so, how does it affect bacterial community composition, organic matter and nutrient cycling? To answer these questions we incubated two strains of axenic *Microcystis aeruginosa* at three different temperatures (20, 26 and 32°C) which represents the range of actual and predicted summer temperatures in temperated lakes. One of the *M. aeruginosa* strains was toxic and one non-toxic. We collected the exudates during late exponential growth period of the cyanobacteria by filtration and analyzed their chemical components. Afterwards, we incubated natural heterotrophic lake bacteria in the soluted exudates (10mg C per L) for one month. The remaining carbon compounds as well as the bacterial community and bacterial cell numbers were analyzed at the end of the incubation. The quantity of exudates increased with increasing temperatures twice as much dissolved organic carbon at 32°C than at 20°C. Interestingly, toxic *M. aeruginosa* only exudated microcystin at the cooler temperatures and this in low amounts which showed no significant effect on the dominant heterotrophic bacteria. However, bacterial community composition was more affected by incubation temperature than by exudates obtained from different temperatures. For example, at 20°C Actinobacteria constituted a large fraction of the bacterial community including the AcI-cluster, at 32°C Proteobacteria especially, Gammaproteobacteria, were dominant. Our results show a significant effect of water temperature on bacterial community composition and prove the importance of cyanobacterial exudates including toxins as a carbon source for a wide range of limnic heterotrophic bacteria.

Abstract Category
02 Climate Change

PS.02.011 ACCLIMATION OF SYMBIODINIUM SP. (KB8) TO HIGH GROWTH TEMPERATURE

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Coral reefs are most biodiverse marine ecosystems on Earth and provide important services to humans such as tourism, fishing, coastal protection and pharmaceutical products, among others. The health and productivity of these ecosystems hinges on a functional symbiosis between the cnidarian hosts and photosynthetic dinoflagellate (*Symbiodinium*) endosymbionts. Coral bleaching is the result of the disruption of this symbiosis. Bleaching is often caused by thermal stress and the symbiont's photosynthetic apparatus is extremely sensitive to thermal fluctuations. In this study, we cultured *Symbiodinium* sp. (KB8) under control (i.e. ambient reef) and high temperature conditions. We found differences in several physiological parameters as growth rate, chlorophyll a and c content, membrane fluidity and electron transport rate of the PSII in the treatment comparisons. Additionally, differential gene expression was assessed by the use of a KB8 gene chip (853 sequences) generated from ESTs sequenced by JGI. This study will be helpful in enhancing our understanding of the molecular and cellular mechanisms involved in *Symbiodinium* temperature acclimation.

Abstract Category
02 Climate Change

PS.02.012 SOIL MICROBIAL COMMUNITY RESPONSE TO DRYING AND REWETTING STRESS: DO MICROORGANISMS ADAPT TO ALTERED RAINFALL TIMING?

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The frequency of drought and floods are expected to increase under future climate regimes. Drying and rewetting events are physiologically stressful to microorganisms. Thus, increases in the frequency of rainfall pulses could cause changes in soil microbial community composition. These changes may alter the soil nutrient fluxes that these organisms control, and display a functional legacy even when normal rainfall resumes. This study asks 1) Does an increase in rainfall variability alter microbial function, biomass, and community composition? and 2) Do microbial communities adapt to drying-rewetting events such that their response differs depending on climatic history? We examined these questions using soils from the Konza Long Term Ecological Research Station that were subject to long-term field manipulations that amplify rainfall variability. In the lab, control and field-manipulated soils were subject to drying-rewetting pulses while a continuously wet treatment served as a control. At the initial wetting up, and after the first and last pulse, we measured respiration, microbial biomass, bacterial species composition and fungal:bacterial ratios of the soil community. Microbial biomass carbon was more reduced by lab moisture pulses in soils from ambient treatments, whereas soils from delayed treatments showed less of a response. Fungal:bacterial ratio increased in response to pulses, suggesting fungi are more tolerant to moisture fluctuations than bacteria. Although field treatments initially responded to pulses differently, both parameters were not significantly different between field treatments after the last pulse. These results suggest that microbial communities may adapt to stressful events such as drying and rewetting, but may converge over time when exposed to similar conditions. Microbial communities, like plant communities, do

display legacies, and this may affect temporal dynamics of ecosystem function and the predictions of ecosystem models.

Abstract Category
02 Climate Change

PS.02.013 IMPACT OF MYCORRHIZAL HYPHOSPHERE CARBON FLOW ON DENITRIFICATION PROCESSES AND ON THE TRANSCRIPTIONAL DYNAMICS OF THE DENITRIFYING MICROBIAL COMMUNITY

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The production of nitrous oxide (N₂O) from soils by denitrification constitutes a major proportion of the global emission of this important greenhouse gas. A major factor influencing denitrification rates in soil is the availability of degradable organic carbon. In mycorrhizal systems, photosynthetically fixed carbon is transported from plants into the mycorrhizal hyphosphere and subsequent exudation of leachates or hyphal biomass turnover may thus contribute significantly to the microbially available soil carbon pool. Currently, neither the contribution of the hyphosphere to the soil carbon pool nor its possible influence on microbial respiration processes are known. The aim of this study was to establish the influence of the arbuscular and ectomycorrhizal hyphosphere on the activity of denitrifying bacteria associated with the hyphosphere and on the resulting evolution of nitrous oxides and N₂. Experimental systems were established using *Plantago lanceolata* and *Populus tremulus* as plant hosts and *Glomus intraradices* and *Paxillus involutus* as arbuscular and ectomycorrhizal mycorrhizal fungi, respectively. Denitrification rates were analysed by GC-MS and the ratio of ¹⁵N₂/¹⁵N₂O production was investigated together with assessment of the *in situ* activity of denitrifying bacteria by quantification of the key functional genes (nitrite reductase: *nirK/nirS* and nitrous oxide reductase: *nosZ*) and gene transcripts. Here we report first results on differences in denitrification rates and transcriptional activities between hyphosphere and non-hyphosphere soil compartments.

Abstract Category
02 Climate Change

PS.02.014 METHANOGENESIS IN A WARMING ARCTIC: RATES, PATHWAYS, AND POPULATIONS

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Methane is next to water vapor and carbon dioxide the third most important greenhouse gas. Northern wetlands are among the most important sources of atmospheric methane. The expected effect of increasing temperature on methane production is often claimed to initiate a feedback mechanism. Here we report about rates and controls of methane production in different organic soils from around the Arctic. Soils were sampled from sites in Svalbard, Finland, West and East

Siberia. Peat from the Boreal (Estonia) and wetland soils from the subtropical Mediterranean served as references. Using a temperature gradient incubator, we studied in vitro the effect of temperature on rates of methanogenesis, on methanogenic populations, and on the microbial pathways that eventually supply methanogens with substrates. The influence of temperature on methanogenesis in the arctic and subarctic soils was similar: starting from a theoretical minimum temperature in the subzero range, methanogenesis was highly active just above zero and had its optimum in the range between 25 and 30°C. In contrast, the temperature optimum for soils from the temperate zone was around 40°C, while activities below 10°C were insignificantly low. While in the Mediterranean samples a moderately thermophilic population co-existed with mesophils, no indication of such a population mix was so far detected in Arctic samples. While population change may happen on the long term, the reaction on increasing temperatures will depend on the yet existing populations. In contrast to a consistent response to temperature, methanogenic populations and pathways were diverse. The cold adaptation of Arctic communities does not exclude functioning up to 20°C and above. The extant populations in Arctic and Subarctic environments will be the main players in a warming environment, too, reacting sensitively even to a minor temperature increase.

Abstract Category
02 Climate Change

PS.02.015 BIOGEOGRAPHY OF ACTIVE AND DORMANT SOIL MICROBIAL COMMUNITIES ALONG A STEEP PRECIPITATION GRADIENT

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Biodiversity of soil microorganisms captivated ecologists for over a century, yet only in the recent decades could their curiosity be gratified, spurred by advances in molecular biology. In this study we extracted DNA and RNA from soils to survey biogeographical patterns in the diversity of archaea and bacteria along a steep precipitation gradient. Thirty soil samples were retrieved from 5 long-term ecological research stations, collected from open patches and from underneath the canopy of the predominant perennial at each site. The molecular fingerprint of the soils was taken using terminal restriction length polymorphism (TRFLP) of 16S rRNA amplified from DNA and reverse-transcribed RNA.

The TRFLP patterns of active (RNA) and total (DNA) microbial community were distinct: DNA-based patterns indicate that diversity within sites is not statistically significant ($P > 0.3$) unlike the diversity among the sites. Conversely, the RNA fingerprints suggest that diversity in arid and semi-arid soils and of open patches at the southern Mediterranean site differ significantly ($P < 0.001$) from the 2 northern sites and from the southern Mediterranean soils retrieved from under plant canopy. These differences could largely be explained by a combination of precipitation and vegetation cover. Although both approaches linked patch type with different microbial populations, the DNA-based patterns pointed to significant differences ($P < 0.0001$) in the community composition of arid, semi-arid and the 3 Mediterranean sites, while the RNA-based fingerprints were found to tightly correlate to soil's water content. The active microbial communities harbored in dry soils (<6%) significantly differed from those detected in

more moist soils (>10%).

Our results suggest that in dry climates biogeographical patterns could not be elucidated by the active microbial communities, but by DNA-based analyses that capture both the dormant and active populations linking them to their ecosystem of origin.

Abstract Category
02 Climate Change

PS.02.016 DISTINCTIVE ASSEMBLAGE OF MICROBIAL COMMUNITY ASSOCIATED WITH THE METHANE FLUX IN SPHAGNUM-COVERED AND SASA-INVADDED WETLANDS IN BIBAI, HOKKAIDO, JAPAN

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Peat bog is the most significant source of atmospheric methane. Over the last decades, wetlands in various parts of Japan have been turning to area of aridity, and thereby resulting in the *Sasa* invasion in those bogs. We have so far analyzed methane flux in the *Sphagnum*-covered (Sp) and *Sasa*-invaded (Sa) wetlands in Bibai, Hokkaido, Japan. The methane emission from the Sp area ($13.2 \text{ g C m}^{-2} \text{ y}^{-1}$) was about twice as high as that from the Sa site ($6.6 \text{ g C m}^{-2} \text{ y}^{-1}$). Yet little is known about the microbes that trigger the differences in the methane flux. The objective of this study was to investigate microbial communities responsible for aerobic methane oxidation and methanogenesis in the Sp and Sa wetlands using molecular analysis targeting particulate methane monooxygenase (*pmoA*), methyl coenzyme M reductase (*mcrA*), and archaeal 16S rRNA genes. The *pmoA* library indicated the type I methanotrophs, relatives of the genera *Methylomonas* and *Methylococcus*, were dominant in the Sp area, while the type II methanotroph related to the genus *Methylocystis* proliferated in the Sa site. Generally, type II has higher affinity for methane than type I. The results suggest the substrate affinity of methanotrophs may play a decisive role in the niche differentiation. With respect to the methanogens as revealed by the *mcrA* and 16S rRNA libraries, the genus *Methanoregula* was the most abundant in the Sp area, indicating their involvement in the methane production. In contrast, in the Sa site, no PCR products of the *mcrA* genes were obtained and there were no 16S rRNA clones affiliated with methanogenic representatives. Given the fact that methane was emitted to some extent from the Sa area, the results suggest the presence of as-yet-unknown methane producer. Novel members within the phyla *Euryarchaeota* and *Crenarchaeota* were frequently retrieved from the Sa and Sp areas. The results strongly indicate the distinctive microbiota associated with the methane flux in the Sp and Sa wetlands.

Abstract Category
02 Climate Change

PS.02.017 RESPONSE OF FOREST LAND MICROBIAL COMMUNITIES TO ELEVATED CO₂ REVEALED BY GEOCHIP ANALYSIS AT THE OAK RIDGE NATIONAL LABORATORY FACE SITE

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It is clear that elevated atmospheric CO₂ has profound effects on ecosystem structure and function. However, the information on belowground soil microbes is still limited though they may play important roles in mediating feedback mechanisms of terrestrial ecosystems to elevated CO₂. To address this issue, we investigated soil microbial communities in a sweetgum monoculture forest land exposed to long-term (10 years) elevated and ambient CO₂ (560 and 405 ppm, respectively) at the Oak Ridge National Environmental Research Park. The GeoChip 3.1, a functional gene microarray which contains 24,676 gene probes in 9 major geochemistry functional gene categories, was employed to characterize the microbial communities. Analysis of variance results of all functional genes detected showed that elevated CO₂ not only increased the abundance of almost all functional genes in C and N cycles ($p < 0.1$) but also significantly increased the richness and diversity (Shannon and Simpson index) of microbial communities ($p = 0.007$ and 0.014 , respectively). Remarkably higher levels of bacteria and fungi ($P < 0.1$) were detected in soil samples with elevated CO₂. The results of detrended correspondence analysis (DCA) indicated significant changes in the composition and structure of microbial communities under elevated CO₂ at a soil depth of 5-15 cm, but less change at a depth 0-5 cm which may be due to an instability in the CO₂ concentration at the soil surface. Dissimilarity test results (mrpp, anosim and adonis) confirmed these findings, with significant differences found between elevated and ambient CO₂ in C, N, P, S cycling genes and in the whole data set at a depth of 5-15 cm ($P < 0.05$), but only no significant differences at the surface level (0-5 cm) ($p > 0.1$). Overall, results revealed a positive response of microbial communities to elevated CO₂ at the ORNL FACE site, suggesting a fertilizing effect of CO₂ on microbial communities.

Abstract Category
02 Climate Change

PS.02.018 GROWTH OF TREES UNDER ELEVATED ATMOSPHERIC CO₂ ALTERS MICROBIAL COMMUNITIES COLONIZING LEAF LITTER IN A TEMPERATE WOODLAND STREAM

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Elevated atmospheric CO₂ can cause increased carbon fixation and altered foliar chemical composition in a variety of plants, which has the potential to impact forested headwater streams because these are detritus-based ecosystems that rely on leaf litter as their primary source of organic carbon. Fungi and bacteria play a key role in the entry of terrestrial carbon to aquatic food webs, as they decompose leaf litter and serve as a source of nutrition for invertebrate consumers. This study tested the hypothesis that changes in leaf chemistry caused by elevated atmospheric CO₂ would result in changes in the size and composition of microbial communities colonizing leaves in a woodland stream. Three tree species, *Populus tremuloides*, *Salix alba*, and *Acer saccharum*, were grown under ambient (360 ppm) or elevated (720 ppm) CO₂ and their

leaves were incubated in a woodland stream. Elevated CO₂ treatment resulted in significant increases in phenolic and tannin content and C:N ratios of leaves. Microbial effects, which occurred only for *P. tremuloides* leaves, included decreased fungal biomass and decreased bacterial counts. Analysis of fungal and bacterial communities on *P. tremuloides* leaves via T-RFLP and clone library sequencing revealed that fungal community composition was mostly unchanged by the elevated CO₂ treatment, whereas bacterial communities showed a significant shift in composition and a significant increase in diversity. Specific changes in bacterial communities included increased numbers of alphaproteobacterial and CFB-group sequences and decreased numbers of betaproteobacterial and firmicute sequences, as well as a pronounced decrease in overall Gram positive bacterial sequences. These results confirm our hypothesis that elevated atmospheric CO₂ can result in changes in the size and composition of microbial communities colonizing leaves in a woodland stream, which could have significant implications for stream food webs.

Abstract Category
02 Climate Change

PS.02.019 SOIL FUNGAL BIOMASS AND ENZYME ACTIVITY RESPOND TO ENVIRONMENTAL MOISTURE GRADIENTS

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As global change begins to affect belowground microbial communities, microbial biomass and function may be altered with ecosystem-scale consequences. We sampled soils from across Southern California to understand the environmental variables that affect belowground fungal biomass and enzyme activity. We hypothesized that soil temperature and moisture are the largest predictors of soil fungal biomass and enzyme activity as they largely control the decomposition processes that determine fungal growth. Soil cores were collected from 13 sites in Southern California from biomes including grasslands, forests, deserts, and shrublands. These sites cover a 100km² area, from Santa Barbara to San Diego. Resin extractable nitrogen and phosphorus, moisture, temperature, texture and pH were measured for all samples. In addition, elevation, mean annual temperature, mean annual precipitation, evapotranspiration, and plant communities were measured at each site. Fungal biomass was measured via hyphal extraction while enzyme activity of cellobiohydrolase, α and β glucosidase and, and n-acetyl-beta-D-glucosaminidase was measured via standard fluorometric techniques. Soil fungal biomass was significantly correlated with soil moisture, but not to any other metrics. Fungal hyphal length ranged from 1000 mm/g dry soil in the driest soils to 2500 mm/ g dry soil in the wettest soils, which represents a change in carbon sequestration from 0.0078g C m⁻² to 0.011g C m⁻². Enzyme activity was also positively correlated with soil moisture, and therefore fungal biomass, for all enzymes assayed. As soil fungal biomass and activity are both affected by soil moisture, global climate change is expected to have a significant effect on below-ground fungal function and soil carbon sequestration. However, the balance between enzymatic soil C cycling rates and sequestration in fungal biomass remains to be determined.

Abstract Category
02 Climate Change

PS.02.020 CHANGES IN PHYTOPLANKTON BIOMASS AND PRIMARY PRODUCTION IN THE WESTERN SCHELDT ESTUARY (THE NETHERLANDS) DURING THE PERIOD 1978-2006

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Changes in annual mean chl_a concentrations were studied the eutrophic and turbid macrotidal Western Scheldt estuary. Three stations were investigated: WS1 (30 PSU at the mouth of the estuary, station WS6 (17 PSU) and station WS11 (10 PSU). No significant long term changes in yearly averaged chlorophyll a (chl_a) concentrations were observed in WS1 and WS6, but in WS11 a large decrease. Spectral analyses revealed a possible periodicity of 7 years in mean chl_a which we could not relate to abiotic factors. Strong phenological responses in the timing of the spring/summer bloom were observed: the center of gravity or the day at which 50% of the cumulative chl_a was reached during the year advanced with 1-2 days per year. All stations showed an earlier initiation of the bloom, whereas the day at which the phytoplankton bloom terminated also moved forward in time, WS11 excepted. As a result the bloom length decreased in station WS1, remained the same in station WS6 and increased in WS11. All these changes also caused a change in the primary production: especially since 2000 the phytoplankton production/biomass ratio decreased significantly. This complicated pattern in bloom phenology demonstrates the complex nature of ecosystem functioning in estuaries.

Abstract Category

02 Climate Change

PS.02.021 METHANE EMISSION AND MICROBIAL COMMUNITY STRUCTURES OF PALM OIL TREATING ANAEROBIC LAGOONS

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Palm oil industry is one of the major industries in Malaysia and Indonesia. Palm oil mill effluent (POME) is discharged about 2.5 m³ from every 1 t of palm oil produced. POME is high COD (chemical oxygen demand) wastewater with high concentration of lipid, high temperature (approximately 70°C), and low pH. Usually, POME is treated in anaerobic open lagoons, from which lots of methane, the global warming gas, is emitted. In this study, we measured methane emission rate by using a closed chamber method from lagoons in a palm oil mill in Malaysia, and microbial communities in the lagoons were investigated by 16S rRNA gene and methyl coenzyme M reductase gene alpha subunit (mcrA) clone libraries. There are four lagoons in the mill and first two lagoons are for acidification (pH was around 4-5) and following two lagoons are for methanogenesis (pH was around 6-8). Methane emission from the third lagoon, the first methanogenesis lagoon, was the most active. Much less, but some methane emission was observed from the second acidification lagoon. Microbial community structure study indicated that lactate is the one of the important intermediate for POME degradation in a lagoon system. Community structures in the second acidification lagoon differed at sampling points whereas

those in first methanogenic lagoon were similar between sampling points. The *mcrA*-based analysis revealed that methanogens in the first methanogenic lagoon were similar to those often found in anaerobic digesters whereas dominant methanogens in the second acidification lagoons were *Methanoculleus* and *Methanosphaera*. These results revealed important microorganisms for POME degradation and unseen methanogenic community structure under low pH and high lipid environments.

Abstract Category
02 Climate Change

PS.02.022 DYNAMICS OF SOIL MICROBIAL COMMUNITIES IN AGROECOSYSTEMS MANAGED FOR BIOFUEL PRODUCTION

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Elevated atmospheric CO₂ concentrations and their link to global climate change are stimulating efforts to reduce dependence on fossil fuels and increase use of alternative energy sources. Initial efforts to incorporate significant amounts of cellulosic ethanol into transportation fuels are focused on utilizing crop residues, primarily corn, from lands under intensive agricultural production management. The removal of crop residues may result in negative impacts to the function and sustainability of these agroecosystems. We are studying the effect of crop residue removal on soil properties, including the dynamics of soil microbial communities using quantitative PCR of rRNA genes from major taxonomic groups and the fluxes of greenhouse gases (CO₂, N₂O, and CH₄). Replicated research plots (24 m x 30 m plots; corn-soybean rotation) were established in 2000 where one primary treatment (biofuel management) is the removal of the corn residue plus grain and the other primary treatment (control) is the removal of corn grain only. In 2005, plots were split to add cover crops as a secondary treatment to examine the potential for cover crops to mitigate decreases in soil carbon observed in the biofuel treatment plots. In soil samples collected following crop harvest in 2009, we have found the highest fungal:bacterial ratios (0.1) in plots with corn and lower ratios (0.05) in the plots with soybean. There were no significant effects on soil fungal:bacterial ratios due to residue removal or the addition of a cover crop apparent at this sampling time, despite changes in the quantity and quality of organic carbon due to both treatments. Ongoing analyses target the quantification of *Basidiomycota*, *Acidobacteria*, *Firmicutes*, *Actinobacteria*, and *Bacteroidetes* in these samples and the compilation of the gas flux data. These data will assist in determining if significant changes may occur in the functioning of agroecosystems managed for cellulosic biofuel production.

Abstract Category
02 Climate Change

PS.02.023 ECOLOGY OF THE ACTIVE METHANOTROPH COMMUNITY: AN ADAPTIVE RESPONSE TO REFORESTATION OF PASTURE IN NEW ZEALAND

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Methanotrophs use methane (CH₄) as their carbon source and are active in temperate forest soils representing a strong CH₄ sink. Afforestation and reforestation of pastures enhances CH₄ oxidation but the transition is apparently a slow process. We conducted a meta-analysis, supported by data from our previous reports, to study long-term effects of afforestation and reforestation of pasture in New Zealand on the structure of the methanotroph community and its consequences on CH₄ flux. Soils were sampled from two sites: a native forest of mixed vegetation (Puruki) and a manuka-kanuka shrubland (Turangi) with stands converted from pasture over 35 and 55 years ago. CH₄ fluxes were measured from intact cores in closed chambers using gas chromatography. Changes in the activity and structure of the methanotroph community were analysed using PLFA-SIP and T-RFLP techniques. CH₄ oxidation rates were significantly higher in the Turangi shrubland stands compared to the pastures and pine forests, but they were similar to the CH₄ sinks in the Puruki native forest soils. PLFA-SIP data suggested that type II methanotrophs were the most active in oxidising atmospheric concentrations of CH₄. T-RFLP data of the functional gene marker pmoA identified a shift in the methanotroph community structure: type I methanotrophs present in pastures and pine forests were progressively replaced by dominance of type II methanotrophs in soils under shrublands and native forest. However, there was no significant difference in the methanotroph community structure between the shrubland and native forest. Our data suggest that reforestation, and afforestation by pines, had a rapid and long-lasting impact on the establishment of a stable and active population of type II methanotrophs. This shift seemed to be independent of the vegetation type. We estimated that only <35 years may be needed for a forested soil to acquire a methanotroph community and reach CH₄ sink capacities similar to a native forest.

Abstract Category
02 Climate Change

PS.02.024 ISOLATION OF SOIL BACTERIA ABLE TO GROW IN SWEET SORGHUM JUICE AND PRODUCE BUTANOL

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Bio-fuel is one of the promising carbon-neutral materials to reduce the atmospheric carbon-dioxide increase due to the use of fossil fuel. However, the bio-ethanol production using food crops such as corn and sugarcane are threat for food crisis and also environmental deterioration including deforestation. To consider those problems, we have developed a new bio-fuel production system which is the cultivation of sweet sorghum, a non-food crop, in the abandoned farm land and the production of bio-alcohol from its sugar-containing juice. In this study we report the bio-butanol production by newly isolated bacteria with sweet sorghum juice because butanol is less corrosive and has a higher caloric value than ethanol. An upland soil was collected from a sweet sorghum field, Field Science Center, Ibaraki University, Japan. The soil was put into 120 ml vial, heated at 80°C for 15 min, added 75 ml of the sweet sorghum juice supplemented with 0.3% cistern hydrochloride, and cultured anaerobically at 35°C for 6 days.

Butanol production in the culture was measured by HPLC. Aliquots of cultures yielding a high concentration of butanol were spread on to peptone-yeast extract-glucose (PYG) agar plates and incubated at 35°C. After 2 days' incubation, several colonies were selected and the isolates were analyzed for their 16S rRNA genes. HPLC analysis showed that isolates SBP1 and SBP2 grew in the juices of several sweet sorghum cultivars and produced butanol at 4.2g/L and 3.8g/L, respectively. It is also found that the butanol-producing reference strain *Clostridium beijerinckii* JCM1390 showed a scant growth in the sweet sorghum juice tested, indicating that our isolates can be a suitable agent for butanol production from the material. Phylogenetic analysis revealed that both strains were closely related to *Clostridium beijerinckii*. Further studies are now in progress to examine the optimal growth condition for the butanol production.

Abstract Category
02 Climate Change

PS.02.025 EFFECT OF CARBON LOADING RATES ON FUNGAL AND BACTERIAL GROWTH IN SOIL

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Growth of heterotrophic soil microorganisms is limited by a lack of available nutrients, especially carbon substrate, but the effects of substrate loading rates on bacterial and fungal growth rates in soil is not well understood. Therefore, a range of concentrations of glucose (100 µg up to 32 mg C g⁻¹ soil) were added to a grassland soil in laboratory experiments (20°C) and monitored at high resolution (bi-hourly measurements) for 200 h. To achieve maximal sensitivity growth rate measurements; the leucine incorporation technique to measure bacterial growth and the acetate-in-ergosterol incorporation technique to measure fungal growth were used. Bacterial growth did not increase until 10 h after glucose addition, although respiration was elevated 6 times higher than basal respiration during this time. Following this stable period respiration rates increased exponentially at loading rates between 2 and 8 mg C, in parallel with simultaneously increased bacterial growth. After 24 h the respiration rate decreased rapidly, while bacterial growth decreased more slowly. Relating the respiration rates to our measurements of bacterial and fungal growth rates suggested that the respiration increase at carbon loading rates from 0.1 to 8 mg C was primarily driven by bacteria. Higher carbon loading rates (above 16 mg C) tended to be more closely related to increases in fungal growth. At very high carbon loading rates (32 mg C) respiration appeared to be entirely due to fungi, since no bacterial growth could be detected. Even at the lowest glucose additions (< 500 µg C) stimulated bacterial growth 24 h after addition, although this increase did not coincide with an exponential increase in respiration, a characteristic often used to indicate microbial growth, could be detected. Thus, respiration measurements did not correlate with microbial growth rates, suggesting rapidly changing growth efficiency after adding substrate.

Abstract Category
02 Climate Change

PS.02.026 PREDOMINANCE OF NITROUS OXIDE-PRODUCING FUNGUS, MORTIERELLA ELONGATA, IN NO-TILLED, LOW-NITROGEN INPUT UPLAND SOILS

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Background and aims: Not only bacteria but also fungi are able to produce nitrous oxide(N₂O) in the denitrification. Previously, it was shown that in situ N₂O producing activity in no-tilled (NT) soils was positively correlated with soil fungal biomass. This study focuses on the identification of fungi contributing N₂O emission in a NT upland soil.

Methods: Soil-in-vial experiments were conducted to determine the activity of N₂O production. Fungi were isolated by the direct inoculation method using cornmeal agar and identified based on morphology of sporulating structures and the sequence data of fungal 18S-26S/28S internal transcribed spacer (ITS) region. For determining the N₂O-producing activity, strains were grown at 30°C in test tubes containing glycerol medium supplemented with either 10 mM NaNO₂ or NaNO₃. Fungal genes of 18S-26S/28S ITS region were targeted to monitor soil fungal diversity by PCR-based terminal-restriction fragment length polymorphism (T-RFLP) profiling and clone library analysis.

Results: Soil-in-vial experiments using acetylene suggested that at 85-100% of soil maximum water-holding capacity, bacterial denitrification was predominant and the one-order lower level of N₂O-producing activity attributed to fungal denitrification. Nineteen fungal strains were isolated and the 16 strains were identified as *Mortierella elongata*. The twelve *M. elongata* strains were examined for the activity of N₂O production and found to have low activities during the growth in cultures containing nitrite but not in cultures with nitrate. The T-RFLP profiling showed that the fungal community was characterized by the dominance of fungi containing 425-base T-RF of ITS region. Based on the ITS clone library prepared from the NT soils, *M. elongata* appeared to account for about 16% of the total fungal community.

Conclusions: From these results, it can be expected that the fungus *M. elongata* contributed in some extent to N₂O emission input NT soils.

Abstract Category
02 Climate Change

PS.02.027 GLOBAL WARMING COUPLED TO DROUGHT ALTERS MICROBIAL COMMUNITIES IN A TALLGRASS PRAIRIE

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Increased atmospheric greenhouse gas concentrations due to anthropogenic activities and the associated rise in global temperatures have been shown to alter regional rainfall patterns. As a result, ecosystem water budgets and timing of rainfall will likely be key mediators of biome productivity, especially in semi-arid environments. The stability of soil microbial communities in response to climatic extremes is likely to be instrumental in preserving long-term ecosystem

function. Here we present results from a two-year study examining the abundances, diversities and community composition of dominant bacterial and archaeal 16S rRNA genes in a tallgrass prairie simulated global warming experiment. During this time, the site experienced a moderate drought, allowing us to address the combined effects of warming and ecosystem water budget on microbial communities. We found that water budgets and more importantly rainfall timing have long-term effects on the population dynamics of bacteria within the soil. When the site received normal precipitation the warming treatment significantly increased population size while simultaneously suppressing diversity and significantly shifting community structure when compared to the unwarmed controls. However in drought conditions, warming did not significantly shift the community structure but did significantly decrease microbial population size while increasing richness, diversity and evenness. We suggest that microbial response to warming is highly dependent upon the amount and the timing of rainfall. Ultimately how plant communities respond to warming and drought will have profound effect on microbial population density by altering the soil microclimate, thereby potentially disrupting nutrient pools and soil microbial processes such as carbon and nitrogen cycling.

Abstract Category
02 Climate Change

PS.02.028 UNRAVELING INTERACTIVE CLIMATE CHANGE EFFECTS ON SOIL DECOMPOSITION: DECIPHERING CLUES FROM MICROBIAL PHYSIOLOGY, FUNCTION, AND COMMUNITY COMPOSITION

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Moisture and temperature are key drivers of soil organic matter decomposition rates, but there is little consensus on how changes in these drivers will affect the degradation of different soil compounds over long-term climate change. Soil enzyme activity and substrate induced respiration are relevant metrics of soil community function because they are related to decomposition of specific substrates in soil. These two methods in conjunction with microbial biomass estimates and fungal:bacterial ratios can reveal how the rates of those reactions may be changing due to altered community size or composition. In June 2009, we collected soils from 3 precipitation treatments and 4 temperature treatments arranged in a full-factorial design at the Boston Area Climate Experiment, Massachusetts. Enzymes activities were measured for 6 common carbon, nitrogen and phosphorus cycling enzymes. Substrate induced respiration was measured using multiple substrates of varying lability. Substrate induced respiration due to additions of glucose, sucrose and yeast extract was significantly affected by both altered precipitation and warming ($p < 0.05$). Specifically under drought there was a significant decline in respiration, while the highest heating treatment caused respiration to significantly increase after labile carbon additions. Substrate induced respiration from drought and heated plots showed decreased temperature sensitivity, likely due to a change in microbial physiology or community structure under increased soil temperatures in the field. Enzyme activity was not significantly affected by either precipitation or warming treatments, and therefore did not explain the differences seen in the substrate induced respiration assay. We hypothesize that the patterns in substrate induced respiration are due to either changes in microbial community composition or

size. We are evaluating these hypotheses using qPCR, 454 pyrosequencing and substrate induced respiration estimates of biomass.

Abstract Category
02 Climate Change

PS.02.029 ISOLATION AND CHARACTERIZATION OF EXTRACELLULAR CARBONIC ANHYDRASE IN BACTERIA INVOLVED IN CARBON CONCENTRATING MECHANISM

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Bacterial strains isolated from soil containing lime were enriched by minimal salt media with different concentration of sodium bicarbonate and characterized by 16S rDNA based denaturing gradient gel electrophoresis (DGGE) and amplified random DNA restriction analysis (ARDRA). One of the isolates survived at 100mM sodium bicarbonate (stressed) and produced extracellular carbonic anhydrase (CA) was identified by 16S rDNA sequence analysis as *Staphylococcus* sp. Stress conditions were responsible for leakage of enzyme from bacterial cell. The enzyme was induced in presence of NaHCO₃ and is envisaged to play an important role in bicarbonate ion transport. The enzyme was initially precipitated by ammonium sulphate (80%), dialysate further purified by Sephadex G-100 and affinity chromatography. It is a monomeric protein having molecular weight of 36.0 kDa and was stable at wide range of pH 5.0-11.0 and temperature 4-60 °C. Optimum pH of this enzymatic activity was 8 and temperature was 37°C. The subsequent actions by carbonic anhydrase in carbon sequestration led to end products which have characteristics beyond human perceptible uses, established potential links to counter environmental degradation. Increased production of Biosurfactants, valuable organic compounds and production of end product as CaCO₃ are intriguing areas of research. This species having above normal capacity to withstand presence of different salts, alkaline pH and wide range of temperature in extracellular conditions and capacity to produce environment friendly end products furrows field for new research models in the field of Carbon Sequestration studies.

Abstract Category
02 Climate Change

PS.02.030 ISOLATION AND CHARACTERIZATION OF LIPID-ACCUMULATING PHOTOAUTOTROPHS FROM YELLOWSTONE NATIONAL PARK

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The increased consumption and dependence of crude petroleum-based fuels has led to an imbalance between supply and demand. As the rate of consumption exceeds production there will be continued shifts in the economic markets, as well as negative environmental impacts. One way to alleviate such influences will be to utilize multiple, renewable biofuels, including

biodiesel produced from biologically-derived oils. Algae will accumulate triacylglycerols under nutritionally stressed conditions that can be converted to biodiesel. In addition, land requirements for algae production are estimated to be far less than that required for plant-based oils (e.g., soybean). The goal of this study is to survey the Heart Lake Geyser Basin in Yellowstone National Park for potential algal strains that can be utilized for biodiesel production. Samples were collected from hot springs, creeks, and run-off streams throughout the Heart Lake Geyser Basin. Obtained cultures were from environments with temperatures between 20°C and 40°C with pH values between 7.4 to 9.3. Microorganisms were cultured in Bold's basal medium and streaked on agar plates for isolation. Based upon partial and full-length SSU rDNA sequences, two organisms were identified as a *Botryococcus* species and a *Chlamydomonas* species. Both organisms grew at 27°C and a pH of 8.5 and accumulated lipids under nutrient-deprived conditions as assayed via a Nile Red fluorescent marker. Two additional isolates have a significant Nile Red fluorescent signal under nutrient limitation conditions at pH 9.0 and 27°C. The isolates represent unique and novel photoautotrophs that accumulate lipids in relation to growth conditions.

Abstract Category
02 Climate Change

PS.02.031 UTILISING A NOVEL CULTURING APPROACH TO ASSESS THE MICROBIAL DIVERSITY OF PETROLEUM HYDROCARBON CONTAMINATED SUB-ANTARCTIC SOIL

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BACKGROUND Persistence of spilled petroleum hydrocarbons in polar soils is extensive. Due to a lack of site-specific data, the environmental impact of petroleum contamination on the sub-Antarctic Macquarie Island is not well established. Our aim was to utilise a novel micro-culturing technique to allow for a realistic assessment of microbial biodiversity in sub-Antarctic soils to be carried out and subsequently characterise the soil ecosystems response to a contamination event. **METHODS** Uncontaminated soil from Macquarie Island was spiked with Special Antarctic Blend (SAB) diesel fuel across a concentration gradient of 0-20,000 mg/kg-1. The SAB spiked soil samples were cultured by using the Soil Substrate Membrane System both aerobically and anaerobically. The resulting mixed communities of microcolonies were analysed by epi-fluorescence microscopy, flow cytometry and molecular assessment, combined with attempts to isolate pure cultures into artificial media. **RESULTS** The aerobic fraction was more susceptible to petroleum hydrocarbon contamination with a significant ($P < 0.01$) decline in total bacterial numbers observed (up to 80%). *Simplicispira limi* and *Rhodoferrax ferrii* within the *Betaproteobacteria* family were observed to dominate uncontaminated soils. Subsequently, SAB diesel fuel addition altered the microbial community with *Burkholderia* re-established as the dominant family in the aerobic portion and *Pseudomonas* as the dominant family in the anaerobic fraction. Sixteen isolates were recovered from the sub-Antarctic soils, including two potentially novel genera and two potentially novel families. **CONCLUSION** By integrating techniques, site-specific contamination data can be obtained in a rapid timeframe. The alteration

of bacterial diversity, abundance and particularly the differing response of the aerobic and anaerobic fractions have relevance to future risk assessment and remediation strategies associated with petroleum contamination in polar soils.

Abstract Category
02 Climate Change

PS.02.032 BACTERIAL DIVERSITY IN TIBETAN LAKES WITH RESPECTS TO GLOBAL CHANGE

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We have investigated the bacterial diversity in more than 50 different Tibetan lakes spanning a large environmental gradient such as i.e. salinity gradient, altitude gradient in the last five years. Different molecular tools combined with isolation of microorganisms have been applied to investigate the diversity and eco-physiology of planktonic bacteria. We found that these Tibetan lakes harbor many novel clusters of bacterioplankton. Even for those well-known clusters of bacteria found in other freshwater habitats, we still found some subclusters which contain exclusively the sequences obtained in the investigated Tibetan lakes. Isolated bacterial strains demonstrated locally adapted characteristics in term of salinity tolerance in the respective Tibetan lakes. Comparison of bacterioplankton diversities estimated by 16S rRNA genes cloning and sequencing for selected Tibetan lakes with reference data for lakes located at lower altitudes indicated relatively low taxon richness in these high mountain lakes. Furthermore, a comparison of the taxonomic compositions of bacterioplankton across the selected Tibetan lakes revealed low overlap between their community compositions. This beta diversity was explained by differences in local environmental change such as salinity between lakes as well as by distance effects. Overall, our study suggested that elevation may have strong impacts on bacterial diversity in lakes and that the observed diversity may reflect local adaptation of various lineages to the environmental conditions in these high altitude lakes. Since lake ecosystems at high altitude are quite sensitive to the impacts of climate change, i.e., global warming, and these rarely explored lakes harbor new and locally adapted microbes, our knowledge of the bacterial diversity in high-altitude lakes will be relevant to species protection and ecosystem conservation of these unique ecosystems.

Abstract Category
02 Climate Change

PS.02.033 POTENTIAL EFFECTS OF SEA SURFACE WARMING AND ACIDIFICATION ON THE AGGREGATION OF EXTRACELLULAR ORGANIC MATTER IN ARCTIC ECOSYSTEMS

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Aggregates play a pivotal role for organic matter cycling and export, as they mediate vertical mass fluxes and elemental cycling in the ocean. Moreover, aggregates harbor a rich and active

community of marine microbes. Aggregation processes in the water column are often initiated by the formation of gel particles, such as transparent exopolymer particles (TEP), from extracellular organic matter produced by phyto- and bacterioplankton. Based on the awareness, that global change will strongly impact arctic marine ecosystems, we conducted a series of perturbation experiments to investigate how increasing sea surface temperatures and pCO₂ may affect the formation, aggregation and microbial turn-over of extracellular organic matter, specifically of TEP. Experiments were conducted on board of the research vessel *Polarstern* at three different locations in the Greenland Sea and in the Fram Strait off Svalbard. First results indicate that organic matter composition as well as microbial activities responded to changes in temperature and pCO₂. Thereby, significant correlations were observed between the concentrations of dissolved organic carbon and TEP, and between TEP and bacterial abundances. We will discuss the potential implications of our findings in the context of cycling and export of organic matter in the future Arctic Ocean.

Abstract Category
02 Climate Change

PS.02.034 FIELD HISTORY IS MORE IMPORTANT THAN SUPPLEMENTAL SUBSTRATES ON INFLUENCING SOIL MICROBIAL COMMUNITIES IN A LABORATORY STUDY

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The understanding of how general ecological principles regulate soil microbial communities is limited despite the fact that soil sustains an immense diversity of microbes. This is possibly due to the complexity of uncultured microorganisms and the limitations of current analysis techniques. Here we adopted a high-throughput microarray-based genomics technique, Geochip, to characterize soil microbial communities at the whole-community and functional gene levels to investigate the impact of supplemental substrates, representing contemporary disturbance, on microbial communities. In this study, soil samples were collected from a long-term warming experimental site (Kessler Farm Field, McClain County, OK, USA) with field treatments of elevated temperature (T) (+2°C) and ambient T, representing historic legacy. By using a respirometer system (Columbus Instruments, Columbus, Ohio, USA) at room temperature, 50 g soil was incubated in the dark in 250 ml bottle for 42 days with 1:100 (w:w) of glucose, ground C3 or C4 plant materials from the same field, or nothing as a control. Each treatment had four replicates. Though supplemental substrates stimulated soil respiration significantly (p<0.05), they did not lead to significantly dissimilar microbial communities based on tests of multiresponse permutation procedure (mrpp), nonparametric analysis of variance using distance matrices (adonis) and analysis of similarities (anosim), while field treatment did. For community richness (detected gene number), abundance (bacteria, fungi or archaea) and diversity (Simpson index), field treatment of elevated T increased these parameters significantly (p<0.05), but supplementary substrates did not change these parameters. Consistently, the abundances of

individual genes in C/N/P/S cycles showed the same trend. All these results demonstrate that historic legacy was more important than contemporary disturbance in influencing soil microbial communities in the case we studied.

Abstract Category
02 Climate Change

PS.02.035 MICROBIAL COMMUNITY RESPONSE TO ELEVATED CO₂ AND O₃

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Elevated levels of the global warming gases carbon dioxide (CO₂) and ozone (O₃) are known to affect plant metabolism and influence the rhizosphere microbial community. Previous research has shown evidence of a feedback mechanism limiting the microbial response to elevated CO₂ and O₃. To understand how elevated levels of CO₂ and O₃ affect the subsurface microbial community, 96 samples were collected from SoyFACE, at the soybean free air gas concentration enrichment facility in Champaign, IL, USA (40°02'N, 88°14'W), under 4 different conditions: 1) ambient (control), 2) elevated CO₂ (550 ppm), 3) elevated O₃ (1.2 x ambient) and 4) elevated CO₂+O₃ with two depths (0-5cm and 5-15 cm) were investigated using functional gene microarray (GeoChip 3.0) approach. Under elevated CO₂ condition, the signal intensities of most detected functional genes were increased, some, such as *rbcL*, *nifH* and *ppx*, were significantly ($p < 0.05$). The opposite response was observed under O₃ treatment, which some functional genes involved in carbon degradation, such as xylanase and cellobiase, decreased significantly, indicating a decrease in microbial metabolism. When treated with both CO₂ and O₃, many of the gene category signal intensities were similar to ambient controls, with the exceptions of *lip*, *mnp*, *nirK* and *phytase*. Compared with O₃ treatment alone, the combined CO₂ and O₃ treatment increased the signal intensity of carbon degradation genes, including xylanase and cellobiase, indicating that elevated CO₂ may mitigate the toxic effect of O₃ on microbial communities. Furthermore, deep depth could reduce the effects of the gases. DCA analysis revealed that the microbial community structure were quite different between the ambient and treatments. The results of this study implying that global warming gases have a significant influence on the rhizosphere microbial activities and communities.

Abstract Category
02 Climate Change

PS.03.001 EXPLOITING AN ECOLOGICAL NICHE AMONG BACTERIA, NEMATODES, AND INSECTS FOR BIOACTIVE SMALL MOLECULE DISCOVERY

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The Gram-negative Gammaproteobacteria *Photorhabdus* and *Xenorhabdus* species are at the heart of a trilateral symbiosis among the bacteria, their mutualistic nematode hosts, and the insect hosts upon which they feed. These bacteria reside in the guts of infective juvenile nematodes that hunt insect larvae. When the nematode infiltrates a larva's circulatory system, it regurgitates the bacteria, which synthesize protein toxins, signaling molecules that govern the symbiosis, inhibitors of the insect's innate immune system, and antibiotics that thwart competitors in the decomposing insect carcass. Bacterial genes encode a virtual armory of bioactive small molecules for these various tasks, and their well-defined ecological niche provides a sound basis for understanding the regulation of their production and the biological functions for which they evolved. We used metabolomic profiling techniques to discover that the high concentration of L-proline in insect hemolymph induces secondary metabolite production in the bacteria. The upregulation of biosynthesized small molecules enabled the identification of a series of new compounds, including indole antibiotics, a phenoloxidase inhibitor, and bacterially produced stilbene development signals for the nematodes. L-proline utilization appeared to enhance the bacteria's proton motive force, which ultimately impacts the downstream regulatory pathways that control virulence and antibiotic production. We also deleted a global regulator important for the developmental switch in the *Photorhabdus*-nematode pair. The *Photorhabdus luminescens* LysR-type transcriptional repressor was identified as a downstream regulator of the stilbene family. The knockout increased stilbene production, which led to the identification of six new stilbene natural products. The stilbene pathway is critical for infective juveniles to develop into reproducing adults, and the new derivatives might act as signals in this developmental transformation. This work illustrates the utility of focusing on the chemical ecology of symbiotic associations to identify new small molecules that hold biomedical potential and to place them in their physiological and evolutionary context.

Abstract Category

03 Microbial Conflict and Conversation

PS.03.002 COMPETITIVE INTERACTIONS AMONG ECTOMYCORRHIZAL FUNGI ARE STRONGLY INFLUENCED BY SOIL SPORE DENSITY

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We investigated the influence of spore density on root tip competition among three ectomycorrhizal species in the genus *Rhizopogon*. *Pinus muricata* seedlings were planted into soils inoculated with all single or pair-wise combinations of each species at low, medium, and high spore densities. Seedlings were harvested at three and nine months and individual ectomycorrhizal species colonization was assessed using molecular identification techniques (PCR and RFLP of ITS rDNA). The outcome of root tip competition was highly asymmetrical when species were inoculated at different densities, with the dominant competitor always being the species inoculated at high density. Seedling biomass did not clearly correlate with spore densities, species composition or colonization levels. This study indicates that previously demonstrated spatial and temporal variation in the ectomycorrhizal soil spore bank may strongly influence the outcome of fungal interactions.

Abstract Category

03 Microbial Conflict and Conversation

PS.03.003 CHEATER CONTROL BY RE-EVOLVED COOPERATION IN PSEUDOMONAS AERUGINOSA

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Social behavior leading to the production of common goods is prone to exploitation. One such behavior in *Pseudomonas aeruginosa* is quorum sensing, by which the bacteria produce signals to regulate hundreds of genes, many of which encode extracellular common goods. Exploitation comes in the form of cheaters which have a mutation in the central quorum sensing regulator LasR. Previously we demonstrated that cheaters arise *in vitro* because they have a growth advantage over the wild-type. In the same experiment we also observed *lasR* mutants that re-evolved cooperative behavior and produced select quorum sensing products, presumably due to a second-site mutation. The objective of this work is to understand the molecular mechanism underlying the revertant "cheater-turned-cooperator" phenotype. The genomes of a revertant and its wild type ancestor were sequenced using Illumina and 454 sequencing. Mutations identified by both techniques and unique to the revertant were confirmed by PCR and dideoxy sequencing. Subsequent genetic analysis included complementation of candidate mutations and construction of respective knock-out mutants in a defined cheater background. Competition assays between the wild-type, cheater, and revertant showed the relative fitness of each. Initial sequencing data revealed a mutation in *psdR*, which encodes a transcriptional repressor that regulates the protease MdpA. The mutation was complemented, but a double mutant did not reproduce the revertant phenotype. The *psdR* locus of a related cheater was sequenced; it was also mutated, although the wild type ancestor's was not. Competition assays demonstrated that the revertant phenotype is superior. The mutation in *psdR* is not the sole cause of the revertant phenotype. It may be the first layer of regulation, with a second mutation required to fully de-repress *mdpA* and other quorum sensing products. Additional candidate mutations are currently being analyzed.

Abstract Category

03 Microbial Conflict and Conversation

PS.03.004 HIGH INTRASPECIFIC DIVERSITY RESULTS IN COLLAPSE OF PLANT-ASSOCIATED *PSEUDOMONAS FLUORESCENS* COMMUNITIES

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Fluorescent pseudomonads are ubiquitous colonizers of plant roots, and exert strong positive feedbacks on the productivity of terrestrial ecosystems. They produce antifungal toxins inhibiting phytopathogens, increasing thus plant health and resistance against diseases. Pseudomonads form diverse communities on plant roots, where they intensely compete for plant-derived exudates. We investigated the effect of intraspecific diversity on the performance of *Pseudomonas fluorescens* communities producing the antifungal compound 2,4, diacetylphloroglucinol (DAPG). We set up artificial bacterial communities ranging from one to eight genotypes, and followed the growth of the communities in batch experiments and on plant roots in a gnotobiotic system. We moreover followed the effect of diversity on the functioning of the community, measured as the antagonistic potential against plant pathogens. Diversity resulted in a collapse of both growth and functioning of the bacterial community, indicating that bacteriocin mediated allelopathic interactions are promoted at high diversity. Diversity only improved growth in conditions favoring complementarity between strains. Our results provide thus important mechanistic explanations on the diversity patterns of microbial communities and suggest that, in opposition to common views, negative diversity effects may dominate in the rhizosphere and be a major determinant of the functioning of natural bacterial communities.

Abstract Category

03 Microbial Conflict and Conversation

PS.03.005 EXTRACELLULAR METABOLITES LIMIT BACTERIAL SUSCEPTIBILITY TO PREDATION IN A PROTIST-SPECIFIC MANNER AND THEIR REGULATION MAY BE PROTIST-INDUCED

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Bacteria employ various strategies to evade protozoan predation, including production and release of bioactive compounds. This capability may be instrumental in determining bacterial resistance to protozoan grazing, thereby enhancing survival of producing strains in soil environments. A limited number of exometabolites have been examined in this regard, and only few studies have demonstrated the distinct nature of the specific response to an individual metabolite. Studies were conducted to assess the capacity of bacterial metabolites to limit predation by the amoeba-flagellate *Naegleria americana* and the ciliate *Colpoda*. Protists exhibited differential sensitivity to diverse cyclic lipopeptides (CLPs) produced by *Pseudomonas fluorescens* strains and to the antibiotic 2,4-diacetylphloroglucinol (DAPG). Strains SBW25 and SS101, producing the CLPs viscosin and massetolide A respectively, were resistant to grazing by *N. americana* but not *Colpoda*. However CLP-deficient mutants of these strains were susceptible to grazing by both protists. The CLP orfamide A produced by strain Pf-5 did not specifically impart predation defense as both the CLP-deficient mutant and parental strain resisted protist grazing. A Pf-5 *gacA*-mutant was susceptible to grazing by the amoeba but not the ciliate. However, with respect to predation DAPG-deficient mutants of strains Pf-5 and Q8r1-96 retained the phenotype of the respective parental strain. *Lysobacter enzymogenes* exhibited resistance to predation, but a mutant deficient in lytic enzyme production was susceptible to grazing by both protists. At the molecular level, enhanced CLP gene expression in the presence of *N. americana* was observed but did not require bacteria-protist physical contact. These studies demonstrate that a diversity of bacterial bioactive compounds may limit susceptibility to predation, that this resistance may occur in a protist-specific manner, and that DAPG production does not limit bacterial predation.

Abstract Category

03 Microbial Conflict and Conversation

PS.03.006 COMPREHENSIVE ANALYSIS OF COOPERATION AND CHEATING IN PSEUDOMONAS AERUGINOSA QUORUM SENSING

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Background and aims: *Pseudomonas aeruginosa* regulates the transcription of hundreds of genes, including many virulence genes, through three hierarchically-arranged quorum-sensing pathways, *las*, *rhl*, and *pqs*, each consisting of genes involved in the synthesis of autoinducers, *lasI*, *rhlI*, and *pqsABC*, as well as their cognate regulatory genes *lasR*, *rhlR*, and *pqsR*, respectively. In the present study, we analyzed the social behavior of signal-blind (*lasR*, *rhlR*, *pqsR*) and signal-negative (*lasI*, *rhlI*, *pqsA*) variants from each quorum sensing system. Due to the large but different number of genes controlled by each quorum sensing system, we hypothesized that all signal-blind isolates behave as social cheaters that exploit the quorum sensing-proficient wild-type to varying degrees.

Methods: We assessed the emergence of *las*, *rhl*, and *pqs*-deficient variants during in vitro-evolution of wild-type *P. aeruginosa* in minimal medium (M9-caseinate) that favors quorum sensing. In addition, we co-cultured defined signal-blind and signal-negative mutants with the wild-type at varying initial mutant frequencies (1, 10 and 50 %) to determine the relative fitness of the mutants.

Results: We found that *lasR* and *pqsR* but not *rhlR* mutants arise during long term growth. In addition, defined *pqsR* and *lasR* mutants enriched in wild-type co-culture, whereas *rhlR* mutants and all signal-blind mutants did not. In wild-type co-culture, both *lasR* and *pqsR* mutants enriched with negative-frequency dependence, although the *pqsR* mutant grew well even without the wild-type. The *rhlR* mutant enriched modestly only when at an intermediate frequency within the population, similar to spiteful behavior.

Conclusions: Only the *lasR* mutant behaved as a typical cheater. The social behaviors of the *pqsR* and *rhlR* mutants, on the other hand, were more complex than predicted from current mechanistic insight. This highlights the utility of evolutionary approaches for a comprehensive understanding of quorum

Abstract Category

03 Microbial Conflict and Conversation

PS.03.007 NUTRIENT LIMITATION AND GROWTH RATE: CUES TO *PSEUDOMONAS AERUGINOSA* QUORUM SENSING GENE EXPRESSION

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Background: Bacteria utilize diffusible chemical signals to monitor cell density and regulate gene expression using quorum sensing. *Pseudomonas aeruginosa* is an ideal model to study quorum sensing. Genetic studies have established that the *P. aeruginosa* quorum sensing circuitry, which controls the expression of 100s of genes, is embedded in a network of global regulation by other factors. The precise environmental conditions that affect quorum sensing have not been comprehensively investigated. We hypothesize that a major trigger of quorum sensing gene expression, in addition to cell density, is slow growth. We predict that this response is nutritionally conditional and stringent-response-dependent. Most quorum-controlled genes are not expressed until the onset of stationary phase during growth in rich medium batch culture, even in the presence of exogenous autoinducer signals. Methods: 1. Investigate the effect of nutrient limitation (C, N, P, S, Fe) in batch cultures on quorum sensing gene expression (*lasB*, *rsaL*, *phzA*) using *lacZ* reporter transcriptional fusion strains. 2. Utilizing growth conditions discovered to induce quorum sensing genes, investigate the effect of slow growth using *lacZ* transcriptional fusion strains in a chemostat culture system. 3. Investigate the role of the stringent response in slow-growth-dependent quorum sensing gene expression using double mutants in stringent response control genes (*relA*, *spoT*) in a chemostat. Results: Limitation in Fe and P results in a 6 to 8 fold increase in quorum sensing gene expression. Slow growth rate in a low phosphate chemostat causes a greater than 10 fold increase in quorum sensing gene expression. We are investigating the role of the stringent response in slow-growth-dependent quorum sensing gene expression. Conclusions: Integration of quorum sensing, nutrient starvation signaling systems, and the stringent response allow *P. aeruginosa* to efficiently respond to environmental change

Abstract Category

03 Microbial Conflict and Conversation

PS.03.008 GROWTH RESPONSE PATTERNS OF TWO PROCHLOROCOCCUS STRAINS TO CO-CULTURE WITH DIVERSE HETEROTROPHIC BACTERIA

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Microorganisms in marine environments live in complex communities, where competition, allelopathy and symbiosis are likely important. The cyanobacterium Prochlorococcus is a dominant microbe in many oligotrophic marine ecosystems, and a number of ecologically distinct strains have been described. Here, we study pair-wise interactions between two ecologically distinct Prochlorococcus strains and a 'strain library' of 392 marine heterotrophic bacteria isolated from seawater. Our goal was to determine to what extent different heterotrophic bacteria affect Prochlorococcus growth.

We analyzed the effect of each heterotroph on the growth of Prochlorococcus strains MED4 and MIT9313 in liquid batch co-culture. To compare the outcome of hundreds of co-cultures, we extracted parameters such as growth rate, growth time and maximal culture fluorescence from the growth curves, and used them to define a parameter space describing the growth and decline of the cultures.

MIT9313 was sensitive to co-culture with heterotrophic bacteria; the growth of ~90% of the co-cultures was either enhanced or inhibited compared to controls. In contrast, the growth of over 98% of the MED4 co-cultures was indistinguishable from that of the controls. Additionally, the magnitude of the effect of co-culture on the various growth parameters was much larger for MIT9313 than for MED4. Finally, bacterial strains whose 16S gene sequences differed by 1-2% tended to have similar effects on Prochlorococcus, suggesting these effects are due to deeply-rooted traits of these heterotrophic clades rather than recently-acquired ones.

Our results reveal different patterns in the outcome of laboratory co-culture between ecologically distinct yet closely related Prochlorococcus strains. Unraveling the mechanisms behind these

differences may provide clues for a deeper understanding of the physiology of different *Prochlorococcus* ecotypes and their dynamics in the wild.

Abstract Category
03 Microbial Conflict and Conversation

PS.03.009 PRODUCTION OF QUORUM SENSING COMPOUNDS BY 'HOEFLEA ANABAENAE', AN ATTACHED SYMBIONT OF ANABAENA SP. SSM-00

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'*Hoeflea anabaenae*' strain WH2K is a novel heterotrophic bacterium that forms an attached, symbiotic relationship with the filamentous, heterocystous cyanobacterium *Anabaena* sp. SSM-00. This symbiosis appears to be species-specific, mutually beneficial, and facultative. Chemical signaling among or between the cells is likely involved in establishing the attached symbiosis and any resulting shifts in metabolism. The research described here sought to detect and characterize any acylated homoserine lactones (AHLs), a class of quorum sensing compounds, produced by WH2K. Another species of *Hoeflea*, *H. phototrophica*, produces AHLs and contains a homoserine lactone synthase gene according to its annotated genome sequence. Polymerase chain reaction primers were developed and used to amplify and sequence the homologous gene in WH2K. AHLs present in the culture medium of WH2K and other species of *Hoeflea* were detected and quantified using a beta-galactosidase-based, cell free assay. These AHLs, along with other metabolites were extracted from culture medium and separated using high-performance liquid chromatography. The presence of AHLs in each fraction was detected with a modified whole-cell assay and confirmed by mass spectrometry. Further chemical characterization of the AHLs produced by each *Hoeflea* is currently underway. After confirming WH2K produces AHLs, their role in the symbiotic relationship can now be investigated. This will be done with studies using homoserine lactone synthase mutants of WH2K and by monitoring shifts in gene expression and metabolism of both symbiotic partners in the presence of purified AHLs. The two-membered symbiotic relationship between '*Hoeflea anabaenae*' strain WH2K and *Anabaena* sp. SSM-00 provides a simple but powerful model system to study the role for cell signaling in other symbioses between heterotrophic and photosynthetic microorganisms.

Abstract Category
03 Microbial Conflict and Conversation

PS.03.010 MOTILITY OF PSEUDOMONAS SPP. IS CONTROLLED BY THE SIGNALING MESSENGER CYCLIC DIGUANYLATE

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Cyclic diguanylate is now recognized as a ubiquitous bacterial second messenger that regulates various functions involved in growth and sustainability, such as developmental transitions, biofilm formation, pathogenesis, and gene regulation, to name a few. Previously, we have described a novel membrane-localized regulator, MorA, which is conserved among diverse *Pseudomonas* species. MorA encodes the GGDEF and EAL cyclic diguanylate signaling domains. In the *Pseudomonas putida* plant growth-promoting rhizobacterial strain (PNL-MK25), MorA affects timing and stage of flagellar development, and a mutation in MorA leads to constitutive formation of flagella and hyperflagellation. MorA is also required for biofilm formation. In the human pathogen *Pseudomonas aeruginosa*, here, we describe that in addition to biofilm formation, MorA affects the flagella motor function, but not the number of flagella. We also show that for in vitro studies, MorA functions as a bifunctional enzyme possessing a strong diguanylate cyclase, but weak yet measurable phosphodiesterase activity. This is the first such regulator known in *Pseudomonas*. Hence, we show that cyclic diguanylate signaling works through a fine balance of cycling, affecting both the number of flagella and its motor function. This second messenger also coordinately controls other microbial functions including biofilm initiation.

Abstract Category
03 Microbial Conflict and Conversation

PS.04.001 MONITORING OF DIARRHEAGENIC *ESCHERICHIA COLI* IN THE AQUATIC ENVIRONMENT OF BANGLADESH

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Diarrheal diseases are major causes of morbidity and mortality among the infants and children in developing countries. In Bangladesh, poor sanitation and hygiene has been responsible for the pollution of the natural aquatic system particularly in and around the big cities. Scarcity of alternative pure drinking sources of water coincides with the prevalence of diarrhea. *Escherichia coli* is a prominent bacterial agent among the diarrheagenic pathogens. Detection and monitoring of different categories of diarrheagenic *E. coli* is important in both fresh and marine waters, which is being used for drinking, household and recreational purposes.

We have used multiplex PCR by combining nine primer pairs specific for enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), and entero-aggregative *E. coli* (EAEC) to facilitate the identification of five different virotypes of diarrheagenic *E. coli* in a single reaction. Special pre-enrichment and enrichment media have been developed to increase the recovery potential of *E. coli* from the water samples to detectable level. Forty six water samples have been collected from major rivers, falls and sea beaches in Bangladesh. Among them, 34 samples were positive for at least one diarrheagenic *E. coli* group. This includes 28 samples (61%) with ETEC, 13 samples (28%) with EHEC and 8 samples (17%) with EAEC. None of the 46 samples were found to be positive for either EIEC or EPEC by PCR. No clear regional distribution pattern was observed for ETEC, but EHEC is mostly found in the North-East and Southern part of the country. Only the overcrowded recreational and commercial zones were found to contain the EAEC, which co-existed with ETEC.

Abstract Category

04 Disease Ecology

PS.04.002 COMPARATIVE ANALYSIS OF THE POPULATION GENETIC STRUCTURE OF A VECTOR-BORNE PLANT PATHOGEN UNDER ENDEMIC AND EPIDEMIC DISPERSAL REGIMES

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The genetic structure of pathogen populations reflects their ecology and provides information that can be used to better interpret patterns of disease spread. We used the vector-borne bacterial plant pathogen *Xylella fastidiosa* as a model system to analyze the population structure of pathogens in two different regimes: endemic— established in a location for several decades, and epidemic— recently emerged and developing rapidly. In northern California where *X. fastidiosa* has caused Pierce's disease of grapevines for

approximately 100 years, disease incidence is low and the pathogen widespread. In Brazil, the same bacterium causes citrus variegated chlorosis, which emerged 20 years ago and now infects approximately 40% (60 million trees) of the country's citrus industry. We used 20 simple sequence repeat markers to type 93 spatially structured isolates from grapevines and 16 markers for 160 isolates from citrus. In the endemic regime, we found extreme diversity (83 different genotypes out of 93 samples) and no spatial structuring among samples. Furthermore, indices of diversity were high and, accordingly, indices of clonality were close or equal to zero. We interpret these results as evidence of spatial mixing of haplotypes over time as a consequence of vector movement in the pathogen's reservoir habitat, effectively resulting in panmixia. The opposite result was observed in the epidemic regime. *X. fastidiosa* was structured regionally and locally, even to the tree level within orchards. Diversity indices were lower and many isolates shared the same haplotype (59 different haplotypes out of 160 samples). Because the citrus disease has continued to expand spatially for several years, population structuring is hypothesized to be a consequence of wave-like dispersal of the pathogen. Altogether our results show that disease ecology is a strong determinant of pathogen population structure and genetic diversity.

Abstract Category
04 Disease Ecology

PS.04.003 TEMPORAL VARIATIONS OF BACTERIAL COMMUNITIES IN TWO TROPICAL FRESHWATER SYSTEMS WITH EMPHASIS ON THE *ESCHERICHIA COLI-SHIGELLA* GROUP

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Environmental water plays a major role in spreading diarrheal diseases caused by certain virotypes of *Escherichia coli* and *Shigella* spp. Although *E. coli* can easily be recovered by cultural means, irrespective of source, isolation of *Shigella* from aquatic environment still remains a challenge. This is probably due to lack of proper resuscitation techniques for recovery of dormant *Shigella* from environmental waters. Still no credible data is available on the distribution of *Shigella* in the environment. The aim of this study was the analysis of temporal variations of the bacterial communities in two tropical freshwater ecosystems in Bangladesh with emphasis on the *E. coli-Shigella* group using denaturing gradient gel electrophoretic analysis of PCR-amplified 16S rRNA gene fragments. The analysis was performed on DNA extracted directly from water samples and after growth in pre-enrichment and enrichment media, including a complex medium for total heterotrophs using water samples obtained 8-9 times in a year. The pre-enrichment and enrichment cultures were successful for detection of the *E. coli-Shigella* group from both the sampling sites. Representatives from a number of common aquatic bacterial genera, e.g. *Aeromonas*, *Acinetobacter*, *Bacillus*, *Comamonas*, *Cyanobium*, *Kurthia* and *Vogesella* were frequently identified, including a variety of other bacterial groups. Both temporal and spatial variations in the community structures were observed, where *E. coli*

and *Shigella* were found to be a prominent group. The *E. coli-Shigella* group was mostly detected during the summer and winter seasons, while their presence could not be detected in the rainy season. No Significant correlations between the presence of enteric bacteria and the environmental community profiles were found. Several bacteria with distant relationship to known bacterial species were identified, demonstrating the presence of a number of novel genera and species in these ecosystems.

Abstract Category
04 Disease Ecology

PS.04.004 BIOGENIC AMINE PRODUCING ABILITY OF ENTEROCOCCI ISOLATED FROM COW'S MILK

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Enterococcus bacteria occur mainly in the intestinal microflora of humans and animals and can also be found in foods as contaminants. In the dairy industry they are used in manufacturing of cheeses or applied as probiotic cultures. Enterococci are also pathogens causing different infections at humans. They can invoke food intoxication by production of biogenic amines. Due to these contrasting characteristics there is little harmony in the acceptance of their presence in foodstuffs. The aim of our study was to isolate enterococci strains from raw cow's milk and to detect their potential ability to produce tyramine and histamine.

90 raw cow milk samples were incubated on Slanetz-Bartley agar (Oxoid, Great Britain) at 36 °C for 48 h. Suspect colonies were isolated on the Blood Agar Base (36 °C for 24 h) and confirmed using STREPTOtest 16 (PLIVA-Lachema, Czech Republic). DNA for identification of specific sequences for tyrosine decarboxylase (tyr_{dc}) and histidine decarboxylase (hdc) was prepared by phenol extraction. PCR was performed in a final volume of 25 µL containing 10 ng of genomic DNA, 10 pmol of each primer TD2/TD5 and HDC3/HDC4 (Coton et al., 2004), and the appropriate amount of HotStar Master Mix (Qiagen, Germany). DNA samples were first denaturated at 94 °C for 15 min. The amplification was done as described by authors. After 0.8% agarose gel separation in 0.5xTBE buffer amplicons were visualized in UV light.

Enterococci were determined in 38 milk samples in which a collection of 31 *E. faecalis* and 7 *E. faecium* isolates was found. PCR analysis shown, that all bacterial isolates were positive for tyr_{dc} gene, but none of them for hdc gene.

Enterococci as potential producers of biogenic amines have been found in 42 per cent of all samples of tested raw cow's milk. Thus, the genus entails disputable aspects for food safety and human health. The work was granted by projects MSMT 2B08069 and LA333.

Abstract Category
04 Disease Ecology

PS.04.005 LANDSCAPE GENETICS OF *ESCHERICHIA COLI* PERSISTING IN SOILS

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Fecal pollution imposes health and economic costs in all nations. Detection of fecal pollution and fecal source tracking (FST) rely on abundance or classification of fecal indicator bacteria (FIB). *E. coli* is a preferred FIB species due to the large variety of means by which it can be assayed in the environment. Persistence in soil has been shown to affect both population structure and attenuation of *E. coli*. Some studies have suggested that commensal *E. coli* may become naturalized to extra-host habitats under conditions favoring persistence. However, quantitative analyses of structural changes imposed by extra-host habitats on *E. coli* populations are lacking. We completed multi-locus sequence analysis of 355 *E. coli* from a recreational meadow. The data show that *E. coli* persist over time in soil, and during persistence environmental gradients are imposed on a patchy spatial structure. The underlying patchiness is attributed to the host-driven spatial pattern of fecal deposits, as are landscape variables that predict *E. coli* distribution in the field. In spite of biotic and abiotic sorting of *E. coli*, soil isolates were neither genetically distinct from fecal isolates nor from a broad diversity of clinical and environmental strains. The *E. coli* species is composed of subpopulations (ECOR groups), and these subpopulations exhibited different genetic structure along abiotic and biotic factors. For example, ECOR D genetic structure was partially explained by pH and organic matter whereas ECOR B2 genetic structure was explained strongly by sample date with a modest effect of pH. The ability of FST methods to attribute FIB to sources will depend on reliable predictions about relevant changes to FIB population structure in extra-host habitats. The persistence of *E. coli* in secondary habitats also raises the possibility of using conservation biology techniques to map its dispersal using genetic information

Abstract Category

04 Disease Ecology

PS.04.006 THE IMPACT OF SOIL COMMUNITIES ON MICROBIAL PATHOGEN SURVIVAL

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Manure and slurry are valuable resources that enhance many soil properties. However, organic amendments can pose a significant health risk to both humans and livestock if not managed correctly, due to the pathogenic load carried within it. Therefore it is crucial to identify the factors that affect pathogen survival in soil, in order to gain maximum benefit from such resources while minimising the threat to public and animal welfare. This research aims to elucidate the impact of microbial community structure on pathogen decline following entry of such organisms into the soil. Initial work based on culture methods showed a significant effect of soil type on the survival of *E. coli* O157.

Microcosms consisting of sterile and non-sterile sand and clay soils were inoculated with known concentrations of the pathogen and destructively sampled over time. Growth was observed in sterile soil in the absence of a microbial community, whereas the pathogen declined in non-sterile soil, hypothesized to be due to microbial antagonism. There was a significant difference in survival between sand and clay soil; however the exact cause of this variation was difficult to discern due to confounding factors such as organic matter, the nature of the communities and soil spatial organization. A subsequent experiment is testing the hypothesis that community structure, particularly microbial diversity, modulates the survival of introduced pathogens. A diversity gradient has been created through the inoculation of gamma-irradiated samples with serial dilutions of field soil, incubated to allow biomass equalization, and the phenotypic structure of the resultant communities characterized using PLFA profiling. To test the hypothesis, the survival characteristics of bacterial pathogens *Listeria*, *Salmonella* and *E. coli* when introduced into the different community contexts, is then established using cultivation and lux-marking techniques.

Abstract Category

04 Disease Ecology

PS.04.007 OVINE PEDOMICS- THE FIRST STUDY OF THE OVINE FOOT 16SrRNA BASED MICROBIOME

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Footrot (FR) and interdigital dermatitis (ID) are highly contagious related diseases which start with the invasion of the epidermis of the interdigital skin producing lameness in sheep. We report the first study of the ovine bacterial microbiome of the interdigital skin based on 16SrRNA deep sequencing and conventional cloning-sequencing of sheep with clinical conditions described as healthy, H; with interdigital dermatitis, ID; and with footrot, FR. Three farms with different disease history were studied; Farm A with healthy animals, Farm B with a history of ID and Farm C with a history of ID and FR. Deep sequencing analysis produced 8 bacterial phyla; Firmicutes followed by Actinobacteria and Proteobacteria were the most abundant across farms and conditions.

Corynebacterium, *Micrococcus* (Actinobacteria), *Macrococcus*, *Peptostreptococcus* and *Streptococcus* (Firmicutes) were the 5 most abundant genera from 110 observed.

Sequence and phylogenetic analyses revealed that the ovine foot bacterial community varied between farms and clinical conditions. The diversity within OTUs for the three conditions was greatest for ID compared to H and FR. Sequence corresponding to *Dichelobacter nodosus* were absent from all libraries due to a double mismatch in the 16SrRNA universal forward priming site (27F). An alternative qPCR assay was

developed based on rpoD which allowed detection of *D. nodosus* in all clinical conditions. Sheep from farms with no prior history of footrot (A and B) had feet colonised by *D. nodosus*. In conclusion, differences in the structure of the bacterial community were observed but core populations were found in all clinical conditions. Representative sequences for the *Corynebacterium* genus were significantly higher in animals with ID diseases status and contrary to previous studies *D. nodosus* was present in all healthy feet.

Abstract Category
04 Disease Ecology

PS.04.008 THE POTENTIAL INHIBITION OF PROBIOTICS IN THE ADHERENCE AND INVASION OF MENINGITIC *ESCHERICHIA COLI* K1 TO MURINE INTESTINAL MUCOSA

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Escherichia coli K1 is the most common bacterial pathogen causing neonatal sepsis and meningitis. Approximately 20% of newborns are colonized in their stool with *Escherichia coli* K1, and approximately one in 200 infants develops an invasive infection. In order to develop probiotics as a better approach for the prophylaxis of bacterial sepsis and meningitis caused by *Escherichia coli* K1, prophylactic efficacy of probiotics in bacterial sepsis and meningitis was tested *in vitro* and *in vivo*.

To evaluate the potential inhibition of the probiotics in the adherence and invasion of *Escherichia coli* K1 to murine intestinal mucosa. The viable bacteria counting was applied to determine the number of colony form unit of *Escherichia coli* K1 adhesion to Lovo cells that were incubated with probiotics *in vitro*. Flow Cytometry analysis were used to investigate probiotics inhibit apoptosis of Lovo cells induced by *Escherichia coli* K1. *In vivo*, neonatal rats randomly assigned to the following experimental groups: probiotics+pathogen group and pathogen only group. The viable bacteria counting was used to detect quantitatively *Escherichia coli* K1 in the intestinal colonization and metastasis in rats of the above groups administered orally.

The results show that the probiotics were observed to competitively suppress the pathogens adhesion to Lovo cells in a dose-dependent manner ($P < 0.01$) *in vitro*. The apoptotic level of Lovo cells induced by *Escherichia coli* K1 in the probiotics+pathogens group was decreased remarkably when compared to the pathogens only group ($P < 0.01$). The number of intestinal *Escherichia coli* K1 and rats with bacteremia in the probiotics+pathogens group was decreased remarkably when compared to the pathogens only group ($P < 0.01$). The above results suggested that probiotics could protect intestinal epithelial cell and inhibit strongly pathogens adherence and invasion ability, and may be effective in preventing bacterial sepsis and meningitis.

Abstract Category
04 Disease Ecology

PS.04.009 COMPOSITION OF THE ORAL LACTIC ACID BACTERIA AND STREPTOCOCCAL FLORA IN HEALTHY CHILDREN, TURKEY

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Oral infections constitute of the most common and costly forms of infections in humans. The concept of microbial ecological change as a mechanism for preventing dental disease is an important one while altered microbial ecology may lead to dental disease. In this study, lactic acid bacteria and streptococcal species in the mouths of healthy children were isolated. The composition of the oral lactic acid bacteria and streptococcal flora were investigated a period of 2 months. The mouth subjects were healthy school children aged between 4 and 16 years. The oral flora was sampled saliva, gingival crevicular fluid, dental plaque, and dental caries. Spread- plated onto MRS agar and M17 agar (Oxoid). The plates were placed in an anaerobic flask (Oxoid) in the presence of a gas-generating kit (Anaerobic System BR0038B, Oxoid) and incubated at 37°C for 2-3 days. Isolates were identified using the following biochemical tests: ammonia production from arginine, CO₂ production from glucose, growth at different temperatures (4, 8, 10, 15 and 45°C), growth at different pH values, and growth at different NaCl concentrations. In addition, the BIOLOG and Ribotyping systems were used to identification of the isolates according to the manufacturer's instruction. Ribotyping was performed with a RiboPrinter Microbial Characterization System (Qualicon Inc., Wilmington, DE) and the standard EcoRI DNA preparation kit as described in the manufacturer's operations and analytical guides. The predominant species among the isolates were identified as *Streptococcus mutans*, *Lactococcus lactis*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lb. paracasei* sp *paracasei*, *Staphylococcus hominis*, and *Staphylococcus epidermis*.

Abstract Category

04 Disease Ecology

PS.04.010 USE OF CULTURE INDEPENDANT METHODOLOGY TO ASSESS BACTERIAL COMMUNITIES IN CHRONIC TONSILLITIS

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Chronic infections of the upper respiratory tract represent a therapeutic challenge. They are mainly caused by biofilm forming bacterial populations showing increased resistance to antibiotics. Precise understanding of inter- and intra-species interactions in biofilms will offer promising targets for innovative antimicrobial drugs. In paediatrics, surgical interventions are often required to cure the patients with adenotonsillitis. In this study, culture independent methodology was used to assess bacterial biofilms in 34 anonymized

tissue samples that were left over following surgical procedures of chronic adenotonsillitis patients (tonsillectomy), mean age of 6 years old. Biofilms were phylogenetically analyzed with primers targeting 16s ribosomal DNA of eubacterial species. Denaturing gradient gel electrophoresis (DGGE) profiles, indicate presence of average of 10 taxa per tonsil. Clone library analysis demonstrated mainly the occurrence of *Streptococcus anginosus*, *Streptococcus pneumoniae*, *Streptococcus constellatus* and *Streptococcus intermedius* associated phylotypes which were previously described as biofilm forming microorganisms of the upper respiratory tract flora. Also *Curvibacter gracilis* and *Oribacterium* affiliated clones were identified which are shown to be present in dental plaque biofilms possibly found its source from surgical contamination. Biofilm based infections is a serious issue of the human throat where culture independent analysis is a promising approach to study bacterial communities specifically to support the design of new drugs targeting quorum sensing. We can expect that the comprehension of biofilm dynamics will propose interesting alternative to overcome the problem of multi resistances.

Abstract Category
04 Disease Ecology

PS.04.011 IN VITRO EVALUATION OF LACTIC ACID BACTERIA AND STREPTOCOCCUS MUTANS BACTERIA ADHESION TO TOOTH SURFACE

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The use of beneficial microorganisms has been proposed to treat a range of clinical conditions that have been linked to bacterial pathogens, including dental diseases like dental caries, periodontal diseases and halitosis. An essential condition for a microorganism to represent a probiotic of interest for oral health is its capacity to adhere to and colonize various surface of the oral cavity. Most probiotic products are consumed orally. Therefore it is thought that the bacteria in these products may also attach to oral surfaces; but the mechanism of action of these lactic acid bacteria on the oral ecosystem is mostly unknown. The aim of this study was to evaluate the adhesion of lactic acid bacteria and *Streptococcus mutans* to tooth surface using two different methods. Thirteen lactic acid bacteria and two *Streptococcus mutans* bacteria were isolated from healthy children and ten lactic acid bacteria were isolated from meat products. Binding of selected microorganisms to extracted tooth surface were investigated by two different methods in vitro.

All microorganisms tested adhered to with/without saliva coated tooth surfaces, but with significantly different binding frequencies. The adhesion of the *Lactobacillus sakei* remained lower in comparison to *Lactobacillus casei* in first test. The adhesion of the *Lactobacillus sakei* remained lower in comparison to *Lactobacillus casei* in second test. *Lactobacillus* and *Streptococcus mutans* species were isolated from healthy mouths, some of them showed higher adhesion capacity to tooth surface. Adhesion to oral soft and hard tissues is of crucial for bacterial colonization in the mouth. *Lactococcus lactis* strains

investigated had a high adhesion percentage. The differences in binding in vitro may indicate that there are also differences in the persistence of the different lactic acid bacteria in the oral cavity in vivo.

Abstract Category
04 Disease Ecology

PS.04.012 BLEACHING IN THE RED ALGAE *DELISEA PULCHRA* LEADS TO PHYLOGENETIC AND FUNCTIONAL SHIFTS IN THE SURFACE-ASSOCIATED BACTERIAL COMMUNITY

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Environmental changes including rising ocean temperatures have been implicated as major causes in the increased incidence of marine diseases. Over the last decade, the temperate-water, red macro-alga *Delisea pulchra* has been observed to undergo bleaching during warm summer periods. This bleaching effect could be reproduced in vitro by a combination of increased temperature and the addition of marine *Roseobacter* strains. In order to better understand the ecology of this disease response, we explored the bacterial community associated with bleached and healthy plants in the field. 16S rRNA gene and environmental shotgun sequencing data were generated for the phylogenetic and functional analysis of the bacterial community associated with healthy and bleached *Delisea pulchra*, as well as for apparently healthy tissue adjacent to bleached regions of the algae. Phylogenetic analysis showed that bleached thalli and tissues adjacent to bleached areas have a similar and consistent community composition, which was distinct from that of healthy plants. While *Roseobacter* strains that induced bleaching in vitro were found exclusively on bleached tissue, they were not numerically dominant. Phylogenetic differences between healthy and bleached communities mainly reflected relative changes in the genera *Thalassomonas*, *Thioclava* and *Parvularcula*. Comparative metagenomics showed a distinct and consistent functional signature for healthy tissue, while a large variability in the functional gene composition was observed for bleached tissue. This variability was mainly due to changes in functions associated with transcriptional regulation, cation/multidrug efflux and non-ribosomal peptide synthesis. Bleaching of *Delisea pulchra* is associated with complex shifts in the phylogenetic and functional community composition. These shifts could be brought about by changes in environmental conditions that allow opportunistic pathogens to cause disease.

Abstract Category
04 Disease Ecology

PS.04.013 EXTRA-VAGINAL RESERVOIRS OF VAGINAL BACTERIA AS RISK FACTORS FOR BACTERIAL VAGINOSIS

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Bacterial vaginosis (BV) is a polymicrobial condition characterized by a shift in vaginal microbiota from one dominated by *Lactobacillus* species to one with diverse anaerobic bacteria. It is unclear why some women develop BV. We sought to determine using quantitative PCR (qPCR) if extra-vaginal bacterial colonization is a risk factor for BV. Conversely, we examined if extra-vaginal presence of *Lactobacillus crispatus* is protective against BV.

In this case-control study, vaginal, perianal, and oral samples were collected from women (n=44) without BV at baseline. Fifteen women subsequently developed BV by Amsel criteria. Controls were women who did not subsequently develop BV and were matched to cases based on follow-up times. Samples were subjected to qPCR assays targeting vaginal bacteria including Bacterial Vaginosis Associated Bacterium-1 (BVAB-1), BVAB-2, BVAB-3, *Megasphaera* spp., *Gardnerella vaginalis*, and *L. crispatus*. Proportions of participants with individual bacteria detected were compared using Fisher's exact test; quantities detected were compared using Wilcoxon rank sum tests.

Participants who acquired BV had higher perianal levels of *G. vaginalis* (p=0.001) and *Megasphaera* spp. (p=0.02). Participants who did not acquire BV had higher perianal levels of *L. crispatus* (p= 0.008). Oral colonization with *G. vaginalis* was associated with acquisition of BV (p<0.001), but BVAB1, BVAB2, BVAB3, and *Megasphaera* spp. were not detected in the mouth of any subject.

Women who acquire BV are more likely to have previous colonization of extra-vaginal reservoirs with key BV-associated bacteria such as *G. vaginalis* and *Megasphaera* species, and reduced levels of *L. crispatus*. These data suggest that some BV-associated bacteria may be vaginally acquired from a woman's indigenous microbiota, and that extravaginal colonization with key bacterial species can be used as one predictor of BV acquisition.

Abstract Category
04 Disease Ecology

PS.04.014 MICROBIAL COMMUNITIES AT VULTURE NESTING SITES AND CONSEQUENCES FOR DISEASE TRANSMISSION

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We are studying how vultures alter the microbial communities at their nesting sites. We are also examining the potential for vulture fecal aggregations to serve as infectious sites of *Bacillus anthracis* in Etosha National Park, Namibia. Scavengers such as vultures may disperse endospores from carcass sites. Vultures are abundant scavengers in Etosha and form stable nests under which their feces collect in high concentrations.

We collected soil samples under nesting sites and at control sites. For the bacterial community characterization, we performed PCR using universal 16S rRNA primers. Using the PhyloChip, we can detect 8,741 bacterial and archaeal operational taxonomic units (OTUs). We used 21 Phylochips to test for an effect of vultures on the distribution of *B. anthracis* DNA and any effects on the microbial community: five white-backed vulture nests, five lappet-faced vulture nests, ten control trees, and one positive control.

We found small but detectable amounts of *B. anthracis* DNA in 84% of samples. Abundance and distribution of *B. anthracis* DNA did not differ between vulture sites and control sites. In the microbial community, there were 2,795 OTUs detected. The soil microbial communities differed significantly with Cyanobacteria being more abundant under white-backed vulture nests and Alpha and Gamma-proteobacteria being more abundant under the lappet-faced vulture nests. The communities associated with the two vulture species also differed significantly from control sites.

The Phylochip provides a highly sensitive method for detection of very small amounts of *B. anthracis* DNA in environmental samples. We found no evidence to support the hypothesis that vultures are concentrating endospores at nesting sites. However, the two vulture species had different soil microbial communities associated with their nesting sites and these communities differed from control sites.

Abstract Category
04 Disease Ecology

PS.04.015 FISH AND WATERBIRDS AS RESERVOIRS AND VECTORS OF VIBRIO CHOLERAE

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Cholera is a life-threatening diarrheal disease that can spread rapidly and in explosive epidemics and pandemics, affecting large numbers of people. *V. cholerae* the causative agent of cholera, is a natural inhabitant of aquatic ecosystems where it can exist in a free-living planktonic form or associate with zooplankton such as copepods and chironomids (Broza & Halpern 2001, Nature). However, the mechanism that enables *V. cholerae* to cross water bodies, including oceans, is still puzzling. Recently we suggested that migratory waterbirds distribute the bacteria among water bodies (Halpern et al., 2008 PLoS Pathog). Our hypothesis was based on Frich et al. (2007) who found that both copepods and chironomids are dispersed by migratory waterbirds. Here we show that in the food chain, fish link chironomids and copepods to waterbirds.

V. cholerae was isolated from fish that were sampled from freshwater and marine habitats.

We found evidence that ten different fish species from freshwater habitats and one marine species, carry *V. cholerae* in their intestine. The fish intestine of *S. galilaeus*

harbored ca. 5×10^3 *V. cholerae* cfu per 1 gr intestine content — high rates compared with known *V. cholerae* cfu numbers in the bacteria's natural reservoirs.

Our results, combined with evidence from the literature, suggest that fish are also reservoirs and carriers of *V. cholerae*. We suggest that the bacteria are introduced into the fish intestine via their invertebrate (copepods and chironomids) prey. As far as we know, ours is the first survey of fish serving as reservoir of *V. cholerae*. As fish carrying the bacteria swim from one location to another, they serve as vectors on a small scale. Nevertheless, fish are consumed by waterbirds, which disseminate the bacteria on a global scale. Better understanding of the epidemiology of cholera can help reduce the times that human beings come into contact with this pathogen.

Abstract Category

04 Disease Ecology

PS.04.016 OCCURRENCE OF CTX-M PRODUCING *E. COLI* IN CATTLE AND THE FARM ENVIRONMENT IN BURGUNDY, FRANCE

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Extended spectrum beta lactamase (ESBL) producing *Escherichia coli* have recently emerged worldwide. The aim of this study was to survey cattle and the farm environment to determine if they are potential reservoirs for these strains. Samples of cattle feces (n=271) were collected from 182 farms in Burgundy (France). ESBL-producing *E. coli* were isolated on selective media containing cefotaxim or ceftazidim. Thirteen ESBL-producing isolates were obtained from 3 farms, and these were characterized for the presence of bla genes. Of the 13 strains, five and eight strains carried blaTEM genes and blaCTX-M genes, respectively. Soil, water and stored manure obtained from the 3 positive farms were evaluated for the presence of ESBL-producing *E. coli*. Ten strains of CTX-M producing *E. coli* were isolated from cultivated and pasture soils and composted manure from 2 of the farms. A genotypic analysis of the strains by rep-PCR using the Diversilab system revealed that the environmental strains were clonally related to the strains isolated from animals. In some cases soils had not been manured for over a year, thus suggesting that some strains may be environmentally adapted. A larger scale study was performed to detect the prevalence of blaCTX-M in soil. Soil DNA from 120 sites in Burgundy (Reseau de Mesure de la Qualite des Sols, 16x16 km grid) was tested using real time PCR with specific primers and probe. The blaCTX-M targets were detected in 20% of the DNA extracts tested. This study confirms the occurrence of CTX-M producing *E. coli* in cattle. To our best knowledge, this is the first report of such strains in cultivated soils. The environmental competence of such strains might explain their long

term survival since CTX-M isolates were recovered from a soil that was last amended with manure one year before sampling. The blaCTX-M gene was found to be very widespread in a regional soil survey. Further studies are underway to determine the spread of blaCTX-M genes.

Abstract Category
04 Disease Ecology

PS.04.017 IN VITRO EVALUATION OF ANTIMICROBIAL ACTIVITY AND ANTIBIOTIC RESISTANCE LACTIC ACID BACTERIA ISOLATED FROM HEALTHY MOUTH

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Effect of probiotic species on oral health is gradually being understood. However, more studies are needed on the characteristics of potential strains before clinical recommendations. The aim of the present in vitro study was to assess the antimicrobial activity of different Lactobacillus strains against Listeria monocytogenes, Staphylococcus aureus, Enterococcus faecalis, Streptococcus mutans and Staphylococcus epidermidis, thus evaluating their prospective use as oral probiotics. And they were tested for resistance to 12 antibiotics. These were Rifampicin (RIF), Lincomycin/Neomycin (LN), Methicillin (MET), Lincomycin/Spectinomycin (LS), Oxacillin (Ox), Penicillin/Novobiocin, Ceftiofur (EFT), Azithromycin (AZM), Gentamicin (CN), Florfenicol (FFC), Ertapenem (ETP), Linezolid (LZD). This testing was performed using the standard disc diffusion method.

Agar spot tests as described by were used to determine the antagonistic activity of the lactic acid bacteria. Agar spot tests were conducted by placing 0.5 µl of an over-night LAB culture onto the surface of a MRS-0.2 (MRS containing 0.2% glucose) with 1.2% agar plate, and incubating for 24 h at 37°C. Anaerobic conditions were maintained to minimize the formation of H₂O₂ and acetic acid. The plates were overlaid with 7 ml of soft TSAYE (0.7% agar) agar seeded with approximately 5×10⁷ cfu of the tested bacteria. After anaerobic incubation for 24 h at 37°C, the plates were examined for an inhibition zone surrounding the colonies of the producer strain.

A total of 13 lactic acid bacteria were isolated from the healthy mouth and all of these isolates showed antimicrobial activity against one or more of test microorganisms under the agar spot test. Lactobacillus paracasei G210-1b and Lactobacillus rhamnosus G27-4 had inhibitor activity against all bacteria. All of the strains were totally susceptible to all antibiotics tested except Gentamicin. Most of the tested strains were resistant to Gentamicin.

Abstract Category
04 Disease Ecology

**PS.04.018 DESIGN OF AN ANTIBIOTIC RESISTANCE ELEMENTS
GENECHIP**

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Background and aims: Healthcare-associated infections [HAI] are a worldwide problem affecting diverse groups of patients. A major challenge in fighting HAI is the rising tide of antimicrobial resistance. Non-clinical environments are the natural origin of most antibiotic resistance elements; however, the use of broad-spectrum antibiotics in the clinical setting places significant selective pressure on microorganisms, altering the functional role of these elements. To further understand the evolution and ecology of resistance in natural and clinical settings, we are developing a resequencing microarray as a sensitive and specific molecular tool for rapid, culture-independent analysis of resistance sequence variation.

Methods: Resistance element gene sequences representing current information on antimicrobial resistance were compiled, and a two-step computational pipeline was built. First, Genbank nr database was searched using Blast to obtain orthologs and all known sequence variants, using a lenient Blast E-value cutoff (10^{-3}) to increase sensitivity as well as to assess cross-hybridization potential, followed by sequence clustering based on an in-house k-mer based clustering algorithm.

Results: We initially compiled 24k sequences spanning 642 species and 145 antibiotics. Using this sequence set as a "seed", a total of 130k sequences were harvested from Genbank nr database representing 6k clusters, each with a minimum of 33% 7-mer identity. Clusters were annotated by resistance element, mechanism, and host, and cluster sequence representatives were chosen for downstream probe design.

Conclusions: We have developed a robust, automated computational pipeline to design a microarray of antibiotic resistance elements. This array will facilitate the study of the epidemiology and microbial ecology of antibiotic resistance and be an invaluable tool to rapidly identify antimicrobial resistance elements in environmental and HAI pathogens.

Abstract Category
04 Disease Ecology

PS.04.019 COMPOSITION OF MUCOSAL ADHERENT BACTERIA IN PATIENTS WITH AND WITHOUT COLORECTAL ADENOMAS

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Colorectal cancer is a major cause of cancer death in the United States. In this study, we characterized mucosal adherent bacteria in normal colon in relationship to colorectal adenomas, which are intermediate precursors to colorectal cancer. Using molecular analysis of 16S rRNA gene by pyrosequencing tags, we assessed bacterial composition and diversity in normal colonic mucosa of 80 adenoma subjects and 87 non-adenoma subjects enrolled in a cross sectional study. Differences in bacterial community composition between cases and controls were evaluated by analysis of similarity matrix. Diversity was measured by Pielou's richness index and Shannon diversity. Overall distribution of bacteria phyla in both groups showed that, *Firmicutes* (54%), *Bacteroidetes* (19%), *Proteobacteria* (18%), *Cyanobacteria* (6%) and *Actinobacteria* (3%) were the most dominant in the mucosa. Compared to controls, cases had lower abundance of *Firmicutes* ($p=0.08$) and higher abundance of *Cyanobacteria* ($p=0.01$), *Actinobacteria* ($p=0.04$), and *Bacteroidetes* ($p=0.08$). At the genus level, case subjects showed increased abundance of *Streptophyta* spp, *Lactobacillus* spp, *Lactococcus* spp, *Acidovorax* spp, *Delftia* spp, *Sphingomonas* spp, and lower proportions of *Bacteroides* spp., *Dorea* spp., *Fecalibacterium* spp, *Streptococcus* spp and *Bifidobacteria* spp. than controls. The Shannon diversity index was significantly higher in cases (5.01) than controls (3.9, $p=0.0006$). Similar observations were noted for bacteria richness comparing cases (21.6) and controls (19.4, $p=0.007$). These findings suggest that alterations in bacterial diversity and composition associated with adenomas may contribute to the etiology of colorectal cancer. (Supported by NIH Grant No. P30 DK034987; P50 CA106991; R01 CA136887)

Abstract Category
04 Disease Ecology

PS.04.020 CHARACTERIZATION OF COLIFORMS IN MANURE APPLIED SOIL.

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From the aspect of materials cycle, using animal waste as fertilizer is an important process. Besides people has interesting in organic products for improving their health. There are two problems for using organic materials which were prepared from animal wastes for producing organic vegetables. First, organic materials contain a large amount of nitrogen or phosphorus but it does not provide sufficient amount of nutrition for plant growth by itself. Second, foodborne pathogen, *E. coli* O157 or *Salmonella* live in intestines of livestock. Ploughing is common process in agriculture and promoting microbial activity in soil. Activated microbes digest organic matter to small molecule and provide nutrition to plant. But it is little known that ploughing promotes microbe except accidentally contaminated foodborne pathogen in soil or not. We applied composted cow manure to lettuce field and immediately ploughed and additional ploughing on two weeks later was carried out. Soil samples were collected on three days before and every week after application for isolating coliforms. Isolated microbes were characterised by using API 20E. Population size of coliform immediately increased and reached highest level in

experimental period after application of manure and ploughing then gradually decreased. After additional ploughing, population size increased again. As a result of identification of each strain by API 20E, *Enterobacter* was most common genera in each sample. Initially, it was not only *Enterobacter* in soil sample, but also *Klebsiella*, *Serratia* in manure applied soil. From these results, we concluded that ploughing is a good attempt for ensuring safety because ploughing promoted activity in *Enterobacter* but little or no effect to *E. coli* in soil.

Abstract Category
04 Disease Ecology

PS.04.021 RELATIONSHIP OF DORSAL TONGUE BACTERIAL COMMUNITIES TO VOLATILE ORGANIC COMPOUNDS IN THE MOUTHS OF HEALTHY SUBJECTS

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Volatile organic compounds (VOCs) produced by bacteria residing on the dorsal tongue surface are often implicated as the cause of halitosis, a condition marked by the increased abundance of sulfur containing volatile compounds in the oral cavity. We know that bacterial communities in the mouth are diverse and highly variable between individuals, yet it is not clear if the structure of the bacterial communities in the mouth influences the types and quantities of exhaled VOCs. To better understand how bacteria in the oral cavity are related to mouth VOCs, we surveyed bacteria on the dorsal tongue surface of 22 subjects using a barcoded pyrosequencing technique with the mouth VOC profiles also determined on these subjects using a proton transfer reaction mass spectrometer (PTR-MS). We found a high level of inter-individual variability in both bacterial community composition and VOC profiles. The dominant mouth VOCs were acetone, methanol, and iso-propanol. The total concentration of VOCs varied by 5-fold between individuals was significantly correlated with bacterial numbers. However, we found no significant relationships between overall bacterial community composition or the relative abundances of individual bacterial taxa and VOC profiles. These results suggest that bacterial community composition is not an important determinant of mouth VOC's and other factors such as diet and host physiology may play larger roles in the formation of volatile compounds in the mouth.

Abstract Category
04 Disease Ecology

PS.04.022 DNA ARRAY BASED DETECTION OF *LEGIONELLA* SPECIES REVEALS SELECTIVITY OF THE CONVENTIONAL CULTURE BASED DETECTION METHOD

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Background and aims - Legionnaires disease is a form of pneumonia caused by *legionellae*, which are ubiquitous in aquatic environments. Out of more than 50 *Legionella* species, *L. pneumophila* is reported as the most common cause of legionellosis. Nevertheless, in addition to *L. pneumophila*, 20 other species have been reported as human pathogens. The aim of this study was to develop and validate a DNA array for the simultaneous detection and identification of all *Legionella* species that have been associated with human infections. Based on macrophage infectivity potentiator and 16S rDNA sequences a DNA array was developed for 20 *Legionella* spp. associated with human disease. Specificity and sensitivity of the assay was evaluated using a large collection of reference cultures. In addition, 183 water samples were processed using the array and classical plating on GVPC agar as described in the ISO 11731 standard protocol. A highly specific DNA array was developed, enabling accurate detection of potentially pathogenic *Legionella* species. Validation of the assay using environmental samples revealed consistent differences with the results obtained by classical plating on GVPC agar. Whereas *Legionella* DNA was detected in approximately 80 % of the samples, plating recovered *Legionella* colonies in only 32 % of the samples. All isolates obtained by culturing were identified as *L. pneumophila* or *L. anisa*. Using the DNA array, a variety of additional *Legionella* species were detected, including *L. birminghamsiensis*, *L. bozemanii*, *L. cincinatiensis*, *L. gormanii*, *L. longbeachae* and *L. sainthelensii*. Consequently, this study illustrates the usefulness of novel detection tools in *legionella* monitoring. The currently used GVPC agar plating method for detection of *Legionella* spp. selects for detection of *L. anisa* and *L. pneumophila*. Screening of a large number of water samples using the DNA array revealed the occurrence of other *Legionella* spp.

Abstract Category

04 Disease Ecology

PS.04.023 PREVALENCE OF SEVERAL HUMAN BACTERIAL PATHOGENS IN FRENCH SOILS

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Soil harboring human bacterial pathogens may contribute to water and food contamination and thus pose a risk for human health. The emergence of these pathogens

might be due to changes in agricultural practices (i.e. application of "fecal" fertilizers, sewage sludge and animal manure). This study evaluated samples (n=1489) from the French soil monitoring network project (RMQS) including cultivated, pasture and forest soils, to evaluate the prevalence of five pathogenic bacteria and establish relationships between prevalence and soil properties and biotic (i.e. abundance and diversity of soil microbial communities) factors. Soils were evaluated for the presence of *Escherichia coli*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella enterica* by quantitative PCR. Species-specific primers and probe were designed. The specificity of the *prs* gene for *L. monocytogenes* and the *ecf* gene for *E. faecalis* was validated using pure strains. Quantitative recovery and method detection limits were established in microcosm assays with soil inoculated with varying amounts of bacteria. The *prs* marker was specific for *L. monocytogenes*, and the detection limit was about 103 copies per gram of soil. Among the 935 soil DNA extract tested to date, only one was positive for *L. monocytogenes* with a concentration of 105 copies of *prs* target per gram of soil. The *ecf* marker was validated for the detection of *E. faecalis*, the detection limit was about 104 copies of *ecf* target per gram of soil. *E. faecalis* was detected in 39% of the studied soils (range of detection from 104 to 107 per g). Despite the fact that *L. monocytogenes* is thought to be able to persist in environment, it was infrequently detected in the French soils tested. Further analyses are underway to monitor levels of the three other bacterial species. Correlation analyses will be undertaken to determine which soil factors are related to the abundance of these bacterial pathogens.

Abstract Category
04 Disease Ecology

PS.04.024 MOLECULAR DETECTION OF CAMPYLOBACTER SPP. IN THE FECES OF CALIFORNIA GULLS

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In southern California, the California gull (*Larus californicus*) is considered a major source of enterococci fecal indicator bacteria. Avian sources of *Campylobacter* spp. are well known, but infection risks to bathers from seagull excreta in waters are poorly understood. Therefore, to better understand the relative risks from *L. californicus* gulls, 159 fresh excreta samples were collected from Doheny Beach from July to September 2009 and used to determine the incidence of *Campylobacter* spp. using genus-specific 16S rRNA gene-based PCR and qPCR methods. Additionally, fecal DNA extracts were tested against enterococci and gull fecal specific *Catelliboccus* qPCR assays to determine if there was a correlation between these fecal bacterial indicators and *Campylobacter* levels. Specifically, 40.4% and 53.1% of the fecal samples were positive by PCR and qPCR, respectively. Sequencing results from clone libraries developed using composite PCR products indicated that the amplicons were closely related to *Campylobacter* spp. The majority of the 255 sequences analyzed were closely related to *C. coli* (55%) and *C. rectus* (24%) at the 95-97% sequence identity. Only a small proportion of the sequences

(i.e., 2-5%) were related to *C. lari*, *C. subanticalis* and *C. jejuni* (99% identity). *Campylobacter* spp. ranged from 340 to 10^8 per gram, but concentrations greater than 10^7 per gram (wet weight) occurred only in four samples. Statistical analysis showed there were positive correlations between log₁₀ qPCR values of *Campylobacter* and the log₁₀ qPCR values of a gull fecal specific *Catellibacterium* marker ($R^2 = 0.45$, $p < 0.0001$) and enterococci ($R^2 = 0.55$, $p < 0.0001$). The relationship between *Campylobacter* spp. and the fecal indicators examined could be explained with a regression model, which may help interpret the source and distribution of *Campylobacter* spp. associated with gull fecal contamination and possible bather risk at coastal southern Californian recreational sites.

Abstract Category
04 Disease Ecology

PS.04.025 SINUS MICROBIOTA DESTRUCTURING AND IDENTIFICATION OF NOVEL PATHOPHYSIOLOGICAL SUSPECTS IN CHRONIC RHINOSINUSITIS.

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Microbial infections are known to play a role in exacerbations of Chronic Rhinosinusitis (CRS), however the etiology of this persistent inflammatory disease remains elusive. Studies to date have primarily used culture-based methods, which do not detect a large diversity of unculturable species. Here we use a culture-independent high-density microarray, the 16S rRNA PhyloChip, to compare bacterial community composition in the sinuses of CRS patients (n=7) and healthy subjects (n=7). Sinus samples possessed a wide diversity of taxa representing 127 classified bacterial families. In both healthy and CRS patients, known or suspected pathogenic members of the Pseudomonadaceae, Helicobacteriaceae, Prevotellaceae and Lachnospiraceae were detected. However, compared with healthy subjects, CRS communities exhibited a significant relative loss of richness ($p < 0.005$), evenness ($p < 0.04$) and diversity ($p < 0.01$), suggesting a dramatic shift in community structure in these patients. Increased severity of CRS symptoms, determined using the Sinonasal Outcome Test-20, correlated with a significant reduction in relative abundance of 227 taxa, including multiple members of the Lactobacillaceae, and an increase in relative abundance of two taxa whose representative species were members of the Corynebacteria. Sinus microbiota destructuring, as a result of repeated antimicrobial administration and/or persistent microbially induced inflammation, represents a hallmark of chronic rhinosinusitis. Use of a culture-independent profiling approach identified members of the sinus bacterial community highly correlated with severity of symptoms implicating them in the pathophysiology of this disease. An animal model of sinusitis is underway to determine the protective effect of the native sinus microbiome against physiologically relevant numbers of *Corynebacterium* species identified in this study.

Abstract Category
04 Disease Ecology

PS.04.026 TRANSPORT OF ESCHERICHIA COLI IN SATURATED QUARTZ SAND SOIL COLUMNS

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The presence of pathogenic microorganisms in the subsurface soil is a threat for public health due to possible contamination of groundwater with fecal originated pathogens. To delimitation of protection perimeter to water capture is better to use the criterion of time of transit in subsurface water to eliminate the contact with pathogenic microbes. Studies of microbial transport behavior are essential to assess contamination potential and to protect drinking water supplies from pathogenic microorganisms. The objective of this research is to study the transport parameters of a Gram-negative enteric bacterium, used as a model, in water saturated quartz sand in tropical conditions. Results showed a 70% adsorption of Escherichia coli in sand during batch experiments. E. coli showed a 100% survival up to 72 hours in distilled water. Columns assays run in a Teflon column (20 cm height x 6.35 cm dia) filled with water saturated quartz sand, with an ascendant flux - continue source - of 4 ml/min of a 10⁸ E. coli cells/ml water suspension. Bacterial suspension from columns effluent, measured by optical density and by microscopy counts showed a delay factor average, in columns, of 4, what means that E.coli, in the conditions tested, can move up to 1.8 m each day and up to 12 m in one week in this quartz sand soil, suggesting a possibility of reaching groundwater reservoirs.

Abstract Category

04 Disease Ecology

PS.04.027 USE OF A MULTI-TEMPLATE CONTROL TO ASSESS T-RFLP FOR HUMAN PEDIATRIC RESPIRATORY SAMPLES

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Background and aims: The microbial ecology of many respiratory infections, particularly in pediatric populations, remains largely unexplored. The aim of this study was to optimise T-RFLP for upper respiratory samples from a pediatric population at high-risk of chronic infections, including otitis media.

Methods: T-RFLP for upper respiratory samples was optimised using a multi-template control containing equal amounts of 13 bacteria commonly detected in the respiratory tract. Optimisation included assessment of primers; enzymes; reaction conditions; and peak-calling methods. Nasopharyngeal and ear discharge swabs collected from Indigenous Australian children with acute otitis media were then tested.

Results: Using the control, differential PCR amplification was noted for some species. This may affect quantitative analysis of clinical samples. Two peaks were detected for *Moraxella catarrhalis* and this needs to be considered when assessing diversity in clinical

samples. Optimal peak-calling was obtained using a constant baseline or constant percentage threshold method. The control was also used to show specific enzymes could differentiate common respiratory pathogens from closely-related commensal species. Clinical samples have shown variation in PCR yield with some samples providing insufficient product for T-RFLP. While primer choice did not affect yield from the control, it was important when amplifying from some clinical samples. Low PCR yield was more common for nasopharyngeal than ear discharge swabs, most likely reflecting smaller sample volume. Testing of clinical samples is on-going. Conclusions: Our data suggest T-RFLP can be useful for upper respiratory swabs with sufficient sample volume. The multi-template control allowed identification of PCR amplification biases; profiling characteristics of important species; and assessment of optimal peak-calling methods. We recommend use of multi-template controls when assessing T-RFLP for other mucosal environments.

Abstract Category
04 Disease Ecology

PS.04.028 COMMUNITY ANALYSIS OF SELECT DRINKING WATER SAMPLES FROM A VIETNAMESE DIARRHEAL DISEASE STUDY

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One of the major weaknesses of the H₂S test for the detection of fecal bacteria is the variation in sensitivity and specificity for bacteria of fecal origin obtained across studies. Previous studies applying the H₂S test to groundwater samples have demonstrated false-positive results, where H₂S -positive samples contained no fecal coliforms or *Escherichia coli*. False-negative results, where H₂S -negative samples were found to contain *E. coli*, have been shown in other studies. This research identified the types of microbial community members, including fecal indicator organisms, pathogens, and other microbes, present in drinking water samples taken from rural households in Central Vietnam. The molecular method we employed characterized the microbial diversity of select drinking water samples using Terminal Restriction Fragment Length Polymorphisms (TRFLP), a quantitative molecular technique developed for rapid analysis of microbial community diversity. Overall, these experiments showed: (1) when a water sample tests positive for H₂S, there are fecal bacteria in the water sample; (2) though community composition changed, there was a fairly stable group of organisms in the water samples; and (3) in all experiments where more than one known pathogen was found, the H₂S test was positive.

Abstract Category
04 Disease Ecology

PS.04.029 RESISTANCE IS NOT FUTILE: SCREENING ENVIRONMENTAL METAGENOME DATASETS FOR ANTIBIOTIC RESISTANCE GENE

DETERMINANTS

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Recent work has revealed that the vast majority of antibiotics currently used for treating infections and the antibiotic resistance gene determinants (ARGD) acquired by human pathogens have an environmental (non-medical) origin. A better understanding of the diversity, prevalence and ecological significance of ARGD may help predict the emergence and spreading of newly acquired resistances and assess the impact of the release of large amounts of antibiotics on microbial communities and the disruption of natural ecosystems. The number of available environmental metagenomic sequence datasets is rapidly expanding and henceforth offer the ability to gain a more comprehensive understanding of antibiotic resistance at the global scale. The objective of our work was to characterize the diversity and prevalence of ARGD in natural and anthropic environments with a particular emphasis on soil environments. The approach developed consisted in the screening of environmental metagenome datasets for antibiotic resistance determinants. A total of 65 metagenome datasets were used, 58 publically available on the MG-RAST server (metagenomics.nmpdr.org) and 7 obtained from the Rothamsted Park Grass soil. Datasets from the different environments (oceans, deep oceans, Antarctic lakes, sediments, sludges, soils..) were compiled into a unique dataset (1.68 x 10⁶ sequences; 7.66 x 10⁹ base pairs) and a local blast was performed using over 23,000 amino-acid sequences obtained from the ARDB-Antibiotic Resistance Genes Database (ardb.cbcb.umd.edu). Results show that about a third of these sequences were detected and unevenly distributed among the different metagenomes, and corresponded to ca 0.3% of the sequences. The approach used in this study is currently completed with the screening of metagenomic DNA libraries with ARGD detected and the sequencing of the clones of interest. Results obtained will be presented and discussed during the symposium.

Abstract Category
04 Disease Ecology

PS.04.030 DETERMINATION OF FECAL CONTAMINATION FLUX FROM WATERSHED ON THE QUALITY OF COASTAL WATERS OF GULF OF AIGUES MORTES (SOUTHERN FRANCE)

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The Mediterranean coast of the Gulf of Aigues-Mortes (GAM) (Southern France) is characterized by a sandy coast, and many lagoons between the Camargue and Thau

lagoon. The seaside tourism represents a major socio-economic issue. The new EU Framework Directive on the quality of bathing water required a better understanding of the origins of microbiological contamination. The objective of this work is to quantify the flux of *E. coli* flowing into the GAM and to model its dispersion.

The bacterial concentrations were measured in the inlets of lagoons and at the mouths of coastal rivers of GAM. Water discharges were determined using ADCP devices (Acoustic Doppler Current Profiler). The instant bacteria fluxes through the river/inlet section is evaluated by multiplying the average concentration by the corresponding discharge. They were measured too in the effluent from the sewage treatment plant of Montpellier city which flows into the GAM by a 10 km pipe located at a depth of 30m. Sampling was conducted at four seasons and during heavy rainstorms.

The first results allow to comparing the bacterial fluxes from the watershed and those from the effluent of sewage plant flowing directly into the GAM. In summer, the bacterial fluxes are $4 \cdot 10^9$ *E. coli*/s. The sewage plant contributes for about 80% of this flux and the watershed for about 20%. In autumn, the flux in *E. coli* is $9 \cdot 10^9$ bacteria/s and effluent from sewage plant contributes only about 30%, 70% of flux are from the watershed. During winter, the contributions of watershed and sewage plant are equivalent and the flux are of $6 \cdot 10^9$ *E. coli*/s. During the periods investigated, the hydrodynamic and dispersion model shows that the coast cannot be contaminated by mean input fluxes of effluent into the sea. The concentration levels of *E. coli* measured in the sea along the coast and around the discharge of effluent are comparable to those predicted by the model. The contamination of the sea along the coast remains low (< 50 *E. coli* / 100 ml). They are due to the contributions of the watershed, especially during periods of strong storms.

These results allow to consider a decision support for managing the quality of bathing water of the Gulf of Aigues-Mortes.

Abstract Category

04 Disease Ecology

PS.04.031 INVESTIGATION OF BACTERIAL PATHOGEN SOURCES AND TRANSFER HYDRODYNAMICS IN RURAL CATCHMENTS.

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Faecal contamination of drinking water poses a serious public health threat. Monitoring and determining the source of faecal pollution to water is imperative for the protection of water quality and human health. Conventional culture methods to detect such pollution via faecal indicator bacteria have been extensively utilised but do not determine the source of pollution. To combat this, microbial source tracking, an important emerging molecular tool, is utilised to detect host-specific markers in faecally contaminated waters. The aim of this ongoing study is to target ruminant and human-specific faecal *Bacteroidales* and *Bacteroides* 16S rRNA genes within rural river catchments in Ireland and investigate hydrological transfer dependencies. Large (5-20 L) untreated water samples were collected from multiple turloughs and freshwater springs within a karst area

in Co. Mayo during storm and non-storm periods. Samples were filtered through 0.2 µm filters to concentrate bacterial cells and total nucleic acids were then extracted. Animal and human stool samples were also collected from the catchment to determine assay sensitivity and specificity following nucleic acid extraction via the Power Soil™ DNA extraction kit. Aquifer response to seasonal events was assessed by monitoring coliforms and *E. coli* occurrence using the IDEXX Colisure® Quanti Tray®/2000 system in conjunction with chemical and hydrological parameters. Host specific primers BacCow-UCD, BacHum-UCD BacUni-UCD and BoBac were then assayed against both faecal and water extracts and quantified using real-time PCR. As expected, the universal primer (BacUni) detected *Bacteroidales* in all faecal samples while ruminant primers (BacCow and BoBac) detected *Bacteroidales* and *Bacteroides* in bovine and ovis faecal samples and faecally spiked water samples. This study is shedding new light on the dynamic between faecal pollution sources and hydrology within agricultural catchment areas.

Abstract Category
04 Disease Ecology

PS.04.032 SEASONAL DISTRIBUTION OF POST-HARVEST FUNGAL PATHOGENS OF POME FRUIT IN BRITISH COLUMBIA ORCHARDS USING DNA MACROARRAY TECHNOLOGY

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Penicillium expansum (blue mold), *Botrytis cinerea* (grey mold) and *Mucor piriformis* (mucor rot) are important post-harvest diseases of pome fruit causing annual losses of 5-20%. Identification and quantification using novel DNA macroarray technology may assist in the development of prediction models and disease forecasting. This study developed and tested a DNA-based assay for rapid and sensitive identification of post-harvest apple pathogens. Macroarray technology is a reverse dot blot technique that is advantageous as it allows for simultaneous identification of numerous fungal pathogens in a single test. Three rDNA ITS probes were selected for pathogen identification: PE-H3u (*P. expansum*), BC-H2d (*B. cinerea*) and Mpir-ITS-414H1 (*M. piriformis*). The array displayed high specificity, no cross-reactivity and detected up to 0.1ng of pure culture fungal DNA extracted from *P. expansum* and *B. cinerea* and up to 0.01ng of pure culture *M. piriformis*. Results indicated a linear relationship between DNA concentration and macroarray hybridization signal intensity ($r \geq -0.962$). Probes became saturated and outside the linear range at >3ng for *P. expansum*, >12ng for *B. cinerea* and >0.5ng for *M. piriformis*. Post-harvest pathogens were monitored throughout the growing season in four apple orchards in the Okanagan Valley, BC in 2007 and 2008. Their detection was variable due to field and year differences. *P. expansum* and *M. piriformis* were primarily detected in the early and mid-growing seasons, with an average percent detection of 27.4±3.4% and 19.2±1.4%, respectively. *B. cinerea* was detected less frequently (6.2±1.4%) and all throughout the growing season. There was a positive correlation between total post-harvest pathogen detection just prior to harvest and after harvest in

naturally infected fruit ($r = 0.79$). These data provide greater insight into predicting post-harvest pathogens as a means of preventing storage diseases in pome fruit.

Abstract Category
04 Disease Ecology

PS.04.033 FIRST GENOME SEQUENCE OF A *VIBRIO VULNIFICUS* PHAGE, Φ VVAW1

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Members of the bacterial genus *Vibrio* are abundant in eutrophic coastal waters, and play an integral role in the ecology and cycling of essential nutrients in these habitats. Vibrios are also pathogens of animals, including humans. *Vibrio vulnificus*, a member of this genus, is a serious human pathogen. Although *V. vulnificus* infections are relatively rare they have a high mortality rate; it has been estimated that *V. vulnificus* is responsible for upwards of 95% of shellfish-related deaths in the US. At present, little is known about phages that infect *V. vulnificus*, but phages are known to be a major contributor of variability in virulence among strains of other pathogenic bacteria, including vibrios, through the processes of horizontal gene transfer and lysogenic conversion. Using the agar-overlay plaque assay, we have isolated the lytic vibriophage ϕ VvAW1, which infects a strain of *V. vulnificus* (V93D1V), from the Ala Wai canal in Honolulu. Electron microscopy images illustrate that the phage has an icosahedral shape with a diameter of 42nm and a small non-contractile tail, suggesting it is a podophage. The complete genome of the phage, determined by shotgun cloning and Sanger sequencing, is 40 Kbp and has a 49.5 % GC content. Analysis of the genome indicates regions of high sequence similarity to another marine phage, BA3, which infects and lyses the coral pathogen *Thalassomonas loyana*. Sequencing the genome of this lytic phage begins the process of placing *V. vulnificus* phages in the evolutionary context of other vibriophages. Model systems, such as this one, are an important resource for investigating the molecular biology, ecology, and evolution of the phage-pathogen interaction. This information is needed to predict pathogen abundance and dynamics in the environment and could contribute to the development of *V. vulnificus* phages as therapeutic tools to combat infections.

Abstract Category
04 Disease Ecology

PS.04.034 ANTIBIOTIC USE AND CONTAMINATION, STRUCTURE AND ANTIBIOTIC RESISTANCE OF E. COLI POPULATIONS : FROM HOSPITAL TO RIVER

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Massive antibiotic use and emergence of bacterial resistance associated are regarded as major problem for public health on world-wide scale. In aquatic environments, antibiotics and their degradation products, and antibiotic-resistant bacteria are rejected via effluents of wastewaters treatment plants (WWTPs) or agricultural runoffs. The aim of this work was to study the relationship between antibiotic consumption with antibiotic contamination and antibiotic resistance of *Escherichia coli* (*E. coli*) population along a longitudinal transect hospital - WWTP- river.

Two sampling campaigns, using autosamplers, were realized during dry weak epidemic period and wet high epidemic period. Quantification of antibiotic was realized in water samples using solid phase extraction (SPE) followed by liquid chromatography-tandem mass spectrometry (LC MS-MS). Macrolides (15 to 18,500ng/L), quinolones and fluoroquinolones (8 to 1189ng/L), sulfonamids (7 to 117ng/L) and tetracyclines (14 to 111ng/L) were detected, but these low antibiotic concentrations did not permit to select corresponding antibiotic-resistant bacteria. From hospital effluents to river, the *E. coli* showed resistances to quinolones & fluoroquinolones (25.9%), sulfonamids (14.5%), tetracyclines (29.5%), penicillins (43.0%), aminosides (7.8%) and phenicols (11.9%). *E. coli* concentrations (p-value<0.0001) and antibiotic-resistance decrease significantly (p-value=0.026 and 0.066) from WWTP influent to river. In addition, the analysis of *E. coli* population structure, based on phylogroups A, B1, B2 and D, shows that the decrease of antibiotic resistant *E. coli* population might be due to higher decrease of *E. coli* belonging to the phylogroup A, resistant to at least one antibiotic (p-value=0.034 and 0.074).

Abstract Category
04 Disease Ecology

PS.04.035 GEOGRAPHICAL EPIDEMIOLOGY OF SALMONELLA IN THE PHYLLOSPHERE OF TOMATOES

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Of the eleven documented outbreaks of *Salmonella* associated with tomatoes grown in the continental United States during a period spanning (1998-2008) where geographic growing region of origin could successfully be traced to a specific location, ten were associated with tomatoes grown in the Eastern United States. Hypothesizing that this might not be coincidence but instead a rare opportunity to explore the epidemiology of tomato contamination by *Salmonella*, a quest that with many cases of fresh produce contamination is confounded by a complexity of variables, we characterized bacterial and fungal species associated with surfaces of tomato plants of eastern and western grown tomatoes (VA and CA) to examine whether phyllosphere microflora may play a role in *Salmonella's* ability to persist in eastern field environments. Characterization was achieved using 'direct capture' DNA recovered from surfaces of Virginia and California grown tomatoes and leaves at time of harvest. PCR product of 16S and 18S was cloned, sequenced and aligned to the Silva SSU reference database and NCBI Genbank to assign

taxonomic identity. Not surprisingly, observed fungal and bacterial taxa from each region were almost completely different from east to west ($P < .05$). Only 3 bacterial OTUs at ($D = .03$) were shared between California and Virginia and only 7 were shared between fungal libraries. Dominant bacterial families in the Virginia libraries were members of Rhizobiaceae while California bacterial families were dominated by Enterobacteriaceae. California fungal libraries were dominated by Erisiphaceae consortia, while Virginia libraries were almost completely dominated by *Alternaria spp.* and other Pleosporaceae. Fungal species have been known to play a commensal role in the ability of bacterial species to survive in certain niches. It is possible that *Alternaria spp.* are contributing to the survival of *Salmonella* in eastern tomato phyllosheres.

Abstract Category
04 Disease Ecology

PS.04.036 THE ANTI-INFLAMMATORY EFFECT OF SURFACTIN, FROM BACILLUS SUTILIS, IN HUMAN MACROPHAGE STIMULATED BY PORPHYROMONAS GINGIVALIS

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Porphyromonas gingivalis is considered the major pathogen of periodontal disease that leads to chronic inflammatory disease in oral tissues. The lipopolysaccharide presented by *P. gingivalis* is a key factor in the development of periodontitis. Surfactin is produced by *Bacillus subtilis* and is reported to exert anti-inflammatory properties. However, the underlying mechanisms that could explain the surfactin induced anti-inflammatory actions by *p. gingivalis* lipopolysaccharide remain poorly understood. We herein investigated whether the regulatory effect of surfactin on *p. gingivalis* lipopolysaccharide stimulated human macrophage THP-1 were mediated by the induction of heme oxygenase-1 signals. Surfactin significantly inhibited the *p. gingivalis* lipopolysaccharide induced pro-inflammatory cytokines. Moreover, the inhibition of the *p. gingivalis* lipopolysaccharide induced pro-inflammatory cytokines by surfactin was mainly mediated by blocking the activation of *p. gingivalis* lipopolysaccharide triggered NF-kappaB. Surfactin also induced heme oxygenase-1 mRNA and protein expression via activation of the NF-E2-related factor-2. Additionally, the knock down of NF-E2-related factor-2 by Small interfering RNA significantly inhibited surfactin induced Heme oxygenase-1 expression. Furthermore, inhibition of PI3K/Akt and ERK significantly decreased surfactin induced Heme oxygenase-1 expression. Treatment with protoporphyrin IX, a selective inhibitor of Heme oxygenase-1, reversed the surfactin mediated inhibition of pro-inflammatory cytokines; these studies suggest that surfactin induced anti-inflammatory effects by activating NF-E2-related factor-2 mediated Heme oxygenase-1 induction via PI3K/Akt and ERK signaling. Taken together, these findings suggest that the surfactin may be a promising possibility for the candidate as both anti-caries and an anti-periodontitis agent or other inflammatory diseases agent.

Abstract Category
04 Disease Ecology

PS.04.037 WIDE-SPREAD PRESENCE OF INTRANUCLEAR BACTERIA IN SHALLOW-WATER BIVALVES

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Bacteria that invade eukaryotic nuclei are commonly found in protists but have rarely been observed in multicellular eukaryotes. Recently, we described intranuclear bacteria in deep-sea hydrothermal vent and cold seep mussels of the genus *Bathymodiolus*.

Phylogenetic analyses showed that these bacteria belong to a monophyletic clade of Gammaproteobacteria associated with marine animals as diverse as sponges, corals, bivalves, gastropods, echinoderms, ascidians, and fish. However, except for bathymodiolin mussels and a shallow water bivalve (the Pacific razor clam *Siliqua patula*) none of these metazoa-associated bacteria have been shown to occur inside nuclei.

It has been suggested that intranuclear bacteria may cause mass mortalities in bivalves but a causal relationship has never been established. Because of their potentially lethal effect on bivalve populations, we specifically looked for the presence of intranuclear bacteria in economically important and commercially available bivalve species, i.e. oysters (*Crassostrea gigas*), razor clams (*Siliqua patula* and *Ensis directus*), blue mussels (*Mytilus edulis*), manila clams (*Venerupis philippinarum*), and common cockles (*Cerastoderma edule*). 16S rRNA phylogenetic analysis and fluorescence in situ hybridization (FISH) revealed the presence of intranuclear bacteria in all investigated bivalves except blue mussels. A FISH probe targeting all currently known intranuclear Gammaproteobacteria was designed for future high-throughput analyses of marine invertebrates.

Furthermore, primers were designed to quantify the abundance of intranuclear bacteria with real time PCR. Preliminary tests with these primers showed massive amounts of intranuclear bacteria in some bivalve species, raising the question if these might significantly affect not only the health of the bivalves but possibly also of the humans that eat them.

Abstract Category
04 Disease Ecology

PS.04.038 ANALYSIS OF BACTERIAL SPECIES ABUNDANCE DISTRIBUTION IN CHRONIC CYSTIC FIBROSIS LUNG INFECTIONS

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Chronic cystic fibrosis lung infections are fundamental in determining morbidity and mortality. In common with the wider analysis of polymicrobial infections, application of culture-independent profiling in this context is revealing a far more complex microbiota than previously described. CF is used here as a paradigm for investigating the microbial ecology of polymicrobial infections through species abundance distribution. Sputum samples from 14 clinically stable adult cystic fibrosis patients were analysed by 16S ribosomal rRNA clone sequencing, with a total of 2137 clones examined. The species abundance distribution for the metacommunity was partitioned into core and satellite species groups using a modification of the method used by Magurran and Henderson (2003, *Nature* 422: 714-716). 16S rRNA clones were found to represent 82 separate species, with Firmicutes, Fusobacteria, Actinobacteria, Proteobacteria and Bacteroidetes species representing ~8%, ~1%, ~1%, ~76% and ~14% of clones respectively. The metacommunity was partitioned into 15 core species that were non-randomly distributed through the patients and 67 randomly distributed species. The core group followed a log normal distribution while the satellite group was better predicted by a log series. Metacommunity distribution was shown to be negatively skewed. Partial Mantel tests revealed that similarities in community composition in the metacommunity and core group were determined by both CF genotype and antibiotic treatment history, whereas members of the satellite group did not correlate with any clinical variables. The dissimilarity commonly observed between any two CF bacterial community profiles results from the randomly distributed species present. Here we demonstrate that disentangling core and satellite species from a CF metacommunity reveals important aspects of species abundance distributions which would otherwise go unobserved.

Abstract Category
04 Disease Ecology

PS.04.039 "EXCEPTIONAL CIRCUMSTANCES", PATHOGEN RISKS, AND MARINE WATER BATHING

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Protecting bathers from pathogens in marine waters requires consideration of high risk "Exceptional Circumstances". This paper will describe how we integrated hydrodynamic modeling, quantitative microbial risk assessment and health protection principles to quantify bather risks arising from the destratification of coastal marine waters and onshore transport of treated wastewater. Levels of index pathogens discharged from an ocean outfall were estimated. Transport and inactivation within the coastal zone was then modeled using finite element analysis. Estimates of illness risk were used to generate exceedence probability plots analogous to those used in flood analysis. 200 exposure scenarios simulated different pathogens, seasons, beaches and user populations. The use of exceedence probability captured the concurrent increases in illness risk probability and decline in likelihood. In some plots a 1-2 log₁₀ step increase in risk probability was seen. These reflected sporadic periods when destratification, onshore winds and surface currents led to plume transport into bathing areas. Though the outfalls were 2km out to

sea, solar inactivation was constrained by short travel times (<6h) and limited dilution of effluent (100-1000 fold). A key concern was the elevated risk posed to the surfer subpopulation whose intake of seawater was assessed to be above the norm. A difficult issue for regulators was what level of transient elevated illness probability is "tolerable"? Routine monitoring based management of marine bathing can be augmented with model based quantification of illness risks reflecting different environmental and social scenarios. Contaminant transport modeling generated detailed information on risk which would be impractical to obtain via monitoring programs alone and facilitated many what-if questions. The primary recommended use of risk modeling is in support of management decisions rather water quality target setting.

Abstract Category
04 Disease Ecology

PS.04.040 SIGNIFICANCE OF WASTEWATER SOURCE ON THE BACTERIAL AND ANTIBIOTIC RESISTANCE DIVERSITY IN THE ENVIRONMENT

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Understanding bacterial diversity in wastewater collection and treatment systems is critical when assessing epidemiological and environmental risks such as dissemination of antibiotic resistant bacteria and pathogens from discharge of insufficiently treated wastewater effluent or biosolids. A typical municipal wastewater treatment system integrates microbiologically diverse sources influenced by the physical and chemical selective stresses imposed on the wastestream. For example hospital wastewaters are expected to favor antibiotic resistant microbes since the appropriate antibiotic selective pressure is usually greater. To evaluate the significance of source on microbial diversity and antibiotic resistant diversity we thoroughly sampled a municipal wastewater system that includes residential areas, a hospital, a university, and an industrial source. Sampling also included all treatment stages at the wastewater treatment plant. DNA was extracted directly from wastewater samples and from cultured populations on antibiotic amended non-selective media. For the latter, antibiotics representative of different antimicrobial mechanisms were added at concentrations above an epidemiological cut-off value for most bacteria. Whole bacterial community diversity was obtained via PCR-DGGE fingerprints using *rpoB* primers. Our results indicate close similarity of fingerprints for samples from certain residential areas. These were distinct from hospital and university source wastewater. Locations that integrate multiple sources tend to produce similar fingerprints indicating a possible bias in favor of abundant versus rare species. Diversity of antibiotic resistant microbial populations indicate that these profiles may be affected by type of waste-source, physical, and chemical stresses imposed during treatment. These risks factors are transferred into environmental receptors as insufficiently treated liquid and solid wastes being discharged to soil and water.

Abstract Category
04 Disease Ecology

PS.04.041 CHANGES IN THE HUMAN VAGINAL BACTERIAL BIOTA: COMPLEMENTARY PERSPECTIVES USING BROAD-RANGE PCR WITH PYROSEQUENCING AND QUANTITATIVE PCR TECHNOLOGIES

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Few studies have used molecular methods to characterize stability of the human vaginal bacterial biota and changes that ensue with development of the dysbiotic condition bacterial vaginosis (BV) or with antibiotic treatment. We sought to systematically catalog daily and weekly changes in vaginal bacterial concentrations using taxon-directed quantitative PCR (qPCR) and species composition using broad-range PCR with pyrosequencing in women with and without BV.

Vaginal samples collected longitudinally over 4 weeks were evaluated using 11 qPCR assays and 16S rRNA gene PCR and pyrosequencing. Participants with BV, by Amsel's criteria, were treated with metronidazole. Taxonomic classification of 1000 reads per sample was performed based on placement of reads in a phylogenetic tree broadly representing vaginal bacteria.

A typical participant without BV was colonized mostly with *Lactobacillus* species, especially *Lactobacillus crispatus* throughout the 32 day study period. However, we detected 22 bacterial taxa by pyrosequencing including *Escherichia coli*, *Staphylococcus* and *Streptococcus* spp. Low levels of *Gardnerella vaginalis* and *Lactobacillus iners* detected by qPCR were not always seen by pyrosequencing. Participants with BV had high levels of diverse bacteria pre-treatment (14-22 bacterial taxa) that rapidly declined and were replaced with lactobacilli by Day 5 post-treatment. Pyrosequencing facilitated detection of up to 34 taxa not assayed for by qPCR. The qPCR assays allowed monitoring of low levels of BV-associated bacteria post-treatment that were not detected with pyrosequencing.

Broad-range PCR with pyrosequencing and qPCR provide complementary perspectives on the composition of the human microbiota. This longitudinal study reveals that the vaginal microbiota is highly dynamic with periodic surges in bacteria like *E. coli* and Group B *Streptococcus* that are associated with urinary tract and neonatal infections.

Abstract Category
04 Disease Ecology

PS.04.042 DOES ORAL BACTERIAL DIVERSITY PREDICT PROGRESSION TO CARIES IN CHILDREN? RESULTS OF A PILOT STUDY

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Dental caries (tooth decay) is a polymicrobial infection resulting from complex interactions between oral microbiota and the host. Although no one bacterial species is exclusively causative of caries, levels of acidogenic and aciduric bacteria are elevated in caries. We used denaturing gradient gel electrophoreses to characterize the dominant oral bacterial communities in caries free and caries active children. Samples were selected from children aged 5- 7 years enrolled in a study on oral health disparities by the Center for Oral Health Research in Appalachia (COHRA) who participated in two consecutive visits. Teeth and tongue scrapings were selected from 8 children caries free at both visit and 8 children caries free at the first visit and caries active at the second visit. DNA was isolated from samples and PCR performed on the V3 region of 16S rDNA using universal bacterial primers. Canonical correspondence analysis was used to identify gel bands that were associated with caries; bands were amplified and sequenced to identify caries-associated bacterial taxa. Resident oral bacterial communities were highly variable within children between visits, and among children from the same visit. Among children that subsequently developed caries compared to those remaining caries free, bacterial communities were less diverse at visit 1 (Shannon diversity index, $H' = 1.90$ vs 2.38 ; $p = 0.05$) but not visit 2 ($H' = 2.29$ vs 2.33) In canonical correspondence analysis, *Streptococcus mitis* and *Streptococcus oralis* were associated with caries free teeth, while *Aggregatibacter actinomycetemcomitans* was associated with caries active Surprisingly, Shannon value at visit 2 was similar between the two groups. Similar experiments on a larger study population are planned. These studies will help understand the dynamics of caries initiation and progress, and identify optimal time periods for intervention. NIH grant #DE-014899

Abstract Category
04 Disease Ecology

PS.04.043 ANALYSIS OF GEOGRAPHICAL DISTRIBUTION OF POTATO SCAB PATHOGENS BASED ON A NEW GENOTYPING METHOD

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Several *Streptomyces* species cause serious plant disease worldwide, known as scab disease that harms a broad range of root crops such as potato, carrot, and sugar beet. So far, molecular mechanisms of bacterial infection and disease development are not fully understood. It has been empirically observed that incidences of the disease were significantly different from area to area. One of the primary reasons for this may be a difference in causative agents in each field. *S. scabiei*, *S. acidiscabiei*, and *S. turgidiscabiei* are the most well-known causative agents of potato scab disease. We have developed a new method for genotyping of these three pathogens using multiplex PCR, which allows simultaneous detection of multiple species. Using the new method, we analyzed geographical distribution of the potato scab pathogens in Japan. A total of 269 lesions were collected from 97 potato fields in major potato production areas around north and south parts of Japan, especially in Hokkaido and Kyusyu. In our analysis, one to three different potato scab pathogens were detected from all 269 lesions. A total number of the pathogens detected from the lesions were 304. The most frequently detected pathogen was *S. turgidiscabiei*, and it accounted for 69% of the total number of the pathogens detected. Especially, *S. turgidiscabiei* accounted for 90% in Hokkaido, the largest potato production area in Japan. On the other hand, *S. scabiei* was also detected at a high rate in Kyusyu area. In most cases, *S. acidiscabiei* were not detected alone in a lesion, but detected together with the other pathogens, *S. scabiei* and *S. turgidiscabiei*, suggesting multiple infections of *S. acidiscabiei* and other pathogens in the same lesion. In conclusion, geographical variation in major causative agent of potato scab disease was found in this study. Such a variation may be associated with environmental conditions of potato production and the region supplying seed potatoes that originally bear pathogens.

Abstract Category
04 Disease Ecology

PS.04.044 IMPACT OF WASTEWATER TREATMENT ON THE AMPLIFICATION AND DISSEMINATION OF A CLASS A β -LACTAMASE GENE USING A FUNCTIONAL METAGENOMIC APPROACH

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The impact of antibiotics in the environment constitutes a topic of great concern and more research is needed to determine how these antibiotics affect public health. Wastewater treatment plants (WWTPs) receive antibiotics and antibiotic resistant bacteria from hospitals, small industries and households. Due to the high level of bacterial activity occurring in WWTPs these facilities may support the generation and

propagation of antibiotic resistance genes that could be released to aquatic ecosystems, transferring resistance to pathogenic bacteria. This represents a risk for aquatic animals and humans. To test the hypothesis that WWTPs act as reservoirs of antibiotic resistance genes, we constructed a metagenomic library from DNA extracted from each treatment process occurring in the Plum Island WWTP, Charleston, SC. The library consists of 7618 clones with an average insert size of 40 kb, representing a total of 0.30 gigabases of cloned WWTP DNA. Clones were screened for resistance to four antibiotics: ampicillin, apramycin, cephalexin, and tetracycline. A total of 27 clones demonstrated antibiotic resistance to these chemotherapeutic agents. To identify the gene sequence conferring ampicillin resistance to one of the 27 clones, we sheared the fosmid insert to 3 kb and subcloned into *Escherichia coli* using the Epicentre® CopyControl™ gene and PCR cloning vector kit. Subclones were screened for ampicillin resistance, plasmids extracted from resistant clones, and inserts sequenced. Bioinformatic analysis of the DNA sequence revealed a class A β -lactamase most closely related to an orthologous gene previously reported in *Legionella drancourtii*. Using gene specific and quantitative PCR, we have identified the presence of this gene throughout the WWTP process, from influent to effluent and surrounding coastal water and sediments, thus indicating that this antibiotic resistance gene survives all treatment processes and is released into the surrounding environment.

Abstract Category
04 Disease Ecology

PS.04.045 TYPIFICATION OF *SALMONELLA ENTERICA* SUBSP. *ENTERICA* ISOLATES FROM ANIMAL SOURCE FOODS

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Different *Salmonella enterica* serotypes are responsible for a great number of food-poisoning outbreaks throughout the world causing diarrhea and other non-diarrheal diseases. The objectives of this study were to evaluate the *S. enterica* serotypes associated to meat and dairy products in the state of Michoacán, Mexico and to assess the genetic diversity in the isolates obtained. 76 isolates of *Salmonella enterica* subsp. *enterica* were obtained from 19 locations. 44.7% of the isolates were obtained from beef meat, 32.8% from pork meat, 13.1% from cheese and the remaining from different products. The Anatum serotype was most abundant (18.4%), followed by Typhimurium (12.0%), Derby (8.0%) and Agona (6.7%); 23 other serotypes were obtained in lesser proportion. Serotype was not determined in two isolates. In general, most frequent serotypes were isolated from all sample types and locations studied. A RAPD amplification assay was made with three primers, from which a dendrogram was generated by Ward's method using Euclidean distances. Four main clusters grouped at the

85% similarity level and 16 genotypes grouped at 95% similarity. Both the distance dendrogram and principal coordinates analysis (PCoA) failed to group isolates by type of sample, location of provenance or serotype. Results show lack of specificity of genotypes for type of sample and suggest a significant dispersion capacity of genotypes between studied locations.

Abstract Category
04 Disease Ecology

PS.04.046 DEVELOPMENT OF BACTERIAL MARKERS FOR TRACKING HUMAN, RUMINANT AND PIG FECAL POLLUTION IN NATURAL SURFACE WATERS

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Fecal pollution affects environmental quality in both inland and coastal areas and is associated with potential spread of diseases, with negative implications for bathing and shellfish harvesting areas. Sewage treatment plants, agricultural runoff and wildlife represent possible sources of fecal pollution. The ability to determine whether fecal pollution present in a water body originates from either a human or animal source would improve management of water pollution. This study addresses the issue of microbial host-specificity and uses this specificity to distinguish human, bovine and pig pollution. In a first step, bacterial markers were identified by analysing the diversity of fecal bacteria (*Bacteroidales*, *Bifidobacterium*, *Clostridiaceae*, *Bacillus-Streptococcus-Lactobacillus*) in different fecal sources (manure, treated wastewater...) using 16S rDNA clone libraries and fingerprint analysis (SSCP). The applicability of these markers to environmental samples was then tested by analysing runoff waters after spreading pig or bovine manure, and watercourses impacted by treated wastewater or animal pollution. *Lactobacillus amylovorus* was selected as a pig marker and a phylotype close to *Bifidobacterium adolescentis* as human marker. Among *Bacteroidales*, human-, ruminant- and pig-specific markers were identified. All markers were quantified by qPCR in waters impacted by fecal pollution from different origins. Pig and bovine markers were only detected in runoff waters after manure application whereas human markers were present in surface waters impacted by human pollution. For example, their quantification along a watercourse impacted by treated wastewater showed that human-specific *Bacteroidales* and *Bifidobacterium* markers were only detected downstream, when *E. coli* was detected both upstream and downstream. In conclusion, this study allowed us to determine efficient markers for identifying the origin of fecal pollution which will now be applied to bathing waters.

Abstract Category
04 Disease Ecology

PS.04.047 ECOLOGICAL IMPLICATIONS OF ENVIRONMENTAL IRON FLUX ON VIBRIO

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Iron is a critical trace element essential for almost all living organisms on earth. Understanding the effects of iron flux on a particular assemblage in the marine environment has been complicated by the lack of understanding of iron chemistry, speciation and its bioavailability in marine waters. As we learn more about iron chemistry and its bioavailability, it is becoming apparent that areas that have not traditionally been thought of as iron limited, are potentially co-limited by iron and other trace elements. Additionally, trophic competition for this limited trace element, has the potential to create iron stressed microenvironments. A major source of iron to the surface waters of the world's oceans is aeolian dust delivered through long-range atmospheric transport from arid continental regions such as those found in Saharan Africa. While much of the work on the ecological effects of desert dust deposition have centered on its potential stimulation of phytoplankton activity, here we demonstrate growth of marine heterotrophic bacteria, including pathogenic *Vibrio cholerae* and *Vibrio alginolyticus* in response to dust from Saharan Africa. In order to study the direct stimulatory ability of iron in aeolian dust, we have created a new formulation of iron-limited media, M9-FeL that successfully induces iron stress. Using this iron-limited media, we evaluated growth characteristics of *Vibrio* seeded into M9-FeL alone and M9-FeL amended with Saharan Dust. Microbial growth was monitored for 48 hours measuring optical density at 600 nm, colony forming units and microscopic direct counts. Cultures amended with dust showed two orders of magnitude more growth compared to control samples. As the global abundance of airborne dust is projected to increase as a result of anthropogenic disturbance and climate change, it is increasingly important to understand the potential effects of iron flux from dust on heterotrophic marine bacteria, especially pathogenic *Vibrio*.

Abstract Category
04 Disease Ecology

PS.04.048 HUMAN SITE USE DRIVES ENTERIC BACTERIAL POPULATION DIVERSITY AND ANTIBIOTIC RESISTANCE IN GALAPAGOS IGUANAS

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The Galapagos Islands are a unique and heavily human-impacted ecosystem. Located off the coast of Ecuador, these islands support a permanent resident human population of about 30,000 people. Additionally, many thousands of tourists visit the Galapagos

annually to see endemic plants and wildlife, which include two species of large, herbivorous iguanas. These animals are often relatively unafraid of humans and live in close proximity to human residences on inhabited islands. However, some of the islands are carefully protected from human visitors. This creates a gradient of potential human influence on bacterial exposures. In this study, we explored population similarity and diversity patterns of *Salmonella enterica* isolated from the feces of wild iguanas at five sites on four islands. We evaluated population patterns using genomic fingerprinting, which revealed that iguana populations living closer to humans or heavy tourist traffic were host to more varied *S. enterica* populations than iguanas living on more isolated islands. The influence of human exposures appeared to overwhelm predicted patterns of bacterial exchange based on natural processes (i.e. distance-decay relationships). In addition, we isolated a related bacterium, *Escherichia coli*, from these same iguanas and tested both species for resistance to a variety of commonly-used antibiotics. Bacteria isolated from iguana populations with increased human contact and more diverse *S. enterica* populations also had higher levels of antibiotic resistance. These findings together suggest that iguanas with increased human contacts have higher exposure to novel bacteria which results in higher strain and trait diversity. This increased "microbial connectivity" of commensal bacteria due to site use by humans may suggest the potential for higher disease risk in these iguana populations.

Abstract Category
04 Disease Ecology

PS.04.049 THE BACTERIAL ECOLOGY OF ACROPORID WHITE SYNDROME IN AMERICAN SAMOA

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In June 2008, coral disease surveys were carried out on reefs around American Samoa to assess the prevalence of Acroporid White Syndrome (AWS) and to characterise the composition and diversity of the bacteria present in AWS lesions, adjacent healthy tissues of diseased colonies, tissues of healthy colonies and the surrounding waters. Samples were analysed by culturable plate counts on Marine Agar and TCBS (Thiosulfate Citrate Bile Sucrose) Agar and morphologically-distinct isolates sequenced using the 16S rRNA gene. A definite partitioning of bacterial genera in lesions and adjacent tissues was observed, with several genera only being cultured from either diseased or healthy samples. A number of the isolates were closely related to known coral pathogens and of these, twenty isolates which were putatively identified as *Vibrio* species (which were found ubiquitously) were further characterised using MLST (Multi Locus Sequence Typing), revealing a diverse *Vibrio* community associated with the coral and surrounding waters. Bacterial community genomic DNA was also extracted and 16S rDNA amplified to construct clone libraries comprising approximately 1500 clonal sequences. These communities were dominated by the *Gammaproteobacteria* and more detailed analyses revealed distinct differences between the bacterial communities found in the healthy and

diseased coral tissues. However, no single candidate for AWS was apparent with a number of causative agents likely responsible for this disease.

Abstract Category
04 Disease Ecology

PS.04.050 OVINE FOOTROT: DETECTION AND QUANTIFICATION OF DICHELOBACTER NODOSUS AND FUSOBACTERIUM NECROPHORUM USING A LONGITUDINAL DATA SET

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Footrot is a highly infectious and debilitating disease of sheep and is estimated to cost the UK agricultural industry £24m p.a. The current consensus is that the onset of disease occurs when the interdigital skin becomes damaged allowing superficial colonization of the epidermis by *Fusobacterium necrophorum*, a ubiquitous soil and faecal anaerobe, which results in inflammation and necrosis (interdigital dermatitis). This permits other organisms to become established, including *Dichelobacter nodosus*, another obligate anaerobe with proteolytic capabilities. The synergistic action of both organisms can ultimately result in the separation of the hoof horn from the sensitive underlying tissue (footrot), causing lameness.

The fusobacteria have been implicated as key components in a number of human and animal diseases. We were therefore interested in the interactions between these two anaerobes and their roles in disease pathogenesis. The aim was to employ a multi-disciplinary approach, combining molecular microbiology with epidemiology to enhance our current understanding of disease progression. Real-time PCR was used to quantify the numbers of both organisms in a longitudinal data set. Fluorescence *in situ* hybridization was then used to examine the spatial distribution and prevalence of footrot-associated organisms in tissue samples from sheep presenting with a range of clinical conditions. The results indicate that the presence of *Dichelobacter nodosus* correlates with interdigital dermatitis severity in a dose-dependent manner, and that colonization with *Fusobacterium necrophorum* appears to be a subsequent process. However, once footrot develops, levels of *Dichelobacter nodosus* detectable in the surface lesion decrease, but *Fusobacterium necrophorum* levels remain high. The results thus far call into question the current understanding of ovine footrot pathogenesis.

Abstract Category
04 Disease Ecology

PS.04.051 TEMPORAL DYNAMICS OF CATTLE AND HUMAN FECAL MICROBIAL COMMUNITIES IN FRESH AND MARINE WATERS

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Urbanization and agricultural runoff have been shown to contribute to non-point source fecal pollution. However, it is unclear to what extent the fecal microbial communities from various sources (e.g. human and cattle) behave differently in the environment. In order to accurately assess the risk of pathogen exposure, we aim to characterize the survival and persistence of fecal bacteria from different sources under various field conditions. We hypothesized that human and cattle fecal wastes have distinct bacterial community compositions and are influenced by fresh water and marine environments differently. We utilized diffusion chambers with 2µm membranes that have 100 ml holding volume. Human septage and cow waste were injected into the diffusion chambers, and immersed in creek water and bay water. Samples were taken at intervals throughout 96 hr incubation period. Community compositions were characterized with a high-density microarray (PhyloChip) targeting the 16S rRNA gene of Bacteria and Archaea. Hierarchical clustering analysis demonstrated that the septage and cow waste clustered separately. Bacteria and Archaea that were unique to each waste type were identified. Septage and cow waste community composition shifted similarly with respect to time. In general, most *Proteobacteria* decreased and *Clostridia* increased in relative abundance over time. Similar responses of cow waste and septage communities to different water types were also observed. *Clostridia* and many *Gammaproteobacteria*, including coliforms, were more persistent in creek water while *Betaproteobacteria* were more persistent in bay water. The results of this research indicate that creek and bay water exert different effects on the fate of waste microbial communities. Therefore, selection of indicators for monitoring should be based on the persistence of fecal taxa under various receiving waters.

Abstract Category
04 Disease Ecology

PS.04.052 RAPID MONITORING OF *LEGIONELLA PNEUMOPHILA* IN AQUATIC ENVIRONMENT BY USING A MICROFLUIDIC DEVICE

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Rapid and simple methods to enumerate *Legionella pneumophila* in aquatic environments are essential for monitoring water quality and prevention of the outbreaks of *Legionella* infections. Conventional plate counting requires more than one week to obtain reliable results. PCR-based method is sensitive and widely used while it requires extraction of DNA from samples. In this study, we applied our microfluidic device to directly enumerate *L. pneumophila* in aquatic environment. *L. pneumophila* serogroup 1 (JCM7571) and cooling tower water samples were used in this study. A microfluidic device (size: 48 mm x 23 mm) was designed and fabricated by polydimethylsiloxane and a thin glass sheet. *L. pneumophila* cells spiked in cooling tower water were stained with anti-*L. pneumophila* fluorescent antibody in microchannel of the device. A fluorescence microscope-based system was used for counting of bacterial cells. The number of *L.*

pneumophila cells in the samples was determined by both this microfluidic system and conventional fluorescence microscopy for comparison. The number of *L. pneumophila* cells determined by the microfluidic device was correlated with microscopic count. The lower detection limit of the system was 10^1 cells/ml and the number of *L. pneumophila* cells in cooling tower water could be determined after concentration. Differences in quantitation of *L. pneumophila* cells between the microfluidic system and microscopy were less than 10% on average. The time required for quantitation of these bacteria by microfluidic devices was less than one hour per sample. The microfluidic system fabricated in this study allowed rapid counting of *L. pneumophila* cells. This method may contribute to reduce the risk of the outbreaks of *Legionella* infections.

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Abstract Category
04 Disease Ecology

PS.04.053 CHARACTERIZATION OF MICROFLORA OF TONGUE COATING IN ELDERLY WITH TUBE FEEDING

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More than 500 species of bacteria inhabit in human oral cavity, but it is unknown what affects individual characteristics of oral microflora. Recently we reported that microflora of tongue coating in elderly were classified into 4 clusters (A, B, C and D) based on terminal restriction fragment length polymorphism (T-RFLP) analysis representing bacterial composition. Interestingly Cluster B was characterized with the presence of unusual oral bacterial species. This study was carried out to clarify the microflora in this unique cluster.

Three hundreds and forty three elderly (65 years or older); long-term hospitalized patients or the person who live in nursing homes were examined in November or December, 2007. Samples of tongue coating collected at the baseline examination were qualitatively characterized by T-RFLP analysis. The T-RFLP profile data were compressed in the lower-number dimensions by principal component analysis (PCA) resulting in a biplot. Detailed sequence data from representing subjects with tube feeding and their matching controls were obtained with pyrosequencing.

Tube feeding was unevenly distributed in cluster B among 4 clusters. The T-RFLP peaks corresponding to the bacteria which are unusual in oral cavity were significantly higher in subjects with tube feeding than those with oral feeding. Seventeen subjects were selected from those with tube feeding and bacterial DNA samples from tongue coating of them and their matching control were analyzed by pyrosequencing. Pyrosequencing revealed that *Corynebacterium diphtheriae*, *C. striatum*, and *C. xerosis* specifically predominated in tongue coating of the subjects with tube feeding.

The floral characteristics of tongue coating is closely related to the mode of feeding in elderly. Some species of *Corynebacterium* which are unusual in oral cavity predominate in oral cavity with the tube feeding resulting in the malfunction of eating.

Abstract Category
04 Disease Ecology

PS.04.054 COMPARATIVE GENOMICS SUGGESTS *GARDNERELLA VAGINALIS*' ROLE IN MICROBIOTA ASSOCIATED WITH BACTERIAL VAGINOSIS IS LINKED TO MUCIN DEGRADATION

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Gardnerella vaginalis is the predominant colonist of the vaginal tract in 95 % of women diagnosed with bacterial vaginosis (BV). Despite this, it has also been isolated from non-BV subjects raising debate as to the role of this organism in the disease. Recently, two strains of *G. vaginalis* were sequenced as part of the NIH-funded Human Microbiome Project (HMP). Strain ATCC 14019 was isolated from a woman suffering from BV. The second strain 409-05 was isolated from a healthy, asymptomatic individual with a high Nugent score of 9, corresponding to a determined increased proportion of *G. vaginalis* relative to the normally dominant *Lactobacilli*. Comparative genomic analyses show significant genomic rearrangement and plasticity between the two strains. A number of metabolic differences were observed; including the ability of *G. vaginalis* strain ATCC 14019 to degrade mucin. This trait is absent from the 409-05 strain that was not associated with symptomatic disease. Previous measures of mucolytic enzymes in the vaginal cavity show strong correlations with BV. The ability to degrade mucins has been proposed as a necessary step in the colonization of vaginal epithelial cells and in the displacement of the *Lactobacilli*. Bacterially colonized vaginal epithelial cells, known to clinicians as clue cells, are one of three determinants used to diagnose BV. Given the importance of mucin to the integrity, growth and function of vaginal epithelial cells, disruption of the mucin-layer may predispose subjects to further complications, such as those associated with BV. These include an increased risk of infection with HIV and other sexually transmitted diseases. This research adds to a growing body of evidence demonstrating substantial genetic heterogeneity between strains of the 'same' species and highlights the disparity between definitions of a species and their metabolic and pathogenic capabilities.

Abstract Category
04 Disease Ecology

PS.04.055 SUPPRESSION OF SOIL-BORNE PATHOGENS OF CUCUMBER BY MICROBIOLOGICALLY IMPROVED PEAT-COMPOST MIXTURES

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Compost-based suppression of a wide range of soil-borne plant pathogens has been demonstrated, and microorganisms are suggested to be one of the driving forces. The aims of this study were to identify and isolate the microbial species associated with the suppressiveness of cucumber disease caused by *Pythium*. If cultivable, they may be chosen as inocula to produce the disease suppressive composts.

21 mature composts of different sources (e.g. sludge, biowaste, manure) were mixed with peat at 1:4 v/v ratios, and screened for their ability to decrease the severity of cucumber wilt disease. Thereafter, the disease suppressive compost amendments were selected for molecular analyses starting by total DNA extraction. The bacterial 16S rDNA and fungal rDNA Internal Transcribed Spacer regions were amplified by Polymerase Chain Reaction. The Polymerase Chain Reaction-generated DNA fragments were separated by Denaturing Gradient Gel Electrophoresis, followed by the DNA sequencing of the excised bands.

After a two-month lasting suppressiveness test, seven amended composts of biowaste and sludge origins revealed strong suppressiveness against *Pythium*. Sterilized peat having no suppressiveness was used as control. The preliminary sequencing results of Denaturing Gradient Gel Electrophoresis bands showed that *Proteobacteria* was the biggest phylum in the beginning of the test, comprising 70% of the bacterial community; in the end *Bacteroidetes* overtook it as the most dominant phylum (54%). The most abundant fungal phylum was *Ascomycota* throughout the test (67% to 58%).

In this study, compost amendments of biowaste and sludge sources suppressed to a high extent disease symptoms on cucumber wilt caused by *Pythium*, which is in line with previous studies. To further reveal the microbial suppression mechanisms to *Pythium* wilt, the massively-parallel 454 pyrosequencing was introduced, and the experiment is currently ongoing.

Abstract Category
04 Disease Ecology

PS.04.056 MICROBIAL DIVERSITY IN HOT SPRINGS OF THERAPEUTIC USE

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The thermal waters known worldwide for ancient civilizations, such as area of recreation and therapeutic use also has variability in mineral content. In Peru there are about 500 hot spring areas in which still has not been reported microbiological studies of these waters, being of importance in public health surveillance.

To isolate infectious and contagious organisms such as Enterobacteriaceae, Estaphylococcus, colony forming units (CFU) and fungi.

Three samples were processed crowded hot springs: Chancos (Huaraz), Los Baños del Inca (Cajamarca) and Churin (Lima). From each sample was taken using a swab from the biofilms attached to the walls of the bathing pools, immediately laid to CFU and differential media for infectious contagious.

Los baños del Inca had a decreased number of infectious organisms infect an average of 1,200 CFU bacteria and 30 fungi. The bathrooms that had the highest number of CFU in mesophiles were Churin with 2,500 and greater chance of CFU of fungi with 1,400.

The study thermal pools have no significant risk of spreading infectious diseases by these microorganisms have not been isolated, is recommended microbial indicators for validation thermal spas.

Abstract Category
04 Disease Ecology

PS.04.057 PHYSIOLOGY AND GENETIC ACTIVITY OF LISTERIA MONOCYTOGENES DURING MULTI-HURDLE STRESS INDUCED CELL DEATH

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The physiological basis of the hypothesis that temperature is the primary governing factor of bacterial cell inactivation under otherwise non-growth permissive conditions was investigated. Application of simultaneous low pH (pH 3.5) and low water activity ($a_w = 0.9$; 2.5 M NaCl) conditions, applied to *Listeria monocytogenes* strains ScottA and FW03/0035, and increasing incubation temperature from 25°C up to 45°C resulted in increased permeability to ethidium homodimer-1 and corresponded to accelerated declines in esterase activity and ATP basal levels but did not result in autolysis. Triphasic survival curves were readily observable when sufficiently large cell populations were inactivated at 25°C and 35°C; indicative of a mixture of sensitive and resistant sub-populations. Enrichment-based recovery experiments however indicate that the stress conditions eventually lead to complete loss of reproductive capacity, potentially corresponding to an irreversible collapse of pH homeostasis. Transcriptomic analyses were used to obtain further insights into the physiology of the inactivation process occurring at 25°C. QPCR, rifampin-enforced decay and microarray experiments revealed

transcripts of *tufA* and other genes become substantially more stable during inactivation resulting from exposure to combined low pH/aw and from non-growth permissive temperature exposure. Gene transcripts were delineated through K-means clustering that appear to be important for initial survival of combined low pH/aw and include an overrepresentation of SigB-activated genes, the response of which fades with increasing time of inactivation exposure.

Abstract Category
04 Disease Ecology

PS.05.001 POTENTIAL FOR NATURAL ATTENUATION OF HYDROCARBONS IN ANTARCTIC SOILS

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Fuel spills on Antarctic soils occur mainly near settlements, including current and former scientific bases and field camps, where fuel is stored and aircraft and vehicles are refuelled. Chemical characterisation of the hydrocarbon contaminants in the soils have revealed that n-alkanes predominate. To investigate those factors that may preclude hexadecane mineralization activity in hydrocarbon-contaminated soils from the Ross Sea region, soils were collected from the existing Scott Base or former bases (Cape Evans, Marble Point, Vanda Station) and two oil spill sites in the Wright Valley (Bull Pass and Loop Moraine). The soils were dry and low in nutrients with a high C/N ratio reflecting hydrocarbon contamination. Following soil water adjustment to 10% (w/v), the influence of hydration with or without nutrient addition (250 mg/kg N added as monoammonium phosphate) and/or inoculation (spiking with soils containing high numbers of hydrocarbon degraders) on hexadecane mineralisation activity was determined. Hydrocarbon degrader numbers were highest in coastal soils from Scott Base and Marble Point (10^7 per gram) and less prevalent in Dry Valley inland soils from Vanda Station and the Wright Valley ($<10^5$ per gram). Following hydration only, hexadecane mineralisation activity occurred in long-term contaminated soils from Marble Point, Cape Evans and Vanda Station. Other soils (Scott Base, Cape Evans and Loop Moraine) required nutrient addition, whereas Bull Pass soil required inoculation, hydration and nutrient addition before hexadecane mineralisation proceeded. Alkane degraders isolated from the soils were identified as Rhodococcus or Pseudomonas species. Results from this study indicate that hydrocarbon degradation may occur at some sites in summer when water is available. Long-term hydrocarbon-contaminated Antarctic soils may provide a valuable resource of hydrocarbon-degrading bacteria that can serve as inocula for more recent oil spills.

Abstract Category

05 Cold Habitats

PS.05.002 CONTRASTING PATTERNS OF ARCHAEAL DIVERSITY IN ANTARCTIC WATER MASSES AS REVEALED BY PYROSEQUENCING

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Although polar oceans harbor remarkably high abundances of Archaea, the patterns of diversity and distribution of these microorganisms remain almost unknown. A few recent studies suggested that the distribution of archaeal groups is linked to oceanic circulation in arctic and North Atlantic waters. However, a detailed description of archaeal diversity in antarctic waters is still lacking. Here, we provide the first exhaustive study of antarctic archaeal communities by using massive parallel pyrosequencing as a fingerprinting tool. A total of 20 samples and 60,000 archaeal reads were analysed across two antarctic regions: the well-studied Ross Sea and the rather unexplored Amundsen Sea. We targeted different antarctic water masses from the surface

to the mesopelagic layer: Antarctic surface Waters (AASW), Shelf Waters (SW), thermocline and Circumpolar Deep Waters (CDW). Archaeal diversity and community composition were strongly affected by hydrography. Particularly, CDW were found to be a hotspot for archaeal diversity in this ecosystem, with significantly higher diversity estimates (such as Shannon and Chao indices) as compared to other waters. Replacement of different euryarchaeal and crenarchaeal phylotypes was also observed among the water masses. The most abundant crenarchaeal phylotype in AASW (contributing more than 60% of total reads) was present in low abundance in CDW (generally less than 10% of reads). Interestingly, a highly similar crenarchaeal phylotype (with 98% similarity over 200 bp reads) contributed up to 30% of reads in CDW while it was almost absent in AASW. Our results show that hydrography strongly shapes patterns of archaeal diversity and composition in antarctic waters and suggest that different water masses could harbour closely related crenarchaeal phylotypes, likely involving an ecological differentiation of these microdiverse groups.

Abstract Category
05 Cold Habitats

PS.05.003 *CELLULOSIMICROBIUM CELLULANS* ISOLATED FROM ANTARCTIC SNOW AND ITS PHYSIOLOGICAL ADAPTATIONS TO LIVE IN EXTREME COLD CONDITIONS

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Strain LH7 isolated from snow collected from the Larsemann Hills region of East Antarctica was found to be affiliated to *Cellulosimicrobium cellulans* (100% similarity). Strain LH7 demonstrated physiological traits that were markedly different from that of the mesophilic *C. cellulans* type strain in terms of the sole carbon sources used for growth, fatty acid profile, cell wall sugars and quinone composition. The major respiratory quinones identified in LH7 were [MK-6(H₁₀) + H]⁺ and its hydroxylated derivative, while MK-9(H₄) was the only characteristic quinone in the type strain. The dominant cell wall sugars in LH7 were glucose, galactose and mannitol whereas rhamnose was the only major sugar in the type strain. The incorporation of these sugars in the cell wall of LH7 may be a mechanism for cold adaptation as sugars have a protective role at low temperatures. Cellular fatty acid patterns in LH7 were dominated by 12-methyltetradecanoic acid, hexadecanoic acid and 14-methylhexadecanoic acid but lacked iso fatty acids unlike the type strain. Anteiso fatty acids comprised about 60% of the total fatty acids. This mechanism would ensure the activity of membrane proteins even at low temperatures as anteiso fatty acids have a lower melting point than iso fatty acids and thus maintain membrane fluidity at low temperatures. Strain LH7 utilized a wide array of carbon substrates like aminoacids, amides, carboxylic acids, carbohydrates and polymers indicating that it could play a potentially significant role in carbon uptake in snow. This is validated by its ability to produce enzymes like cellulase, amylase and protease. The ability of strain LH7 to survive in Antarctic snow could be due to these modified physiological and metabolic properties that distinguish it from its mesophilic counterpart. However, the presence of *Cellulosimicrobium* sp. known for its cellulolytic activity in Antarctic snow devoid of celluloses is intriguing.

Abstract Category
05 Cold Habitats

PS.05.004 MICROBIAL SPATIAL HETEROGENEITY IN ARCTIC SOILS

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Soil attributes are typically heterogeneous as they vary from location to location and in most of the cases, this spatial heterogeneity is non-stochastic and tends to decline as distance decreases between points in space. Due to the characteristic variability of soil physical and chemical properties soil microbial abundance may vary across multiple spatial scales. Approximately 26% of world's land area is underlain by permafrost, however, in Canada permafrost affected soils comprise 3.7 million km² or about 40% of the land area. Despite the growing interests in microbial spatial variability, studies investigating this issue in Arctic soils are extremely limited. In this study we elucidated the spatial heterogeneity of archaeal, bacterial, and fungal populations in three high Arctic ecosystems. At each site 93 soil samples (in total 279) were collected following a variable lag-distance design along three transects. For each soil sample moisture, pH, dissolved organic carbon and nitrogen, total organic carbon, and total nitrogen contents were measured. Microbial abundance was examined by real time PCR. Spatial heterogeneity and spatial correlation was estimated by semivariogram and cross-semivariogram analyses respectively. The overall abundance of archaeal (10^8 - 10^{11}), bacterial (10^8 - 10^{10}), and fungal (10^8 - 10^{10}) phylogenetic gene copies is similar to agricultural or grassland soils. In spite of the extreme conditions microbial spatial distribution is well structured in Arctic soils and demonstrates high spatial dependency (0.54-0.99). The range, zone of spatial dependency, of microbial spatial variability varies between 0.2 and 7.24 m. Microbial spatial distribution was significantly ($P < 0.01$) correlated to soil attributes. Although the strength and range spatial variability differ among sites, the overall spatial structure was similar.

Abstract Category
05 Cold Habitats

PS.05.005 ENVIRONMENTAL DRIVERS OF BENTHIC BACTERIAL DIVERSITY ALONG THE ARCTIC CONTINENTAL SLOPE

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One of the big challenges in ecological research is to understand patterns of biodiversity in their environmental context, so as to eventually be able to predict how ecosystems and their components may respond to changing environmental conditions. Systematic assessments of bacterial community patterns along spatial, temporal and environmental gradients to test ecological hypotheses on what shapes microbial diversity are still scarce. In this study we examined changes in bacterial communities in Arctic Ocean sediments along three water depth transects on the Laptev Sea continental slope, covering water depths from 37 m to 3427 m and encompassing ice-covered and ice-free areas. We demonstrate how two molecular techniques,

i.e. Automated Ribosomal Intergenic Spacer Analysis and 454 massively parallel tag sequencing, can be advantageously combined to describe variations in bacterial communities in their environmental context. Overall, bacterial communities significantly changed along the Laptev Sea continental slope, with a clear correlation to pigment gradients among many contextual parameters available, suggesting that phytodetritus input is an important driver of bacterial diversity in the oligotrophic Arctic deep sea. Relationships between bacterial taxa and phytodetritus input were investigated from the phylum down to the genus level. The significant relationships between taxon sequence abundance and pigment concentrations varied from positive to negative, suggesting that broad-scale patterns of co-existence and avoidance between bacterial groups may exist. In the future our results may serve as a baseline for comparative studies that could provide valuable insights into bacterial community dynamics in a region dramatically influenced by global change over a time frame of almost two decades.

Abstract Category
05 Cold Habitats

PS.05.006 THE ENIGMATIC MUMMIFIED SEALS OF THE MCMURDO DRY VALLEYS: MICROBIOLOGICAL CONSEQUENCES WITH IMPLICATIONS TO CLIMATE CHANGE PREDICTIONS

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Since Scott first observed the presence of their weather-worn carcasses on his 1901-1904 Discovery expedition to Antarctica, mummified seals were thought to be rare, isolated natural artefacts peculiar to the ice-free areas of the continent. Our recent discovery of over 100 mummified seal carcasses concentrated on a precipitous portion of a small remote cliff over 10 km inland in the McMurdo Dry Valleys, deepens the mystery of their provenance and raises unexpected questions concerning the significance of the phenomena and its impact on a severely oligotrophic ecosystem. Dry Valley organisms typically endure extreme temperature and light regimes, high soil salt concentrations, and near-negligible levels of organic C and N; but a single carcass, by providing protection, substantially alters this inhospitable microenvironment, stabilizing temperatures, raising relative humidity by up to 230%, reducing UV exposure, and increasing organic C levels by over 500% in underlying soil. To investigate the biological consequences and timing of these disturbances we transplanted a seal carcass to a pristine patch of valley floor. Remarkably, we found that within only two Antarctic summer seasons, genetic and stable isotopic analyses of bacterial communities taken from the new seal-augmented site resemble those of samples taken from the original site, occupied for >400 years. Moreover, the composition and structure of these communities is unique to the amended environment and is significantly less diverse than those of control sites. Furthermore, microbial biomass is an order of magnitude greater and CO₂ respiration 4-5x higher, indicating rates of potential metabolic response and C turnover in the Dry Valleys are far greater than widely believed. This paradigm shift indicates that increases in water availability and C as a result of warmer predicted regional temperatures could radically reduce biodiversity on the continent's ice-free areas on a near-immediate time scale.

Abstract Category
05 Cold Habitats

PS.05.007 HYPOLITHIC MICROBIAL DIVERSITY IN HYPER-ARID POLAR DESERT

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The Dry Valleys in Antarctica are hyperarid polar desert that present extreme challenges to life. Recent study of microbial biodiversity in McKelvey Valley has shown highly-specialized communities in distinct lithic niches occurring concomitantly within this terrain. Here we focus on the biodiversity of the hypolithic niche. Hypoliths are colonization on the beneath-ground surfaces of rocks. They occur in McKelvey Valley with a mean frequency of 4.9% (n=1 260). Samples were collected from 5 polygons across the landscape. Bacterial diversity of the hypolithic communities were accessed by terminal restriction fragment length polymorphism (t-RFLP) on the hypervariable 16S-23S ITS region. Based on the t-RFLP profiles, similarities between communities were analyzed statistically. The Bray Curtis similarities were then visualized by nonmetric multidimensional scaling ordinations. No significant difference in community structure was found within or between locations. Clone libraries based on full-length 16S rRNA gene sequences reveal that communities are dominated by cyanobacteria *Oscillatoriales*. Other bacterial phylotypes also present but in a relatively low abundance, including Acidobacteria, Actinobacteria and Alpha proteobacteria. The lack of significant variation in microbial communities is probably due to the high selective pressures of this extreme environment.

Abstract Category
05 Cold Habitats

PS.05.008 BAR-CODE PYROSEQUENCING OF ARCTIC MICROBIAL COMMUNITIES

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Recent evidence has shown that microbial communities in the Canadian Arctic are responding to the gradual temperature increase and freshening of Arctic surface waters during this last decade. Smaller cells, picophytoplankton (0.2-2 μm) and bacterioplankton, appear to be increasing, whereas nanoplankton (2-20 μm) appear to be decreasing while total biomass (measured by chl a) remains constant. The full implications of such changes on Arctic food webs are not known, partly since it is unknown what community species changes underlie such size shifts. Changes in the photosynthetic communities will have a direct effect on zooplankton communities, equally changes in bacterial and archaeal communities could be important since these organisms are the agents of biogeochemical cycling of nutrients and carbon. It is therefore imperative to determine whether these size-shifts in the community are accompanied by changes in species composition/diversity of the communities or whether the same organisms are becoming more (or less) abundant. To answer this question, we have utilized bar-coded tag sequencing approach

targeting a variable region of the 16S (bacteria and archaea) and 18S (picoeukaryotes) rDNA gene followed by ~400 bp-length pyrosequencing. We have analyzed the <3 µm size-fraction of the community sampled from the deep chl a maximum of Amundsen Gulf, Beaufort Sea (Canada). Samples were from the end of the summer growing season into fall (July to November) taken over 7 years, from 2003 to 2009. We report the complex community dynamics revealed by this method which generated ~2,000-3,000 reads per Domain of life per site.

Abstract Category
05 Cold Habitats

**PS.05.009 AUTOMATED RIBOSOMAL INTERGENIC SPACER ANALYSIS
(ARISA) LIBRARY OF ANTARCTIC SOIL FUNGI**

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A major stumbling block in determining the relationship between fungal communities and their potential influence on ecosystem function is the determination of which species are present. Several molecular methods have been used to assess community composition. Of these, automated ribosomal intergenic spacer analysis (ARISA) has been shown to give the greatest number of bands (e.g. ITS diversity) and can give a good "snapshot" of fungal diversity. This method is based on the heterogeneity of length of the entire ITS region, and utilizes PCR amplification of the spacer regions and automated fluometric analysis on a fragment analyzer. In a single assay it provides an entire picture of the targeted microbial group, where DNA fragment lengths produced are characteristic of the taxa present in the sample. A problem with this method is that some species may give the same fingerprint, thus underestimating diversity. Our study sites are located in the Antarctic Dry Valleys, an ecosystem lacking vascular plants yet inhabited by a diverse community of microfungi. Previous work demonstrated that a high percent of species identified in genomic studies are also culturable. We applied ARISA to cultured fungi using ITS-F1 and ITS-4 primers. This allowed for the generation of an ARISA fingerprint library from a vast number of the species present in the ecosystem. The fingerprint library was directly compared with an ARISA profile from a field sample. Thus, we rapidly identified not only which individuals were present but also those fingerprints that represented several species for further analysis by DNA sequencing. A variant of the profile was generated using cDNA from field samples to identify active members of the community.

Abstract Category
05 Cold Habitats

PS.05.010 BIOGEOGRAPHY OF MARITIME ANTARCTIC SOIL BACTERIA

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Knowledge of microbial biogeography is poor despite awareness that microbial diversity can influence ecosystem function and stability. This study aimed to establish whether bacteria in maritime Antarctic soils exhibit biogeographical variation with respect to latitude, and if so, to determine whether the pattern was related to contemporary environmental conditions. Soils were collected from 70 locations along the Antarctic Peninsula (53-74°S), a range of soil physicochemical parameters were measured, and 600,000 bacterial 16S RNA genes were pyrosequenced. We quantified alpha diversity (richness and equitability) using Simpson's index and beta diversity (taxonomic variation between samples) using multivariate ordinations. Alpha diversity was not related to latitude, however, beta diversity was. By removing variation in the community data that was attributable to local environmental factors (e.g. pH, total nitrogen and organic carbon) we demonstrated that the relationship between latitude and beta diversity was related to differences in habitat types. Substrate quality rather than quantity was identified as a key factor determining the structure and composition of communities. This study indicates that bacterial diversity in maritime Antarctic soils is determined by contemporary environmental conditions. Given that this region is among those experiencing the most rapid warming on Earth it is likely that bacterial diversity will change profoundly in the future and influence the functioning and stability of ecosystems.

Abstract Category
05 Cold Habitats

PS.05.011 BIOGEOGRAPHY AEROBIC ANOXYGENIC PHOTOTROPHIC BACTERIA IN ATHALOASSOHALINE WETLANDS IN THE CHILEAN ALTIPLANO DETERMINED BY *PUFLM* AND *BCHY* GENES

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The extreme environmental conditions (e.g. high solar radiation, low temperatures, negative water balance) and the geographic isolation of wetlands located in the Chilean Altiplano are associated with the presence of an endemic and diverse biota. Previous microbial diversity studies based on 16S rRNA gene revealed the presence of highly diverse microbial communities and the wide distribution of aerobic anoxygenic phototrophic bacteria (AAPB) in these environments. Nevertheless, the role and function of these bacteria in the primary productivity in these environments is still unknown. We examined the diversity of AAPB using a functional gene approach utilizing specific primers for *pufLM* (encoding photosynthetic reaction centers of AAPB) and *bchY* (chlorophyllide oxidoreductase subunit Y) genes as molecular markers in samples of water, sediment and microbial mats collected from Salar de Huasco (20°S) and Salar de Ascotán (21°S) located at altitudes of 3800 and 4200 meters above sea level respectively. *BchY* and *pufLM* sequences were frequently associated with members of the *Roseobacter* clade (*Roseobacter*, *Roseovarius*, *Loktanella*) in microbial mats from Salar de Huasco and water samples from Salar de Ascotán. Other AAPB (*Rhodospirillum*, *Erythrobacter*) were detected from Salar de Huasco, including a phylotype highly similar with the recently described gammaproteobacterium *Congregibacter litoralis*. No members of *Chlorobi*, *Chloroflexi* and *Heliobacteria* were found. Many of the *bchY* sequences displayed limited similarity with available sequences, or could not be identified using public databases, indicating the novelty of

phototrophic bacteria inhabiting the inland waters of the Chilean Altiplano. This study was supported by the Fondecyt project N°11080228.

Abstract Category
05 Cold Habitats

PS.05.012 BIOGEOGRAPHY VULNERABILITY OF PERMAFROST SOIL CARBON TO CLIMATE WARMING: EVALUATING MICROBIAL CONTROLS ON DECOMPOSITION

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Permafrost soils contain a large portion of the terrestrial carbon stocks, which are threatened by rapid warming at high latitudes. However, the accuracy of our carbon efflux predictions is constrained by our poor understanding of the controls on decomposition in thawed permafrost. Global climate models assume that decomposition increases linearly with temperature, yet decomposition in thawed permafrost may be strongly controlled by the adaptations of decomposer organisms, such as the temperature sensitivity of enzymes. The objective of this study is to understand the contribution of physiological controls to decomposition in active layer and permafrost soils. We used freeze-thaw cycles, not experienced by permafrost microorganisms in nature, to investigate differences in physiology between the active layer and permafrost microbes. Permafrost and active layer soils were collected from Caribou Poker Creeks Research Watershed, Alaska in July 2008 and subjected to 5 freeze-thaw cycles in the lab. Although 4 times more carbon was respired from the active layer than the permafrost ($p < 0.001$), the permafrost microorganisms respired 6 times more carbon than active layer microbes per unit microbial biomass ($p < 0.001$) indicating a critical difference in physiology, community structure, or substrate availability. DNA fingerprinting indicates that permafrost communities are more diverse than the active layer; 454 pyrosequencing is underway to examine differences in community composition. Carbon:nitrogen ratios indicate soil organic matter has undergone less microbial degradation in the active layer than permafrost (42.1 ± 6.5 versus 14.4 ± 1.2). Soil organic matter quality is being studied in greater detail using mid-infrared spectrometry. Differences between carbon release per unit biomass between the permafrost and active layer suggest that models predicting the soil response to temperature change should consider differences in microbial physiology and community structure.

Abstract Category
05 Cold Habitats

PS.05.013 BIOGEOGRAPHY CAROTENOID PIGMENTATION IN ANTARCTIC BACTERIA AS A STRATEGY TO WITHSTAND ENVIRONMENTAL STRESSES

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Organisms that exist in icy environments must possess mechanisms to protect themselves from extreme environmental conditions that would otherwise cause severe damage to non-adapted organisms. Bacterial strains isolated from Antarctic environments were used in this study to assess the role of carotenoid pigments as cryo- and solar radiation protectants. Isolates were subjected to one hundred 12 hr freeze-thaw cycles and exposed to ambient simulated solar radiation (300 Wm^{-2}) with growth recovery evaluated after pre-set time intervals. Differences in survival were observed between carotenoid pigmented and non-pigmented strains in response to the different stresses based upon the enumeration of colony forming units. On average, carotenoid pigmented strains were more resistant to freeze-thaw cycles as compared to the non-pigmented strains. Survival for non-pigmented strains decreased precipitously from 2×10^7 to 1.5×10^4 cells ml⁻¹, on average, within the first 20 cycles. Similar results were found in the solar radiation experiments. After 2 hrs of solar radiation exposure, 61% of the pigmented organism survived versus 0.01% for the non-pigmented isolates. We applied an additive mixed model to estimate differences between the carotenoid pigmented and non-pigmented bacterial groups. Modelled results confirmed a positive effect of pigmentation on survivability and provide evidence that carotenoid pigmentation in heterotrophic bacteria isolated from Antarctic environments increases resistance to environmental stressors.

Abstract Category
05 Cold Habitats

PS.05.014 BIOGEOGRAPHY THE THERMAL REGIME AND MICROBIAL ECOLOGY OF TWO NATURAL COLD TRAPS IN PENNSYLVANIA (USA)

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Natural cold traps in the Mid-Atlantic region of the eastern United States are known to serve as localized refugia for boreal plants and lichens typically found hundreds of kilometers further north. These cold traps often represent sites where relatively porous talus slopes accumulate cold air and ice in winter, and retain their cold temperatures in summer due to thermal stratification. Such sites are locally known for the persistence of cold temperatures, and sometimes ice formations, well into the summer. In this study, we report on the thermal and preliminary microbial characteristics of two central Pennsylvania cold trap sites: The Trough Creek ice mine and the Spruce Creek ice caves. The ice "mine" is a tectonic cave located at the base of a large talus slope, and the ice caves are smaller air drainage vents similarly located along the bottom of an extensive talus slope. Temperature data loggers were installed at both sites for up to 3 years time, and the results were compared to ambient temperatures. The ice mine was cooled to the lowest recorded ambient winter temperatures due to displacement by dense cold air, but slowly warmed through the summer and fall, reaching a maximum temperature of ca. 4°C in late fall. Patterns were similar at the ice caves location, where coldest annual temperatures were measured at the base of the talus slope. Bacterial populations isolated from both locations tended to be psychrotolerant, demonstrating good but suboptimal growth rates at in situ temperatures. Boreal lichens unique to the state of Pennsylvania were found on the ice caves talus slope, and the

bacterial population associated with locally-abundant *Stereocaulon glaucescens* was examined in greater detail.

Abstract Category
05 Cold Habitats

PS.05.015 BIOGEOGRAPHY METAGENOME PYROSEQUENCING OF HÅKON MOSBY MUD VOLCANO SEDIMENTS

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The Håkon Mosby Mud Volcano is an active methane driven cold seep located in the Barents Sea. Methane and hydrocarbons in the emitted volcano fluid provide enough energy to sustain a highly diverse microbial community in the subseafloor sediments. Here we present the metagenomic analysis of the prokaryotic community found in a sediment sample of the Håkon Mosby Mud Volcano. The metagenomic dataset was created using 454 pyrosequencing. The resulting metagenome was studied to understand the species diversity and metabolic capacity of the microbial community. Analysis of the metagenome indicated a high microbial diversity. *Bacteria* and *Archaea* made up 94.6 and 5.4% of the microbial reads, with *Proteobacteria* and *Euryarchaeota* being the dominant phylum in the distinct kingdoms. The metabolic capacity was studied using both the MG-RAST server and the KEGG Automatic Annotation Server (KAAS). Since metagenomic reads could be binned into different groups such as *Proteobacteria*, *Archaea* and *Bacteroidetes*, we could annotate reads belonging to a specific taxon. This combination of taxonomic binning followed by metabolic annotation of metagenomic reads enabled us to link metabolic routes to specific microbial taxa. Thus taxa specific genes were identified belonging to specific metabolic routes like: CO₂ fixation, nitrogen cycling, sulfur cycling and anaerobic oxidation of methane. For instance, our analysis showed that many of the important enzymes in anaerobic oxidation of methane are only found among archaeal sequences in the Håkon Mosby Mud Volcano metagenome. Our approach shows how metagenomic analysis can be used to unravel the metabolic capacity of different taxa within the same sample. Thereby our approach can provide a better understanding of the ecology and relationships within complex microbial communities.

Abstract Category
05 Cold Habitats

PS.05.016 BIOGEOGRAPHY PRESENCE AND ACTIVITY OF HYDROCARBON-DEGRADING MICROBIAL POPULATIONS IN CONTAMINATED SOILS FROM AN AIRFIELD IN THE GREENLAND HIGH ACTICS

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In this study, we sampled contaminated soils from the airfield at Station Nord with the aim of assessing the intrinsic attenuation potential by quantifying the presence and activity of specific hydrocarbon-degrading microbial populations at temperatures $\leq 0^{\circ}\text{C}$. Microbial populations metabolically active at -5°C were detected in the contaminated soils by measuring $[14\text{C}]$ glucose mineralization to $^{14}\text{CO}_2$ and sizable naphthalene, undecane, biphenyl and phenanthrene-degrading populations were measured in contaminated soil by most-probable-number analysis. Phylogenetic analysis of the phenanthrene-degrading populations from the contaminated soils, based on cloning and 16S rRNA gene sequencing, revealed the dominance of *Pseudomonas* spp., *Sphingomonas* spp. and *Variovorax* spp. closely related to strains previously described from cold and/or contaminated environments. Mineralization of $[14\text{C}]$ phenanthrene was measured in contaminated soils at temperatures down to 0°C but no mineralization of $[14\text{C}]$ pyrene was detected during the 150 days experiment. Attempts to enhance the mineralization activity by increasing the temperature and adding an inorganic nutrient solution to the soils were successful for phenanthrene but did not stimulate any pyrene mineralization. This study provides the first indications of the presence and activity of degradative microbial populations in soils from the Greenland High Arctic suggesting that natural attenuation could be an approach for remediation of some oil components in these remote regions.

Abstract Category
05 Cold Habitats

PS.05.017 BIOGEOGRAPHY MICROBIAL ECOLOGY OF JET FUEL DEGRADATION IN SOIL FROM HIGH-ARCTIC GREENLAND AT LOW TEMPERATURES

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Fuel handling and storage at the military base Station Nord (NE Greenland, 81°N) has caused several severe fuel spills over the years. The natural in-situ degradation of oil compounds is slow under high-arctic conditions. However, if stimulated, existing degraders may be sufficient to perform bioremediation of fuel contaminated soil. Degradation distribution and density were described in five soil profiles from terrain to the permafrost. This was done by qPCR to quantify specific fuel degradation genes, and by counts of the numbers of culturable degraders, grown on selected fuel compounds and jet fuel, by a most-probable-number (MPN) method. Mineralisation of $[14\text{C}]$ -n-dodecan in 20 g samples of polluted soil was used as a model system for investigating the effects of fertilizer addition and temperature. Mineral stimulated the mineralisation, compared to unfertilised controls, with optimum concentrations of 250-500 mg N kg⁻¹ soil. Fertiliser concentrations of 1000 mg N kg⁻¹ or more were toxic for the degrader organisms. The effect of temperature on mineralization of $[14\text{C}]$ -n-dodecan was tested and the highest mineralisation was observed at 5°C , which corresponds to the soil temperature at the depths where the largest microbial degrader populations was found. Degradation of authentic fuel contamination, and the developments in degrader populations, were investigated in 4.7 kg soil microcosms at 5°C and with the optimal fertiliser concentration. These microcosms simulated stimulated biodegradation under natural conditions to explore bioremediation strategies to be used in field-scale tests at Station Nord. Changes in total degraded oil components (GC-FID)

was followed over time, and correlated to changes in degrader populations (qPCR and MPN). Birgitte Weber-Blicher the Danish Defence Estates and Infrastructure Organisation is thanked for partly funding and discussions.

Abstract Category
05 Cold Habitats

PS.05.018 BIOGEOGRAPHY PROKARYOTIC COMMUNITY STRUCTURES IN THE ANOXIC SEDIMENT OF ANTARCTIC MEROMICTIC LAKE NURUME-IKE

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In order to expand knowledge of the biodiversity of Antarctic prokaryotes, archaeal and bacterial community structures were revealed by the analysis of 16S rDNA clones derived from the Lake Nurume-Ike sediment. We also compared the communities of Nurume-Ike with other Antarctic meromictic lakes located in Vestfold Hills, Eastern Antarctica, previously reported.

Anoxic sediment at 13 m depth of the lake was taken using an Ekman-Birge-type bottom sampler. Community DNA sample was extracted and purified. Archaeal and bacterial 16S rDNA were amplified by PCR, and individual clones were sequenced and identified by BLAST search.

The Archaeal clones (205 total) consisted of only three phylotypes, and were dominantly affiliated with uncultured euryarchaeotes. Specifically, 93% of the clones were identified as marine benthic group-D archaeal phylotype. In contrast to the limited archaeal diversity, 53 phylotypes were detected within 312 bacterial clones. Major bacterial phylotypes were affiliated with α -Proteobacteria (20% of clones), δ -Proteobacteria (9%), Planctomycetales (7%), and Cyanobacteria (7%). The bacterial community of Lake Nurume-Ike sediment consisted of numerous phylogenetic groups and had a diversity comparable to the diversity of other Antarctic lakes communities previously reported. Interestingly, however, there seemed to be very few phylotypes shared between the communities of lakes Nurume-Ike and five other lakes located in Vestfold Hills.

This is the first comprehensive study to analyze more than 500 16S rDNA clones for microbial community analysis of an Antarctic lake sediment sample, and the results significantly expand current views of Prokaryotic diversity in Antarctic lakes.

Abstract Category
05 Cold Habitats

PS.05.019 BIOGEOGRAPHY MICROBIOLOGY OF SEASONAL LAKE ICE COVER

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Studies of bacterial survival in icy biomes typically focus on polar environments. Seasonally ice-covered lakes and seasonal sea ice, on the other hand, are widespread ecosystems that require microorganisms to survive in an icy environment and have the further stress of annual freeze/thaw cycles. This study focuses on the inter- and intra-seasonal microbial assemblage composition within the lake ice of Miquelon Lake, a shallow saline lake in Alberta, Canada. A single ice core from the 2008/2009 field season and eight ice cores, snow cover, and lake water samples were collected approximately every two weeks over the 5 month 2009/2010 winter season. Microbial diversity within the samples was characterized using denaturing gradient gel electrophoresis (DGGE) of small subunit rRNA genes of Bacteria, Archaea, and Eukaryotes. The microbial assemblage at all depths of the ice was essentially identical to that found in surface water at the ice/water interface with the exception of the uppermost 0-5 cm sample, which was partially influenced by the assemblage in the overlying snow layer. Furthermore, the assemblage was highly temporally stable, showing no significant differences either within the 2009/2010 season or between years. Low richness was observed, with ~10 Bacteria DGGE bands, ~5 Archaea DGGE bands, and ~3 Eukaryotic DGGE bands observed. We are currently in the process of obtaining sequence to determine the identity of these dominant DGGE bands. This study represents the first characterization of microbial diversity in the ice covered lakes of central Alberta; and though the prokaryotic and eukaryotic populations appear stable in these environments, the impact of ambient temperature, snow cover, and ice thickness variations, and other parameters on the composition of the assemblages have yet to be examined.

Abstract Category
05 Cold Habitats

PS.05.020 BIOGEOGRAPHY IMPACT OF EXPERIMENTAL WARMING AND FERTILIZATION ON GREENHOUSE GAS FLUX BUT NOT BACTERIAL COMMUNITY COMPOSITION IN ARCTIC SOILS

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Warming due to global climate change is more pronounced in the Arctic than in more temperate regions. The response of Arctic soil biogeochemical cycling of the greenhouse gasses carbon dioxide, methane, and nitrous oxide to climate warming is still poorly understood due to complex interactions between warming temperatures, changing precipitation, and changing nutrient availability. We examined changes in greenhouse gas flux and microbial community composition in a factorially designed experimental system in high Arctic soils from Alexandra Fiord, Ellesmere Island, Canada. Selected plots at this site have been experimentally warmed, fertilized, and/or irrigated for 16 years. No clear trends in community composition shifts with warming, irrigation, fertilization, or any combination of these factors were observed via 16S rRNA DGGE. Furthermore, there were no observable changes between samples in functional

guilds as assessed by DGGE of the functional genes nosZ (denitrifiers) or bacterial or archaeal amoA (nitrifiers). We were unable to obtain PCR product for the pmoA gene (methanotrophs) from any sample. CO₂ fluxes increased with soil temperature and water additions, whereas CH₄ and N₂O fluxes increased in low fertilizer treatment relative to the control and high fertilizer treatment. No other treatment effects on greenhouse gas flux were observed. Vegetation height increased in high fertilizer relative to low fertilizer or control samples, while both live and total vegetation cover increased in warmed and fertilized plots. No effects were observed on plant community composition. Overall, these data show that while high Arctic soils respond to climate change parameters by increased greenhouse gas flux and changes in vegetation cover and height, there is little to no effect either on overall soil microbial community structure or specific functional guilds.

Abstract Category
05 Cold Habitats

PS.05.021 THE MICROBIAL DIVERSITY AND ACTIVITIES OF CHANNEL SEDIMENTS FROM A SUBZERO HYPERSALINE HIGH ARCTIC SPRING

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Axel Heiberg Island contains the only known cold perennial springs underlying thick permafrost on Earth and represents extremely cold and saline habitats useful for expanding our knowledge of microbial extremophiles. Lost Hammer (LH) spring is the most unique spring on the Island and is characterized by hypersalinity (20-25% salinity), subzero temperatures, reducing, oligotrophic conditions and is rich in sulfates and dissolved H₂S/sulfides. The outflow channels from LH remain unfrozen in winter because of their high salinity with in situ subzero temperatures of -18°C. CO₂ and methane flux from the channel exceed 0.81 μmol/m²/s and 15 μmol/g sediment, respectively.

We investigated the microbial composition, biodiversity and activity of LH channel sediments using catalyzed reporter deposition fluorescent in situ hybridization (CARD-FISH), bacterial cultivation/characterization, 16S rRNA clone libraries, and mineralization of 14C-acetate in microcosms.

On average, the abundance of bacteria was 4.51×10^7 cells/g sediment while archaeal abundance was 3.99×10^6 cells/g sediment using CARD-FISH enumeration. A total of 22 isolates were identified and characterized as belonging to different phyla including: Firmicutes, Actinomycetes, and γ-proteobacteria. The bacterial 16S rRNA clone libraries revealed that all three samples contained Bacteroidetes, α-proteobacteria, Actinomycetes, and Firmicutes. Phylotypes of methanotrophic and sulfur reducing/oxidizing bacteria were also identified. Phylotypes representing methanogens were found in the archaeal 16S rRNA clone library. The mineralization rates of 14C-acetate by the channel sediments exceeded 29.58% and 10.00% at 5°C and -5°C, respectively, after 6 months incubation in microcosms.

This unique methane/sulfur ecosystem supports a diverse and metabolically active microbial community within this extreme hypersaline cryoenvironment.

Abstract Category
05 Cold Habitats

PS.05.022 PHYSICAL AND MICROBIAL CHARACTERISATION OF MARINE SEDIMENT FROM ROSS SEA, ANTARCTICA

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Very little is known about the bacterial diversity and activity in Antarctic and Ross Sea sediments. Bacteria are known to play a major role in the cycling of nutrients and are responsible for the breakdown of most of the organic matter. The focus of this research was to determine bacterial diversity in the sediments and to link bacterial diversity to the physical characteristics and specific bacterial processes in the sediments. Physical characteristics included: sediment grain size, chlorophyll *a*, calcium carbonate content and total organic matter. The following bacterial processes were measured using exo-enzyme assays: breakdown of protein, lipid, cellulose, chitin and inorganic phosphate cycling and bacterial production was measured using tritiated thymidine. Sediment samples were collected by multi-corer from a total of 11 sites in the Ross Sea, Antarctica, around Scott Island and Admiralty Seamount. Three cores at each site were sub-sampled aseptically and a surface sample was frozen at -80°C for subsequent post voyage analysis. In addition, a known volume of sediment was diluted with an equal volume of sterile PBS for exo-enzyme and bacterial production analysis on board. Exo-enzyme activity was determined using standard methods. DNA was extracted from each core at each site using the FASTDNA SPIN kit for soil (MP Biomedicals, USA). Bacterial community profiles were obtained for each core using fluorescent 16S rRNA gene primers. Physical sediment characteristics were measured using standard methods. The exo-enzyme data showed relatively high exo-enzyme activity in the sediments at the sites close to the Antarctic continent and in shallow water (400-600m) compared to the continental slope sites and abyssal sites (>3000m) around Scott Island and Admiralty Seamount. The community profiles show that a large number of peaks are present showing a large microbial diversity and that the diversity decreases between the shelf and abyssal sites.

Abstract Category
05 Cold Habitats

PS.05.023 ABIOTIC AND BIOTIC CONTROL ON MICROBIAL COMMUNITIES IN ARCTIC ALPINE TUNDRA SOIL

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Tundra biome stores a large proportion of global soil carbon which is potentially mineralized at an increasing rate. Factors controlling the tundra soil microbial community composition and function are thus of increasing interest. Besides temperature, soil microbial communities are influenced by interactions between above- and belowground biota as well as soil chemistry such as nutrient content, pH and organic matter. To study different factors that drive the microbial

community composition in Arctic tundra, a field experiment with factorial treatments of fertilization, liming and herbivore exclusion was conducted in two contrasting tundra vegetation types, non-acidic (N rich) and acidic (N poor) tundra heaths. Microbial community composition was characterized by phospholipid fatty acid (PLFA) analysis, terminal restriction fragment polymorphism (T-RFLP) and clone libraries. Habitat (acidic - non-acidic) was the main controller of the microbial community structure. Acidic tundra contained relatively more fungi where as the non-acidic sites contained more abundant and diverse bacterial communities. The bacterial communities of both habitat types were dominated by members of Acidobacteria and Alphaproteobacteria. However, different subgroups of Acidobacteria dominated in the acidic vs. non-acidic soils. Both fertilization and liming increased the proportion of bacteria in relation to fungi and either increased or decreased the relative share of certain bacterial phylotypes. The effect of fertilization on bacterial community composition interacted with herbivore exclusion which coincided with the stronger fertilization effect inside the exclosures. These results indicate that besides pH, soil N availability has a strong effect on microbial community composition and function in the tundra. Moreover, herbivory interact with nutrient concentrations in structuring the soil microbial communities similarly as has been shown in plant communities.

Abstract Category
05 Cold Habitats

PS.05.024 METHANOTROPHIC BACTERIAL DIVERSITY IN TUNDRA SOILS FROM THE CANADIAN HIGH ARCTIC

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Methane is an important greenhouse gas and permafrost thaw following global warming will probably lead to increases in its atmospheric concentration. In view of this potential large release of methane from Arctic soils, it is crucial to understand the local methane sinks that could mitigate net methane emissions. Dry high Arctic tundra soils could have the capacity to oxidize atmospheric methane and to be such a sink. Previous studies of various upland soils indicated that yet uncultured methanotrophic bacteria containing novel *pmoA* gene variants are dominant in these soils and might be implicated in the process of atmospheric methane oxidation. However, the ecology of these atmospheric-level methanotrophs in high Arctic tundra soils remains unexplored. The current research project aimed therefore at understanding the ecology of Canadian high Arctic tundra soil methanotrophic bacteria. In order to do so, soil samples (top soil and 40 cm depth) were collected from Expedition Fjord, Axel Heiberg Island, Nunavut and the methanotrophic community was analysed using *pmoA*-based qPCR, clone libraries and functional gene microarrays. Incubation of soils with methane showed that top soils had higher methane oxidation rates than soils from the 40 cm depth. This higher activity in the top soils was linked to a higher abundance of the *pmoA* gene. Microarray and clone library analyses revealed some similarities between the methanotrophic bacterial populations from the top soils and soils from the 40 cm depth. However, methanotrophs related to the 'upland soil cluster γ ' (USC γ), which has previously been detected in upland soils that consume atmospheric methane, were

only detected in the top soils. These results indicate that high Arctic tundra top soils harbour methanotrophic bacterial populations potentially capable of atmospheric methane oxidation. These tundra Arctic soils could become important methane sinks following global warming.

Abstract Category
05 Cold Habitats

PS.05.025 N₂-FIXATION ACTIVITIES AND *nifH* GENE ANALYSES OF MICROBIAL MATS FROM WETTED ANTARCTIC DRY VALLEY SOILS

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Water availability is a vital driver of microbial activity in the McMurdo Dry Valleys of Antarctica as evidenced by the formation of microbial mats in hyporheic zones associated with ephemeral melt-water glacial runoffs and lake-edges during the summer months. It has been postulated that these microbial mats are an important source of organic matter to the entire Dry Valley ecosystem through aeolian transportation of detritus to low productivity areas, yet descriptions and identification of the microbial communities responsible for primary-production within these mats are limited. In this study, microorganisms responsible for N₂-fixation from four wetted soils of the Antarctic Dry Valleys were examined and *in situ* N₂-fixation activities were linked to *nifH* gene data at both the DNA and RNA level. A high diversity of *nifH* genes were revealed (Shannon-index values of 1.8-2.6) including four major clusters with two clusters related to *Geobacter* sp., and sulfate-reducers within the delta-proteobacteria, a group related to the cyanobacteria, and a group most closely related to the beta-proteobacteria. Some *nifH* phylotypes were also represented within the alpha- and gamma-proteobacteria. N₂-fixation rates ranged between ~ 0.5 to 6 nmol N cm⁻³ h⁻¹ with sulfate-dependent N₂-fixation accounting for ~ 50% of that total activity for two of the sites. Comparison of the microbial communities to proximal dry soils via 16S rRNA and *nifH* gene T-RFLP-based AMMI ordinations also suggested that these communities are not ubiquitously distributed and local conditions unrelated to moisture content may define the composition, diversity or habitat suitability of microbial communities within hyporheic zones and lake margins of the McMurdo Dry Valleys.

Abstract Category
05 Cold Habitats

PS.05.026 BACTERIAL ENDOPHYTE COMMUNITIES OF ARCTIC PLANTS

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Plants in all climates and ecosystems are associated with a diverse array of microbes. Endophytic bacteria inhabit the internal plant tissues, where they form non-pathogenic, often mutualistic, relationships with their hosts. Awareness of abundance and potential benefits of endophytic bacteria has grown rapidly in recent years. Currently the diversity and functions of endophytic

bacterial communities in arctic flora are virtually unknown.

The aim of this study is to characterize the taxonomic and functional diversity of endobacterial flora of three arcto-alpine plant species (*Oxyria digyna*, *Diapensia lapponica* and *Juncus trifidus*) in the low Arctic in Kilpisjärvi area, northwestern Scandinavia (69°03'N). The endobacterial flora was isolated from plant samples collected from three distinct sites, and endophyte community diversity was analyzed by cultivation and DNA-based methods. Functional diversity of cultivable endobacteria was analyzed by activity assays and by PCR amplification of functional genes.

Analysis of over 600 endobacterial isolates revealed a wide diversity, most commonly representing Proteobacteria, Actinobacteria and Bacteroidetes. Both the taxonomic and functional diversity was more dependent on plant species than sampling site, suggesting specific plant-endobacterial interactions. Several fungal-associated species were isolated from *D. lapponica*; *Variovorax* sp. and *Collimonas fungivorans* were common, very likely associating with the ericoid mycorrhizal partners of *D. lapponica*. Additionally, several isolates have not been reported as endobacteria previously, including acidobacterial isolates of *D. lapponica* and *J. trifidus*, most closely related to *Terriglobus roseus*. The isolated bacteria are well adapted to low temperatures; this is reflected in both taxonomy, with the closest relatives often representing psychrophilic ecotypes, as well as in their physiology: most isolates grow normally at +4 °C.

Abstract Category
05 Cold Habitats

PS.05.027 TENTATIVE MECHANISMS AND SIGNIFICANCE OF BELOW-ZERO MICROBIAL ACTIVITY

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Background and aims. Last decade it was found measurable subzero activity (SZA) of microbial communities in permafrost, tundra and polar ice which changed dramatically view on geochemistry of permanently cold ecosystems. Earlier (Panikov, Sizova, 2005-2007), we developed sensitive techniques to measure SZA and found that preferential substrates are gases and volatile compounds (CH₄, ethanol). Several fungi and bacteria were isolated which displayed SZA in vitro and in situ by using molecular probes. This communication further explores SZA with test of wide range of C-substrates and evaluation of significance of biogenic heat production. Methods: permafrost samples (Fairbanks) were amended with various C-substrates and incubated at -5 to -30°C. Response of microbial community was followed by uptake of ¹⁴CO₂ and 16S rRNA survey. Heat generation was measured by microcalorimeter. Results. Aerobic permafrost community was active in consumption of volatile and some mobile non-volatile compounds (succinate, citrate) but was not able to degrade sugars and any polymeric C-substrates (cellulose, starch and proteins). The rate of polymers degradation abruptly stopped just below the freezing point. Anaerobic Yedoma permafrost did not respond to ethanol and CH₄ but was stimulated by fermentable substrates, e.g. glucose. Growth and consumption of added compounds was accompanied by release of measurable heat which warmed up the micro-environment around microbial colonies. Conclusion. Obtained experimental data confirmed our early conclusion that SZA is true growth (not just maintenance) supported by delivery of volatile substrates via aeration pores. The polymeric compounds remain intact because of lack of mass-

transfer. Biogenic heat can be of considerable importance in supporting of SZA at thermally insulated microsites, e.g. in organic permafrost.

Abstract Category
05 Cold Habitats

PS.05.028 ANTARCTIC SUBGLACIAL LAKE EXPLORATION: A NEW FRONTIER IN MICROBIAL ECOLOGY.

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Antarctic subglacial lake ecosystems have the potential to be one of the most extreme environments on Earth, with combined stresses of high pressure, low temperature, permanent darkness, low-nutrient availability and variable oxygen concentrations, where the predominant mode of nutrition is most likely to be chemoautotrophic. For microbial ecologists, they provide a unique opportunity to address some very interesting scientific questions, such as: can these extreme ecosystems sustain life, and if so, in what form, where exactly and how? What can any subglacial lake microorganisms found tell us about the distribution and evolution of microbial life in on Earth? What are the potentially novel biogeochemical resources of this unique gene pool? What potential historical climate change record is locked within subglacial lake sediments, and how do Antarctic subglacial lakes interact with and influence the rest of the biosphere? Results from a preliminary search for microbial life in former subglacial Lake Hodgson, Antarctica, will be used to illustrate the opportunities and challenges of working in such remote environments.

Abstract Category
05 Cold Habitats

PS.05.029 STRINGENT STARVATION PROTEIN SSPA POSITIVELY REGULATES COLD ADAPTATIONS IN *SHEWANELLA PUTREFACIENS* W3-18-1

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More than 80% of biosphere is permanently cold. Respiratory versatility and psychrophily are hallmarks of the *Shewanella* genus (Hau and Gralnick, 2007; Fredrickson *et al.*, 2008). A series of *Shewanella* strains have been isolated from permanently cold environments (<5°C) including deep-sea, marine sediments, and polar and subpolar areas, and were able to grow well at low temperatures. The psychrophily of *Shewanella* strains is highly desirable for the in-situ bioremediation of cold environments such as terrestrial subsurface and marine sediments. To identify the cold adaptation-related genes and mechanisms, we utilized mariner transposon mutagenesis in *S. putrefaciens* W3-18-1, a strain isolated from the marine sediment off the Washington coast. More than 80 cold sensitive mutants, exhibiting defective or slow growth at 4°C and normal growth at 25°C, have been isolated. The disrupted genes were identified and the

involvement of several genes in cold adaptation was further confirmed by in-frame deletion and/or genetic complementation. In agreement with previous findings, some cold sensitive mutants harbor the transposon insertion in the genes involved in replication, transcription, and translation processes and their mutations caused temperature sensitivity in other bacteria, including *Escherichia coli* and *Pseudomonas syringae*. Two of the previously unidentified genes, encoding the stringent starvation protein A (SspA) and a ribosome 50S subunit associated protein (YjgA in *E. coli*), may play a crucial role in the bacterial growth of *Shewanella* at low temperatures. Furthermore, the pleiotropic regulatory factor SspA may regulate a series of cellular processes such as the sodium motive force dependent pH homeostasis and nutrient uptake as well as tRNA modification, which are essential for bacterial cold adaptation.

Abstract Category
05 Cold Habitats

PS.05.030 FUNGAL COMMUNITY OF ANTARCTIC DRY VALLEYS SOIL AS REVEALED BY CULTIVATION, ENVIRONMENTAL DNA AND RNA ANALYSES

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The McMurdo Dry Valleys in Eastern Antarctica represent extreme challenges to life. Microbial communities are therefore represent the key feature of the Dry Valleys biota. Studies have generally focused upon prokaryotic assemblages whilst relatively little is known about the fungi. In this study, we used a combination of cultivation, DNA and RNA (cDNA) approaches to compare diversity estimates for Dry Valleys soil using these techniques. Cultivation under a variety of conditions and growth media revealed only two ascomycete taxa were able to grow under laboratory conditions, *Debaryomyces hansenii* (a yeast) and *Zalerion varium*. The environmental DNA library was dominated by several *Zalerion*-like phylotypes and Heliotiales teleomorph phylotypes. The environmental cDNA library was also dominated by *Zalerion* phylotypes, but interestingly supported Heliotiales anamorphic phylotypes, indicating the a teleomorph-anamorph connection between DNA and cDNA libraries. Yeasts were not recovered from any of the libraries. The dominant taxon retrieved by all methods is noted for its halophilic representatives and our data shows that Dry Valleys soils support high salt levels. The differences between the various approaches for diversity estimation suggest that cultivation may markedly under-estimate diversity, whilst DNA and cDNA libraries have potential in indicating fungi at different life stages.

Abstract Category
05 Cold Habitats

PS.05.031 PREHISTORIC ARCTIC AND HIGH ARCTIC ICE WEDGES: POSSIBLE ANALOG SITES FOR LIFE ON MARS

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Polygon features are characteristic of wedge ice in permafrost on Earth and have been recently described by the Phoenix Lander on Mars. Formed during freeze-thaw cycles, their presence on Mars is highly suggestive of present or past surface and subsurface liquid water. This study examined the microbiology of two acidic ice wedges found on Axel Heiberg Island in the Canadian High Arctic (AHW1 and AHW2) as well as that of Arctic ice from the Yukon: a buried glacier at Chapman Lake CL, a pingo at Eagle River ER, two ice wedges at Old Crow OC and Moose Creek MC and a snow bank at Red Creek RC. Viable heterotrophic plate counts were done. Unique colonies were isolated, identified via 16S rDNA sequencing and tested for salt tolerance, growth temperature and oxygen requirements.. Card-FISH was used to quantify Archaea and Bacteria while DAPI staining was used for total cell counts. DGGE is being used to create a depth profile of the AHW1 and AHW2 samples. Clone libraries were constructed for ER (Bacterial) and CL (Archaeal). Plate counts yielded concentrations of 3.3×10^3 , 3.0×10^3 and 2.2×10^2 CFU / ml of melted ice for MC, OC and ER, respectively, while viable microorganisms were not clearly detected in RC and CL. AHW1 and AHW2 yielded maximal counts of 2.7×10^4 and 2.8×10^5 CFU/mL respectively, though counts varied with core depth. Salt tolerance of the isolates varied by origin. In ER, MC, OC and RC, the main phyla represented were *Proteobacteria*, *Actinobacteria* and *Firmicutes*. Evidence of Archaea and Bacteria was found in all samples. Total cell counts were significantly higher than viable plate counts. The CL library was dominated by clones related to marine methane oxidizers, whereas the ER library clones were related to *Alpha*- and *Betaproteobacteria* from soil, permafrost and ice environments. Preliminary results suggest that compared to other ground ice, wedges harbour a higher viable biomass and a greater microbial diversity.

Abstract Category
05 Cold Habitats

PS.05.032 TEMPERATURE OR CARBON AVAILABILITY — WHAT CONTROLS CARBON MINERALIZATION IN PERMANENTLY COLD HABITATS?

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Understanding the time scale and magnitude at which carbon mineralization increases with temperature is important to predict carbon cycling in marine habitats subject to global warming. Regional comparisons of different temperature regimes suggest that the availability of reactive organic matter rather than temperature limits carbon mineralization rates. By contrast, long-term temperature up-shift experiments with Arctic sediment indicated sustained stimulation by temperature over more than a year. To address this discrepancy, temperature shift experiments were performed with permanently cold shelf sediment from the high Arctic off Svalbard and with shelf and slope sediments from the South Atlantic (MARUM cruises M76 and M78).

Temperature response curves of carbon mineralization were established in temperature gradient block experiments by measuring ^{35}S -sulfate reduction rates as the terminal step of carbon mineralization. Temperature response curves reflect microbial community adaptation to ambient temperature and provide information on the sensitivity of metabolic rates to increased temperature. In South Atlantic shelf sediments sulfate reduction rates showed a mesophilic response while South Atlantic slope sediments and Arctic sediments showed a moderate to strong psychrophilic response. In the Arctic, *in situ* rates were 20% of those at T_{opt} and activation energies of 30-55 mol kJ-1 indicated good adaptation of the active community. In shallow, organic-rich sediments of the South Atlantic, *in-situ* rates were 10% of those at T_{opt} , while in the cold slope sediments they were 50%. Thus, while deep-sea sediments host a cold-adapted community rates of carbon mineralization were not significantly stimulated by temperature, probably because organic carbon availability was the overriding regulating factor. Our conclusions are that environmental temperature increase stimulates organic matter mineralization most strongly in sediments with abundant reactive organic matter.

Abstract Category
05 Cold Habitats

PS.05.033 HISTORICAL PATTERNS IN STREAM FLOW INFLUENCE DIATOM COMMUNITIES IN ANTARCTIC STREAMS

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Polar regions are susceptible to climate change and ecological indicators are vital to track these ecosystem changes. The McMurdo Dry Valleys Long-term Ecological Research site in Antarctica is an ideal place to study the effects of climate change due to its reduced trophic complexity and geographic isolation. Diatoms are sensitive ecological indicators and occur in relatively high diversity in Dry Valley stream algal mats. The goal of this study was to expand our current knowledge of diatom community responses to environmental parameters in this environment. Specifically, this study determined spatial and temporal trends in diatom communities in algal mat cores collected from 5 streams in Taylor Valley over 15-years. Biological, chemical, and physical measurements were collected and analyzed during each sampling event. Relative abundances of diatom species were counted microscopically and the resulting counts data were correlated to environmental parameters using numerous univariate and multivariate techniques. Ordinations of diatom communities demonstrate that Antarctic streams have distinctive diatom communities that resist seasonal changes. These ordinations also show that streams with more similar flow regimes have more similar diatom communities. Distance matrices of species and environmental data were generated and used to develop a model to identify explanatory variables of species composition using Mantel-like tests. In this model, the parameters with the highest explanatory power were total annual discharge and historical variation in discharge ($p=.001$). Discharge was also negatively correlated with sample biovolume, suggesting that higher stream flow may select for smaller diatom species. These species tend to be cosmopolitan in distribution and size may therefore be a general adaptive mechanism for life in lotic ecosystems.

Abstract Category
05 Cold Habitats

PS.05.034 REGULATION OF MICROBIAL PROCESSES BY SOIL NUTRIENT AVAILABILITY, SOIL pH AND HERBIVORY IN THE TUNDRA

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Soil microbial processes in tundra ecosystems are strongly influenced by soil nutrient availability and abundance of herbivores. By factorial treatments of fertilization, liming, and herbivore exclusion at two contrasting tundra habitats, acidic and non-acidic tundra heaths, we investigated how these factors interact to regulate the soil microbial C and N and activities of polyphenol-degrading microbial enzymes. We found a strong interaction between the effects of fertilization and herbivore exclusion on the activity of polyphenol oxidase (PO): the PO activity was unaffected by fertilization inside the herbivore exclosures, but fertilization drastically increased the PO activity in the areas that were open to grazing. Liming, by contrast, reduced the PO activity. Changes in the microbial enzyme activities were also reflected in the chemical composition of soil organic matter. Fertilization increased the microbial N in both grazed and ungrazed areas, but did not influence the microbial C. Our results demonstrate that soil N availability exerts strong control over the microbial processes, which interacts with the mammalian herbivory. Combined fertilization and grazing increases microbial degradation of polyphenols, most probably through physiological plant responses to the increased grazing pressure which influence the availability of labile C for the soil microorganisms. Increases in the soil pH, by contrast, seem to favour microbial taxa with limited capability for polyphenol degradation.

Abstract Category
05 Cold Habitats

PS.05.035 NITRIFYING COMMUNITIES IN A SUB-ARCTIC DIAMOND MINE WATER COLLECTION POND

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Diavik Diamond Mines Inc. in the Northwest Territories, Canada produces eight million carats of diamonds annually. Excavation of the kimberlite pipes requires ammonium nitrate explosives. Residual N washed into the mine pit is piped to a nearby mine water collection pond where N removal is accomplished by microbial activity prior to a final water treatment step and release into the pristine sub-Arctic lake, Lac de Gras. In the pond, microbial removal of ammonium and nitrate is rapid during the brief ice-free summer, but slows considerably under ice cover that persists up to nine months of the year. Water samples were collected across the length of the collection pond. Community analysis of Bacteria by DGGE showed generally homogeneous populations of relatively low diversity across the length of collection pond with major shifts correlating to changes in temperature, pH, and dissolved oxygen. Archaeal abundance increased

from 2% to 17% of the microbial population from April to December and did not correspond to temperature. Crenarchaeal ammonia monooxygenase genes were not detectable by PCR. Two major OTUs of bacterial ammonia monooxygenase genes were identified with 96-97% sequence identity (521 bp) to the uncultivated "fresh water habitats cluster" and 97-98% sequence identity to *Nitrosospira* cluster 0. Sequences from *Nitrosospira* cluster 0 dominated April samples, whereas the fresh water habitats cluster dominated September sample clone libraries. Maximum potential nitrification activity (PNA) occurred at 12°C and did change significantly month to month, indicating that temperature likely limited ammonium removal rates rather than changes in the microbial community. PNA rates of enrichment cultures were significantly accelerated at both 4 and 12°C by providing zeolite as a cell-attachment and ammonium chelating substrate. Addition of zeolite to the collection pond may provide a relatively inexpensive method to increase ammonium removal year round.

Abstract Category
05 Cold Habitats

PS.05.036 INSIGHTS INTO HAPTOPHYTE BLOOM DYNAMICS IN AN ARCTIC LAKE USING MASSIVELY PARALLEL TAG SEQUENCING

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Increasingly warmer annual temperatures in the Arctic are having a profound effect on the timing of lake ice melt and the resulting ecosystem response. Lake BrayaSo in southwestern Greenland hosts an annual haptophyte algal bloom in late June; the biological and geochemical controls of this bloom remain undefined. This haptophyte is a novel species unique in its colonial physiology and massive alkenone lipid production. In order to identify putative bloom triggers or requisite biological cofactors, we analyzed the spring bloom eukaryotic communities from 2007 and 2009. We extracted DNA from the water column depth corresponding to peak alkenone lipid concentrations and sequenced the V9 variable region of the 18S rRNA gene to infer species' identities. We compared these two bloom communities and their individual water column geochemical profiles to better understand the controls on haptophyte bloom events and how temporal variability in ice-off have impacted these microbial communities. These data will help us predict variability in bloom dynamics with continued seasonal warming.

Abstract Category
05 Cold Habitats

PS.05.037 CHARACTERIZATION OF BIOLOGICAL SOIL CRUSTS FROM MIDRE LOVÉN GLACIER FORELAND, SPITSBERGEN, SVALBARD

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Global change models predict that the speed and magnitude of climate warming will be greatest at the highest latitudes. Glacial regression makes new ground surface available to biotic colonization and establishment of vegetation. In these polar environments pioneering microorganisms such as Cyanobacteria, green algae, lichens, mosses and heterotrophic bacteria are typically the first to colonize the substrate as biological soil crust (BSCs). This study describes community assembly of different types of BSCs on typical high Arctic glacier foreland, to gain a broader understanding about dynamics and development of these important systems.

Sampling sites were situated close to Ny-Ålesund, Svalbard, on the foreland of the Midre Lovén glacier. A general insight into microorganisms colonizing the crusts was gained with microscopy observations and molecular fingerprints (ARISA and T-RFLP) both for Cyanobacteria and Eubacteria. Physical (pH and water content) chemical (C/N, Chl A, Cation Exchange Capacity) and granulometric characteristics of soil were analyzed. Pictures of surfaces were taken in order to determine the heterogeneity and biomass with image analysis. Furthermore nitrogen fixation, oxygen concentration and maximum quantum efficiency of Photosystem II (Fv/Fm) were measured.

The morphological analysis by microscopy allowed us to identify several species of Cyanobacteria and algae. Communities fingerprints were compared and statistically analyzed. Eubacterial community resulted different from site to site, whereas Cyanobacterial one was rather uniform and similar. Nitrogen fixation rate, C/N concentration and granulometric characteristics of soil, confirmed the low nutrients availability of the high Arctic ground and the extremely slow pedogenesis of these soils. Detected biological activities were low.

The phototropic component of these communities was always present. Establishment and development of BSCs is controlled by substrate and microclimatic factors in the earlier stages, eventually these communities are able to affect and enrich the surrounding microenvironment.

Abstract Category
05 Cold Habitats

PS.05.038 CHARACTERIZATION OF BACTERIAL STRAINS ISOLATED FROM ANTARCTIC SOIL

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Psychrophiles can be for study of biological adaptation to the cold environment, and are potential resources for cold adapted enzymes which can be applied for research, industry or bioremediation. In order to obtain a variety of psychrophiles, the Antarctic soil was subjected to cultivation experiments.

The Antarctic soil associated with terrestrial moss vegetation was suspended in saline, and the diluted suspension was spread onto solid medium (pH 7.6) including minerals and 0.1% yeast extract. The plates were incubated at 4 or 15°C for 20~42 days. The colonies appeared were re-inoculated onto same medium to purify the isolates. The isolates were identified by 16S rDNA sequences. The strains assumed to be novel species were then characterized.

A total of 91 strains were isolated, and were classified into 13 genera. 10 strains of them showed less than 97% 16S rDNA sequence similarity, and were assumed to classify into three novel species. The representative strains (*Aurantimonas* sp. SA4125, *Frigoribacterium* sp. SA4127, and *Devosia* sp. SA4129) were further characterized. These three strains were aerobic rods and grew at 5°C. The strain SA4127 grew optimally around 10°C, and no growth occurred at 25°C, indicating that strain SA4127 is psychrophile. Strain SA4127 also showed relatively strong amylase activity at 15°C.

Three bacterial strains isolated from Antarctic soil were characterized. They were assumed to be novel species based on the 16S rDNA sequences. The two of them showed Psychrophilic growth property and amylase activity at 15°C. In order to validate these strains, morphological and chemotaxonomic characterizations are now in progress.

Abstract Category
05 Cold Habitats

PS.05.039 METHANE CYCLING AND MICROBIAL COMMUNITIES IN HIGH ARCTIC WETLANDS

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A considerable part of natural methane emissions originates from Arctic and sub Arctic wetlands. An increase of wetland formation in permafrost areas as a result of warming at high latitudes could cause an increase in the emissions of this potent greenhouse gas. Arctic soil microbial communities are poorly studied, and to be able to predict future fluctuations in methane gas fluxes one has to understand the underlying microbial processes. Studies on methane cycling communities in cold habitats have so far been limited to the diversity and dynamics of the two key-groups methanogenic archaea and methane oxidizing bacteria. However, the net methane emissions are controlled by a complex interplay between these groups and various fermentative microorganisms. To shed light on the complex microbial interactions an in-depth, nontargeted, metatranscriptomic approach was initiated. Samples were collected from a depth profile at two peat wetland locations close to Ny Ålesund, Spitsbergen 78°N, in August 2009. cDNA generated from total community RNA and the enriched mRNA fraction from both sites was subjected to 454-Titanium pyrosequencing together with parallelly extracted metagenomic DNA. In addition, the key enzymes particulate methane monooxygenase and methyl coenzyme M reductase were targeted by microarray analysis and RT-qPCR, respectively. The generated data enabled the first assessment of the abundance of organisms from all three domains of life as well as expressed functional genes related to metabolic processes relevant in the methane cycle. Preliminary results indicate a comparably high microbial activity, even in the highly degraded lower peat layers, with similar taxonomic groups and transcriptional profiles between sites. The present study combines a set of methods for the detailed study and elucidation of microbial networks driving methane cycling in High Arctic wetlands.

Abstract Category
05 Cold Habitats

PS.05.040 DENSITY AND PHYLOGENETIC DIVERSITY OF CULTURABLE POPULATIONS OF COLD-ACTIVE ANAEROBES IN THE TEMPERATE SOIL ENVIRONMENT OF RICE FIELD IN JAPAN

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Psychrophilic and psychrotrophic, *i.e.*, cold-active anaerobes in the soil of rice field were enumerated, and diversity of the anaerobes was phylogenetically analyzed. Soil samples were taken from a rice field during the period from December 2007 to August 2009. Anaerobes were MPN-counted in diluted PY4S medium at 5, 10, 20 or 30°C under O₂-free N₂/CO₂ (95%/5%) atmosphere. 16S rDNA V3 region sequences of cells collected from positive MPN cultures were PCR-amplified with the primer set B341fGC/534r or ARC344fGC/A533r. Amplificates were analyzed by denaturing gradient gel electrophoresis (DGGE). Amplificates recovered from DGGE bands were sequenced and phylogenetically analyzed. The MPN counts (/cm³ fresh soil) at 5 and 10°C were at the level of 10⁶-10⁷, while those at 30°C were at the level of 10⁷-10⁸. The sequence of 16S rDNA V3 region was amplified only with the *Bacteria*-specific primer set. Ten or more bands were detected in DGGE profiles of 5°C MPN cultures inoculated with 10⁻³ dilutions of soil samples. Of 16 dominant bands in DGGE profiles of 5°C MPN cultures, 10 bands were most closely related to known species of cold-active anaerobes including *Clostridium bowmanii* and other species isolated from an Antarctic lake with high sequence similarities. From MPN-cultures at 5 and 10°C, 52 strains of anaerobes were isolated. The strains grew well at 5°C, but fell into three groups according to the range of growth temperature. The groups included that of psychrophilic strains growing only at the temperature below 20°C. The 16S rDNA phylogeny based on the full-length sequence indicated that psychrophilic strains were affiliated with *Clostridium* cluster I, being closely related to psychrophiles including *C. bowmanii* with the similarity of 98.5-99.5%. These results indicated that anaerobic microbial community in the rice field soil includes culturable populations of psychrophilic and psychrotrophic anaerobic bacteria at a high proportion.

Abstract Category
05 Cold Habitats

PS.05.041 MICROBIAL DIVERSITY OF GLERÁ RIVER, NORTHERN ICELAND: EFFECTS OF A LANDFILL SITE

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Northern Iceland lies in the subarctic climate zone. Its microbial biotopes are thus expected to be characterized by low species diversity and low metabolite degradation rates. The effects of domestic and industrial contamination on the natural microbiota in northern Icelandic habitats have thus far received very little scrutiny from the research community. This study aims to characterize the diversity and composition of microbial communities in Glerá river with particular emphasis the effects of landfill leachate thereon.

Water and riverbed sediment samples for culturing and total environmental DNA-extraction were obtained from 16 pristine and contaminated sampling points spanning most of Glerá's 18-km length. Culturable bacteria were described in terms of colony and cell morphology, and subjected to a battery of characterization tests, including degradation of several polymers and known contaminants. Selected strains were identified by 16S rDNA sequencing. The total microbiota is currently being characterized by denaturing gradient gel electrophoresis patterns and by SYBR Green melting curve analysis of real time PCR products targeting intergenic spacer regions adjacent to the 16S rRNA gene.

Enumeration of culturable bacteria on PCA medium yielded colony counts of 10^2 to 10^4 CFU/mL of river water. Sediment counts were substantially higher. Colonial diversity was considerable and increased downstream of the landfill site, as did colony counts on mercury-agar (0.05 mM $MgCl_2$) and on defined media with surfactants as the only available carbon source. A collection of 260 isolates was prepared and subjected to characterization tests with emphasis on biodegradative properties. Selected representatives were identified by 16S rDNA sequencing. Further characterization work on both the culturable and unculturable microbiota is ongoing.

The Glerárdalur landfill site has a substantial impact on the Glerá microbiota.

Abstract Category
05 Cold Habitats

PS.05.042 BIOACTIVE BACTERIA FROM ARCTIC MARINE ENVIRONMENTS

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Bacterial antagonism plays a key role in microbial interactions, being a competitive advantage but also mean of communication. Bacterial metabolites with antagonistic activity could also have biotechnological applications. The present study describes the isolation of marine bacteria from high Arctic environments and their screening for antagonism against pathogenic bacteria.

Environmental samples were collected between 84 and 89° N. Bacterial isolates were obtained from Marine Agar 2216, chitin agar, two low-nutrient agars and an actinobacterial-selective mixture at different temperatures, and screened for bioactivity by replica-plating onto a bacterial pathogen.

Fifty-eight samples, including 17 seawater, 20 sea ice, 11 zooplankton, 3 sediment, and 7 freshwater-related samples (snow, meltwater and frost flowers) were used for isolation of bacteria. Culturable counts for ice samples were above 10^4 CFU/mL, compared to 10^2 CFU/mL for water and zooplankton and below 10^1 CFU/mL for all other samples. Counts on nutrient limited agar did not significantly differ from counts on nutrient rich agar. Also the incubation temperature did not influence the culturable count. 81 strains (representing 0.3% of all tested colonies) antagonized *Vibrio anguillarum* 90-11-287, of which 9 strains also inhibited *Staphylococcus aureus* 8325. The most pronounced bioactive strains are presently characterized with respect to identity and phenotypic characteristics. In addition, several strains with chitinolytic activity were isolated and are also being identified.

This study shows that culturable bacteria in high Arctic marine environments harbor strains with bioactive properties. These findings contribute to the knowledge of bacterial antagonism and the diversity of culturable bioactive bacteria in polar environments. The antagonistic isolates are implemented in an ongoing research project on bioactive bacteria from the World's Oceans.

Abstract Category
05 Cold Habitats

PS.05.043 TINY GIANTS THAT WERE OVERLOOKED: PROTEOGENOMIC ANALYSIS OF A VIRALLY-DOMINATED HYPERSALINE ANTARCTIC LAKE

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Organic Lake is a hypersaline, meromictic (stratified) lake in the Vestfold Hills, Antarctica. Due to its high salinity (6Xs seawater) lake biota is entirely microbial. This perennially cold (-13°C), low-complexity system is particularly amenable to community molecular-based analyses. Its isolation and the paucity of species data make it a possible reservoir of novel taxa. This study aimed to characterise the microbial community of Organic Lake and to develop a global understanding of microbial diversity and dynamics within this extreme environment.

Water samples were collected from Organic Lake during the austral summer. Biomass passed through a 20µm pre-filter, then sequentially filtered onto 3.0µm, 0.8µm and 0.1µm pore-sized filters. Environmental DNA was sequenced by the J. Craig Venter Institute from all size fractions using a combination of Sanger and 454 sequencing technologies and the resulting reads were used to generate hybrid assemblies. Metaproteomic analysis was conducted on protein extracted from the same samples used to generate the metagenomic data.

Microbial diversity of Organic Lake is low with the surface community dominated by large algal viruses related to marine Phycodnaviruses. The viruses likely infect unicellular algae of the phylum *Haptophyceae*. A novel virus type was also detected that is a putative relative of the recently described virophage family (La Scola et al. 2008). Near-complete genomes have been assembled of both viral families and corresponding proteins were identified.

The proteogenomic analysis of Organic Lake has revealed the diversity and structure of a previously undescribed viral population while simultaneously allowing for its functional characterisation. The role of viruses in this low-complexity system may offer insights into their function and dynamics in the marine environment.

La Scola B et al. (2008). The virophage, a unique parasite of the giant mimivirus. *Nature* 455:100-104

Abstract Category
05 Cold Habitats

PS.05.044 METAGENOMIC ANALYSIS OF DIESEL BIOREMEDIATION IN CANADIAN HIGH ARCTIC SOILS

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With the opening of the Northwest Passage, human activities in the Canadian high Arctic will increase, thereby increasing the risks of accidental pollution. Soil bioremediation in the high Arctic is complicated by the low temperatures and the low availability of nutrients and water. Large-scale bioremediation field experiments were set-up at Eureka and Alert, Ellesmere Island, Nunavut, Canada to test the effects of nutrient addition on diesel degradation by indigenous soil microorganisms. Microarray and RT-qPCR analyses indicated that large functional and phylogenetic shifts were associated with efficient pollutant removal following nutrient addition. To further our knowledge of the microorganisms and associated functional genes involved in cold-temperature hydrocarbon degradation, the metagenome of soils from the Alert bioremediation experiment were sequenced its metagenome through a time course (before nutrient addition, after 1 month, after 1 year) and compared this to the metagenome of uncontaminated control soils. Results confirmed that large shifts in the microbial community occur immediately following nutrient addition, steering the community toward more efficient hydrocarbon degradation. Bacteria known for their hydrocarbon degradation capacity (e.g. *Pseudomonas*, *Rhodococcus* and *Sphingomonas*) were detected in the time zero and the control soil bacterial communities and became dominant at different time points after nutrient addition. A wealth of hydrocarbon degrading genes were also identified, many of which could be potential candidates for highly efficient cold-adapted hydrocarbon-degrading enzymes. In summary, the metagenomic approach undertaken here gives an unprecedented view of the ecology of hydrocarbon degradation in Canadian high Arctic soils and suggests that the indigenous bacterial communities possess hydrocarbon degradation capacity that may be limited by nutrient availability.

Abstract Category

05 Cold Habitats

PS.06.001 STRATEGIES FOR SELECTING HYDROGEN PRODUCING MICROORGANISMS AFFECT MICRO AND MACRO STRUCTURE OF GRANULAR SLUDGE

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Mixed-culture dark fermentation is an environmentally friendly bio-hydrogen production process. In this work we study the potential for directing microbial anaerobic mixed communities towards improved hydrogen production. Strategies applied for promoting the selection of hydrogen-producing bacteria in anaerobic granular sludge consisted of heat treatment and chemical treatment with 2-bromo-ethane sulfonate (BES) and with BES+Chloroform. Three expanded granular sludge bed reactors, R_{Heat} , R_{BES} and $R_{BES+Chlo}$, were inoculated with the treated sludges and fed with synthetic sugar-based wastewater. Hydrogen production was monitored. Morphological integrity and microbial diversity of the granules were studied by using image analysis technique and molecular techniques (DGGE, cloning and sequencing), respectively. Hydrogen production in R_{Heat} was below $300 \text{ mLH}_2\text{L}^{-1}\text{d}^{-1}$. In $R_{BES+Chlo}$ hydrogen production rate never exceeded $300 \text{ mLH}_2\text{L}^{-1}\text{d}^{-1}$. In this sludge, a physical deterioration of the granules was observed with a decrease of their density and microbial diversity. In R_{BES} , a transient period of unstable H_2 production was observed but an additional pulse of BES triggered hydrogen production rate to an average value of $700 \pm 200 \text{ mLH}_2\text{L}^{-1}\text{d}^{-1}$, which was kept for 30 days. This strategy did not affect significantly granular sludge structure. The dominant bacterial ribotypes displayed in the DGGE profiles along the R_{BES} operation were found to be closely related to *Clostridium* species, e.g. *Clostridium ljungdahlii* (99% identity), *Clostridium drakei* (94% identity), and to uncultured microorganisms belonging to *Clostridiaceae* and *Ruminococcaceae*. The strategies used for selecting hydrogen producing microorganisms affected both, micro and macro structure of granular sludge. Image analysis allowed the identification and quantification of changes on the morphological properties of granular sludge that could be related to bacterial community dynamics and diversity.

Abstract Category

06 Engineered Environments

PS.06.002 SCREENING OF AMMONIA-OXIDIZING ARCHAEA ABUNDANCE IN DOMESTIC AND INDUSTRIAL WASTEWATER TREATMENT PLANTS

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We investigated the occurrence and abundance of Ammonia Oxidizing Archaea (AOA) in wastewater treatment plants (WWTPs). Samples were collected from anaerobic reactors

of 16 different domestic and industrial WWTPs, including petroleum and oil refineries; food, alcohol and chemical industries and landfill leachate treatment plant. All the genomic DNA were extracted from activated sludge using a commercial kit for soil and later the samples were tested using PCR primers targeting archaeal ammonia monooxygenase subunit A (amoA) genes. In addition to WWTPs, numerous environmental soil samples were collected and screened for AOA occurrence. We report that there is no molecular evidence on AOA abundance in WWTPs. Considering AOA grows most efficiently between 1 to 3 mM (~25mg N/liter) ammonium concentrations. Landfill leachate treatment plant contains 100 to 200mM (~2500 mg N/liter) ammonium, which is considered as very high for the reactor to harbor AOA; and other anaerobic reactors contains 0.2 to 0.8 mM (~5 mg N/liter) ammonium, which is very low. Sludge retention times of the reactors which are screened are either too high or too low for AOA to grow efficiently. It is clear that conventional parameters of WWTPs inhibit growth and activity of AOA. Results indicate that AOA are not abundant in engineered systems as it has been widely distributed in both pristine and agricultural soil samples but their activity yet shall be studied. Acknowledgment: This project is supported by The Scientific and Technological Research Council of Turkey (TUBITAK- Project No: TBAG 107T680)

Abstract Category
06 Engineered Environments

PS.06.003 TARGETED DISCOVERY OF GLYCOSIDE HYDROLASES FROM A SWITCHGRASS-ADAPTED COMPOST MICROBIAL COMMUNITY

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Development of cellulosic biofuels from non-food crops is currently an area of intense research interest. Tailoring depolymerizing enzymes to particular feedstocks and pretreatment conditions is one promising avenue of research in this area. Here we added a green-waste compost inoculum to switchgrass (*Panicum virgatum*) and simulated thermophilic composting in a bioreactor to select for a switchgrass-adapted community and to facilitate targeted discovery of glycoside hydrolases. Small-subunit (SSU) rRNA-based community profiles revealed that the microbial community changed dramatically between the initial and switchgrass-adapted compost (SAC) with some bacterial populations being enriched over 20-fold. We obtained 225 Mbp of 454-titanium pyrosequence data from the SAC community and conservatively identified 800 genes encoding glycoside hydrolase domains that were biased toward depolymerizing grass cell wall components. Of these, ~10% were putative cellulases mostly belonging to families GH5 and GH9. We synthesized two SAC GH9 genes with codon optimization for heterologous expression in *Escherichia coli* and observed activity for one on carboxymethyl cellulose. The active GH9 enzyme has a temperature optimum of 50°C and pH range of 5.5 to 8 consistent with the composting conditions applied. We

demonstrate that microbial communities adapt to switchgrass decomposition using simulated composting and that full-length genes can be identified from complex metagenomic sequence data, synthesized and expressed resulting in active enzyme.

Abstract Category
06 Engineered Environments

PS.06.004 THREE-DIMENSIONAL STRATIFICATION AND STRUCTURE OF AOB-ANAMMOX BIOFILMS OF DIFFERENT AGE FROM INTERNAL BIOCARRIER COMPARTMENTS

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In situ studies of aerobic ammonia oxidizers (AOB) and anaerobic ammonia oxidizers (anammox) biofilm structure and population distribution are crucial for understanding and improving biological nitrogen removal in wastewater treatment. Biocarriers with high protected internal surface area, such as the AnoxKaldnes Biofilm-Chip carriers, are often used to facilitate biofilm growth. However, the dimensions of the small internal compartments hamper in situ biofilm studies of this important environment. Using a cryosectioning approach combined with Fluorescence *in situ* Hybridization (FISH), intact biofilms of different ages from biocarrier compartments in a moving bed biofilm reactor were examined with regard to their structure and population distribution in three dimensions. These multi-species biofilms contained anammox related to *Brocadia* sp., *Nitrosomonas europaea*-related AOB and a diverse community of putative heterotrophic bacteria. High-resolution composite FISH-images of entire biofilm sections in the xy-dimension ("wall to wall") from different depths (z) revealed stratification in all dimensions in mature biofilm, probably due to oxygen gradients. In these square-shaped sections, at least three regions of varying population distribution were observed in the xy-dimension. On average anammox was ~40% of total bacteria, increasing with the overall thickness of the biofilm and with depth, in the z dimension. AOB on the other hand displayed the reverse pattern. Younger biofilms (4 months old) were dominated by AOB (~80% of all bacteria) compared to the older biofilms (~12% AOB). However, anammox made up less than one percent of the bacterial population and only a few small microcolonies were observed, suggesting that the anammox population had just recently begun to establish on these carriers. These observations increase our general understanding of AOB -anammox biofilm growth and may impact the future design of biocarriers.

Abstract Category
06 Engineered Environments

PS.06.005 DIVERSITY OF DENITRIFYING REDUCTASE GENES IN A MARINE METHANOL-FED DENITRIFICATION REACTOR

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The Montreal Biodome operated a methanol-fed fluidized denitrification reactor to remove nitrate from a 3 million-liter seawater aquarium. The composition of the microbial communities of the denitrifying biofilm was previously determined in our laboratory. Nitrate respirers such as *Methylophaga* sp. JAM1 and *Nitratireductor aquibiodomus* NL21 and denitrifying bacteria such as *Hyphomicrobium* sp. NL23 were identified. However, we believed that more denitrifying bacteria exist in the biofilm. We investigated the diversity of the *narG*, *napA*, *nirS*, *nirK*, *norB* and *nosZ* that encode for the reductases involved in the different steps of nitrate reduction to N₂ in the biofilm but also in the bacterial strains that we isolated from the biofilm. Different sets of degenerated primers were used to detect, clone and sequence these genes. Depending of the denitrifying genes, between 1 and 9 different sequences were PCR-amplified from the DNA extracted from the biofilm. All these sequences were affiliated to Proteobacteria. Our results showed that the diversity of the denitrifying bacteria was dominated by *nirS*-type denitrifiers. Also we found a higher diversity of *narG* than *napA* in the biofilm. Furthermore, we detected *napA*, *nirK*, *norB* and *nosZ* in *Hyphomicrobium* sp. NL23, *narG* and *nirK* in *N. aquibiodomus* NL21 and two different *narG* in *Methylophaga* sp. JAM1. Expression assays suggested that *Hyphomicrobium* sp. NL23 and *Methylophaga* sp. JAM1 could contributed to the nitrate reduction activity in the biofilm. Further studies will be achieved to determine the abundance of these genes by quantitative PCR.

Abstract Category
06 Engineered Environments

PS.06.006 EVALUATION OF ANAMMOX ACTIVITY AND DISSIMILATORY NITRATE REDUCTION IN THE PRESENCE OF ACETATE OR PROPIONATE.

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Anaerobic ammonium oxidation (Anammox) is effective nitrogen removal process which has possibility of applying to anaerobic digested effluent. Anammox bacteria are recognized to have poor resistance to organic compounds. Consequently, we investigated the influence of short-chain fatty acids which contained in digested effluent to Anammox activity. Batch tests were conducted with anammox granule to evaluate the anammox activity in the presence of acetate or propionate. The evaluation of anammox activity was based on the measurement of ammonium removal rate. In this granule, anammox bacteria detected with the Amx820 probe accounted for more than 73% of total bacteria. Predominant species in anammox granules were closely related to *Canadidatus "Brocadia fulgida"* with 94% sequence similarity. To confirm whether nitrate reduction occurs or not, batch tests with a medium (ammonium, nitrate, and acetate without adding nitrite)

were carried out. In addition, to investigate which bacteria carried out nitrate reduction, anammox bacteria were inhibited by 1 M methanol. Ammonium activity with or without nitrate were $105.2 \text{ mg-N L}^{-1} \text{ day}^{-1}$ and $82.5 \text{ mg-N L}^{-1} \text{ day}^{-1}$, respectively. The difference between these two anammox activities might attribute to endogenous denitrification. The anammox activity increased about 10-20% compared with normal anammox series with a medium (ammonium, nitrite, nitrate, and acetate/propionate). Furthermore, anammox activity was also detected with a medium (ammonium, nitrate, and acetate without adding nitrite), though anammox activity was not detected when using anammox granules inhibited by methanol. These results indicated that the anammox bacteria can supply nitrite derived from nitrate with acetate and perform anammox reaction even if nitrite was absent in the medium. We speculated that increase of anammox activity in the presence of acetate is due to the dissimilatory nitrate reduction by dominant anammox bacteria in granules used in this study.

Abstract Category
06 Engineered Environments

PS.06.007 QUANTITATIVE ANALYSIS OF METHANOGENIC COMMUNITIES IN INVERTED FLUIDIZED BED (IFB) AND EXPANDED GRANULAR SLUDGE BED (EGSB) REACTORS AT 37°C, 25°C, 15°C

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Psychrophilic anaerobic digestion (PAD) represents a cost-effective wastewater treatment option, by comparison to mesophilic AD. Reactor configuration can be a key driver for enhanced PAD, but little is known about quantitative methanogenic community dynamics associated with diverse reactors. This study aimed to: 1. Quantitatively compare methanogenic community structure and dynamics in two different anaerobic reactor types: IFB and EGSB. 2. Investigate the effect of temperature decrease on reactor methanogenic community structure. Lab-scale IFB and EGSB reactors, treating dairy wastewater, were operated identically for 630 days and the temperature was reduced from 37°C to 25°C and finally to 15°C in three stages. Methanogenic community analysis was performed using quantitative real-time PCR. Non-metric multidimensional scaling (NMDS) analysis was employed to visualize community shifts, in response to temperature changes. In the range of 37-25°C both reactors exhibited similar performance. At 15°C, however, the IFB demonstrated better stability and process performance, while a significant deterioration in the treatment efficiency of EGSB occurred. The methanogenic communities of the two reactors were strikingly different, particularly at 15°C. Methanosarcinaceae were detected only in the IFB reactors. Methanobacteriales was the most dominant group at 37°C and 25°C in both reactors but Methanomicrobiales prevailed at 15°C and were more abundant in IFB. The Methanosaetaceae population remained at a similar level in both reactors, at all temperatures studied. NMDS revealed that methanogenic communities evolved in

different manner in each reactor with decreasing temperature. Consequently: 1. Methanogenic communities in the IFB and EGSB reactors displayed significantly different quantitative profiles, suggesting that reactor configuration influences methanogenic community structure. 2. Better stability and process performance of IFB at 15°C favours this configuration for PAD

Abstract Category
06 Engineered Environments

PS.06.008 FACTORS INFLUENCING SUNLIGHT INACTIVATION OF INDICATOR MICRO-ORGANISMS IN AQUATIC ENVIRONMENTS

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Sunlight is considered the predominant factor causing micro-organism inactivation in surface and wastewaters. Attenuation especially of the more lethal wavelength ranges e.g. UVB, is rapid in turbid environments where environmental factors such as pH, dissolved oxygen (DO) and photosensitizers may have a synergistic effect on sunlight inactivation. A study on the affect of pH and DO on UVA, UVB and photosynthetically active radiation (PAR) inactivation of the coliphages MS2, ΦX174 and *Escherichia coli* and *Enterococcus* sp. in optically clear water was conducted as part of a larger study on the role photosensitizers present in turbid waters.

Micro-organisms were spiked into 300 mL solutions of sterile distilled water at various DO concentrations and pH. The vessels, with quartz lids were irradiated with normally incident light (UVA, UVB or PAR), at environmentally relevant doses, at 20° C for up to 48 hrs. Numbers of surviving organisms were quantified over time and inactivation rate constants calculated.

For all wavelengths the relative sensitivity of micro-organisms to inactivation was ΦX174 > *E. coli* > *Enterococcus* sp. > MS2. UVB incubations produced inactivation rates 10 to 200 times faster than for the other wavelengths. Increasing pH had a significant, positive affect on inactivation rates for all micro-organisms ($P < 0.05$) except ΦX174 at all wavelengths, but not in the dark. The affect of DO was small, but significant for *E. coli* which experienced increased inactivation with increasing DO ($P < 0.001$).

Synergistic affects on inactivation rates were observed for DO and to a greater extent pH with light in optically clear water even in the absence of sensitizers; however this observation was organism dependent. This implies that micro-organism inactivation rates would vary depending on the particular freshwater environment and more specifically the values of DO, pH and the amount of sunlight entering the system.

Abstract Category
06 Engineered Environments

PS.06.009 BIOREACTOR SIMULATING NITRATE INJECTION IN A LOW-TEMPERATURE OIL RESERVOIR.

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Water injection in oil reservoirs stimulates sulfate-reducing bacteria (SRB). The sulfate in the injection water is reduced to sulfide promoting reservoir souring. H₂S production is costly to both inland and offshore operations. Nitrate injection is useful in promoting nitrate-reducing bacteria (NRB) to counter SRB activities in situ via molecular mechanisms. Nitrate is effective in high temperature reservoirs while low temperature fields are more challenging. Field data prompted an investigation into the effects of a continuous and pulsed nitrate injection regime on NRB and SRB community dynamics at the near injection well-bore region. Two up-flow packed-bed bioreactors (at 22°C) were used to simulate an inland, low-temperature and low-sulfate field under anaerobic conditions. The continuous nitrate treated bioreactor was supplied with 2 mM sulfate, 4 mM nitrate and 3 mM volatile fatty acids (acetate, propionate and butyrate). The pulsed nitrate treated bioreactor was subjected to continuous 2 mM sulfate and 3 mM VFA with discontinuous 4 mM nitrate at 2-week intervals. We used both analytical and molecular tools to analyze these systems. Sulfide output remained constant at 2 mM with continuous nitrate injection, while a pulsing regime was effective in lowering sulfide to zero but this was followed by recovery. The microbial activities in both bioreactors were present as a biofilm at the influx point where the limiting electron acceptor and excess electron donor mixed. Community biofilm analysis revealed that microbial zonation developed during continuous injection, with nitrate being reduced close to the influx point by NRB, while sulfate passed through to adjacent SRB-inhabited zones. Sulfide was thus continuously produced from these outlying areas and no nitrate control was demonstrated. In contrast, a pulsed nitrate treatment was able to effectively lower sulfide output because it disrupted zonation allowing nitrate to penetrate the SRB-inhabited zone.

Abstract Category
06 Engineered Environments

PS.06.010 MICROBIAL COMMUNITY COMPOSITION OF SULFIDE OXIDISING BIOFILMS RESPONSIBLE FOR SEWER CORROSION.

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Microbially induced concrete corrosion in sewer networks is a worldwide problem with significant economic and environmental consequences. In order to develop effective mitigation strategies, it is essential to characterise the microbial biofilm communities responsible and elucidate how their activities promote concrete corrosion. Previous

studies have revealed that the sulfur cycle is central to this corrosion, with hydrogen sulfide undergoing microbial oxidation to corrosive sulfuric acid. As a result, management strategies have focused on controlling hydrogen sulfide levels through the use of chemicals to oxidise or precipitate hydrogen sulfide to prevent its availability for microbial oxidation. Historically, *Acidithiobacillus thiooxidans* has been considered the main sulfur oxidising microorganism responsible for this process. We aim to perform the first comprehensive analysis of microbial communities associated with concrete corrosion. In September 2009 and March 2010 sewer biofilms were collected from the same main gravity trunk site within Sydney sewer network. Community diversity and composition was assessed by high-throughput 16S/18S rRNA gene amplicon sequencing using universal primers. Preliminary phylogenetic analysis revealed higher than expected bacterial and eukaryotic diversity. Only 10 of the 110 phylotypes were represented by >1% of the sequences, these 10 accounted for >85% of all sequences. The three most dominant phylotypes represented greater than 70% of sequences generated and clustered with the *Acetobacteraceae* (~21%), *Mycobacteriaceae* (~25%) and *Xanthomonadaceae* (~25%). *A. thiooxidans* was detected at relatively low abundance (~4%). The dominance of *A. thiooxidans* has been overstated in previous research and concrete corrosion is more likely the result of the activity of a diverse microbial community. Further research will reveal what role these new organisms play and allow for an improved understanding of the corrosion process.

Abstract Category
06 Engineered Environments

PS.06.011 MICROBIAL ECOLOGY AND BIOKINETICS OF GLYCEROL INDUCED DENITRIFICATION IN A MOVING BED BIOFILM REACTOR

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As a principal component of biodiesel waste, glycerol can support higher rates of nitrate removal when compared to methanol. However, little is known about the microbial ecology and biokinetics of glycerol induced denitrification. Moreover, the characterization of the niche differentiation of the suspended phase and biofilm bacteria in a moving bed biofilm reactor is essential to describe the constituent dynamics of microbial activities and substrate utilization. This study was aimed at investigating the biofilm and suspended microbial communities assimilating glycerol to support heterotrophic denitrification in a sequencing batch moving bed biofilm reactor. Glycerol assimilating bacteria in both phases were investigated using ¹³C-DNA stable isotope probing (SIP). Based on ¹³C DNA clone libraries, dominant species in both phases were identified and then quantified via 16S rRNA based real-time PCR assays to study the dynamic changes of their abundance in both phases during one year of operation. Denitrification performance was indicated by measuring effluent nitrate, nitrite and COD concentrations, and biokinetics were determined via extant batch assays. *Comamonas badia* and *Diaphorobacter sp.* related species were dominant in the suspended phase. Biofilm communities were more diversely dominated by *Comamonas badia*, *Bradyrhizobium sp.* and *Tessaracoccus bendigoensis* related populations. Near complete

nitrate removal was achieved during steady-state operation and the specific denitrification rate of suspended biomass (4.6 ± 1.6 mg-N/gCOD/h) was higher than that of the biofilm (3.9 ± 1.2 mg-N/gCOD/h, $P=4.33E-4$). Niche differentiation between two phases possibly resulted from different affinities to nitrate and glycerol after long-term adaptation to the gradients of substrate concentrations in the biofilm. Lower affinity to substrate (carbon and nitrate) in the suspended phase possibly resulted in lower biokinetics compared to the biofilm.

Abstract Category
06 Engineered Environments

PS.06.012 MICROBIAL ECOLOGY OF AMMONIA OXIDIZING BACTERIA AT A FULL-SCALE DRINKING WATER-SHED IN RESPONSE TO ALTERNATING CHLORINATION AND CHLORAMINATION

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Nitrification is one of the most prevalent triggers for deterioration of drinking water quality in chloraminated drinking water distribution systems. Short-term chlorination is a known remedy to curb nitrification as a result of chloramination. The overall goal of this study was to examine changes in ammonia oxidizing bacteria (AOB) community structure in response to chlorine shocks at a full-scale water distribution system over a one and half year monitoring period. During this period, AOB populations were monitored at several locations of an entire county in the State of Florida from 2007-2009, which had been subjected to chloramination with one month of free chlorination. *It was hypothesized that the imposition of chlorination would result in the reduction of overall AOB diversity and result in selective proliferation of these 'persistor' AOB upon return to chloramination.* Based on phylogenetic analysis of several DGGE bands, two novel AOB, distantly related to *Nitrosomonas marina*, and *Nitrosomonas nitrosa* (83% and 82% similarity, respectively), were dominant throughout the water distribution system and intermediate storage tanks. The widely documented AOB related to *Nitrosomonas oligotropha* were notably absent throughout the monitoring period at all locations. In addition, the imposition and recovery from the shock chlorination episode did not change the AOB community structure. Thus, our initial hypothesis was rejected. It was further determined that two specific upstream sites were the source of the novel AOB species, from where they seemed to be transported downstream. Based on the *amoA* abundance monitoring using quantitative PCR (qPCR), a more efficacious disinfection strategy is proposed consisting of an earlier chlorine shock during the summer with additional chlorination in winter, especially focusing on dead-end branches, to improve the biological stability and drinking water quality.

Abstract Category
06 Engineered Environments

PS.06.013 INTRODUCING A COMPUTER PROGRAM FOR SELECTING RESTRICTION ENZYMES FOR TERMINAL RESTRICTION FRAGMENT LENGTH POLYMORPHISM BASED ON PHYLOGENETIC DISTANCE

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Recently, attention on rational enzymes selection for terminal-restriction fragment length polymorphism analysis is increasing. However, suitable computational tools in optimizing congruency between terminal-restriction fragment sizes and phylogenetic distance-based operational taxonomic units were lacking. To address this problem, we developed a computer program that helps selecting appropriate restriction enzymes sets from user-provided sequences. Briefly, our program compares the congruency between operational taxonomic units and terminal restriction fragments sizes of each sequence pairs, and the program provides the score based on congruency for each enzyme or enzymes sets. To explore potential enzyme or enzymes set, users can decide several parameters such as phylogenetic distance levels, degree of enzyme combination and weighted methods. We tested the program with archaeal ammonia monooxygenase alpha-subunit gene sequences retrieved from wastewater treatment plants. With different phylogenetic distance levels including 0%, 3%, 7% and 12%, we analyzed the sequences against 189 restriction enzymes from REBASE for both single enzyme and double enzymes combinations. With the parameters tested, double enzymes generated highest score. We also verified the *in silico* results using BsaAI-HinP1I, one of the highest-scored combination obtained with unweighted method at 0% level, towards clones and environmental samples, and demonstrated applicability of this enzymes set for archaeal ammonia monooxygenase alpha-subunit. In sum, our program is both efficient and feasible in exploring the potential enzymes combinations towards provided sequences, we also suggest further application of the program towards other target genes with larger sequence database in future study.

Abstract Category

06 Engineered Environments

PS.06.014 KINETICS STUDY OF SULFIDE OXIDATION BY NITRATE REDUCING-SULFIDE OXIDIZING BACTERIA IN WASTEWATER BIOFILMS

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Kinetics of net sulfide oxidation by nitrate reducing-sulfide oxidizing bacteria in wastewater biofilms was studied in the laboratory, in biofilms grown *in situ* at the "Guadalete"-Waste Water Treatment Plant (Jerez de la Frontera, Spain). Sulfide and pH microelectrodes were used to monitor quick changes in both variables at the water-

biofilm interface and within the biofilm. Nitrate addition reduced significantly sulfide concentration within the biofilm and increased the net rate of sulfide oxidation, calculated from the changes in the vertical profiles of sulfide. Both processes were dependent on the added nitrate concentration. The decreases in sulfide concentration and the increase in the net rate of sulfide oxidation respect to nitrate concentration followed a Michaelis-Menten kinetics, with a half saturation constant of 21 and 17 $\mu\text{M NO}_3^-$ for total sulfide and net rate of sulfide oxidation respectively. The stimulation of the net sulfide oxidation by nitrate was a clearly reversible process. Net sulfide oxidation rate decreased to initial values in 3-6 h after the suppression of nitrate addition. Net sulfide oxidation rate and the recovery rate of sulfide levels after the suppression of nitrate addition were dependent on total sulfide and on nitrate concentrations, suggesting a double-substrate kinetics. Mass balance analysis and the stoichiometry of the process showed that neither sulfide oxidation nor nitrate reduction were completed to H_2S and N_2 respectively, what suggested that intermediary compounds as elemental sulfur and nitrite accumulated in our experimental system.

Abstract Category
06 Engineered Environments

PS.06.015 HYDROGEN PRODUCING MICROBIAL COMMUNITIES OF THE BIOCATHODE IN A MICROBIAL ELECTROLYSIS CELL

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In the search for alternatives for fossil fuels and the reuse of the energy from waste streams, the microbial electrolysis cell is a promising technique. The microbial electrolysis cell is a two electrode system in which at the anode organic substances, including waste water, are used by microorganisms that release the terminal electrons to the electrode. These electrons are subsequently used at the cathode resulting in the production of a current. By addition of a small voltage, hydrogen gas can be produced by combining electrons and protons at the cathode. To catalyse the hydrogen evolution reaction at the cathode, expensive catalysts such as platinum are required. Recently, the use of biocathodes has shown great potential as an alternative for platinum. The microbial community responsible for the hydrogen evolution in such systems is, however, not well understood. In this study we focused on the characterization of the microbial communities of the microbial electrolysis cell biocathode using molecular techniques. The results show that the microbial community consists of 44% *Proteobacteria*, 27% *Firmicutes*, 18% *Bacteroidetes* and 12% related to other phyla. Within the major phylogenetic groups we found several clusters of uncultured species belonging to novel taxonomic groups at genus level. These novel taxonomic groups developed under environmentally unusual conditions and might have properties that have not been described before. Therefore it is of great interest to study those novel groups further.

Within the *Proteobacteria* a major cluster belonged to the *Deltaproteobacteria* and based on the known characteristics of the closest related cultured species, we suggest a mechanism for microbial electron transfer for the production of hydrogen at the cathode.

Abstract Category
06 Engineered Environments

PS.06.016 ARE ACTIVATED SLUDGE PHOSPHATE-STORING BACTERIA DISSIMILATORY METAL (IRON) REDUCERS?

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Despite thirty-five years of research in microbiology, biochemistry and lately molecular microbial ecology of enhanced-biological-phosphorus-removal, this important wastewater treatment process remains poorly understood. Lack of fundamental knowledge is commonly attributed to the difficulty to obtain pure cultures of responsible phosphorus-accumulating microorganisms, disabling the option to assess key features of their metabolism *in vitro*. The objective of this critical paper is to strike the observed knowledge gap at the root by highlighting a pivotal but neglected step in research methodology and general microbial ecological approach.

Compared to the Anammox case the research trajectory reveals indeed a crucial difference with far-reaching implications. Failing is an initial step of postulating the ecological niche that phosphorus-storing bacteria are assumed to occupy. This inadvertence in methodology has deprived research of a fundamental premise to guide cutting-edge theoretical and experimental work. It explains the overall empirical character of scientific progress so far, its weak foundation in microbial and theoretical ecology and the inability of powerful biomolecular tools to answer most generic questions with respect to the organisms involved.

Application of the ecological niche concept was found to open a new dimension in the fundamental understanding of enhanced biological phosphorus removal from wastewaters. Based on the well-known impact on process performance of environmental parameters, gradients and limitations, the responsible microbes could be assigned a conjectural position within the broad metabolic classification of organo-heterotrophic bacteria that significantly differs from common belief. From this rational approach it was inferred that the mechanistic clue behind the process is not a distinctive feature or pathway in the carbon metabolism of the bacteria involved, but rather a peculiarity of their respiratory ability: metal respiration.

Abstract Category
06 Engineered Environments

PS.06.017 INVOLVEMENT OF THERMOPHILIC ARCHAEA IN THE BIOCORROSION OF OIL PIPELINES

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Sulfide accumulation resulting from microbial metabolic activities is an important mechanism of biocorrosion. In addition to sulfate- and thiosulfate reducing bacteria, thermophilic sulfate- and sulfur reducing Archaea can contribute to sulfide production in high-temperature petroliferous environments. A hyperthermophilic sulfur reducing archaeon, strain PK, was isolated from solids scraped from inner surface of a hot oil pipeline. We characterized the isolate to test its prospective role in pipeline corrosion processes.

The organism was cultivated under strictly anaerobic conditions and isolated by serial dilution in a basal marine medium containing yeast extract and elemental sulfur instead of sulfate. All incubations were at 80°C. Growth was followed by turbidity, cell counts and sulfide production. Sulfide was determined colorimetrically and iron reduction was assayed as ferrous iron accumulation. The organism was identified by phylogenetic analysis based on 16S rRNA gene sequencing.

Cells of strain PK are coccoidal and growth was observed within a temperature and pH range of 55-85°C (opt. 80°C) and 5-8 (opt. 6.5-7.0), respectively. The isolate is a strict anaerobe that can ferment organic substrates, and grow with the reduction of elemental sulfur or iron. It does not reduce sulfate, thiosulfate or nitrate. Phylogenetic analysis revealed that strain PK is most similar to *Thermococcus aegaeus* strain DSM 12767 and to uncultured clones from a North Sea high temperature oil reservoir.

Sulfide and ferrous iron resulted from metabolic activity of *Thermococcus*, str. PK and thus this organism can contribute to biocorrosion in high temperature oil processing and transport facilities.

Abstract Category

06 Engineered Environments

**PS.06.018 MICROBIAL COMMUNITY DYNAMICS FROM
GROUNDWATER AND SURROGATE SEDIMENTS DURING HRC[®]
STIMULATION AT A CHROMIUM CONTAMINATED FIELD SITE**

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The Hanford 100-H site is a chromium-contaminated site that has been designated by the Department of Energy Environmental Management as a field study site for *in situ* chromium reduction. In August 2004, the first injection of hydrogen release compound (HRC[®]) resulted in an increase of microorganisms and reduction of soluble chromium(IV) to insoluble chromium(III). Little is understood about the microbial community composition and dynamics during stimulation. The aim of this study is to

compare microbial communities of groundwater and soil samples across time and space during a second injection of HRC[®]. A second injection occurred November 2008 and geochemical data collected throughout the study showed an overall decrease in nitrate, sulfate, and chromium(IV). Spatial and temporal water and soil samples were collected pre- and post-injection from four wells at the field site. "Bug traps" constructed from stainless steel mesh were lined with nylon mesh and filled with Hanford soils from the 100-H site. The "bug traps" were used to represent not only the microbes flowing through the soil via groundwater, but the microbes that require a matrix in order to grow. DNA was extracted from each of the samples and the SSU rDNA was sequenced via multiplex pyrosequencing. Preliminary results do not indicate a large shift in dominant organisms in soil from pre- to post-injection, and this may be due to the organisms remaining dominant from the first stimulation. However, there was a shift from *Acidovorax* to *Aquaspirillum* from upstream (non-stimulated) to downstream soil both pre- and post-injection. Furthermore, while post-injection soil samples indicate a continuing dominance of *Aquaspirillum*, corresponding water samples indicate *Pseudomonas* as a dominant genus. These results demonstrate community differences based on water and soil samples and elucidate community dynamics upon stimulation for chromium reduction.

Abstract Category

06 Engineered Environments

**PS.06.019 MICRODIVERSITY OF NITROSPIRA-LIKE NITRITE-
OXIDIZING BACTERIA IN ACTIVATED SLUDGE**

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The genus *Nitrospira* represents the dominant nitrite-oxidizing bacteria in most wastewater treatment plants (WWTPs). Two *Nitrospira* lineages were previously found in WWTPs, but more detailed data about *Nitrospira* diversity in engineered systems are still lacking. In this study, the population structure and function of *Nitrospira* in activated sludge was investigated at a very high resolution by a multiphasic approach that included marker gene phylogeny, deep sequencing, metagenomics, and isotope-labelling techniques. We designed new FISH probes targeting single nucleotide polymorphisms (SNPs) in highly similar *Nitrospira* 16S rRNA genes, which had been retrieved from a nitrifying WWTP. Sequences containing SNPs formed distinct phylogenetic clusters. Intriguingly, quantitative FISH with the new probes revealed the stable coexistence of these very closely related *Nitrospira* in the same WWTP during a five-year period. Their ecophysiology (carbon source usage) was studied by isotope-labelling methods. To further assess *Nitrospira* microdiversity, we performed high-throughput pyrosequencing of nitrite oxidoreductase (*nxrB*) genes from *Nitrospira* that were PCR-amplified from another WWTP. Using a 97% sequence similarity threshold, we detected more than 600

operational taxonomic units, representing an astonishing diversity of these genes in the same activated sludge. This finding was supported by the metagenomic analysis of a third nitrifying WWTP. Here, paired-end BAC sequences were mapped to a previously sequenced *Nitrospira* genome and screened for SNPs in open reading frames. Interestingly, a high number of SNPs was found in genes encoding ecologically relevant functions such as siderophore synthesis. Altogether, our data show a previously overlooked microdiversity of *Nitrospira* in WWTPs and the coexistence of closely related ecotypes that differ in key functional genes, suggesting small-scale niche differentiation of these highly specialized microbes.

Abstract Category
06 Engineered Environments

PS.06.020 MICROBIAL SURFACE COLONIZATION IN A WORKPLACE AND RESIDENTIAL INDOOR ENVIRONMENT

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Reliable and precise methods for characterization of microbial contamination of surfaces is critical to developing hygiene and decontamination plans to ensure public health as well as understanding what constitutes a healthy indoor microbiome. We are investigating surface colonization and the parameters that impact sampling performance, including sampling bias, in an effort to improve surface sampling methodologies used to characterize unknown microbial populations in indoor environments. Microbial community populations native to inhabited office and residential spaces were recovered from coupons (Teflon, aluminum, copper, stainless steel) after 1, 5 and 7 weeks exposure to normal operational environments. Coupons were processed in phosphate buffered saline plus 0.04% Tween 80 (PBST) to remove microbial growth. Additional 7 week samples were collected by wipe sampling. Eluants were filtered for plating on two media types (i.e. TSA, R2A). Distinct colony morphologies were further characterized by 16S rRNA sequence analysis and fluorescent *in-situ* hybridization was performed on replicate coupons to investigate structural relationships. Colonization varied based on surface type and location, however, surface coverage increased over time for all surfaces in a residential kitchen (ranging from 0.5 to 2.5 CFU/cm²) and on steel and Teflon in an office environment (<1.0 CFU/cm²). Sequencing results from nearly 100 isolates indicated a predominance of gram positive bacteria belonging to the Firmicutes and Actinobacteria phyla. This work is an initial effort to understand surface colonization in the indoor environment and the fundamentals critical to the successful sampling of microbes relevant to a range of environmental, clinical and public safety sector stakeholders.

Abstract Category
06 Engineered Environments

PS.06.021 NEUTRAL AND NICHE-ORIENTED ASSEMBLY OF ACTIVATED SLUDGE FLOC

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Understanding the processes that generate patterns of community structure is a central focus of ecological research. With that aim, we manipulated the diversity of bacterial activated sludge to test the influence of the diversity and composition of source bacterial communities on the dynamics of activated sludge floc assembly in lab-scale bioreactors. Three sets of experiments were performed. In the first set of experiments four replicate lab-scale reactors were seeded with sludge from a full-scale wastewater treatment plant (WWTP). Secondly, four laboratory scale reactors were replicates of a mixture of sludge from four different full-scale WWTP, added in equal proportions. In the third experiment, each of four lab-scale reactors contained 70% of sludge from a single full-scale WWTP, and 10% of sludge from the remaining 3 plants. Changes in bacterial community profiles were analyzed for a period of 10 solid retention time using denaturing gradient gel electrophoresis (DGGE) of RT-PCR amplified 16S rRNA. Fingerprinting of each set of reactors, including those started with the same source communities added in different proportions, converged to patterns that were more similar than expected by chance, suggesting a deterministic selection in floc development. Evidence for neutral dynamics was suggested by the dependence of the rate of replacement of species on the number of available species in the source community. Further indication of stochastic dynamics was obtained by the application of the Sloan neutral model for prokaryotes. The fitting of the observed data to the model predictions revealed that the importance of the stochastic component increased with higher diversity. Our results illustrate how both neutral and deterministic dynamics operate together in the assembly of the bacterial floc, and show that the balance of the two depends on the diversity of the source community.

Abstract Category

06 Engineered Environments

PS.06.022 NITRIFYING BACTERIA AND ARCHAEA IN AGRICULTURAL AND INDUSTRIAL WASTEWATER TREATMENT PROCESSES

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Linking microbial communities' structure and function is a challenge for all microbial ecologists but also for civil engineers that rely on measurement of active biomass and metabolic activities for model development and process design. In the field of wastewater nitrogen removal the limiting process is nitrification. Numerous studies proposed that it is done by various nitrifying *Bacteria*, but recently, ammonium monooxygenase genes (*amoA*) from *Archaea* were detected in wastewater treatment plants asking the question of their implication in nitrification.

Activated sludge was taken from 19 plants treating either piggery, food industries or urban wastewaters. Nitrifying activity was studied by respirometric assays based on oxygen uptake rate. The *Bacteria*, *Archaea* and *Nitrospirae* communities were quantified by PCR amplification of 16S rRNA genes while the *amoA* gene was used for ammonia-oxidizing communities.

Considerable variability in profiles and in maximum oxygen uptake rates of ammonia and nitrite oxidation was observed between sludge. Real-time PCR indicated that the ammonia-oxidizing *Bacteria* abundance differed and represented less than 1.4% of the total *Bacteria*. Their concentrations were low but correlated positively with the maximum oxygen uptake rates of ammonia oxidation and active biomass concentrations approximated by model based interpretation of respirograms. The *Archaea amoA* gene was found in most of plants at about 5×10^4 copy/mL but as high as 2×10^7 in one plant treating piggery wastewater where it reached 66% of the *Archaea*. Statistical analyses showed a correlation between ammonia-oxidizing *Bacteria* and *Archaea* and nitrification activities. The abundance of *Nitrospirae* ranged from 9×10^3 to 3×10^7 copy/mL when it was detected. A relationship existed between the *Nitrospirae* cell numbers and the maximum oxygen uptake rate of nitrite oxidation.

This study suggests that ammonia-oxidizing *Archaea* could play an important role in some treatment processes.

Abstract Category
06 Engineered Environments

**PS.06.023 DISTINCT ECOLOGICAL NICHE OCCUPIED BY TWO
ARCOBACTER SPP. ISOLATED FROM A MICROBIAL FUEL CELL**

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Microbial fuel cells (MFCs) are devices catalysing the degradation of organic waste materials with concomitant production of electricity. To date, many different electrogenic bacteria which colonise, and transfer electrons to, a solid MFC anode have been identified, but the ecology of the interactions of these organisms with those in the MFC liquid phase is poorly understood. In this study, electrogens from a marine sediment sample were selected in an MFC fed with acetate in batch mode. Culture-independent molecular methods were used to identify the predominant organisms in this MFC, which were then cultured on selective media. Complete genome sequencing of these organisms followed by metabolic reconstruction were then used to rationalise the different niches which they occupy within the MFC. The primary electrode-attached organism within the MFC is the *Arcobacter butzleri* strain ED-1, the first ϵ -proteobacterium found to act as an exoelectrogen, while the liquid phase is dominated by a different *Arcobacter* sp., *Arcobacter-L*. Together, these organisms comprise >90% of the fuel cell community.

Genomic analysis showed that strain ED-1 has high homology with the type strain RM4018 throughout its genome, while *Arcobacter-L* has a much larger and more diverse genome, containing several large genomic islands. Metabolic reconstruction suggests that the central metabolism of ED-1 is geared towards anaerobic or microaerobic growth, whereas *Arcobacter-L* has a metabolism more suited to growth under aerobic conditions. Tests with pure cultures grown anaerobically in the presence of an anode show that ED-1, but not *Arcobacter-L*, can transfer electrons derived from acetate degradation to a solid extracellular electron acceptor. These results suggest a model in which planktonic *Arcobacter-L* cells scavenge oxygen within the MFC and maintain its anaerobic state, while *A. butzleri* ED-1 grows primarily on the anode and transfers directly to it electrons derived from acetate.

Abstract Category

06 Engineered Environments

**PS.06.024 CHARACTERISTICS OF AMMONIA OXIDIZERS
ACCLIMATIZED UNDER DIFFERENT SALINITY CONDITIONS**

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Ammonia oxidizers play key role in the first and rate-limiting step of nitrification. While ammonia oxidizing bacteria (AOB) have been considered major players for ammonia oxidation, the recent discovery of ammonia oxidizing archaea (AOA) revealed their potential for ammonia oxidization in natural and engineered systems. Salinity is recognized as an important factor determining abundance of AOA and AOB in natural environments such as marine, estuary and soil. However, the details of ecology and physiology of ammonia oxidizers under different salinity conditions remain unclear. To characterize ammonia oxidizers enriched under different salinity conditions, we operated laboratory-scale reactors with high (3.5%) and low (0.4%) salinity conditions for more than 300 days. The morphological characteristics of ammonia oxidizers in both reactors were observed using fluorescence *in situ* hybridization. To evaluate sensitivity of ammonia oxidizers to salinity, batch tests with high or low salinity conditions were conducted using mixed liquors containing enriched cultures in both reactors. Archaeal and bacterial amoA mRNA were quantified using real-time reverse transcription quantitative PCR.

AOB in both reactors formed cell aggregates. Archaea, possibly AOA, in the high salinity reactor also formed cell aggregates, while those in the low salinity reactor were dispersed. The results of batch tests showed that ammonia oxidizers in the low salinity reactor could not oxidize ammonium under high salinity condition, while those enriched in the high salinity reactor could oxidize ammonium in both low and high salinity conditions. The quantification results of amoA mRNA suggested that only AOB was active in batch experiments, although the reason is unclear.

In this study, characteristics of ammonia oxidizers acclimated under high and low salinity conditions were investigated. This study will provide the fundamental information of salinity effect on AOA and AOB.

Abstract Category
06 Engineered Environments

PS.06.025 CHARACTERIZATION OF ACTIVATED SLUDGE FROM A SEAWATER-PROCESSING WASTEWATER TREATMENT PLANT: A COMPARISON OF DIFFERENT MOLECULAR TECHNIQUES

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Relatively few works in the past two decades have assessed the study of activated sludge microorganisms by a full-cycle rRNA approach, and never before the diversity of a marine activated sludge has been detailed. In our study, the objective was to obtain a comprehensive picture of the diversity of the procariotic assemblage in a seawater-utilizing waste water treatment plant (Almería, Spain), combining and comparing different molecular approaches (DGGE, clone library and CARD-FISH). Most of the sequences retrieved in the DGGE and the bacterial clone library were similar to uncultured members of different phyla. The most abundant sequence recovered from Bacteria in the clone library corresponded to a bacterium from the Deinococcus-Thermus cluster (almost 77% of the clones), although the library included also members from other groups such as the Alpha-, Gamma- and Delta- subclasses of Proteobacteria, the Bacteroidetes and Firmicutes. Concerning the archaeal clone library, we basically found sequences related to different orders of methanogenic Archaea, in correspondence with the recovered DGGE bands.

Enumeration of DAPI (4', 6-diamidino-2-phenylindole) - stained cells revealed a total cell count of 1.6×10^9 /ml, being 89% in the form of aggregates and the rest as free cells. Around 80% and 96% of DAPI counts of free and aggregated cells respectively hybridized with a Bacteria specific probe. Alphaproteobacteria were the dominant bacterial group while Beta-, Delta- and Gammaproteobacteria, Bacteroidetes, Actinobacteria and Firmicutes contributed to lower proportions. Archaea accounted only for 2 and 4% of DAPI counts for free and aggregated cells respectively. Overall, it was obvious that particular groups were over or underrepresented depending on the method, proving that no single technique alone can be trusted to cover all aspects of an acceptable description of the procariotic phylogenetic diversity.

Abstract Category
06 Engineered Environments

PS.06.026 STIMULATION OF NITRATE REDUCERS AND NITRATE-REDUCING SULFATE REDUCERS RESULTS IN A DECREASE IN NET H₂S PRODUCTION IN A NITRATE-TREATED OIL RESERVOIR

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Sulfate-reducing prokaryotes (SRP) cause severe problems like microbial corrosion and reservoir souring in seawater-injected oil production systems. One strategy to inhibit the activity of SRP is the addition of nitrate to the injection water and thereby favoring the growth of heterotrophic, nitrate-reducing bacteria (hNRB) and nitrate-reducing, sulfide-oxidizing bacteria (NR-SOB). Using a combined molecular, microbiological and biogeochemical approach, we investigated the diversity, abundance and activity of SRP, hNRB, and NR-SOB in water samples from two adjacent, high-temperature oil fields in the North Sea. Nitrate has only been added to one of them regularly. Effects of nitrate addition could be inferred from both injection (IW) and production water (PW) samples. In the IW, mesophilic hNRB related to the genus *Arcobacter* were identified as potential competitors to SRP. They were highly enriched compared to untreated IW, but not detected in PW samples. It is indicated that their activity resulted in a decrease in net sulfide production. PW samples from the nitrate-treated system lacked SRP activity and exhibited significantly lower relative abundances of SRP, but higher abundances of putative hNRB and/or NR-SOB. The latter were affiliated with members of the *Epsilonproteobacteria* and *Deferribacterales*. Most SRP were related to thermophilic representatives of the genus *Archaeoglobus* and the spore-forming bacterial genus *Desulfotomaculum*. Thermophilic SRP were successfully enriched from PW samples, whereas the cultivation of their nitrate-reducing counterparts failed. We hypothesize that the detected putative nitrate reducers (1) did not originate from the reservoir, but were introduced from the production-site facilities, and (2) were not able to compete with thermophilic SRP for substrates. Therefore, the decrease in net sulfide production might rather be due to the presence of SRP using nitrate as an alternative and more energy-yielding electron acceptor.

Abstract Category

06 Engineered Environments

PS.06.027 EFFECTS OF THERMAL ENERGY USAGE ON SHALLOW GROUNDWATER ECOSYSTEMS

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The use of groundwater as a carrier of thermal energy is becoming more and more important as a sustainable source of heating and cooling. At the same time, the present understanding of the effects of aquifer thermal usage on geochemical and biological aquifer ecosystem functions is extremely limited. Recently, in a pioneering study, we have assessed the effects of groundwater heat discharge on the ecological integrity of an oligotrophic, energy-limited, shallow aquifer. We have monitored hydrogeochemical, microbial, and faunal parameters in groundwater from the vicinity of an active thermal discharge facility. The observed seasonal variability of abiotic and biotic parameters between wells was considerable. Yet, no significant temperature impacts on bacterial or faunal abundances and on bacterial productivity were observed. Also, standard microbiological drinking water analyses did not indicate an improved survival or growth of faecal bacteria with temperature. In contrast, the diversity of aquifer bacterial T-RFLP fingerprints and faunal populations was either positively or negatively affected by

temperature, respectively. Statistical analysis indicated that both, the impact of infiltrating surface water from a nearby river (seasonal hydrology) and temperature were important drivers for the observed variations in aquifer biota. In subsequent laboratory column experiments temperature induced effects, simulating a cooling and heating mode, were investigated for (i) non energy-limited conditions, (ii) the fate of a model contaminant (toluene) amended and (iii) the retention and survival of *E. coli*. Now, under these conditions, significant changes were found not only for the composition of bacterial communities but also for further microbial patterns. These results demonstrate that aquifer thermal energy usage can affect intrinsic aquifer microbial populations and ecosystem functions.

Abstract Category
06 Engineered Environments

PS.06.028 EFFECTS OF AERATION INTENSITY ON ORGANIC MATTER DEGRADATION AND MICROBIAL COMMUNITY DYNAMICS DURING LIQUID COMPOSTING OF COW MANURE SLURRY

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Aerobic treatment was recommended to minimize odor nuisance of animal manure slurry during land application. Organic matter degradation and microbial subpopulation evolving during aeration may be largely influenced by aeration intensity. This study aimed to determine changes in organic matter degradation and simultaneous dynamics of microbial community at different aeration intensities. Batch aerobic treatment was performed in a 5-L jar fermentor containing manure slurry (3 L; temperature, 40°C; stirring rate, 350 rev•min⁻¹). The slurry was aerated using low, medium, and high (50, 150, and 250 mL•min⁻¹, respectively) aeration intensities for 9 days. Periodically collected slurry samples were analyzed for soluble total organic carbon and microbial community by using terminal restriction fragment length polymorphism and clone library. The amount of soluble total organic carbon linearly decreased and stabilized on day 2 (high-aeration) and day 4 (medium-aeration). On day 9, however, 90% of the initial soluble total organic carbon was retained in low-aeration conditions. During active stages of organic carbon decomposition, the microbial community comprised many lineages of organisms of the class Bacilli (>90% of the total clone). Bacilli were predominant in the slurry subjected to high-aeration on day 0.5; however, this phenomenon was observed on day 1 in the slurry subjected to middle-aeration. Moreover, many identical operational taxonomic units defined as sequences with >97% similarity were recovered from these slurry samples. High aeration intensity greatly accelerated the organic matter degradation and drastically altered the microbial community. Similar microbial communities-involved in the organic matter decomposition-were noted in the samples of slurries subjected to medium- and high-aeration; however, there was a lag in the time of appearance of these microorganisms between the 2 slurries.

Abstract Category
06 Engineered Environments

PS.06.029 COMPLEXITY AND HETEROGENEITY IN BIOSTIMULATED SEDIMENT AND GROUNDWATER COMMUNITIES DURING IRON, SULFATE AND URANIUM REDUCTION

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A phylogenetic microarray investigation into biostimulated iron- and sulfate-reducing bacterial (SRB) communities revealed unexpected similarity between sediment and groundwater fractions, variability in key functional groups, and an insight into potentially important low-abundance organisms. Bacterial communities from a range of acetate- and un-amended samples from U(VI) bioremediation experiments in Rifle, CO, were compared using a high density LBNL PhyloChip, which detects 16S rRNA genes from thousands of bacterial groups, including low-abundance taxa not typically detected by more traditional techniques (e.g. clone libraries, TRFLP). Geochemical monitoring showed that acetate added to Rifle groundwater stimulated consecutive Fe(III), sulfate and concomitant U(VI) reduction. PhyloChip data indicated that abundant *Geobacter* spp. were enriched and evidently responsible for Fe(III) and U(VI) reduction during early biostimulation, while later sulfate reduction was primarily performed by *Desulfobacteraceae*. Data also suggested minor enrichments of other SRB families, *Peptococcaceae* and previously undetected, largely non-acetate-oxidizing *Desulfovibrionaceae*. Both families include potential competitors to residual *Geobacter* for enzymatic U(VI) reduction in late biostimulation. Communities overall were highly similar within sample treatments (acetate-amended subsurface [a] groundwater or [b] sediment; acetate-amended [c] bench-top or [d] in-well field column sediment/quartz; [e] naturally reduced subsurface sediment), although *Geobacter* demonstrated a strong preference for Fe(III)-bearing sediment over quartz sand (c). Curiously, a subset of sulfate-reducing sediments (d) displayed greater similarity to Fe(III)- and sulfate-reducing groundwater communities than to other sulfate-reducing sediments (b-e). This may be due partly to the broad overlap in elevated *Geobacter* and SRB in groundwater and sediment, and to increases in *Peptococcaceae* in selective sediments (c, e).

Abstract Category
06 Engineered Environments

PS.06.030 OPTIMIZING MICROBIAL ELECTROSYNTHESIS WITH *CLOSTRIDIUM LJUNGDAHLII*

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Microbial electrosynthesis (ME) produces organic compounds via the same net reaction as photosynthesis: carbon dioxide and water are converted to organic compounds and oxygen. One key difference is that electricity is the energy source for ME. Thus, when photovoltaics serve as the source of electricity, ME is an alternative form of photosynthesis. Another key difference is that in ME carbon and electron flow is diverted primarily toward the production of extracellular organic products rather than biomass. This offers the possibility of directly producing desirable chemicals and fuels from carbon dioxide and water with ME. *Clostridium ljungdahlii* represents a potentially attractive catalyst for ME because it already contains the Wood-Ljungdhal pathway for carbon dioxide reduction and it has recently been demonstrated as feasible to engineer this organism to produce butanol. In order to determine whether *C. ljungdahlii* could use electrons derived from an electrode to support carbon dioxide reduction, biofilms were grown on a graphite electrode poised at -400 mv (versus standard hydrogen electrode), a potential too high to support electrolytic hydrogen production. *C. ljungdahlii* biofilms steadily produced a mixture of acetate, formate, and 2-oxobutryate with the poised electrode serving as the sole electron donor and carbon dioxide as the sole electron acceptor. Electron recovery in these three products accounted for $86 \pm 8\%$ of the electrons consumed with 85% of these electrons appearing in acetate. Confocal scanning laser microscopy and scanning electron microscopy revealed that *C. ljungdahlii* forms an incomplete monolayer of uniformly active cells on the electrode surface. The addition of soluble electron shuttles did not stimulate ME. *C. ljungdahlii* is only the second microorganism shown to be capable of ME and the first gram positive. Strategies to engineer *C. ljungdahlii* to produce products other than acetate with ME are being designed.

Abstract Category

06 Engineered Environments

PS.06.031 MICROBIAL DIVERSITY IN THE MUNICIPAL COMPOSTING PROCESS AND DEVELOPMENT OF DETECTION METHODS

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Composting refers to aerobic degradation of organic material and is one of the main waste treatment methods used for treating separated organic waste. The composting process allows converting organic waste to a humus-like end product, which can be used to increase the organic matter in agricultural soils, in gardening, or in landscaping. Microbes play a key role as degraders during the composting-process, but there are open questions regarding the microbiota in industrial composting processes.

Here, the presence of microbes in full-scale and pilot-scale composting processes were first characterised with cloning and sequencing. Several clone libraries were constructed and altogether nearly 6000 clones were sequenced. The microbial communities detected were found to differ from the compost microbes observed in previous research with cultivation-based methods or with molecular methods from processes of smaller scale, although there were similarities as well. In some libraries there were a few abundant

phylotypes present that masked the rare ones. The rare phylotypes were of interest and a method for collecting them for sequencing was developed.

Since microbes play a central role in composting and in many other biotechnological processes, rapid methods for characterization of microbes would be of value. Microarrays have been used in microbial ecology for a decade to study the microbes of interest in a multiplex manner. The sequence database collected was used for microarray development. The enzyme assisted detection method, ligation-detection-reaction (LDR) microarray, was adapted for species-level detection of microbes characteristic of each stage of the composting process. The microarray can be used to monitor composting processes or the hygienization of the compost end product in a sensitive and specific manner. The platform is universal and the method can easily be adapted for studying microbes from environments other than compost.

Abstract Category

06 Engineered Environments

PS.06.032 BACTERIAL COMMUNITY ANALYSIS OF ANAEROBIC SLUDGE IN AN UPFLOW ANAEROBIC SLUDGE BLANKET (UASB) REACTOR TREATING MUNICIPAL WASTEWATER.

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Upflow anaerobic sludge blanket (UASB) system is one of well-established anaerobic wastewater treatment technologies for high-strength organic wastewater, and it has also been used for treating low-strength organic wastewaters like municipal wastewater. A number of such full-scale, municipal wastewater-treating UASB systems are successfully used in tropical and subtropical countries, in which they are operated without temperature control. However, the system is known to be susceptible to temperature variation, and therefore seasonal modulation in temperate climate countries often makes the system unstable. To understand the mechanisms underlying the instability, we surveyed the microbial community structure and its shift by seasonal modulation in granular sludges taken from a pilot-scale municipal sewage-treating UASB reactor based on 16S rRNA genes. The UASB reactor inoculated with digested sludge was operated at an HRT (Hydraulic retention time) of 8 hours under ambient temperatures, ranging from 10 to 28°C, over 700 days. Bacterial 16S rRNA gene clone libraries were constructed from granular sludge samples collected in summer and winter. Phylogenetic analyses revealed that the most predominant phylotype in the clone libraries was the one affiliated with the phylum *Caldiserica* (formerly known as the Candidate phylum OP5). Fluorescence *in situ* hybridization (FISH) with a probe specific for the most dominant phylotype revealed

that the probe-positive cells had coccoid morphologies and were abundant in the sludge samples. These results suggest the importance of the phylotype in municipal sewage-treating UASB systems.

Abstract Category
06 Engineered Environments

PS.06.033 ELECTRICITY-GENERATING MICROBIAL COMMUNITY IN AN AIR-CATHODE MICROBIAL FUEL CELL FED WITH PRIMARY CLARIFIER EFFLUENT

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Microbial fuel cells (MFCs) are devices that exploit microorganisms as "biocatalysts" to recover energy from organic matter in the form of electricity. Optimizing conditions for current production will lead to accelerated organic matter-degradation. In this study, electricity-generating microbial communities of anode biofilms were analyzed in wastewater-fed MFC operating under three different conditions. For all experiments we used primary clarifier effluent collected from the North City Water Reclamation Plant (San Diego, CA) as the sole carbon and microbial source for the air-cathode MFCs. Three different operating conditions were explored: 1) MFC operation using a 750 ohm external resistor (wMFC); 2) Open circuit operation with no current generation (ocMFC); and 3) set-potential operation with the anode electrode controlled to +100 mV vs SHE using a potentiostat (spMFC). The current production was 0.3 mA for wMFC, 0 mA for ocMFC, and 4 mA for spMFC, respectively. During the batch operation, nearly all organic matter was consumed after a residence time of 8-12 days for wMFC, 15-20 days for ocMFC, and 4-6 days for spMFC. These results indicate that higher current generation accelerates organic matter degradation. We conducted microbial community analysis using 16S rRNA gene sequencing. Although the primary clarifier effluent was dominated by ϵ -Proteobacteria, γ -Proteobacteria, and Bacteroidetes species, the electricity-generating biofilms of wMFC and spMFC were dominated by σ -Proteobacteria and Bacteroidetes. In the ocMFC, β -Proteobacteria, σ -Proteobacteria, Firmicutes, and Bacteroidetes related species were dominant; however, on the genus level, the microbial community of the ocMFC was completely different from that of the wMFC. These analyses suggest that phylotypes classified as Desulfuromonadales and Bacteroidales were correlated with electricity generation. This work was supported by Grant-in-Aid for JSPS Fellows and the NSF-BBBE (award No. 0933145).

Abstract Category
06 Engineered Environments

PS.06.034 STEAM AUTOCLAVING WASTE PRIOR TO ANAEROBIC DIGESTION (AD) ENHANCES BIOGAS PRODUCTION

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The preferred disposal method for municipal solid waste (MSW) is sanitary landfill but this is not necessarily the most effective option. An attractive alternative is steam autoclaving technology that diverts 80 per cent of waste from landfill. One of its outputs is an organic fibre which could be used as substrate for anaerobic digestion (AD). In this paper we report on the development anaerobic digestion biotechnology to convert this fibre into biogas. Since the autoclaving process sterilises the MSW we initially focussed on isolating a microbial inoculate capable of degrading the fibre. We then compared the AD efficiency between autoclaved waste (fibre) and non-autoclaved waste (MSW) using gas chromatography and molecular fingerprinting approaches. A series of microcosms were set-up to monitor changes in biogas production and microbial community composition over a 40-day period. We found that autoclaving the MSW had a significant impact on the microbial community which was accompanied by a 10-fold increase in methane production. From the different microbial successions during fibre and MSW digestion, key components of the microbial community responsible for driving AD pathways were identified using multivariate approaches and Structural Equation modelling. Totally different bacterial degradation pathways were involved in fibre digestion compared to MSW digestion and this probably reflects autoclaved-induced changes in substrate quality. Hydrogenotrophic methanogenesis prevailed in both systems but was catalysed by different archaea. The methanogens were supported by different syntrophic bacteria including fatty acid, propionate and methanol degraders. Syntrophy was identified as the most important factor driving system dynamics and biogas production. The resolution of information gained regarding the response, interactions and succession of these communities provided valuable insights into the dynamics of anaerobic environments.

Abstract Category
06 Engineered Environments

PS.06.035 BIOELECTROCHEMICAL PRODUCTION OF BUTANOL FROM BUTYRIC ACID

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The need to reduce greenhouse gas emissions and the depletion of fossil fuels is driving the development of alternative fuels. While bioethanol has been introduced to the market, biobutanol's greater mileage and compatibility with current infrastructure makes it a more promising renewable fuel. Traditional biobutanol production using *Clostridium acetobutylicum* fermentation is marred by the high cost of fermentable carbon source and low yields. In an alternative alcohol production method, bacteria are cultured in bioelectrochemical systems to use the cathode as an electron donor to drive the reduction

of the carboxylic group of volatile fatty acids to the corresponding alcohol. Previous research has employed mediators such as methyl viologen to integrate reducing equivalents into the central fermentation pathways. The purpose of this study was to investigate the production of butanol in bioelectrochemical systems without mediators, instead using the inherent extracellular electron transfer capabilities of the microbial population. The bioelectrochemical system used had a sacrificial anode and biocathode with a minimal media electrolyte containing 4.4 g/L butyric acid. A mixed culture was used and the cell current was 100 mA. Butyric acid consumption and generation of products was analyzed by gas chromatography and high performance liquid chromatography enabling an electron balance calculation. Preliminary results show that the microbial community produced at least 0.2 g/L butanol, concomitant with an increase in acetate, due to alternative butyrate utilization. Using *C. acetobutylicum*, the model organism for butanol production, a parallel experiment was performed in pure culture. The rate of growth was slow in this experiment, leading to low butanol yields. This indicates that direct interaction between the existing *C. acetobutylicum* type strain and cathodes cannot readily be established. Community analysis on the butanol producing population is presently underway.

Abstract Category
06 Engineered Environments

PS.06.036 FUNGAL AND BACTERIAL COMMUNITY DYNAMICS DURING THE FERMENTATION OF KOREAN TRADITIONAL ALCOHOLIC BEVERAGE DETERMINED WITH THE BARCODED PYROSEQUENCING

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'Makgeolri' is the Korean traditional alcoholic beverage manufactured with alcohol fermentation starter 'nuruk' and grains. Nuruk is generally formed as starchy disk or tablet containing various fungal and bacterial strains transferred from natural environment which participates in the fermentation process. Initially, filamentous fungi such as *Aspergillus* sp. or *Rhizopus* sp. induce saccharification of the grains and yeast strain, *Saccharomyces cerevisiae*, consequently produce alcohol from the saccharide. In addition, lactic acid bacteria (LAB) in the nuruk prevent growth of the spoilage bacteria by producing lactic acid. In this study, six different nuruk were used for making the makgeolri and eight samples from each fermenter were periodically collected for the analysis of microbial community dynamics during the makgeolri fermentation. In order to compare the microbial community, five commercial brand of makgeolri were also collected. Parameters such as pH, glucose and alcohol concentration were examined to determine the degree of alcohol fermentation. Fungal and bacterial community change during the fermentation of makgeolri were investigated using the barcoded pyrosequencing with primers targeting nuclear ribosomal internal transcribed spacer 2 (ITS 2) region for fungi and V1 to V3 hypervariable region of the 16S rRNA gene for bacteria, respectively. The detailed results of this study are described below. This is the first molecular ecological study investigating the microbial dynamics of the Korean

traditional alcoholic beverage and will provide us the basic and detailed information of the microbiome in naturally fermented alcoholic beverages.

Abstract Category
06 Engineered Environments

PS.06.037 QUANTIFICATION OF RESISTANCE GENES FOR LAST RESORT ANTIBIOTICS FROM WASTEWATER TREATMENT PLANTS

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Antibiotic resistance is increasing rapidly among bacteria as antibiotics are used in increasing quantities in treating humans and animals. Due to bacterial multiresistance to different antibiotics, we are heading back to pre-antibiotic era where there is no treatment for common bacterial infections. One major concern is the microbial resistance for the last resort antibiotics, such as vancomycin, carbapenems and quinolones. In wastewater treatment plants antibiotics, other pharmaceuticals, heavy metals and xenobiotics mix up with bacteria from human, animal and environmental origin. Wastewater treatment plants are known reservoirs for various resistance determinants and serve as hotspots for horizontal gene transfer. Resistance genes for different antibiotics are usually associated with mobile genetic elements that are able to horizontally transfer between different bacteria. Dense bacterial populations and biofilms in the sludge promote horizontal gene transfer and different chemicals can pose a selection pressure promoting the survival of the resistant bacteria. Horizontal transfer of certain mobile genetic elements, such as Tn916, has also been shown to be inducible by antibiotics. The resistant bacteria from the wastewater treatment plants may eventually end up in the environment either in the effluents or in the sludge. In this study we quantified different resistance genes for the last resort antibiotics, such as carbapenems, 3rd & 4th generation cephalosporines, quinolones and vancomycin, from different compartments of the wastewater treatment plant using quantitative PCR. The antibiotic resistance genes were quantified from the total microbial community DNA extracted from the sludge. Our results show that many resistance determinants can be found in the wastewater treatment plants, including recently described resistance genes for clinically relevant last resort antibiotics.

Abstract Category
06 Engineered Environments

PS.06.038 STUDY OF ELECTRON FLOW DURING ACETATE OXIDATION IN A MICROBIAL FUEL CELL

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Microbial fuel cell (MFC) is a device that could convert chemical energy of organic compounds into electricity using microorganisms. Its power generation is influenced by many factors such as microbial electron transfer. Of these, acetate oxidation and methanogenesis are thought to be one of the most critical bottle-neck steps in an

anaerobic digestion. Therefore, in this study, we investigated the electron flow using acetate as an electron donor and characterized the microbial communities involved in acetate metabolism. Continuous two-chambers MFC reactors were operated with acetate loading rate of 4 mmol d⁻¹, with hydraulic retention time of 5 days, and with 300 ohm external resistance. A proton-exchanged membrane separated each chamber, and the carbon sheets were used as the anode and cathode electrodes. Microbial communities were analyzed by stable isotope probing (SIP), denaturing gradient gel electrophoresis (DGGE) and fluorescence *in-situ* hybridization (FISH). Using SIP and DGGE analyses, we found that *Geobacter* and *Hydrogenophaga* contribute to the electricity generation. In addition, FISH analysis indicated that *Geobacter* predominate in our reactor and is a major acetate decomposer. However, the coulombic efficiency was only 10%, and the methane conversion rate was 30%, suggesting that coulombic efficiency was not in the proportion of bacterial activity, and *Geobacter* might be able to use other electron acceptors beside the electrode. Altogether, the electron flow during acetate fed was formed only by *Geobacter*. However, the electron balance showed that the electrode is not a main electron acceptor for *Geobacter*.

Abstract Category

06 Engineered Environments

PS.06.039 INTERACTIONS OF ZERO VALENT IRON NANOPARTICLES WITH NATIVE AQUIFER MICROBIAL COMMUNITIES AND DEHALOCOCCOIDES CONTAINING CULTURES

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Nanoscale zero valent iron (NZVI) particles are a promising technology for the remediation of chlorinated solvent contaminated groundwater. These particles are small enough to transport through aquifer pores, and the zero valent iron core rapidly reduces chlorinated ethenes to nontoxic products. NZVI addition to groundwater rapidly changes the site geochemistry and generates cathodic hydrogen. These particles are toxic to planktonic cultures of *E. coli*, and therefore there are legitimate concerns over injecting NZVI into the environment. The objective of this study was to determine how the addition of NZVI to aquifer materials changes the microbial community. Microcosm experiments were performed with aquifer material from a TCE contaminated site in Alameda Point, CA. Quantitative polymerase chain reaction (qPCR) and denaturing gradient gel electrophoresis (DGGE) were used to assess changes in the microbial communities after the addition of NZVI. The strongly reducing conditions and hydrogen evolution dramatically changed microbial populations, stimulated sulfate reduction, and in some cases stimulated methanogenesis in the microcosms. Despite dramatic changes in the major populations, the overall Eubacterial abundance did not decrease with the addition of nanoparticles. The addition of a polyaspartate coating (designed to aid particle transport) increased Eubacterial populations an order of magnitude. These results have

positive implications for the field scale application of NZVI. Ongoing investigations are focusing on whether synergism (for chlorinated ethene dechlorination) can be achieved between NZVI and communities containing *Dehalococcoides sp.*

Abstract Category
06 Engineered Environments

PS.06.040 EFFECTS OF ANAEROBIC-AEROBIC PERIOD ON THE MICROBIAL COMMUNITY STRUCTURE OF POLYPHOSPHATE ACCUMULATING ORGANISMS

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Poly-phosphate accumulating organisms (PAOs) are bacterial group responsible for phosphorus removal in wastewater treatment plants (WWTPs). PAOs release phosphate under anaerobic condition, while PAOs take up it under aerobic condition. "*Candidatus Accumulibacter Phosphatis*" (*Accumulibacter*) is one of the PAOs frequently detected in WWTPs, it is not available in pure culture and it is difficult even to obtain within enrichment culture. A lot of previous studies reported the influence of the carbon source, pH and temperature on community structure of PAOs, while little of them researched about the influence of anaerobic-aerobic period. Therefore, in this study we investigated how anaerobic-aerobic period affects community structure of PAOs. PAOs were enriched from activated sludge with different 3 anaerobic/aerobic time ratios and 5 cycle durations. After 3 to 5 months incubation, quantification of *Accumulibacter* by FISH with PAOmix probes and phylogenetic analysis based on 16S rRNA gene were conducted. The results of PAOs enrichment, in the 4 hours of anaerobic and 12 hours of aerobic condition (4 hours-12 hours condition), *Accumulibacter* accounted for 55.6% of total bacteria, which is the highest composition of *Accumulibacter* in all enrichment condition. The highest amount of phosphate uptake and release were also obtained under this condition. Even though the phosphate uptake and release were confirmed in all conditions, the amount of PAOs determined by FISH was less than 1% under the 6 hours-12 hours and 8 hours-16 hours condition. Phylogenetic analysis of the biomass under the 6 hours-12 hours condition revealed that only few clones were closely related to *Accumulibacter*, while almost others were closed to *Acidovorax* or *Mycobacterium* groups. Under these conditions, the bacteria other than *Accumulibacter* might be acting as PAOs. Thus, anaerobic-aerobic period is also one of factor that strongly affects community structure of PAOs.

Abstract Category
06 Engineered Environments

PS.06.041 BIOREMEDIATION OF CONTAMINATED HARBOR SEDIMENTS USING EFFECTIVE MICROORGANISMS CARRIED ON LOESS

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Effective microorganisms (EM) have been applied in agriculture, medicine and environmental remediation although their mode of action has not been well proven. In this study, we applied EM carried on loess in the treatment of sediments contaminated with organics. We measured the physiochemical, biochemical and microbial parameters during the remediation process at a lab scale. Treatment with higher concentrations of EM loess balls (4%) and with a lower concentration of EM (0.1%) containing molasses (0.05%) contributed to a more rapid removal of malodor. Gas chromatographic analysis of the various treatments as well as the EM fermentation pure culture showed the variable profiles of organic acids produced. Acetic acid, propionic acid, valeric acid, capionic acid, and lactic acid were rapidly removed when molasses (0.05%, w/w) was used as a supplementary nutrient source, indicating the EM activity enhanced by molasses. However, isobutyric acid and isovaleric acid did not change in quantity, and in fact isocapionic acid and butyric acid concentrations increased in the all treatments, indicating these compounds could be produced non-specifically in the sediment. Fermentation of molasses by EM showed that acetic acid was produced much higher than the other organic acids and most of the organic acids were eventually converted through intermediate metabolites to acetate. This shows that the rapid reduction in malodor could be due to the reduction of these organic acids by the molasses-assisted EM. In situ remediation carried out at a Youngdo port showed the presence of acetic acid and propionic acid after one month. Besides, acetic and propionic acids, isobutyric, butyric, isovaleric, valeric, isocapionic, capionic and lactic acids were found after two months with lactic acid appearing in excess compared to the others which were not present on the first month. This might be due to the presence of EM capable of converting the organic constituents into lactate.

Abstract Category

06 Engineered Environments

PS.06.042 FILAMENTOUS BACTERIA IN WASTEWATER TREATMENTS PLANTS: NOVEL INSIGHT IN DIVERSITY, ABUNDANCE AND FUNCTION

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Activated sludge wastewater treatment plants world wide often experience problems such as bulking and foaming due to excessive proliferation of filamentous bacteria. The result is evident as deteriorated settling properties and compromised effluent quality.

Unfortunately, most of the previous studies on the ecology of filamentous organisms in wastewater treatment plant have not been based on reliable culture-independent methods. Therefore, during the past 3 year we have carefully studied 30 full-scale Danish wastewater treatment plants with nutrient removal and obtained new understanding about identity, function and abundance of filamentous bacteria using culture-independent methods. Information about temporal variations in abundance, treatment plants

characteristics and wastewater compositions were obtained allowing search for interesting statistical correlations. The filamentous community constituted between 20-30 % of the total bacterial community in most plants which is significantly more than hitherto expected. The dominating filamentous organisms in most plants were identified as species within the phylum Chloroflexi, encompassing several morphotypes (Type 0041, 1851, 0803 and 0092) together with Candidatus *Microthrix parvicella*. Some seasonal variation in the abundance of Chloroflexi could be observed as well as some variation of species composition between the different sampling years. Also present in many samples were other filamentous bacteria, some with epiphytic growth identified as filaments belonging to phylum TM7 or *Curvibacter*-related species as well as other commonly but less abundant species. Comprehensive statistical measures (principle component analysis and cluster analysis) were applied for further investigation of probe-defined filamentous bacteria and treatment operation and design. Several correlations were revealed e.g. the abundance of filamentous Chloroflexi and known protein hydrolysers identified as epiphytic members of Saprospirace.

Abstract Category

06 Engineered Environments

PS.06.043 EFFECT OF SALINITY ON DENITRIFICATION IN SALINE AND NON-SALINE GROWN BIOFILMS

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Fresh water shortage in many parts of the world and salt water intrusions into freshwater systems, have increased the eco- and socioeconomic importance of desalination for potable water production. One of the most common desalination methods is reverse osmosis. This method produces brine as a by-product that is usually contaminated with nitrate, which might be eliminated before brine disposal by denitrification. Denitrification efficiency may decrease with increasing salinity, which could pose problems on its application on the brine treatment. In this study, we investigated the nitrate- and nitrite-reducing activities in saline and non-saline grown biofilms aiming to get a better insight of salinity effects in biofilm denitrification. We obtained biofilms from model upflow-sludge bioreactors. Incubation experiments with ¹⁵N-labeled substrates were used in combination with membrane inlet mass spectrometry to assess N-cycling processes, in particular denitrification rates under different salinities (0-3%). Additional microsensor measurements were performed to examine the detailed stratification of the saline grown biofilms. Our experimental results showed a rapid decrease of denitrification rates in non-saline grown biofilms. In comparison, salinity increase had only little effect on denitrification rates measured in saline-grown biofilms. Intriguingly, dissimilatory nitrate reduction to nitrite was not affected by salinity for either of the biofilms, resulting in an accumulation of toxic nitrite when activities of subsequent reactions of denitrification were reduced. The decrease in denitrification rate of the non-saline grown biofilm suggested only low abundance of halotolerant denitrifying bacteria. The microbial community within the saline grown biofilms appeared to be more adapted to saline

conditions. This salinity dependent difference in denitrification rate contrasts sharply with the observed salinity independence of dissimilatory nitrate reduction to nitrite.

Abstract Category
06 Engineered Environments

PS.06.044 CHARACTERIZATION OF MICROBIAL COMMUNITY OF CATHODE BIOFILM IN SINGLE-CHAMBER AIR-CATHODE MICROBIAL FUEL CELLS DEGRADING TEXTILE DYES

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Microbial fuel cells (MFC) represent a new approach for clean energy production with their capability of bioelectricity generation from organic matter. Determination of microbial community profiles in MFCs using various substrates can provide vast amount of information towards a better energy yield. Various studies about increasing the efficiency of MFC devices were published recently, however, the importance of microbial dynamics of electrode biofilms, particularly on the cathode, in MFC performance, remain to be better understood. Thus, the main goal of this study is to characterize bacterial species on the cathode biofilm in identical single chamber air-cathode MFCs degrading various reactive textile dyes, using cultivation-independent methodology. Despite their identical inocula, electrode materials and architecture, the maximum power generated varied significantly. Maximum power densities of 322,7mW/m², 349,1 mW/m², 516,3 mW/m² and 297,7 mW/m² were calculated in four identical fuel cells operating under same conditions. According to the DGGE profiles, the MFC with the maximum power output housed less number of taxa on the cathode biofilm (varied between 11-13) in comparison to that in cells generated lower power output (14-17). The DGGE profile of the MFC generating the maximum power exhibited high dissimilarity (38-53%) with other MFCs (dice's similarity coefficient). Community members of the cathode biofilm were identified phylogenetically using 16S rRNA gene clone-library analyses. Clone library analysis showed the presence of *Aquamicrobium*-, *Mesorhizobium*-, *Ochrobactrum*-, *Thauera*-, *Paracoccus*-, *Achromabacter*-, *Chelatacoccus*- and *Methanobacterium*- affiliated phylotypes as major phylotypes, according to the clone frequencies. It is clear that the distribution of microorganisms in cathode biofilms is important for the overall MFC performance and will be the further research focus.

Abstract Category
06 Engineered Environments

PS.06.045 NOVEL NITRITE OXIDIZERS RELATED TO *NITROTOGA ARCTICA* FREQUENTLY APPEAR IN WASTEWATER TREATMENT SYSTEMS

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During the last two decades, many studies have shown that members of the genus *Nitrospira* are the most important nitrite-oxidizing bacteria (NOB) in wastewater treatment plants (WWTPs). Only recently, the novel nitrite oxidizer *Nitrotoga arctica* was identified in permafrost soil and close relatives were enriched from wastewater. The aim of this study was to investigate the in situ distribution, abundance, and spatial localization of *Nitrotoga* in different WWTPs by PCR, FISH, and digital image analysis. For this purpose, *Nitrotoga*-specific new PCR primers and FISH probes were developed and applied to a range of wastewater samples. By using PCR, *Nitrotoga* were detected in approximately half of the WWTPs analyzed, whereas quantitative FISH revealed a high abundance of *Nitrotoga* only in some of these plants. Surprisingly, however, in some systems *Nitrotoga* coexisted with *Nitrospira* and even were the sole detectable NOB in one of the WWTPs. Quantification revealed that they accounted for nearly 2% of the total bacterial community in this reactor, a number resembling the abundance of *Nitrospira* in other WWTPs. Furthermore, statistical analysis of spatial distribution patterns in sludge flocs revealed that *Nitrotoga* co-aggregated with ammonia oxidizers of the *Nitrosomonas oligotropha* lineage, strongly suggesting that they indeed were responsible for nitrite oxidation. Cultivation showed that main factors favoring *Nitrotoga* are low temperatures and low nitrite concentrations. However, the conditions under which they outcompete *Nitrospira* in full-scale WWTPs are not yet understood. Our PCR results indicate that *Nitrotoga* are present in many plants at least as part of the "rare biosphere", but their amounts may increase in response to changing conditions in the reactors. Thus, deeper insights into the physiological differences between *Nitrotoga* and *Nitrospira* will help to understand and predict the population dynamics of these functionally important NOB in engineered systems.

Abstract Category
06 Engineered Environments

PS.06.046 INFLUENCE OF CARRIER BACKGROUND AND DESIGN ON THE MICROBIAL POPULATION IN A ONE-STAGE NITRITATION-ANAMMOX MBBR PROCESS

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A Moving Bed Biofilm Reactor pilot-plant, designed for N-removal was monitored for one year. It was fed with an ammonium-rich anaerobic digester effluent. In this process, partial nitrification and anammox N-removal occur within the aerobic and anoxic zones of the carrier biofilm resulting from limited dissolved oxygen condition. Due to the slow growth rate of anammox bacteria (AnAOB), carriers with previous AnAOB biofilm were added to the reactor to reduce the start-up phase. The aim of this study was to define the best operating conditions to quickly establish well-performing biofilm with nitrifying bacteria and AnAOB. The impact of 2 different parameters on biofilm development and composition was assessed: a) carrier design: different types of brand-new carriers were

used; b) carrier background: carriers previously colonised with nitrifying biofilm were used. Behaviour of different bacterial groups involved in autotrophic N-removal was evaluated and several parameters were measured: a) total biomass for estimation of the carrier colonisation; b) quantification of AnAOB, ammonium oxidising bacteria (AOB), nitrite oxidising bacteria (NOB) using qPCR for estimate the biofilm microbial composition; c) diversity was estimated for total bacteria and AnAOB by using DGGE. qPCR analyses showed: a) a rapid and important colonisation of all carriers including brand-new carriers; b) at the end of this study, an equivalent AnAOB concentration was observed on all carriers independently from the carrier design; c) carrier colonisation by AOB seemed to be related to carrier design; d) even if a significant NOB concentration is detected on all carriers it did not seem to influence N-removal performances. DGGE analyses revealed that if the carrier design had no significant impact on the AnAOB diversity, the latter was strongly influenced by the carrier background. Actually, carriers with previous nitrifying biofilm showed reduced AnAOB diversity compared to brand-new carriers.

Abstract Category

06 Engineered Environments

PS.06.047 MICROBIAL COMMUNITIES IN RELATION TO BIOLOGICAL STABILITY IN A NON-CHLORINATED DRINKING WATER SYSTEM

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In Switzerland, multistep ozonation-biofiltration treatment processes are used to convert surface water to biologically stable drinking water, which is then distributed without the addition of disinfectant residuals. Indigenous microbial communities proliferate in the water during the treatment process and as a result are present in the distributed water. Biological stability refers to the inability of drinking water to support microbial growth, which implies that the amount and composition of these indigenous bacteria do not change during distribution until consumption. However, varying residence times and elevated temperatures might influence microbial cell concentrations and composition, especially on household level where the water often stagnates. We have analyzed a full-scale drinking water system from source to tap, while the influence of stagnation on household level was specifically assessed. Changes in bacterial cell concentrations, activity and composition were determined with flow cytometry, adenosine-trisphosphate measurements and denaturing gradient gel electrophoresis. The results showed considerable changes in bacterial numbers and composition during the various treatment steps. Bacteria in the final drinking water originated from the biofilters and cell concentrations and composition did hardly change during distribution. However, overnight stagnation in household taps caused at least a two-fold increase in total bacterial cell concentrations, with a clear shift in the community composition. This study

has highlighted the need to understand the factors influencing microbial composition and stability in drinking water during treatment and distribution.

Abstract Category
06 Engineered Environments

PS.06.048 CHARACTERISTICS OF MICROBIAL FUEL CELL ENRICHED WITH SLUDGE USED LEATHER WASTEWATER TREATMENT

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Researches of Microbial fuel cell (MFC) were approached from various fields, one of them is wastewater treatment capability. MFCs were enriched with sludge used leather wastewater treatment, tested Cr(VI) removal activity, and the microbial communities were analyzed by PCR -DGGE. The sludge from biological treatment tank for leather tanning wastewater treatment was used as inoculum and synthetic wastewater was sequentially added to the anode as fuel. The current profile was increased and stabilized in a week and the stable coulombs were $0.51 \text{ C} \pm 1.16\%$. The SEM and CLSM distinguished that the electrode was covered with bacteria consortium. After enrichment, the device was used to Cr(VI) removal test. The results were found to 93% of 5 mg/L Cr(VI) and 61% of 25 mg/L Cr(VI). The microbial structure was examined. DNA extracted from the anodic electrode in the microbial fuel cell and the inoculated sludge was amplified with GC-clamped primer by PCR and resolved on the denaturing gradient gel. DGGE profiles from the anodic electrode showed significant change in the microbial population when compared with the inoculated sludge. The dominant bacteria were found to be to *Betaproteobacteria* sp., *Actinobacter* sp. and *Acinetobacter* sp., of which *Acinetobacter* sp. is known to be a Cr(VI)-reducing bacteria. Taken together, these results suggest that Cr(VI) removal by MFC enriched with sludge using leather wastewater treatment could be one of the properties using MFC.

Abstract Category
06 Engineered Environments

PS.06.049 PULL THE TRIGGER: STRIKING EMERGENCE AND ABSOLUTE DOMINANCE OF AEROMONAS DURING THE INITIAL PERIOD OF ANAEROBIC DIGESTION

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Anaerobic digestion has been widely used to treat various wastewaters due to the production of methane and the low generation of sludge. This useful process is underpinned by a series of reactions performed by various microorganisms, grouped into acidogens and methanogens. Acidogens hydrolyze and ferment organic materials to

produce organic acids and alcohols, which are then converted by methanogens to methane. It is thus desirable to understand the microbial background of anaerobic digestion in light of the behavior of acidogens as well as methanogens. Imbalance or overgrowth of acidogen community can cause an accumulation of unfavorable intermediates to methanogens, resulting in inhibition of methanogenesis. This is why we often see digester upset during start-up. This study aimed to identify and monitor key acidogens during the initial anaerobic digestion period, which likely trigger the overall process. Three anaerobic batch bioreactors were operated on synthetic glucose wastewater, whey permeate, and liquefied sewage sludge, respectively. Bacterial community structures were investigated using denaturing gradient gel electrophoresis, and *Aeromonas*-like and *Clostridium sticklandii*-like groups were prominent and common in all reactors. The real-time PCR primer/probe sets specific to *Aeromonas* and *Clostridium sticklandii* were developed and used to monitor the quantitative dynamics of target populations. Intriguingly, *Aeromonas* population surged up to 86.6-95.3% of total bacteria in 16S rRNA gene copy concentration, indicating its critical function in the initiation. Given its ability to use various carbohydrates, *Aeromonas* was likely to be responsible for the rapid initial fermentation of carbohydrate. *C. sticklandii*, utilizing amino acids, occupied up to 8.58-55.2% of total bacteria. The overall results suggest that both *Aeromonas* and *C. sticklandii* populations were functionally critical in three reactors regardless of substrate composition.

Abstract Category

06 Engineered Environments

PS.06.050 CONTROL OF *MICROTHRIX PARVICELLA* AND SLUDGE BULKING THROUGH LOW-OZONE DOSE EXPOSURE

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The problem with sludge bulking is common in wastewater treatment plants (WWTP) and is often caused by increased growth of filamentous bacteria. One of the most common filamentous bacteria involved is *Candidatus Microthrix parvicella*. These filamentous bacteria have a negative impact on the sludge settling properties in the activated sludge process. It has been shown that a continuous ozone dosage in an activated sludge process can control sludge bulking. Ozone stresses the filamentous bacteria causing inactivation through cell wall disintegration. The aim of this study was to evaluate the low dosage of on-site generated ozone in the recycled activated sludge flow on a full scale plant, Himmerfjärden WWTP. The evaluation of the experiment was done by using process data, microscopic analysis and microbial screening on the experimental and control line before, during and after the period of operation. By FISH analysis *Microthrix parvicella* was identified in both the experimental and control line. The ozone treatment of the recycled activated sludge resulted in decreased abundance of *Microthrix parvicella* and improved settling properties of bulking sludge. However, it did not interfere with other important processes e.g. nitrification. As the ozone treatment was

stopped the problem with sludge bulking reappeared indicating the importance of continuous evaluation of the process.

Abstract Category
06 Engineered Environments

PS.06.051 EFFECT OF INORGANIC CARBON ON NITRIFYING COMMUNITY STRUCTURES

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In this study, we examined the hypothesis that different inorganic carbon concentrations would enrich different nitrifying populations (ammonia-oxidizing and nitrite-oxidizing bacteria) by operating two chemostat bioreactors fed with different influent carbonate concentrations at 15 and 100 mg-inorganic carbon/L, respectively. During the operation period, both bioreactors were able to maintain satisfactory nitrification efficiency higher than 95% at an influent ammonium concentration of 250 mg-N/L, and nitrate was the major end product without nitrite accumulation. Based on cloning/sequencing and terminal restriction fragment length polymorphism results targeting on ammonia monooxygenase gene subunit A functional gene, *Nitrosomonas nitrosa* lineage was the dominant ammonia-oxidizing bacteria population in the high-inorganic carbon bioreactor, while *Nitrosomonas europaea* lineage was dominant in the low-inorganic carbon bioreactor. Furthermore, results of batch experiments on effects of inorganic carbon concentration on ammonia-oxidation rate indicated that low inorganic carbon conditions can cause negative impact on *Nitrosomonas nitrosa*-like ammonia-oxidizing bacteria dominated in the high-inorganic carbon bioreactor, but not on *Nitrosomonas europaea*-like ammonia-oxidizing bacteria enriched in the low-inorganic carbon bioreactor. The community structures of nitrite-oxidizing bacteria were also analyzed in the two reactors by cloning and sequencing, and terminal restriction fragment length polymorphism. To know more about the community structures, the abundance and diversity of nitrifiers were analyzed by fluorescence *in situ* hybridization. In this study, we confirm that different inorganic carbon concentrations would influence nitrifying community structures and the associated kinetic characteristics.

Abstract Category
06 Engineered Environments

PS.06.052 MICROBIAL ECOLOGY IN REMEDIATION OF PETROLEUM HYDROCARBON CONTAMINATED SOILS - EFFECTS OF SOIL ORGANIC MATTER AND AGEING

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Though information on bioavailability of single hydrocarbons of polycyclic aromatic hydrocarbons is available, less is known bioavailability of petroleum hydrocarbons impacted by aging and soil organic matter (SOM) in soil. Two bio-pile batches were conducted for remediation of total petroleum hydrocarbon (TPH) contaminated soils, designed as different SOM (Batch I) and ageing time (Batch II). The study aimed at investigating the microbial ecology during the TPH remediation.

The soils in Batch I contained SOM 70.5% (P), 9.0% (STP), and 2.3% (STC). Batch II soil samples were artificially contaminated with diesel for 0 day (ST0), 38 days (ST38), and 101 days (ST101). Microbial ecology was analyzed with terminal-restriction fragment length polymorphism (T-RFLP), followed by statistical multivariate analyses: non-metric multidimensional scale (MDS) and cluster analysis.

The 1st order kinetic rate (k) of P, STP, and STC, were 0.0106, 0.0188, and 0.0133 day⁻¹; respectively. The TPH degradation efficiency presented a similar trend: P (72.7%) < ST (84.4%)~STC (85.8%). The k values in Batch II were decreased with ageing (ST0: 0.0245 day⁻¹, ST38: 0.0128 day⁻¹, ST101: 0.0090 day⁻¹). The similar degradation pattern was found in the TPH degradation efficiency (ST0: 87%, ST38: 71%, ST101: 58%). The MDS plot indicated 1) two different bacterial communities in P and ST, 2) different bacterial communities among ST0, ST38, and ST101 on the first day of remediation, and 3) the bacterial community in ST101 produced the communities with the least diversity. 14 and 6 species which caused the clusters in the MDS plot were identified with an analysis of variation.

SOM content in soils could affect the rate and the extent of TPH degradation. Aged soil is more difficult to be remediated than freshly-contaminated soil. Bacterial community structures and diversity in contaminated soil could be affected by SOM and TPH ageing.

Abstract Category

06 Engineered Environments

PS.06.053 MICROBIAL COMMUNITY STRUCTURES OF POISED ELECTRODES IN MICROBIAL FUEL CELLS

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Microbial fuel cells (MFCs) take advantage of exoelectrogens, or microbes that can reduce electrodes, to create electricity via biochemical reactions. The electricity production can be coupled with the degradation of organic substrates, such as in a wastewater treatment plant. Researchers have shown that poisoning an electrical potential at the anode can improve the electrical output of the MFC by favoring colonization by certain exoelectrogens from pure and mixed culture inocula, such as wastewater. In this

research, we correlate the power production in poised MFCs with the anodic and cathodic microbial communities. We applied a floating potential (ranging from -400 to +400 mV) between the anode and cathode in acetate-fed wastewater single-chamber MFCs, which resulted in consistent anodic potentials and varying cathodic potentials. After 8 days, during which time the anodic and cathodic biofilms were established and power production had stabilized, the MFCs at +200 mV or - 200 mV showed the highest power production. Anodic microbial community structure, as assessed using Ribosomal Intergenic Spacer Analysis (RISA), did not differ greatly between the samples. The floating potential was subsequently removed from the MFCs, and they were monitored for another 6 days during which time all the MFCs reached the same power output. A second RISA showed that the anodic microbial community structure was consistent among the different MFCs, but the cathodic community structures varied greatly. While previous research has demonstrated changes in anodic biofilm due to an applied potential, our research demonstrated that the microbial communities varied most at the cathode. We hypothesize that the application of a floating potential between electrodes may encourage different redox functions in the colonizing microbes, or that the cathodic microbial community plays a greater role in determining power production than previously appreciated.

Abstract Category

06 Engineered Environments

PS.06.054 MICROBIAL COMMUNITIES ANALYSIS OF IN BIOAUGMENTED MUNICIPAL WASTEWATER TREATMENT SYSTEM USING PCR-DGGE AND BIOLOG TECHNIQUE AT HILLY AREAS

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Bioaugmentation was applied for the treatment of municipal wastewater plant at low temperature ($T < 14^{\circ}\text{C}$) in hilly areas of China. The main study objective was to characterize microbial community structure in bioaugmented system by culture-independent techniques, and dynamically monitor the pollutants removal efficiency of the wastewater treatment plants. The results showed that the microbial community structure was stable and the average concentrations of COD, $\text{NH}_4^{+}\text{-N}$ meet the discharge standards from the influent to the effluent in bioaugmented system at low temperature. The average removal efficiency of effluent COD and $\text{NH}_4^{+}\text{-N}$ was more than 80.81% and 76.24%, respectively. Culture-independent microbial community produced by using PCR-denaturing gradient gel electrophoresis (PCR-DGGE) technique showed that the bacterial community was rich in diversity. Data produced by using Biolog technique showed that the microbial metabolic activity was strengthened. Sequencing of partial 16S rDNA genes fragments revealed that the total microbial community composition was dominated by *Pseudomonas* sp., *Aeromonas* sp., *Nitrospira* sp., other flocculating bacteria and other soil bacteria. Functional analysis demonstrated that some of the microorganisms were related with low temperature. Different bacteria played important roles to the efficient and stable performances of the bioaugmented systems. In

conclusion, the analysis of microbial community and function with bioaugmentation laid a solid theoretical basis for promoting the development of bacterial agent at hilly areas of China.

Abstract Category
06 Engineered Environments

PS.06.055 CHINA'S WASTEWATER TREATMENT: CHANCES AND CHALLENGES

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China's environmental problems dominate those of the world, not only because China accounts for a fifth of the world's population, but also because China's economy is growing in an unprecedented speed. Water pollution and water scarcity are burdening the economy, rising levels of air pollution are endangering the health of millions of Chinese, and much of the country's land is rapidly turning into desert. Among all the natural elements to sustain human being's survival, ensure economic construction and maintain social development, the importance of water is beyond question. China is now confronted with double challenges of water resources shortage and severe water pollution. With the objectives to resolve China's water pollution problems from a systematic and ecological perspective, the wastewater treatment general situation, the wastewater treatment technologies, the treatment and disposal of excess sludge and green house gases emitted from waste and wastewater treatment of China were surveyed. Many issues normally ignored by the public, such as the green house gases emission from wastewater treatment field, were advanced. Besides enhancing the disposal efficiency of wastewater and sludge, recycling treated wastewater and biomass, choosing appropriate processes and operation parameters for wastewater treatment facilities, innovative concept characterized as microbial oriented conversion for the future wastewater treatment in China were also presented. It is hoped that conclusions arrived in this paper would be helpful to solve China's water pollution problems and alleviate its impacts on human being, economic growth and social development.

Abstract Category
06 Engineered Environments

PS.06.056 EVALUATION OF INTER-RELATIONSHIP BETWEEN NUTRIENTS IN SUBSTRATES AND DE-EMULSIFICATION CAPABILITY OF DEMULSIFYING BACTERIA (A *Bacillus* sp.)

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Biological de-emulsifier will become a kind of de-emulsifier instead of chemical de-emulsifier for its high efficiency, low cost, high adoptability, environment-friendly and repeated use. This work investigated the effects of nutrients in substrate (glucose, liquid paraffin, NH₄NO₃ and yeast) on de-emulsification capability and the growth of a bacillus sp. (isolated from petroleum-contaminated soil by our laboratory). The de-emulsification capability of the broth was evaluated by the demulsifying ratios of oil-in-water model emulsion system and the biomass was represented by absorbance (620nm) during 24h feed with substrates for different compositions. Based on the results obtained from the orthogonal tests, the significant of de-emulsification capability in turn was glucose>liquid paraffin>NH₄NO₃>yeast and the significant of the growth in turn was glucose>NH₄NO₃>liquid paraffin>yeast. Compare with other three nutrients, the highest effect on de-emulsification capability and growth was glucose. The highest demulsifying ratio (87%) was reached when the glucose (10g/L) was used up in substrate. The liquid paraffin contents (0%, 1%, 2%, 3%, 4%, 5%, 6% (v/v)) in substrates had no obvious influences on the growth. The de-emulsification capability was enhanced at liquid paraffin contents of 2%~5% (v/v), while in absence of liquid paraffin, the demulsifying ratio was 0%. Measurements of the liquid paraffin residue indicated only 30.1% liquid paraffin (initial value was 4%(v/v) in substrate) were consumed and this part was related to demulsifying compounds production. The highest demulsifying ratios (90%) and biomass were appeared at yeast extract concentration of 0.8mg/L~1.0mg/L. The main conclusion of this study was that the de-emulsification capability of the broth was induced by liquid paraffin, while the highest efficiency of de-emulsification ability was obtained by glucose.

Abstract Category
06 Engineered Environments

PS.06.057 URANIUM REDUCING MICROCOSMS REVEALED COMMUNITIES DOMINATED BY *GEOBACTER* AND *PELOSINUS*

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To identify uranium reducing bacterial populations and the structure of strongly reducing communities, enrichments of U-contaminated sediments were performed using modified Freshwater Medium (FWM) and PIPES Buffered Artificial Ground Water (PBAGW). Three actively reducing, uranium contaminated sediments (FW107, FW102-2 and FW102-3) from the S3 area of the Field Research Center at Oak Ridge National Laboratory were used as the initial inocula. Amplicon pyrosequencing targeting 16S rRNA genes revealed substantial differences between inocula from FW107 and FW102-2 or FW102-3, where the latter two were from different depths of the same uranium-reducing sampling well. Known Fe/U-reducing bacteria *Geobacter* and *Desulfovibrio* were detected in all three original sediment samples at low abundance (1-5% for *Geobacter* and 0.4-5% for *Desulfovibrio*). Despite these shared populations, in U(VI)-lactate supplemented FWM microcosms, FW107 inoculated enrichments were dominated by *Pelonsinus* (84.86%) while *Geobacter* was dominant in FW102-2 (84.41%) and

FW102-3 (59.45%). After incubation, *Geobacter* was no longer detected in the FW107 uranium microcosm while *Pelonsinus* increased significantly in both FW102-2 and FW102-3 uranium microcosms (from undetected to approximately 10%). To diminish the formation of uranium precipitates, modified PBAGW microcosms supplemented with U(VI) and pyruvate were used in similar enrichments of FW107 and FW102-2. During the first ten days of uranium reduction, cell protein of both communities increased from 0.1µg/ml to 8µg/ml accompanied by the depletion of pyruvate and accumulation of acetate. Within 20 days, U(VI) in solution decreased from approximately 250µM to 196µM and 86µM in FW107 and FW102-2, respectively. In addition, the abundance of *Pelonsinus* increased from 19% to 43% in FW107, and from undetectable to 2% in FW102-2, respectively. Transcriptomics will be used to better understand the functioning of these uranium-reducing communities.

Abstract Category
06 Engineered Environments

PS.06.058 NATURAL OCCURRENCE OF RESISTANCE GENES: RISK ASSESSMENT ON THE USE OF NPTII GENE IN TRANSGENIC FIELD

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One of the risks associated with the use of transgenic plants in the field is the transfer of transgenes to soil bacteria that could lead to a wide and uncontrolled dissemination of unwanted traits among bacterial communities. The main concern is about the transfer of transgenic plant cell selection genes, such as the antibiotic resistance genes, to human pathogens. Several laboratory and greenhouse studies demonstrated that such gene transfer events could happen under natural conditions, but that their potential impact would strongly depend on the natural occurrence of these genes in soil bacterial genomes. For example, the widely used *nptII* gene confers resistance to kanamycin. The risk of modifying the antibiotic resistance genetic determinants of soil bacteria is qualitatively greater in the absence of naturally-occurring *nptII* gene in soil bacteria if the gene is efficiently transferred while the risk would be much lower if the gene was already widely spread among soil bacteria. In our study we combined in silico analysis of available metagenomic sequences and qPCR measurements of DNA extracted from both control and transgenic plant field soils to determine the occurrence of the *nptII* gene in various environments. Our objective was to provide an estimation of the theoretical risk of the use of the *nptII* gene in transgenic plants and to compare it with other available marker genes to determine the safest environmental alternative for plant construction.

Abstract Category
06 Engineered Environments

PS.06.059 REPRODUCIBILITY OF ELECTROCHEMICAL CHARACTERISTICS AND MICROBIAL COMMUNITIES IN MIXED-CULTURE MICROBIAL FUEL CELLS

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The Microbial fuel cell (MFC) has received a lot of attention in recent years as a candidate for alternative energy generation. In order to optimize the MFC process, several studies have been conducted studying the parameters affecting the power output such as cell design, operation conditions, electrode materials, and most importantly the microorganisms that are active in the MFC environment. However, comparison of the results provided is not always possible because of the variation of the experimental parameters. In addition, the results reported are not necessarily representative if they are not shown as reproducible. In this study we attempt to address a number of reproducibility issues in MFCs including electrochemistry and microbiology. We operated, in parallel, three identical but independent MFC units under the same environmental conditions for 90 days. Each MFC unit comprised of autoclavable compartments, a cation exchange membrane and flat-plate graphite electrodes. Each anodic electrode was overlaid with carbon paper to accommodate biofilm collection and visualization. The system was assembled under sterile conditions and each MFC anode was inoculated with the same mixed bacterial culture. The MFC units were operated in a semi-batch mode, at a temperature of 32°C and circumneutral pH with acetate as the electron donor in the anolyte and $\text{Fe}(\text{CN})_6^{3-}$ in the catholyte for a fast and stable cathode reaction. Anode potential, cell voltage, bacterial cell growth and substrate consumption was monitored over time. Electrochemical comparison studies included maximum power output determination, cyclic and potential step voltammetry at different activity stages of the anodic biofilm and impedance spectroscopy. The MFC design of choice allowed for a uniform biofilm formation enabling quantitative assays of the biofilm and phylogenetic characterization of the dominant microbial populations of the biofilm with denaturing gradient gel electrophoresis.

Abstract Category

06 Engineered Environments

PS.06.060 QUANTIFICATION OF DISSIMILATORY (BI)SULFITE REDUCTASE GENES AND TRANSCRIPTS DURING IN SITU BIOREMEDIATION OF URANIUM-CONTAMINATED GROUNDWATER

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Studies at the Department of Energy Integrated Field Research Challenge site in Rifle, CO, have demonstrated that addition of acetate to the subsurface initially stimulates dissimilatory metal reduction by *Geobacter* species and effective removal of uranium from the groundwater. However, after approximately thirty days of acetate additions, the

system typically undergoes a transition from Fe(III) reduction to sulfate reduction and the effectiveness of uranium removal diminishes. During the 2008 field study at the site, the diversity of sulfate-reducing microorganisms and their metabolic state was investigated to better understand the microbial community dynamics involved in the transition to sulfate reduction. Clone libraries based on the *dsrAB*, which encode the α and β subunits of the enzyme dissimilatory (bi)sulfite reductase, indicated that there were three major phylogenetic clusters of sulfate reducers in the groundwater at the Rifle site: *Desulfobacteraceae*, *Desulfobulbaceae*, and the *Syntrophaceae*-related sulfate reducers. The recovered *dsrAB* sequences were used to design primers to assess the metabolic state of the sulfate-reducing community from estimates of *dsrAB* transcript abundance. Quantitative PCR assays revealed that the *Desulfobacteraceae* cluster was the most active, as the number of *dsrAB* transcripts rapidly increased and remained at high levels (1.0×10^4 transcripts/cell) during the sulfate reduction phase. *Desulfobulbaceae* and *Syntrophaceae* were present at the site, but their level or *dsrAB* expression remained significantly lower than that of the *Desulfobacteraceae* (1.0×10^1 - 1.0×10^2 transcripts/cell). These results suggest that diversion of acetate flux from *Geobacter* species to non-U(VI)-reducing *Desulfobacteraceae* is a likely explanation for the poor performance of the uranium bioremediation strategy under sulfate-reducing conditions.

Abstract Category

06 Engineered Environments

PS.06.061 CHRONIC BACTERIAL INFECTION OF A PAPER MILL?

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Paper makers pay substantial amounts for controlling biofouling and microbial contamination of their facilities. When successful anti-fouling strategies are in place, these costs are justified by improved paper quality and reduced operating costs. Two contamination scenarios are conceivable in a paper mill: (1) Transport of high pulp- and water volumes through the plant leads to high migration rates of microorganisms and consequently to the spread of related bacterial communities in all areas of the plant. (2) Environmental conditions select for adapted communities in unit operations, independent of volume flows. These two scenarios have major implications for treatment strategies. We follow bacterial communities using Single Strand Conformation Polymorphisms of PCR-amplified bacterial 16S rDNA. Two sets of bacterial communities along the production lines could be detected. Within the sets, the communities are related to each other. Between the sets, the relatedness of the communities is weak. When following pulp flow in the paper mill, the disconnect between the community sets coincides with two samples from the entry and exit of one specific storage silo for pulp. Bacterial communities in this storage silo have undergone a dramatic shift, possibly because of a strong selection for a locally adapted community as hypothesized in scenario (2). A long retention time in the silo facilitated growth of this community to sufficiently large numbers to outweigh the entering community. Bacterial communities downstream the silo are more related to each other, possibly following contamination scenario (1).

Treatment upstream of the storage silo will not affect downstream communities as the silo divides the paper mill into two separate entities from a bacterial perspective. A chronically contaminated storage silo is comparable to an infection of an implant or a lung in that the source of the infection has to be treated rather than the contaminated bloodstream or the pulp circuit.

Abstract Category
06 Engineered Environments

PS.06.062 NITRIFICATION AND DENITRIFICATION COMMUNITIES ASSOCIATED WITH A SEMI-PERMEABLE SWINE WASTE LAGOON BIOCOVER

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Ammonia emission from swine waste lagoons presents a serious environmental challenge to pork producers. Semi-permeable swine waste lagoon biocovers have been developed to serve as a physical barrier and as an attachment site for biofilm development, but microbial analysis of the biocover technology is needed to validate their usefulness. Samples of mature biocover and its associated biofilm and lagoon liquids were collected from a swine waste lagoons, and their potential nitrifying and denitrifying activities were determined in laboratory aerobic and anaerobic incubations. Potential nitrification activity for the mature biocover samples produced $1.9 \mu\text{moles nitrite L}^{-1} \text{ hr}^{-1}$ during a 24-hour period, whereas new biocover (no biofilm) and lagoon supernatant samples produced $< 0.008 \mu\text{moles nitrite L}^{-1} \text{ hr}^{-1}$. Potential denitrifying activity for the mature biocover samples consumed $25 \mu\text{moles nitrate+nitrite L}^{-1} \text{ hr}^{-1}$. In the supernatant and new biocover (no biofilm) samples $< 1.6 \mu\text{moles nitrate+nitrite L}^{-1} \text{ hr}^{-1}$ was consumed. Molecular analyses of PCR products by DGGE indicate that nitrifiers belong to *Betaproteobacteria* were present. We conclude that the biocover served as a useful support for the production of a beneficial biofilm community capable of transforming ammonia to nitrogen gas via a coupled nitrifying and denitrifying process.

Abstract Category
06 Engineered Environments

PS.06.063 TREATMENT OF XENOBIOTICS IN ACTIVATED SLUDGE PROCESS AND HYBRID BIOREACTOR: IMPACT OF PROCESS PERFORMANCES ON BACTERIAL COMMUNITY STRUCTURE

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In conventional wastewater treatment plants based on the activated sludge process, biodegradation of aromatic hydrocarbons (BTEX, PAHs) can be difficult due to the propensity of the compounds either to transfer to the gas phase (e.g., BTEX, naphthalene), absorb in the complex sludge matrix (e.g., other PAHs), or inhibit microbial activity (e.g., phenol derivatives). In this work, the addition of mobile carriers in activated sludge was investigated for improving the biological degradation of xenobiotic compounds. This strategy leads to hybrid processes including microbial flocs and biofilms in the same reactor. The objective of this study was to compare the microbial community structure and diversity in the different compartments of the conventional activated sludge and hybrid processes in relation to dynamic performances. Experiments were performed in laboratory-scale bioreactors and a pilot-scale bioreactor set up at an oil refinery wastewater treatment plant. Laboratory-scale bioreactors were fed in batch and submitted to a specific feeding scenario with a stable phase followed by a disturbance. In all experiments, biomass activity, BTEX and PAH concentrations as well as overall process performances were monitored. In addition, microbial community structures were characterized as a function of time using RISA and 16S-based taxonomic microarrays. Results show that bacterial community structures differed drastically between the different processes and as a function of performances (i.e., degradation of xenobiotic compounds). Microbial diversity was increased in the hybrid reactors due to the presence of carriers supporting biofilm growth. Interestingly, profiles of functional community could be established with identification of taxonomic groups responsible for the degradation of different PAHs and BTEX. Data obtained suggested that bacterial community structure could be used as a bioindicator of process performances in regard to PAHs and BTEX degradation.

Abstract Category

06 Engineered Environments

PS.06.064 METAGENOMIC AND METAPROTEOMIC ANALYSIS OF ANODIC COMMUNITIES RESPONSIBLE FOR ELECTRICITY PRODUCTION IN MICROBIAL FUEL CELLS

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Replacement of fossil fuels with renewable energy sources is one of the greatest challenges we will have to meet in the coming years. One of the most promising approaches developed is the use of microorganisms to convert biomass into valuable energy such methane, biodiesel, hydrogen and electricity. Microbial production of electricity via microbial fuel cells has been intensively investigated these recent years and major advances have been achieved through improvement of reactor architecture, membranes and electrodes types. Less effort has been put on the microbial compartment and the comprehension of mechanisms leading to the development of electroactive biofilms in such systems. Development of microbial bioelectricity as a renewable energy sources will require in-depth knowledge of the microbial communities developing at the

surface of the electrodes and identifying the processes leading to the development of electroactive consortia. The main objective of this project was to use cutting-edge metagenomic and metaproteomic technologies on bacterial communities responsible for electricity production in microbial fuel cells in order to characterize the diversity and adaptive features of bacteria in these systems as well as the identification of mechanisms involved in electron transfer. Metagenomic and metaproteomic data were obtained from biofilms developed at the surface of the anodes and exhibiting different electrical performances in microbial fuel cell reactors. This study represents the first in-depth functional analysis associating metagenomic and metaproteomic datasets of complex communities involved in bioenergy production. Results obtained demonstrate the power of combining the two approaches to study complex communities and should prove useful to assessing the significance of extracellular electron transfer in shaping microbial communities.

Abstract Category
06 Engineered Environments

PS.06.065 USING ECOLOGICAL PRINCIPLES TO SELECT FOR SUPERIOR PHA-PRODUCING BACTERIA

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Polyhydroxyalkanoates (PHAs) are polyesters that are synthesized as energy and carbon source by many bacteria. PHAs ('bioplastics') can also be used to replace traditional plastics. A limitation of pure culture-based production of PHAs is the high substrate price and high energy consumption. Production of PHAs from waste by mixed cultures as proposed here could drastically reduce the price. By using a so-called 'feast-famine' strategy we were able to select a microbial community that was capable of accumulation of more than 90% of the cell dry weight as pHA. Phylogenetic analysis based on 16S rRNA sequences showed that the strongly dominant bacterium, *Plasticumulans acidovorans*, belonged to a novel lineage within the Gammaproteobacteria. Here we describe the conditions under which *P. acidovorans* could successfully be enriched. An aerobic sequencing batch reactor inoculated with activated sludge was operated at different temperatures (20°C and 30°C) and cycle lengths (1, 4, and 12 h), but at a fixed solid retention time (SRT) of 1 day. Sodium acetate was used as substrate. The microbial communities established in the different conditions were first analyzed by PCR-DGGE. Subsequently, the dominance of *P. acidovorans* was estimated using qPCR. The results clearly showed that cultivation temperature was more important for the selection of *P. acidovorans* than cycle length. At 20°C the reactors were dominated by a *Zoogloea*-related species, while at 30°C *P. acidovorans* was dominating. Kinetic analysis demonstrated that the PHA consumption rate was considerably lower than that of the *Zoogloea*-related species, thereby losing the competition at 20°C and 1day SRT during the famine phase. A thorough understanding of the conditions at which *P. acidovorans* could outcompete other bacterial populations would be essential for a commercial success of bioplastics from waste.

Abstract Category
06 Engineered Environments

PS.06.066 THE GENETIC BASIS OF ADAPTATION TO FORMALDEHYDE IN EXPERIMENTALLY EVOLVED POPULATIONS OF METHYLOBACTERIUM.

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Methylotrophs are a class of microorganisms that are specialized to grow on single carbon substrates. Most aerobic methylotrophic bacteria produce formaldehyde as a central metabolic intermediate. To grow on formaldehyde, these bacteria have to withstand its non-specific toxicity that causes DNA-DNA/ DNA-protein cross-linking. In this study we have evolved strains of *Methylobacterium extorquens* (strain AM1 and PA1), that are incapable of growth at formaldehyde concentrations ~1mM, to grow on 45 mM formaldehyde. *M. extorquens* (strains AM1 and PA1), evolved in liquid media (15mM methanol + 2.5mM formaldehyde) for 30 generations, were transferred to gradient plates with methanol + formaldehyde initially and formaldehyde as the sole carbon source later on. A band of cell material from the region of highest non-lethal concentration of formaldehyde on the plate was used as the inoculum for the next transfer. 15 transfers have been performed so far. Trade-offs and phenotypic parallelism are being investigated by examining the growth characteristics of replicate populations of the evolved and ancestral strains on single-carbon and multi-carbon substrates. The minimum inhibitory concentration (MIC) of formaldehyde increased from 1mM to 25mM after seven transfers. After fifteen transfers the evolved populations are capable of growing on 45mM formaldehyde. As a trade-off the evolved populations grow 2-5 times slower in methanol than the ancestor. Evolved populations of *M.extorquens* are likely to have accumulated adaptive mutations that confer resistance to formaldehyde toxicity and the ability to use it as a substrate. The nature of these mutations will be revealed by genome resequencing of isolates from the evolved populations. Replicate populations will reveal the extent of , both genotypic and phenotypic, parallelism during adaptation to growth on formaldehyde.

Abstract Category
06 Engineered Environments

PS.06.067 DEVELOPMENT OF A METABOLIC MODEL FOR TETRASPHERA INVOLVED IN ENHANCED BIOLOGICAL PHOSPHORUS REMOVAL PLANTS

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Enhanced biological phosphorus removal is an environmental and cost-effective phosphorus removal process from wastewater. It is believed to be carried out mainly by certain polyphosphate accumulating organisms called *Candidatus Accumulibacter*. Recently, *Actinobacteria* belonging to the genus *Tetrasphaera* has been identified as putative polyphosphate accumulating organisms. New analyses show that they comprise a larger percentage of the biomass in full-scale Danish wastewater treatment plants than *Accumulibacter* (8-12% and 3-7%, respectively) and may therefore be more important in the phosphorus removal process. Little is known about the physiology of *Tetrasphaera* but preliminary results indicate that its ecological niche is different from *Accumulibacter*. In order to understand their role in the biological removal of phosphorus, the aim of the project is to develop a metabolic model for phosphate-removing *Tetrasphaera* based on genomic investigations of different *Tetrasphaera* species. The genomes of three *Tetrasphaera* isolates from wastewater treatment plants (*T. australiensis*, *T. japonica*, *T. elongata*) have been sequenced using the Illumina platform. This was done by paired-end sequencing, producing short reads of 2x36 bp giving up to 540x coverage. The sequences have been subjected to de novo assembly using CLC Genomics Workbench and ABySS. After assembly, the genomes have been annotated using the MaGe annotation platform (<http://www.genoscope.cns.fr/agc/mage/>) and genome specific metabolic models were reconstructed. Several fundamental differences between *Tetrasphaera* and *Accumulibacter* have been identified, mainly related to the ability to take up substrate under anaerobic conditions and formation of storage material (polyhydroxyalkanoate). Several differences in the genomes have also been observed between the different *Tetrasphaera* species. Key physiological features are presently being tested using single cell in-situ microbiology in full-scale treatment plants.

Abstract Category

06 Engineered Environments

PS.06.068 MICROBIAL AND PHYSICOCHEMICAL DYNAMICS IN AN ARTIFICIAL GROUNDWATER AQUIFER

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Boreal surface waters are characterized by high concentrations of natural organic matter (NOM) which must be removed during drinking water production. In artificial groundwater recharge (AGR) NOM is removed during passage of surface waters through a natural aquifer. Adsorption and biodegradation are crucial for NOM removal but their mechanisms, along with the underlying microbial communities, remain poorly characterized. To improve the understanding of NOM biodegradation, microbial community and physicochemical dynamics were monitored at a Finnish AGR site during and after a transient pause in surface water availability, at which time chemically pre-treated water was infiltrated. Five points along the aquifer flow path were sampled over a period of six months. Microbial dynamics studied in the attached and water phases included total cell counts and bacterial community analysis (using the semi-quantitative method of length heterogeneity PCR). Chemical parameters measured from water

samples included total/dissolved organic carbon (TOC/DOC) and DOC molecular size fractions. Total water-phase cell numbers were similar (10⁴-10⁵ cells/ml) in both recharge situations. Bacterial communities in the surface and pre-treated waters were different and diverse. Mixing of the waters in the aquifer was clearly demonstrated as a progressive shift towards the surface water bacterial community along the flow path over time. The attached phase community profile differed markedly from that of both water phases and remained stable during the water change. After completion of the transient water change, the DOC removal profile along the flow path was altered and the produced water DOC had increased (from <2 mg/l to ~2.7 mg/l). This study clearly showed the responses of the aquifer to a change in infiltrated water. Ongoing work focuses on identifying the major and metabolically active bacterial groups in the aquifer and on clarifying biodegradation/NOM-removal mechanisms.

Abstract Category

06 Engineered Environments

PS.06.069 BIOAUGMENTATION OF CR(VI) TRANSFORMATION ON AN INDUSTRIAL WASTE BY CHROMIUM RESISTANT FUNGUS STRAIN AND MICROBIAL CONSORTIA

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The bioaugmentation is the stimulation of the remediation of a contaminated site by the introduction of microorganisms capable of transforming metal or organic compounds. This technique has been used efficiently to degrade different pollutants, e.g., in the petrochemical industry. One of the greatest environmental problems of Guanajuato state (Mexico) is the contamination by chromium (Cr) from industrial waste, mainly close to its most populated city, León. To supply the necessity of this metal for shoes and automobilist factories, a Cr processing industry was installed in the city on the 50's, which accumulated a huge mountain of industrial waste oversaturated on hexavalent chromium [Cr(VI)], besides other minor contaminants. In the last decade this industry began to investigate the best methods for remediating their wastes, including cleaning approaches such as the ones that use microorganisms capable of transforming Cr(VI) to Cr(III). Both a fungi strain (Ed8), isolated from another Cr(VI) polluted site, and a microbial consortia that flourished on a wastewater contaminated with Cr(VI), both from León city, were used for bioaugmentation tests. The Cr(VI) was measured by the diphenylcarbacide method and the microbial activity by the FDA method. We first probed an organic compost (in proportions of 90, 75, 50 and 25%) as the carbon source for the Ed8 strain seeded on a Cr(VI) contaminated waste. After 3 months, the largest effect was obtained with the 25:75 fraction of waste to compost (about 30% reduction), an unsatisfactory result. The following approach was to use wastewater as carbon source and probe both the Ed8 and the microbial consortia as inoculates, alone and combined. The wastewater alone stimulated a Cr(VI) reduction of 30%, probably by the action of its

endemic microorganisms. With the inoculates, the best results [65% of Cr(VI) reduction] were obtained with the wastewater and the consortia together.

Abstract Category
06 Engineered Environments

PS.06.070 AMMONIA REMOVAL LIMITED BY INHIBITION OF METABOLISM AND GROWTH

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Ammonia removal in biotrickling air filters takes place through dissolution in a biofilm with ammonia oxidizing bacteria (AOB) which partially oxidize ammonia to nitrite. Products of protonation (NH₄⁺) and oxidation (NO₂⁻) wash out via filter water drainage. AOB activity is inhibited by availability of their substrate NH₃ and concentration of the oxidation product HNO₂. Mechanisms controlling AOB activity in a biotrickling filter remains only partially understood, but recent studies of inhibition kinetics for activity and growth suggest that when growth is fully inhibited by HNO₂, activity remains only 40-50% inhibited. Ammonia removal, filter water pH and conductivity, were measured on line during experimental manipulations of ammonia load in a full scale biotrickling filter constructed for removal of NH₃ and odor from ventilation air at a pig farm. On line recordings were supplemented by point measurements of odor removal. Effects of changes in NH₃ load were studied over short periods (hours) and long periods (weeks). Results showed that 1) The AOB biomass stabilized relative to the average load of NH₃. If NH₃ load decreased, inhibition increased and biomass decreased. If NH₃ load increased, inhibition decreased and biomass increased. Gain and loss of biomass happened surprisingly slow over weeks. 2) AOB activity responded immediately to changes in NH₃ load, at constant biomass. 3) Outlet air NH₃ concentration was approximately in equilibrium with filter water and therefore governed by its [NH₄⁺] and pH, which again was controlled by filter water conductivity and AOB activity. Implications of the function of the AOB community in bio trickling filter is that biomass adapt to the average load of NH₃, and adjust activity to most of the short term (hours) variation seen in livestock ventilation. The activity of the heterotrophic microbial populations removing odor compounds was largely unaffected by varying inhibition by NH₃ and HNO₂.

Abstract Category
06 Engineered Environments

PS.06.071 ANAEROBIC BIOREMEDIATION OF VOLATILE ORGANIC COMPOUNDS IN A FRACTURED BEDROCK SYSTEM: INSIGHTS FROM A BIOAUGMENTATION TRIAL IN CANADA

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Anaerobic in situ bioremediation has proven to be a reliable and low-cost technique for treatment of numerous sites contaminated with chlorinated solvents, mainly in the US. There is, however, little knowledge of the effectiveness of this technique at sites that tend to have both colder temperatures, with slow microbial growth, and contaminants in fractured bedrock, which presents significant hydrological challenges. In this study, we aim at demonstrating the effectiveness of bioaugmentation using a stable microbial consortium called KB-1 at a Canadian site characterized by cool groundwater temperatures and contaminants in fractured bedrock. To prove the success of bioaugmentation, strategic wells are being periodically monitored and investigated using a combined approach including general groundwater chemical analysis, compound specific isotope analysis and molecular biomarkers (16S rRNA general and specific genes, *vcrA* gene). Results from the first stage are discussed. These will help to develop general approaches for other bioaugmentation projects at similar sites.

Abstract Category

06 Engineered Environments

PS.06.072 CHARACTERIZING MICROBIAL POPULATION STRUCTURE AND DYNAMICS IN MESOPHILIC AND THERMOPHILIC PRETREATMENT OF TWO STAGE ANAEROBIC DIGESTION

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Anaerobic digestion (AD) is a microbially mediated process becoming the preferred method of treating biosolids due to energy recovery, minimal sludge production, cost effectiveness and low environmental effect. Thermal pretreatment is used to enhance AD, especially of sludge from nutrient removal plants, for producing class A solids. Pretreatment is the first stage (hydrolysis/fermentation) in a two stage AD process. However, the detailed understanding of the microbiology of this important and often rate limiting stage is lacking. We aimed to determine the microbial constituents important for the thermophilic pretreatment stage of AD. The bacterial communities in two functionally different laboratory scale pretreatment reactors, one thermophilic (>500C) and one mesophilic (350C), in two stage anaerobic digestion systems were evaluated for 340 days. Bacterial community structures were determined by 16S rRNA gene terminal restriction fragment length polymorphism (tRFLP) and clone sequencing. Community profiles were correlated to reactor performance by multivariate statistical analysis. Analysis of 16S rRNA gene clones demonstrated that the thermophilic pretreatment bacterial community is mostly composed of populations affiliated to Firmicutes,

Proteobacteria, Thermotogae, Actinobacteria, Bacteroidetes and Chloroflexi. Statistical analysis of tRFLP profiles showed that bacterial populations in both reactors were dynamic, although for thermophilic reactor community the diversity was lower and increasing temperature had a significant positive effect on the abundance of some particular dominant ribotypes within Thermotogae and Firmicutes. This study provides previously unseen insight of microbial populations important during an enhanced thermophilic hydrolysis-fermentation stage of AD. Our improved conceptual understanding will be important for optimizing the two stage AD process. This work was funded by Environmental Biotechnology Cooperative Research Centre, Australia

Abstract Category
06 Engineered Environments

PS.06.073 ENVIRONMENTAL SELECTION PRESSURES TO MAINTAIN A NON-STERILE CULTURE OF HIGH-PHB PRODUCING METHANOTROPHIC BACTERIA

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Poly- β -hydroxybutyrate (PHB), a storage polymer produced by many bacteria, can be harvested and used as a biobased, biodegradable plastic. Due to high production costs, PHB-based plastics been kept out of most markets by their relatively high production cost. One goal of this research is to reduce production costs by using a less expensive carbon substrate, methane, and non-sterile culture conditions to produce PHB. Not all methanotrophs produce PHB, and among those that do, production rates and yields differ. In order to maintain a non-sterile, PHB producing culture of methanotrophs, the function of PHB in methanotrophic cells is investigated and used to design selection pressures that favor the growth of high-PHB producing methanotrophs. Previous work has demonstrated that when carbon is lacking and all other nutrients are present, some bacteria replicate using stored PHB as a carbon source. Other studies have suggested that PHB plays a role in the long-term survival of cells under starvation conditions. This study strives to understand the role of PHB in methanotrophic cells by comparing the behavior of the PHB-producing, Type II methanotroph *Methylocystis parvus* OBBP with the non-PHB producing Type I methanotroph *Methylomonas* sp. LW13. Both strains are subjected to nutrient deprivation to induce formation of PHB or other storage polymers. They are subsequently subjected to various conditions of carbon/nutrient excess or deprivation to determine what effect PHB has on cells. It is determined that neither strain can replicate using only a storage polymer in the absence of an exogenous carbon source and that the presence of PHB decreases the time required for cells to recover from nutrient deprivation. Competition experiments between the two methanotroph strains are carried out to determine if the advantages identified can be effectively used to select for PHB-producing methanotrophs.

Abstract Category
06 Engineered Environments

PS.06.074 ASSESSING THE MICROBIAL DYNAMICS OF A DRINKING WATER TREATMENT SYSTEM TO ENHANCE BIOLOGICAL DRINKING WATER TREATMENT POTENTIAL AND WATER QUALITY.

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Traditional drinking water treatment relies primarily on energy intensive physico-chemical processes. An alternative to this approach is to promote the development of microbial communities in parts of the drinking water treatment plant to exploit metabolic capabilities of microbes to accomplish pollutant removal, including removal of trace levels of organic compounds, such as pharmaceuticals, herbicides, etc, which recently have been found in many water sources and finished drinking waters. In this study, we are exploring the potential of promoting microbial activity in a dual media filter in a full-scale drinking water treatment plant to enhance finished water quality. To this end, we are conducting a sampling campaign to determine the level of microbial activity and characterize the microbial communities at multiple points along the treatment train and the water distribution system. Despite non-ideal conditions, including high operational pH and suboptimal filter backwashing strategy that uses water containing disinfectant, the granular activated carbon part of the dual media filter was found to contain a diverse biofilm community using 16S and 18S rRNA gene based methods. Microbial community analysis of samples collected after the filtration step using terminal restriction fragment length polymorphism (T-RFLP) and quantitative PCR (Q-PCR) will help determine if detected microbial populations found after disinfection and have the potential to seed the distribution system. The phylogenetic information collected on the filter microbial community will be used to infer potential metabolic capabilities and explore strategies to promote additional biological activity, including reducing filter influent pH, dechlorinating the backwash water, and adopting nutrient supplementation strategies. In addition to reducing the levels of trace organics in the finished water, such strategies also have the potential to minimize microbial proliferation in the distribution system.

Abstract Category

06 Engineered Environments

PS.06.075 IMPACT OF CHEMICAL PERTURBATION ON TROPHIC INTERACTIONS AND ITS IMPLICATIONS FOR ECOSYSTEM FUNCTION IN AN ENGINEERED ENVIRONMENT.

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The application of macro-ecological principles in engineered systems has largely focused on bacterial communities. However, engineered ecosystems present a complex interactive network, which include predatory pressures. In this study, we evaluate the impact of perturbation on mixed bacteria and protozoa communities and its influence on bioreactor function. Experimental systems were inoculated with microbial community from a biological wastewater treatment plant. Cadmium was used as a model toxin to induce chemical perturbation. Experiments were conducted multiple times under continuous flow and sequencing batch reactor (SBR) modes. The SBR mode also included additional reactors where protozoa were selectively inhibited. Bioreactor function was monitored by quantifying maximum specific substrate utilization rates (MSSUR). Bacterial community structure was determined using 16S rRNA based T-RFLP and Q-PCR. Bacterivorous protozoa were quantified by microscopic counting. Bacterial abundance increased despite initial reduction in MSSUR. Post cadmium washout, the increase in bacterial abundance was also accompanied by an increase in MSSUR above unperturbed control levels. This trend was replicated for all independent experiments. Increase in bacterial abundance or MSSUR did not correlate with changes in community structure. Stress induced reduction in predator abundance correlated with increase in total bacterial abundance and MSSUR. This correlation was also observed when protozoa were selectively inhibited. Trophic interactions play a critical role in regulating bacterial community function in bioengineered ecosystems. Low level external perturbation can reduce ambient stress for bacteria resulting from predator grazing. This causes bacterial proliferation despite short-term toxic inhibition. A complete evaluation of ecosystem community structure and function must include trophic interactions and address the balance between ambient and novel bacterial stressors.

Abstract Category

06 Engineered Environments

**PS.06.076 HYDROGEN PRODUCTION BY THE JOINT ACTION OF
CYANOBACTERIAL AND PURPLE NON-SULFUR SPECIES IN LATEX
COATINGS**

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Biocatalytic latex coatings are stable, nanoporous matrices ideal for photosynthetic hydrogen production because they provide an increased surface area for incident light, increased rates of gas diffusion compared to liquid-based systems, and can increase cell longevity by protecting the microorganisms. Various cyanobacterial isolates and mixed cultures were embedded in latex coatings and screened for nitrogenase catalyzed H₂ production. A nonaxenic culture of the filamentous soil cyanobacterium *Calothrix* sp. MCC-3 was identified as a promising candidate. MCC-3 coatings (n=4) produced 78 mmoles m⁻² H₂ over an 8 day period in BG11° medium supplemented with 20mM α-

ketoglutaric acid. Latex-embedded cells of the *Calothrix* sp. MCC-3 culture exhibited H₂ yields that were approximately 3x greater than an equal number of planktonic cells. Rates of H₂ production by the MCC-3 coatings exhibited a biphasic pattern, increasing after approximately 8 days at which time the coatings began to take on a red appearance. A red pigmented strain was isolated on PMNF agar medium and identified as a *Rhodopseudomonas* sp. by 16S rRNA gene sequencing. This strain, *Rhodopseudomonas* MP, grows slowly on PMNF agar medium, but cultivation attempts in PMNF or BG11 liquid media augmented with various carbon sources have been unsuccessful - unless *Calothrix* sp. MCC-3 is included in which case abundant growth of *Rhodopseudomonas* sp. MP occurs after approximately one week. We are currently testing the hypothesis that H₂ production in MCC-3 coatings is initially carried out by *Calothrix* sp. MCC-3 and later supported by the syntrophic action of *Rhodopseudomonas* sp. MP and *Calothrix* MCC-3. By combining phototrophs that contain light harvesting pigments that effectively absorb different wavelengths of light, e.g. cyanobacteria and purple non-sulfur bacteria, a greater fraction of natural light can be utilized to power H₂ production.

Abstract Category

06 Engineered Environments

PS.06.077 FISH FARMING AFFECTS THE ABUNDANCE AND DIVERSITY OF THE BACTERIAL MERCURY RESISTANCE GENE

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Fish farming in natural waters is an open system that has an influence on chemical and ecological balance of the farming area. Fish feed, pharmaceuticals and anti-foulants used in aquaculture affect microbial communities in the sediments under the fish cages. For example, fish feed have reported to contain considerable amounts of metals, even mercury. The aim of this study was to determine whether fish farming affects the abundance and diversity of bacterial mercury resistance gene *merA* in sediments. The sediments were collected from four fish farms and from four pristine sites near the farms. Sensitive PCR-primers that are suitable for quantitative PCR were designed to detect *merA* without need of enrichment. The primers were used to sequence *merA* clone libraries from fish farm and pristine site sediments. Potential selection pressure for *merA* was evaluated by determining chemical and bioavailable concentrations of mercury from the sediments. Our results showed that *merA* was more abundant in the fish farm than in the pristine site sediments. In addition, there was a difference between farm and pristine site *merA* phlotypes. Pristine site phlotypes represented two novel *merA* clades. Concentrations of chemical and bioavailable mercury in the sediments were low and did not differ between farm and pristine site sediments. This study shows that fish farming increases the abundance of the *merA* in sediments and affects the diversity of the *merA* pool. Because the concentrations of mercury were low in all sediments, we hypothesise that selection for *merA* takes place before sedimentation. One of the possibilities is co-selection with other resistance genes that are, similarly to mercury resistance genes, often

found in readily transposable genetic elements. Other hypothesis is that the selection pressure for merA is in the gut of the farmed fish as there have been reports about high concentration of mercury in fish feed.

Abstract Category
06 Engineered Environments

PS.06.078 TEMPORAL DYNAMICS OF FUNGAL COMMUNITIES IN DENITRIFYING BIOFILTERS RECEIVING AGRICULTURAL RUNOFF

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High nitrate loads from agricultural runoff play an important role in eutrophication of aquatic ecosystems. One way to reduce the entry of anthropogenic nitrate into natural waterways is to engineer denitrifying biofilters to treat effluent from agricultural tile drainage systems. In this system, the denitrifying biofilters contain a woodchip medium that serves as a carbon substrate for microorganisms, and the hydraulic retention time is controlled to maximize denitrification. Previous results indicate that fungal activity enhances denitrification rates, while bacterial activity is essential. This study focused on the temporal relationship between biofilter nitrate reduction performance and fungal populations. Woodchip and water samples were collected every two weeks for one year at distinct spatial locations, at both 0.75m and 1.5m depths, from 3 biofilters. Following DNA extraction, Fungal Automated Ribosomal Intergenic Spacer Analysis (FARISA) was performed to examine fungal community structure and dynamics. Time-series analyses suggest that fungal populations vary significantly by season, however, this variance is not strictly temperature dependent. In addition, distinct fungal assemblages are observed at different depths in each biofilter. Current analyses are focused on the identification of environmental and biological parameters that correlate with, and could potentially influence these dynamics; these parameters include chemical data, hydrology, and bacterial community analysis. Furthermore, analogous analyses performed on the bacterial communities within each biofilter have demonstrated similar temporal and spatial patterns. Understanding the dynamics of microbial communities in these biofilters will assist in the development of models to predict the functionality and long-term reliability of denitrifying biofilters as a solution to agricultural nitrate pollution.

Abstract Category
06 Engineered Environments

PS.06.079 FUNCTIONAL BACTERIAL POPULATION DUALISM IN HYDROGEN DARK FERMENTATION PROCESSES

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Hydrogen (H₂) is one of the most promising energy carriers that can be produced by dark fermentation of renewable organic wastes using anaerobic mixed cultures. Since H₂ is a metabolic intermediate in anoxic ecosystems, the H₂ production performances in bioprocessing are not only influenced by the metabolic switches but also by population shifts between H₂ producing bacteria (HPB) and H₂ consuming bacteria (HCB). The aim of this study was to gain a better understanding of the bacterial population dynamics and the metabolic changes in dark fermentation.

A series of batch tests were performed at different pH (3.5 to 6), temperatures (25°C to 45°C) and with different substrates (mono- to tri-saccharides). Bacterial population dynamics (structure/diversity) were monitored by CE-SSCP fingerprinting using bacterial-specific 16S rDNA primers and new HPB-specific primers targeting *hydA* genes, encoding the catalytic sub-unit of [FeFe]-H₂ases.

Changes of environmental conditions influenced the H₂ production performances and had a higher impact on the functional population (*hydA* genes, ANOSIM R=0.34, p<0.001) than on the total bacterial population structure (16S rDNA, R=0.17, p<0.02). Since the ecosystem functionality correlated with *hydA* diversity (0.76<R²<0.96, p<0.05), it was concluded that the *hydA* marker was more suitable as an indicator of H₂ production. The lower H₂ productions observed at extreme temperatures or with the increase of substrate complexity corresponded to different *hydA* population structures (R=0.84, p<0.001). Greater *hydA* diversity seemed to correlate with higher H₂ production rates in the pH range (5-6), probably with the establishment of positive bacterial interactions, showing the development of specific *Clostridium*. On a longer term, the higher diversity impacted negatively the stability of H₂ production through the increase of the HCB activity. Further studies on the nature of the bacterial and metabolic interactions are in progress.

Abstract Category

06 Engineered Environments

**PS.06.080 TRANSCRIPTOME ANALYSIS OF CO-CULTURE
ENTEROCOCCUS FAECIUM AND GRAM-NEGATIVE BIOFILMS ON ANODE
ELECTRODES**

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Bioelectrochemical Systems (BESs) can use bacteria as catalysts for oxidation and/or reduction reactions at electrodes. The best known example is the microbial fuel cell (MFC), where net power is produced from e.g. wastewater. The bacterial catalysis is achieved through extracellular electron transfer (EET), a process allowing the transfer of electrons from the bacterial membrane to the surface of an insoluble conductive material. While numerous studies have investigated pure cultures, co-culture interactions have been given less focus. The aim of this project was to investigate the transcriptome and biofilm development of co-cultures to elucidate the mechanism of this interaction.

We performed whole genome mRNA microarrays to investigate the transcriptome, biofilm development and possible EET mechanisms using Gram-positive *Enterococcus faecium* with Gram-negative *Geobacter sulfurreducens*, *Shewanella oneidensis* and *Pseudomonas aeruginosa* separately. Additionally we performed biofilm studies using FISH, 3D confocal imaging and COMSTAT quantification to compare the developmental and structural differences between pure (we have performed previously) and co-cultures on the anode electrode.

The co-culture biofilms generated more current than the pure cultures while also providing a more comprehensive coverage of the electrode which is demonstrated by the decreased COMSTAT scores. Transcriptome results identified a range of genes which highlight several possible EET mechanisms employed within co-cultures, these did not include those already known from previous pure culture studies. Examples are possible colicin shuttling, modified biofilm conductivity and other indirect EET mechanisms. Additionally, the biofilm study revealed a defined segregation of Gram-negative and Gram-positive within the co-culture biofilm with *E. faecium* colonising the electrode and the Gram-negative staying on top of the biofilm.

Abstract Category
06 Engineered Environments

PS.06.081 THE INFLUENCE OF HYDROPHOBIN-COATED SURFACES ON MICROBIAL BIOFILM FORMATION

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Biofilms cannot be avoided on a great variety of natural and synthetic surfaces in unsterile habitats. However the characteristics of the material and its corresponding surface properties affect the biocompatibility and consequently bacterial adhesion and biofilm growth.

In this approach hydrophobins are used as a novel modification of materials to change the surface properties and thus to influence microbial biofilm formation. Hydrophobins are non-toxic fungal proteins which self-assemble on different surfaces into extremely stable monolayers in an amphiphilic manner. Recombinant hydrophobins provide the opportunity to use these highly surface-active proteins for large-scale surface modification and functionalization (with e.g. enzymes) of industrial and medical relevant materials.

Thus, hydrophobin coating protocols were developed for different materials. Quartz crystal microbalance measurements were used to analyze the adsorption behavior and contact angle measurements, immunofluorescent labellings and atomic force microscopy were applied to characterize the properties of the protein coatings. The recombinant hydrophobins self-assembled on the surfaces depending on different parameters such as

incubation temperature or incubation time.

The growth behavior of various microorganisms was studied on hydrophobin modified versus unmodified surfaces. Single bacteria strains as well as natural bacterial communities were used to analyze biofilm formation. Apart from conventional plating experiments, fluorescent microscopy and molecular-biological methods such as denaturing gradient gel electrophoresis were applied to determine differences in the biofilm growth.

Recombinant hydrophobins are well suited for surface modification and functionalization. To enhance the effects on the biofilm growth further modifications of the recombinant hydrophobins are necessary by e.g. antimicrobial peptides.

Abstract Category

06 Engineered Environments

PS.06.082 NOVEL DIVERSITY OF PUTATIVE GEOSMIN-PRODUCING BACTERIA IN FRESHWATER AQUACULTURE

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The compound geosmin gives soil its pleasant "earth" odor but can also cause problems with off-taste in drinking water and in freshwater fish, particularly in aquaculture. Two bacterial groups are thought to be the significant geosmin producers based on culture-dependent studies: freshwater *Cyanobacteria* and the genus *Streptomyces*. In this study, the gene encoding geosmin synthase (*geoA*) was used to investigate the diversity of putatively geosmin producing populations in water and in biofilms from a nitrifying biofilter from two recirculating aquaculture plants, one indoors and one outdoors. Most of the diversity (>90%) was not related to any previously known geosmin-producing bacteria. The identity of the bacteria from which these sequences are derived is unknown, but sequence diversity formed three novel clades branching with about the same depth as the other clades known to represent separate phylum-level groups. Using qPCR, the relative proportion of each putative geosmin producing group was estimated by measuring the abundance of *geoA* genes compared to total bacterial 16S rRNA genes. Each of the newly discovered clades accounted for approx. 1% of the bacteria in both plants and appeared therefore to be more significant than a) *Streptomyces*, which were not detected, and b) *Cyanobacteria*, which were only represented in the water phase from the outdoor plant. These findings suggest that the abundance and diversity of bacteria that can produce geosmin is considerably greater than was previously estimated based on culture-based studies and enables the detection of the true diversity geosmin-producing bacteria. Such molecular methods must therefore be used as a diagnostic tool in studies to develop strategies to limit geosmin production in aquaculture.

Abstract Category

06 Engineered Environments

PS.06.083 UV INDUCTION AND REPAIR OF CYLOBUTANE PYRIMIDINE DIMERS AND (6-4) PHOTOPRODUCTS FROM REFERENCE BACTERIA AND NATURAL AQUATIC POPULATIONS

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UV light-caused DNA damage is a widespread field of study. As UV light has the biological effect of inactivating or killing bacteria, it is used for water disinfection. Due to this application, it is important to study the DNA damage efficiencies and regeneration capacities in bacteria after UV irradiation. Two monoclonal antibodies, anti-CPD and anti-(6-4) PP, were applied for an immunoassay of UV-induced DNA modifications. Cyclobutane pyrimidine dimer (CPD) and 6-4 photoproduct (6-4 PP) were detected in the reference bacteria *Pseudomonas aeruginosa* and *Enterococcus faecium*, and in natural water communities. The antibody-mediated detection signals increased with the UV fluences from 100 — 400 J/m². Here, the CPD-specific signals were stronger than the (6-4) PP-specific signals. These immunological results were in accordance with parallel cultivation experiments. All UV-irradiated bacteria showed a reduction of their growth rate depending on UV application by several orders of magnitudes. The immunoassay was also applied to three types of natural aquatic habitats with different cell densities. Besides artificial UV irradiation, it was possible to visualize natural sunlight effects on these natural bacterial communities. Light-dependent and dark repair processes were distinguished using the established immunological assays. The antibody-mediated analyses presented are fast methods to detect UV-induced DNA lesions and repair capacities in selected bacterial species as well as in entire natural mixed populations.

Abstract Category

06 Engineered Environments

PS.06.084 POTENCIAL USE OF HUMAN URINE IN BACTERIAL SAND BIOCONSOLIDATION

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Calcium carbonate precipitation by ureolytic bacteria has been studied worldwide for many types of application: restoration of historic buildings, protection of building materials, calcium recovery from waste water rich in calcium, and soil improvement, among others. On the other hand, calcium carbonate can be a problem when produced in urine collection systems, leading to pipe clogging. We studied the potential use of human urine as a medium for bioconsolidation of sand. An ureolytic strain, *Bacillus sphaericus*

LMG 22257, was used as inoculums. Human urine autoclaved at 110°C for 20 minutes was used as a culture medium and a sterile solution of CaCl₂·2H₂O, 50 grams per liter was added after bacterial growth. The test flow system was composed of 20 milliliter syringes filled with quartz sand of grain size between 150 and 300 micrometers. Environmental Scanning Electron Microscopy/Energy Dispersive Spectroscopy and thermogravimetric analysis were used to detect calcium carbonate bioprecipitation. Preliminary results showed that calcium carbonate was precipitated between the sand grains. Only a few millimeters of sand were biocemented. It was observed that organic matter and cells in the urine were responsible for clogging the system, as well as the upper layer of calcium carbonate. This did not allow adequate bacterial flow and consequently reduced ureolysis in other parts of the system. Although many adaptations are necessary to implement the system, the main novelty of this work is that for the first time sand bioconsolidation has been produced by ureolytic bacteria using solely human urine plus a single calcium source as culture medium. This research opens a new perspective in soil bioconsolidation in a sustainable context, with potential applications in geotechnical engineering (erosion control, slope stabilization, soil treatment).

Keywords: Soil bioconsolidation, ureolytic bacteria, human urine. Acknowledgement: FAPESP, CNPq.

Abstract Category
06 Engineered Environments

PS.06.085 COMPOSITION AND STABILITY OF BACTERIAL COMMUNITIES IN GRANULAR ACTIVATED CARBON AND ANTHRACITE FILTERS AT A PUBLIC DRINKING WATER TREATMENT FACILITY

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Treatment alternatives for long-term regulatory compliance with drinking water standards are continually being investigated by drinking water utilities. Granular activated carbon (GAC) is a potentially cost-effective alternative for the municipal treatment of drinking water. GAC provides a high surface area suitable for microbial colonization in biofilter systems. The resulting microbial growth promotes biodegradation of organic materials and other contaminants from influent waters, increasing the overall efficacy of the filter system. To monitor changes in bacterial community composition and to ensure human pathogens were not colonizing the filter matrix, the community structure of the bacteria in 3 GAC pilot filters and 2 anthracite filters was examined over a 12-month period. Total DNA was extracted from monthly samples and nearly complete 16S rRNA genes were PCR amplified with a fluorescently labeled primer for terminal restriction fragment length polymorphism (T-RFLP) analyses. The identities of bacteria responsible for commonly occurring individual peaks were determined through the construction of 5 16S rRNA clone libraries. Based on sequence analysis, the bacterial communities associated with both GAC and anthracite filters appear to be dominated by environmentally derived bacteria, with no common human pathogens detected. Statistical analyses from T-RFLP

data suggest that a diverse community of bacteria is associated with both filters. Analysis of similarity tests revealed significant differences in bacterial community structure occur over time with filter substrate type playing an important role in determining community composition. The GAC filters exhibited the greatest degree of bacterial community variability over the sampling period, and the anthracite filters showed a lower degree of variability and less overall change in community composition. These results suggest that GAC is a suitable filter matrix for the treatment of municipal drinking water.

Abstract Category
06 Engineered Environments

PS.06.086 MICROBIOLOGICAL INFLUENCED CORROSION OF LIGHT ALLOYS

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Pipe systems are part of our manmade environments. They can be found in urban environment where it is usually used to transfer water or sewage, in industrial environment where it can carry hazardous waste (for instance in the chemical industry), or oil and its products in the petroleum industry. Leakage in all pipe systems is an unwelcome event. The physical presence of microbial cells on a metal surface, as well as their metabolic activities, can cause Microbiologically Influenced Corrosion (MIC) or biocorrosion. It is caused usually by chemoautotrophs microorganisms. MIC is a problem in many industry areas and can apply to both metals and non-metallic materials. While normal corrosion rate in water is about 2 - 4 milli-inch per year (mpy), The presence of bacteria cause about tenfold increase in corrosion rate or even higher. Such corrosion rates would definitely cause to a decrease within the pipelines life time. We have investigated the phenomenon of MIC by testing several parameters. We have tested the effect of different bacteria (*Bacillus thuringiensis*, *Staphylococcus aureus* and mixture of bacteria). We have tested two incubation conditions: 37°C and environmental temperature. Two metals were tested, Aluminum and Steel. These metals are commonly used in chemical industry, construction and piping. We have determined corrosion rates by using three different measurements: weight loss, Potentiodynamic and Impedance spectroscopy in order to have a comprehensive and accurate data. We have found that *Staphylococcus aureus* is more corrosive to steel then *Bacillus thuringiensis* or the bacterial mixture. We have found higher corrosion rates when tested MIC phenomena at higher temperature (37°C). We would like to suggest the idea of "probiotic" bacteria to piping, i.e. "good bacteria" which will generate biofilm inside piping and will form a better protective surface regarding the MIC phenomena.

Abstract Category
06 Engineered Environments

PS.06.087 MONITORING MICROBIAL ACTIVITY OF A REVEGETATED COAL MINE IN CENTRAL QUEENSLAND, AUSTRALIA

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Research to improve rehabilitation of disturbed mine sites have escalated over the last two decades. However, the techniques of assessing rehabilitation success are yet to be refined, although some researchers have attempted to use ants, diazotrophic bacteria, spiders and enzymes as bioindicators of rehabilitation success. The current study explored the possibility of using microbial activity as an indicator of rehabilitation success of a coal mine site in Central Queensland, Australia. Soil samples were collected from sites that were progressively rehabilitated using different techniques (eg with or without topsoil) and from adjacent undisturbed natural sites. The soils were analysed for pH, electrical conductivity, organic matter and plant nutrients. The same samples were assessed for microbial counts, basal and substrate induced respiration, fluorescein diacetate hydrolysis and microbial biomass carbon.

The results showed that the mine spoils had higher pH and EC, but were markedly low in microbial activity compared to natural sites. The freshly dumped spoils had the lowest microbial biomass carbon, microbial count, substrate induced respiration and fluorescein diacetate hydrolysis compared to recently revegetated spoil, indicating the importance of using top soils in mine site rehabilitation. The similarities between the 10-year-old spoils (that were revegetated but had no top soil) and those of recently top soiled revegetated spoils, suggest that the microbial activity could improve over time. However the presence of markedly higher activities in the natural sites indicate that the mine spoils might take a long time to build up microbial activity, or they may require some intervention to promote their activity in mine spoils. Several studies are currently under way to enhance microbial activity on rehabilitated coal mine spoils.

Abstract Category

06 Engineered Environments

PS.06.088 FUNCTIONAL DIVERSITY IN FOSMID LIBRARY FROM REFINERY WWTP SLUDGE ANALYZED BY 454-PYROSEQUENCING TECHNOLOGY

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Wastewater treatment plants (WWTPs) represent habitats of continuous change in chemical composition. In petrochemical refinery, different concentrations of pollutant compounds are received daily in the influent stream. It is well known that WWTP has

high microbial diversity, and that these represent a huge source of genes, metabolic pathways and potentially bioactive end-products. In this sense, our goal was to evaluate the functional diversity present in one WWTP system by using the pyrosequencing approach, focusing mainly on novel genes or metabolic pathways involved in the degradation of pollutant and recalcitrant compounds. The sludge sample was collected from a pilot membrane bioreactor of a petroleum refinery WWTP in Brazil. Sludge DNA of high molecular weight was isolated, ligated into the pCC2Fos fosmid vector and cloned into *E. coli* EPI300TM-T1R cells. The fosmid DNA was extracted and run on 454-FLX sequencing. The singlets and contigs obtained were analyzed using BLAST, BASC and MEGAN programs. The pyrosequencing run yielded 122,053 sequences, 2,202 of these were large contigs of 744 bp on average. The BASC/BLAST analyses revealed 76 positive hits related with pollutant and recalcitrant degradation, such as phenol hydroxylase, alkane hydroxylase, naphthalene and catechol dioxygenase, biphenyl degradation enzymes, among others. The MEGAN analysis showed that the general community profile is aerobic, gram-negative, aquatic, motile, mesophilic and unknown salinity. Hundred of positive hits were grouped in COGs categories defined by NCBI. These results showed that the refinery bioreactor sludge community have a complex functional profile consistent with the activities performed by microorganisms in WWTP. Yet, a significant proportion of the genes found is of unknown function according to the Genbank database and may possibly represent enzymes playing key roles in the WWTP system under study.

Acknowledgments: FAPESP, PETROBRAS

Abstract Category
06 Engineered Environments

PS.06.089 INVESTIGATING BACTERIAL COMMUNITY RESPONSE DURING DISCOLOURATION EVENTS IN AN EXPERIMENTAL WATER DISTRIBUTION SYSTEM

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Discolouration of potable water, due to fine insoluble particles, is a major cause for customer contacts to water companies. Within water distribution systems (WDS) particulate material accumulates on pipe walls. The stability and amount of material within these layers is known to be influenced by the maximum shear stress exerted by daily flow profile but the processes and mechanisms explicitly involved are poorly understood. Mobilisation of material into the bulk water occurs when shear stress exceed the conditioning values. Whilst discolouration is predominantly an aesthetic issue association with microbiological content has been made. Planktonic and biofilm microbiological loads are known to be significant within WDS. It seems likely that microbial biofilms may be important in understanding causes and consequences of discolouration. The aim of this study was to examine the response of WDS bacterial communities to an experimental discolouration event. Utilising a unique temperature controlled 3 x 200m pipe-loop test facility, material layers were accumulated under three

different steady state boundary shear stresses over a 28 day period at 8°C. Pipe wall material was subsequently mobilised via incremental increases in boundary shear stress. Changes in bacterial biofilm community structure and abundance were monitored by T-RFLP and Q-PCR throughout the mobilisation event using coupons inserted along the length of the pipe. For all three experimental shear stress conditions, water turbidity, Mn and total DAPI counts increased with each mobilisation step. In contrast no significant change was observed in biofilm community structure or the abundance of 16S rRNA genes before and after the mobilisation event. Future experiments will include higher temperatures and variable conditioning shear stresses to determine factors affecting microbial community structure and the role of WDS microbiology in discoloration.

Abstract Category

06 Engineered Environments

PS.06.090 ANAEROBIC DIGESTER METAGENOME REVEALS MICROBIAL COMMUNITY STRUCTURE AND GENETIC POTENTIAL

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Anaerobic digestion is an effective method for reduction of organic waste related pollution while providing a sustainable means of bioenergy production. Although anaerobic digestion technologies have been in development for over a century, much of the microbial diversity responsible remains unknown. Even less well known is the genetic potential of these engineered microbial communities. The goals of this study were to characterize the microbial community structure as well as the genetic potential of the microbial community involved in the thermophilic anaerobic digestion of poultry farm waste. These objectives were accomplished by total community genomic DNA pyrosequencing. Comparison of the 16S rDNA fragments retrieved from the metagenome to the RDP database indicates that the bacterial community is dominated by Firmicutes (40%), Proteobacteria (23%), and unclassified Bacteria (22%). Metabolic analysis by the MG-RAST server revealed an abundance of gene clusters of unknown function within the "Carbohydrates" metabolic subsystem. This subsystem is otherwise dominated by functional gene clusters involved in the metabolism of mono-, di-, and oligosaccharides. Among the most abundant of these is xylose utilization (26% of the monosaccharide subsystem), L-arabinose utilization (19% of the monosaccharide subsystem) and maltose and maltodextrin utilization (38% of the di- and oligosaccharide subsystem). Recruitment plot comparison of the digester metagenome to the whole genomes of known organisms revealed that *Clostridium thermocellum* ATCC 27405 and *Clostridium phytofermentans* ISDg had the highest levels of mapped fragments (18% and 5% of classifiable fragments respectively). Both *C. thermocellum* and *C. phytofermentans* are important organisms for biofuel production due to their cellulolytic capabilities. The abundance of fragments which map to these genomes indicates the presence of microbes with similar potential metabolic functions.

Abstract Category
06 Engineered Environments

PS.06.091 STABLE ISOTOPE PROBING TO IDENTIFY CARBOXYLIC ACIDS ASSIMILATING BACTERIA ASSOCIATED WITH BIOLOGICAL ACTIVATED CARBON USED IN DRINKING WATER TREATMENT

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Control of assimilable organic carbon (AOC) level has gained an increasing attention to prevent bacterial regrowth in drinking water distribution system. Biological activated carbon (BAC) process can efficiently remove AOC through biological oxidation. The AOC biodegradation is normally promoted by ozone oxidation prior to BAC process to convert refractory organic matter into more readily biodegradable organic compounds such as carboxylic acids including formate, acetate and oxalate. However, little is known which bacteria are responsible for degrading AOC in BAC filter. This study aimed to identify carboxylic acids assimilating bacteria associated with BAC by stable isotope probing. BAC samples were individually incubated with ^{13}C -formate, ^{13}C -acetate, and ^{13}C -oxalate at 20°C. Control experiment was conducted by adding unlabeled-substrate. Two substrate concentrations were compared (0.5 and 10 mgC/l). DNA extracted from each sample was separated by isopycnic centrifugation. Bacterial 16S rRNA genes were evaluated by terminal restriction fragment length polymorphism analysis. Results showed that bacterial community profiles of all treatments were similar in light DNA fraction. At both concentration levels, terminal restriction fragment of 330 bp was observed in heavy fractions of ^{13}C -formate, ^{13}C -acetate and ^{13}C -oxalate samples, suggesting that some common bacteria corresponding to the fragment have versatile function of assimilating three carboxylic acids. On the other hand, some fragments were observed in the specific samples. The fragment of 200 bp was detected in only ^{13}C -acetate sample at 10 mgC/l. In addition, two fragments of 358 and 499 bp were found in ^{13}C -oxalate treatment while the latter fragment was only detected at lower concentration. These results indicate that bacteria associated with BAC play different roles in removing carboxylic acids depending on their types and concentration levels.

Abstract Category
06 Engineered Environments

PS.06.092 PREDOMINANCE OF *SYNTROPHOMONAS ZEHNDERI* IN OLEATE-FED BIOREACTORS AND ITS POTENTIAL AS BIOAUGMENTING STRAIN

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Denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S rRNA genes was used to follow the changes in bacterial communities during anaerobic continuous and fed-batch reactors operation with oleate, an unsaturated long-chain fatty acid (LCFA).

Throughout continuous operation, bacterial profiles of the sludge showed an average similarity with the inoculum of $9\pm 5\%$. Bacterial profiles were also altered during fed-batch operation; similarity between sludges before and after fed-batch cycles was 50%. A predominant DGGE-band was identified in all the DGGE profiles of sludges contacting with oleate. 16S rRNA gene sequences retrieved from the different sludges, and corresponding to this position in the DGGE profiles, were found to be closely related to *Syntrophomonas zehnderi* (99% identity). *S. zehnderi* is known to degrade saturated and unsaturated LCFA [1], and could potentially be used to bioaugment anaerobic sludge for improving methane recovery from LCFA. This hypothesis was tested in batch assays performed with and without the solid microcarrier sepiolite. Oleate was added to the medium at a final concentration of 1 mM. Methane production and volatile fatty-acids were monitored throughout the experiment and LCFA were quantified at the end of the assays. The results obtained show that methane production from oleate was enhanced through *S. zehnderi* addition to the anaerobic sludge. After 15 days of incubation, methane yield in bioaugmented assays with sepiolite was as high as $71\pm 3\%$, whereas in non-bioaugmented bottles only $27\pm 1\%$ of the theoretical methane could be accounted. Methane yield in bioaugmented bottles without sepiolite was also lower, i.e. $36\pm 12\%$. The use of a microcarrier might facilitate interspecies metabolite exchange, favoring faster methane production. This approach can be potentially useful for a faster reactor start-up or recovery of an LCFA-inhibited anaerobic bioreactor.

[1] Sousa DZ, Smidt H, Alves MM & Stams AJM (2007) Int J Syst Evo

Abstract Category

06 Engineered Environments

PS.06.093 MICROBIAL STABILITY AND FEED SAFETY OF A SINGLE CELL PROTEIN FISHFEED PRODUCT FROM A BREWERY WASTEWATER PILOT PLANT

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Single cell protein (SCP) produced from brewery wastewater has been proposed as a protein alternative in animal feeds. But when studying reuse of such biological wastes, a life cycle approach over a linear "factory-to-consumer" approach is warranted since the very act of reuse raises questions relating to the stability and safety of the process. Here we examine the microbial stability and safety of a bacterial SCP product produced over 1 year in a pilot scale aerobic reactor at New Belgium Brewing (Fort Collins, Colorado). This product is intended as an aquaculture feed replacement. Dried feeds are typically stable in storage, but when applied to aquaculture may become more active and potentially pathogenic. To understand what organisms are preferentially activated, SCP samples before and after hydration were pyrotag sequenced on a Roche 454 platform using bacterial 16S SSU rRNA gene primers. Pyrotags from this run were used to evaluate microbial variability within the SCP. Results show no sequences were identified from *Salmonella* and *Clostridium perfringens*, but sequences from *Lactobacillus* were detected from dried SCP product. To examine the safety concerns of dried SCP before

and after hydration, real-time PCR was conducted for the presence of *Salmonella* spp., *Bacillus cereus* and *Clostridium perfringens* using previously tested gene fragments as standards. Tests of re-hydrated dried SCP cultures were shown to be positive for *Salmonella* spp. and *Bacillus cereus*. These results suggest that pyrotag surveys may not sequence deep enough to reveal low-count pathogens particularly when SCP is exposed to humidity or water. On-going work will apply similar methods on conventional animal feeds to investigate what differences in pathogenicity and microbial profiles might be significant in feeds as currently used. Further, culture based techniques will also be used to verify these results. Technical but no financial support was received from Oberon, FMR.

Abstract Category
06 Engineered Environments

PS.06.094 EXPLORING NITRITE OXIDATION IN A MARINE RECIRCULATION AQUACULTURE SYSTEM BY CHARACTERIZATION OF A NEW *NITROSPIRA*

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Beginning in 1998, several authors identified representatives of the genus *Nitrospira* to play the major role in the important step of nitrite oxidation in biofilters of marine recirculation aquaculture systems. Here, we describe a novel bacterium closely related to *N. marina* which was highly enriched from HDPE (High Density Polyethylen) biofilter material of a recirculation aquaculture plant in Büsum, Germany.

Cultivation, 16S rRNA analyses, fatty acid profiling and labeling, electron microscopy, physiological investigations.

In electron microscopic ultrathin sections dense colonies of diverse ammonia oxidizing bacteria and nitrite oxidizing bacteria of the genus *Nitrospira* were observed in close vicinity within the thick biofilm on the filter material. Complete fatty acid profiles of the biofilm revealed *Nitrospira*-specific compounds (cis 7 and cis 11 isomers of hexadecenoic acid) with a percentage of 6 - 8%. Experiments with ¹³C-labeled carbonate and nitrite as substrate indicated the presence of a metabolically active *Nitrospira*-population with a degree of labeling from 13 up to 21%. In nitrite-oxidizing enrichment cultures both fatty acids (16:1 cis 7 and cis 11) increased to amounts of 42% respectively 31% identifying *Nitrospira* as the dominant organism. Cells exposed a strong capability to develop biofilms with a high affinity to glass and isolation of the new *Nitrospira* was possible by serial dilutions of biofilm material grown at the air/water interface of the bottle. 16S rRNA gene analyses of the new strain revealed a sequence similarity of 98% to *N. marina*. Physiological tests exhibited elevated tolerances against nitrite, nitrate and ammonium as well as the capacity for mixotrophic growth.

The new *Nitrospira* strain seems to be well adapted to the attached lifestyle on biofilter

material and to the high nitrogenous load prevailing in the effluents of the fish tanks.
Funded by the DBU and DFG

Abstract Category
06 Engineered Environments

PS.06.095 FREE LIVING PROTOZOA IN DISTRIBUTED WATER FROM DIFFERENT SOURCES IN THE US AND CANADA

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Background and aims: Free living protozoa (FLP) are bacteriovores increasingly recognized for harbouring pathogens. In drinking water they propagate on filters and are shed into the distributed water where they graze on biofilms. Their potential to protect pathogens from disinfection is an area of great interest for water utilities and public health. This study was initiated to investigate the diversity of FLP from drinking water and pipe biofilms using different methods of collection. *Methods:* Water concentrates were collected from 10 µm cotton filters installed in different locations in the distribution systems of four utilities across North America; warm and cold regions as well as chlorine and chloramine disinfection were represented. Pipe biofilms and custom 'fish foam' biofilm traps were also sampled. A few samples were concentrated by a novel method without filters. Adaptations of classical methods were used to grow the FLP using four different bait bacteria. After optimizing DNA extraction, PCR for 18S rDNA followed by DGGE, cloning and sequencing were used to characterize FLP. Morphology and PCR with some specific primer sets were also used. *Results:* A wide variety of FLP were obtained by all methods, with more isolated from cotton filters. Acanthamoebae were predominant in some samples; some samples contained predominantly Cercozoa. Many fungi were co-isolated but were often not obvious in the cultures. Where hyphae were seen, they often served as 'amoeba highways'. It is not clear that any specific relationships existed between fungi and FLP. Some FLP grew very slowly and needed 'adaptation' to bait bacteria. Many FLP that were seen could not be identified. *Conclusions:* FLP are present in all sample types, Those cultured are only a small fraction of those present; considerable inhibition from the concentrates was likely. More information is needed on whether specific predator-prey relationships can exist in drinking water ecosystems.

Abstract Category
06 Engineered Environments

PS.06.096 IMPACT OF DREDGED MATERIAL ON BACTERIAL COMMUNITIES OF THE BENTHOS IN THE GERMAN BIGHT

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Bacteria of marine sediments play an important role in carbon and nutrient cycles. Due to their disposal of various genetic information and their competence for gen transfer bacteria have the capability to adapt to changes in their environment very fast. Thus investigating the bacterial community allows following changes, as result of anthropogenic origin in many cases, very early. Waterways form important networks in transport systems but due to tidal pumping effects sediment accumulates in coastal rivers intercepting them. Dumping this sediment into the sea is a common procedure to prevent its accumulation. The impact of these potentially polluted sediments on bacterial communities of unpolluted environments is barely investigated. In this study the dumping site "Tonne E3" in the German Bight (North Sea, Germany) was examined. From 2005 to 2007 4.2 Mio m³ of excavated material from the Hamburg port area have been dumped into the North Sea. Further activities followed in the last years. In August 2009 sediment samples were taken at "Tonne E3" to analyze the bacterial community via automated ribosomal intergenic spacer analysis (ARISA). The dumping area and a reference region were investigated. ARISA focuses on the length polymorphism of a highly conserved region in the bacterial genome providing a highly resolved community pattern. The results revealed three community structures. One pattern occurred in the samples of the reference region and two others in the samples of the dumping site which differed to the community pattern of the reference. The different patterns in the samples suggest an impact of other variables such as the composition of the sediment. For a detailed insight into the community structure the patterns were further analyzed via distance matrices. Correlations with additional parameters, e.g. heavy metal contamination or CHN values were analyzed by gradient analyses to show relations with variables explaining the different patterns.

Abstract Category

06 Engineered Environments

PS.06.097 TOTAL MICROBIAL COMMUNITY PROFILING OF OIL SANDS TAILINGS PONDS AND MICROCOSMS

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Our study aims are to better understand the microbial ecology and bioremediation potential of oil sands tailing ponds. During oil sands exploration and processing, waste water slurry is formed and is called tailings. Composed of the waste from bitumen processing, hydrocarbons, salts, and trace metals, tailings are toxic to aquatic organisms and cannot be discharged. Tailings are, therefore, stored within artificial ponds. After oil sands exploration, these sites, including the tailings ponds, must be rehabilitated to functional ecosystems. Microbial communities in oil sand tailings have the potential of aiding site rehabilitation. We are studying 7 experimental tailing ponds, each with different biological characteristics. Using water and sediments from these ponds, aerobic and anaerobic microcosms were set up to mimic sediment conditions under shallow and

deep water. After 3 months, visual changes in the sediment structure were observed. Distinct layers of colour, potentially iron oxides, were visible in the sediments of the aerobic microcosms. In addition to visual changes in sediment structure, the total microbial community in the water and sediment of these tailings was investigated using DNA profiling. Similar studies usually examine only a particular microbial group, typically bacteria; however, because bioremediation involves multispecies microbial consortia, we are also analyzing the profiles of archaea, fungi, and algae from the sites. Bacterial and archaeal community profiles from tailings' water and sediments were analyzed. No archaea were detected in the water samples from the tailings ponds; however, they were detected in tailing sediments and in the water and sediments in both sets of microcosms. Overall, the bacterial profiles of the tailing ponds were similar to one another and did not show substantial seasonal changes. Study results will be discussed, providing insights into oil sands tailing ponds microbial ecology and bioremediation potential.

Abstract Category
06 Engineered Environments

PS.06.098 BACTERIA OF NEUTRALISED ACID MINE DRAINAGE

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Acid rock drainage (ARD) occurs when sulfidic ore bodies are exposed to oxygen and water. Management of this acidic metal rich discharge is one of the most difficult problems for the mining industry. Treatment systems such as reactive barriers, neutralisation with lime and wetlands can be used to manage ARD. Addition of lime to drainage or lime capping increases the pH, which results in the precipitation of metals as hydroxides. We examined the microbiology of a waste rock dump neutralised with lime. Soil and rock samples were collected from the waste rock dump (WRD), the WRD capped with lime and from the tailings. Drainage was collected from two water bodies (S1 and S2), which collect seepage from the lime capped WRD, and the evaporation dam (S3). Reference soil and water samples were taken. Principal component analysis, based on physiochemical data, showed that the seep S2 (pH 8.2), the evaporation dam drainage S3 (pH 7.38) and reference water were more similar to each other than to the seep S1 (pH 6.05). In addition to the lower pH, S1 drainage had higher cadmium, zinc, copper, cobalt and nickel than S2 and S3. For S1 the dominant phyla in rank order were the *Gammaproteobacteria* followed by the *Firmicutes*. In contrast, the *Alphaproteobacteria* were dominant in S2 and S3 drainage. These findings support previous studies in which the *Gammaproteobacteria* and *Firmicutes* dominate samples with elevated metals. The same bacteria community changes were not observed for the WRD, the limed capped WRD and tailings samples. The untreated WRD and tailings were chemically similar, and although most of the bacteria in these samples could not be classified, of those that could be, most were assigned to the *Actinobacteria*, *Alphaproteobacteria* or *Gammaproteobacteria*. The same community shift does not occur in lime capped WRD

samples and in fact, the bacterial community composition may be dominated by bacteria present in the lime from the site of origin.

Abstract Category
06 Engineered Environments

PS.06.099 PROKARYOTIC AND EUKARYOTIC COMPONENTS FROM THE CATHODE OF A PHOTOSYNTHETICALLY DRIVEN BIOELECTRIC SYSTEM

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Biocatalyzed cathode reactions of microbial fuel cells (MFCs) are important for power production, bioremediation, and potentially biofuels production. However, little is known about the mechanisms of biocatalyzed cathode reactions or the organisms that are capable of mediating them. We have previously reported the development of a self-sustained, solar microbial fuel cell (SMFC). This closed system continuously generates power throughout a 12 hr light cycle without additional fuel or oxidant. Catalytic activity of the illuminated cathode based on slow scan voltammetry, and light-dependent fluctuations in power suggested 1) a photosynthetically driven, biologically mediated reaction, and/or 2) increased oxidant from photosynthetic O₂ generation. In order to evaluate the microbial community of the SMFC cathode, the 16S and 18S rRNA gene profiles of the cathode biofilm from a 2nd generation SMFC were monitored over time. The 2nd generation SMFC was established in a sealed container with an anoxic anode ca. 1-2 cm below the sediment surface and a cathode set in overlying seawater. The cathode biofilm from a previously established SMFC served as the inoculum. The SMFC was maintained in an illuminated incubator (6000 lux, 30°C) with a 12 hr light cycle. Temporal analysis of the cathode biofilm of the 2nd generation SMFC revealed a complex microbial community enriched in *Cyanobacteria* and *Alphaproteobacteria*. Subsequent cathode transfers to identical reactors were performed that were either light exposed or maintained in darkness. Dissolved O₂ was regenerated in illuminated SMFCs and a consistent V_{cell} could be maintained for 3 months, while O₂ was depleted in dark cells with V_{cell} approaching zero. Sediment-free, potentiostat-poised SMFCs sustained photo-dependent current fluctuation in seawater. Here we report our latest results on the microbial community profile of SMFC cathode enrichments and the role of this community in enhanced catalytic activity at the cathode.

Abstract Category
06 Engineered Environments

PS.06.100 454-PYROSEQUENCING AND QUANTIFICATION ANALYSES OF BACTERIAL AND METHANOGENIC ARCHAEA DIVERSITY IN 22 FULL-SCALE BIOGAS DIGESTERS

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Biogas is one of the alternative greenhouse gas neutral energy sources. Its production has developed from using relatively simple substrates, e.g. manure, towards more complex mixtures of high biogas production potential. However, intensive utilisation of these more energy-rich substrates may result in less stable process conditions with foaming and suboptimal use of the organic material. During the optimization the biogas yield, studies of the composition and shifts in the microflora linked to the measures taken to improve the process performance were undertaken. Accordingly, samples from 22 full-scale biogas digesters using different substrates (slaughterhouse- municipal household- and industrial process waste, energy crops and sewage sludge) and process configurations were subjected to FLX genome sequencing and real-time PCR. Largest bacterial diversity occurred in sewage sludge digesters with 27 identified classes and many unclassified sequences. Industry waste digesters showed less diversity on the class level whether manure was included or not. Clostridium was the dominating bacterium on class level for all substrates and Bacilli, Shingobacteria and Erysipelotrichi were identified in all digesters. Among Archaea, aceticlastic Methanosarcinales was the dominating order in sewage sludge digesters and hydrogenotrophic Methanobacteriales in industry-derived substrates with manure. In industry waste digesters without manure hydrogenotrophic Methanomicrobiales or Methanobacteriales dominated. Industrial waste digesters bacterial communities were of the same size, while the Archaea community was larger when manure was included. Overall, lower abundance of Bacteria and Archaea occurred in sewage sludge digesters. Substrate characteristics were likely the strongest factor affecting the microflora composition.

Abstract Category
06 Engineered Environments

PS.06.101 SYSTEM FOR HIGH THROUGHPUT GROWTH AND CHARACTERIZATION OF POLY- β -HYDROXYBUTYRATE (PHB) PRODUCTION IN METHANOTROPHIC BACTERIA

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Biologically produced poly-hydroxyalkanoates (PHAs) represent a biodegradable and renewable alternative to petroleum based plastics. A variety of species and consortia are under investigation for potential large scale PHA production, including methanotrophic bacteria known to store methane gas as poly- β -hydroxybutyrate (PHB). PHAs are produced naturally, with production and consumption dependent on a complex set of conditions. Complete study of the factors affecting PHA production is limited by the modest throughput of available culture and analysis methods. The standard method for PHB detection requires microgram quantities of biomass and 30 minutes of analysis time per sample. By contrast, flow cytometric analysis of cells stained with Nile Red dye reveals the same information but requires only a fraction of the biomass and handling

time. Reduced biomass allows for high throughput growth in aerated microtiter plates, resulting in two order of magnitude savings in handling time per replicate. This system opens up a variety of applications, including media optimization, large scale selection studies, expedited screening of archived strains, and isolation of promising mutants. In this study, the system above is described in detail and used to analyze the effects of media composition on PHB production in *Metholycystus parvus* OBBP. Optimization of media composition results in substantial increases in both the percentage of PHB by dry cell weight and the total concentration of PHB per liter. PHB production was found to be highly dependent on the identity of the limiting nutrient as well as the concentration of calcium and copper. In particular, highly enhanced PHB production is observed during growth with low but non-limiting levels of calcium in the media. PHB is a known component of some bacterial calcium transport channels, and exposure to low levels of calcium may therefore stimulate PHB production as a means of maintaining intracellular calcium concentrations.

Abstract Category
06 Engineered Environments

PS.06.102 NUMERICAL ANALYSIS FOR EVALUATION OF BACTERIAL DIVERSITY BY PCR-DENATURING GRADIENT GEL ELECTROPHORESIS USING INTENSITY INFORMATION OF GEL IMAGES

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PCR-denaturing gradient gel electrophoresis (DGGE) is a useful method for looking over the microbial diversity in environmental samples. To analyze DGGE banding patterns for comparison of microbial compositions, numerical evaluation methods such as diversity index and similarity index are available. For evaluation of the similarities of each banding patterns, similarity indices such as Dice's and Jaccard's indices which compare the presence or absence of each DNA band without intensity information are popularly used. However, in consecutive bioprocess samples such as bioremediation, dynamics of microbial population should be concerned, so that similarity index using intensity information of the DNA bands is required. The aim of this study is to apply similarity indices using relative intensities of each DNA bands in DGGE gel images for analysis of microbial population dynamics in composting samples.

Food waste composting process were performed using commercially available biological composting processor in three different conditions. PCR-amplified bacterial 16S rDNA fragments were applied for PCR-DGGE. The electrophoresis images were digitized, and positions and intensities of the DNA bands were used for numerical analyses.

During the composting processes, unexpected system troubles such as irregular heating and system fall down occurred in several times. The compositions of microbial consortia in the reactor were affected by these system troubles, since DNA banding patterns of PCR-DGGE images were changed obviously. However, clustering analyses of PCR-DGGE images using similarity indices of Dice's and Jaccard's were inadequate to distinguish the change of microbial consortia by the system troubles. In contrast, cluster

analyses using similarity indices with intensity information of DNA banding patterns such as Renkonen's and Kimoto's successfully separated the clusters of microbial consortia before and after the system troubles.

Abstract Category
06 Engineered Environments

PS.06.103 MICROBIAL COMMUNITY DYNAMICS DURING PROPIONATE ACCUMULATION IN A LOW TEMPERATURE ANAEROBIC DIGESTER TREATING SWINE MANURE

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Volatile fatty acid accumulation is associated with loss of optimal anaerobic bioreactor performance. We investigated the microbial community dynamics in a low temperature anaerobic digester treating swine manure over a 5-month period, by monthly sampling 2 months before, 2 months during, and 1 month after propionate accumulation. Amplicon length-heterogeneity PCR (LH-PCR) and terminal restriction fragment length polymorphism (T-RFLP), based on the bacterial and archaeal microbial community 16S rRNA gene, were used to determine the microbial community dynamics. Nonmetric multidimensional scaling (NMS) and multi-response permutation procedure (MRPP) showed that, from both the rRNA- and rDNA-based analyses, the propionate crisis perturbed the bacterial community structure with no return to the initial bacterial community structure. Indicator species analysis (ISA) combined with phylogenetic assignment of the peaks from clone libraries suggested that the main groups, *Bacteroidetes* and *Spirochetes*, remained predominant through the 5-month period. Based on rRNA ISA, some particular phylotypes belonging to the *Spirochetes* significantly decreased during the propionate crisis and remained at low level thereafter. *Syntrophomonas* decreased but *Desulfotomaculum* increased during the propionate accumulation period. *Methanoculleus* were predominant in the archaeal community. However, ISA suggested that the appearance of *Methanosaeta* during propionate accumulation contributed to archaeal community changes. This study illustrates how multivariate statistical analyses combined with clone library analysis helps to determine how the microbial communities are changing and provides insights to further investigate the metabolic pathways involved.

Abstract Category
06 Engineered Environments

PS.06.104 OPENING THE BLACK-BOX: METAGENOMIC ANALYSIS OF A CONSTRUCTED WETLAND DESIGNED FOR METAL LEACHATE TREATMENT

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Passive treatment systems are defined as natural or engineered systems that make use of naturally occurring geochemical and biological processes for detoxification of water and soils. Mine drainage and other similar wastewaters are a significant source for potential contamination of aquatic environments in Canada and the world. Thus, there is a need for effective, inexpensive and sustainable approaches to remediate metal leachate and acid mine drainage (AMD) from mine sites. Processes involving microbial communities are critical for successful and sustainable performance of passive treatment systems. These communities and their metabolisms have been mainly characterized using cultivation-dependent methods and as most organisms from the environment cannot be cultured in the laboratory, we propose to open the "black-box" of passive treatment systems by developing and using culture-independent metagenomic approaches to study microbial community structure and metabolism in passive treatment systems. The studied passive treatment system efficiently removes arsenic and zinc from seepage of a landfill used by a mining company and has been successfully running for almost a decade; however its mode of operation varies by seasonal changes. Genomic DNA was extracted from core samples and culture-independent approaches were applied to study microbial community structure and functional gene characterisation. For microbial community analysis, bacterial and archaeal 16S rDNA libraries were constructed and PhyloChip G3 probing was performed. Fosmid library end-sequencing was applied to characterise the metabolic potential within the treatment system and extensive metadata generation for geochemical characterisation of the environment was performed. These data will help to understand the processes for efficient heavy metal removal in passive treatment systems and to improve the knowledge about how future wetland systems need to be designed for efficient and reliable operation.

Abstract Category

06 Engineered Environments

PS.06.105 EFFECT OF CULTURE CONDITIONS ON MANGANESE OXIDATION OF MICROBIAL CONSORTIA FROM BIOLOGICAL FILTER MEDIA

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Biological filtration is a cost-effective and eco-friendly technology for removal of manganese (Mn) from groundwater. However, the complex mechanisms for removal of Mn by biological processes are not well-understood. Mn oxidation in biological filtration plant is thought to be mediated by microbial consortia containing various kinds of Mn-oxidizing bacteria such as *Leptothrix* and *Hyphomicrobium* commonly found in groundwater. To study the mechanism of microbial Mn-oxidation in biological filtration system, microbial consortia which can oxidize soluble Mn^{2+} were cultured from biological filter media of groundwater treatment plant in Joyo, Kyoto.

Microbial consortia which can oxidize soluble Mn^{2+} was cultured using PYM medium with or without anthracites and sea sands as carriers for fixing microbial cells. Tetravalent Mn-oxides were confirmed using leuco-crystal violet staining method. Total DNAs of microbial flocs were extracted using FastDNA SPIN Kit for Soil.

11-days cultivation produced microbial flocs with dark-brown particles of Mn-oxides. Interestingly, the cultures with carriers produced larger amount of tetravalent Mn-oxides than the cultures without carriers. PCR-DGGE analysis indicated that molecular diversity of 16S rDNAs of microbial consortia in the culture with carriers was higher than that of the cultures without carriers. Among the DNA fragments separated by DGGE, rDNA sequences similar to *Leptothrix* and *Hyphomicrobium* were observed. The amount of total 16S rDNAs from each culture were analyzed by real-time PCR, however, no significant difference was found between the culture conditions with and without carriers. Effect of pH on Mn oxidation was studied, and raise in pH caused increase of Mn oxidation of the microbial consortia cultured from biological filter media.

Abstract Category

06 Engineered Environments

PS.06.106 DEVELOPMENT OF BACTERIAL COMMUNITIES DURING SPONTANEOUS SUCCESSION ON SPOIL HEAPS AFTER BROWN COAL MINING

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In central Europe, large areas of soils are used for spoil deposition after brown coal mining. Artificial ecosystems are established where initial substrate is almost free of microbial life. Such sites represent a unique opportunity to study soil development during primary succession or revegetation. The aim of this work was to characterize the succession of bacterial communities along a chronosequence of spoil deposits after brown coal mining in the Sokolov area, Czech Republic. We selected sites developing spontaneously for 6, 12, 21 and 45 years. The 6-year-old site had no vegetation cover, the 12-year-old site was covered by grasses and herbs; shrubs of *Salix* and *Populus* dominated the 21-year-old site and the 45-year-old site was covered by *Betula* and

Populus trees. A 21-year-old reclaimed with *Alnus* was used for comparison to primary succession. Microbial biomass was quantified using PLFA analysis and qPCR and bacterial communities were characterised using a phylogenetic microarray combined with sequencing. The content of Corg and N increased while pH decreased in the course of succession. Bacterial biomass was always higher than fungal and bacteria dominated the 6-year-old site. Fungal/bacterial biomass ratio increased from 0.11 over 0.25 and 0.32 to 0.20 along the succession. Microarray data showed a clear separation of the youngest 6-year-old site from the other sites, which were much more similar for each other. Subsequent cloning confirmed microarray data. The 6-year-old site was dominated by e. g. *Spiroplasma*, *Paracoccus*, *Desulfosarcina*, *Propionibacterium*. The site revegetated with *Alnus* was similar to the spontaneous succession site of the same age showing high effect of soil C accumulation and low effect of vegetation. Our results show that a specific bacterial community develops in initial stages of succession under low C conditions and in the virtual absence of fungi. Among its members, most do not occur in the later stages of success

Abstract Category

06 Engineered Environments

PS.06.107 GEOGRAPHICAL VARIATION OF FUNGAL AND BACTERIAL COMPONENTS IN MATTRESS DUST ACROSS EUROPE

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Exposure to environmental microbes may affect human health both harmfully and beneficially. Dampness and mold in the home environment are associated with respiratory symptoms and asthma. The causative agents behind the effects are not clear. New methods for microbial exposure assessment, such as qPCR, can give more detailed information.

The European Community Respiratory Health Survey (ECRHS) is a large adult cohort study on asthma, allergies and their risk factors. In this study we evaluated indoor microbial exposure assessed by molecular methods in subpopulations of asthmatics and non-asthmatics of the ECRHS.

Mattress dust samples from 200 asthmatics and 200 control subjects were obtained from 14 ECRHS study centers across Europe. DNA was isolated from 20 mg of dust. Nine different bacterial or fungal qPCR assays (*Aspergillus versicolor*, *Alternaria alternata*, *Cladosporium cladosporioides*, *C. herbarum*, *C. sphaerospermum*, *Trichoderma viride/atroviride/koningii*, the group of *Penicillium/Aspergillus/Paecilomyces varioti*, *Mycobacterium* spp. and *Streptomyces* spp.) were performed.

Based on preliminary results, *Penicillium/Aspergillus* group was found in all samples. For some microbes, such as *A. versicolor*, the overall prevalence was very low (6%). Detection of *A. alternata* was rare in Northern Europe, whereas it was found in one third of the samples in several Southern European centers. Similar differences were visible for other species. Microbial concentrations varied strongly between the centers, showing as high as 100 fold differences in the median concentrations.

As a conclusion, we found large geographical variation in mattress dust microbial content across Europe, which may help to explain conflicting results obtained in various studies concerning environmental microbial exposure and health. Prevalence and concentration of some species was very low, indicating that mattress dust might not be the best sample to study all types of environmental microbial exposure at home.

Abstract Category
06 Engineered Environments

PS.06.108 USE OF FLUORESCENT MICROBEADS TO UNDERSTAND AEROBIC GRANULE FORMATION FOR ACTIVATED SLUDGE WASTEWATER TREATMENT

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Aerobic granular sludge is an emerging technology for wastewater treatment. Aerobic granules form larger biofilm aggregates than conventional sludge floccular biofilms, settle much faster than flocs, and can maintain higher biomass levels. Thus, there are potential operational and cost saving advantages using aerobic granules. However, long start-up periods are required for the development of aerobic granules from a floccular-based system, and loss of biomass and can occur. In a recent study using an innovative seeding strategy, addition of crushed granules to a floccular sludge significantly reduced the start up period (Pijuan, submitted). However, currently there is poor understanding of how granules form and such conceptual information is important for improving start-up strategies. This study aims to determine mechanisms of granule formation and understand the accelerated process. A novel methodology was used to follow the granule formation. Granular and floccular biofilms were labelled with differently coloured fluorescent microbeads (4 μm diam); these were then added to a laboratory scale wastewater treatment reactor. Confocal laser scanning microscopy, incorporating image analysis using the daime program, was used to monitor the granule formation period. Labelled biofilms were successfully detected in samples from the activated sludge reactor over the 80-day period. In the early stage (first 20 days) there was evidence that flocs were attaching to the surface of the granules, and further analysis indicated this attachment was permanent. The results imply that the granules act as nuclei for floccular particle attachment, which accelerates the granule formation. This provides important supporting evidence for this innovative strategy and for the full-scale application of this technology. Additionally, our novel use of fluorescent microbeads to monitor biofilms for an

extended period in a reactor, provides foundation methodology for studies of biofilm dynamics.

Abstract Category
06 Engineered Environments

PS.06.109 MICROBIOLOGICAL STUDY OF THE INFLUENCE OF AMMONIUM CONCENTRATION ON METHANE BIOFILTRATION ON AN INORGANIC BED FILTER

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The effect of ammonium on the oxidation of methane (CH₄) in complex environments such as soil is still a matter of discussion. While most authors consider that moderate or high ammonium concentrations (1-10 mM) have inhibiting effect on methanotrophy, stimulation has been observed in some studies. Our study was performed in a "controlled" ecosystem: an inorganic bed biofilter dedicated to CH₄ biooxidation. We determined the effect of increasing ammonium : nitrate mass ratio on CH₄ removal, carbon dioxide production and bed nitrogen retention. Bacterial communities were followed by denaturing gradient gel electrophoresis and metagenomic libraries. It was found that the CH₄ removal efficiency and carbon dioxide production tended to decrease with the increase of ammonium concentration with an important drop of removal efficiency at 0,2 gN-NH₄⁺/L - a concentration that seemed to be strongly limiting for the bacterial microbiota. The 16s rRNA gene fragment denaturing gel electrophoresis patterns were continuously evolving as ammonia concentrations increased, implying various changes in the microbial community. Methanotrophs closely related to *Methylocystis sp.* were identified in 16s rRNA gene libraries throughout the 485 days-long experiment. Globally, increasing concentrations of ammonium significantly reduced the removal efficiency of CH₄ biofiltration. A succession of methanotrophs and methylotrophs was observed, but this adaptation of the structure of the bacterial community was not sufficient to maintain the initial biofilter performance, observed at the initial concentration of NH₄⁺ of 0,05 gN-NH₄⁺/L.

Abstract Category
06 Engineered Environments

PS.06.110 NITRATE DOSAGE OPTIMIZATION TO CONTROL NET PRODUCTION OF SULFIDE IN EXPERIMENTAL BIOREACTORS. INTERACTION BETWEEN N AND S CYCLING IN WASTEWATER.

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In anaerobic conditions, sulfate-reducing bacteria oxidize organic matter with sulfate, producing sulfide, which is toxic, corrosive and usually reduces the efficiency of the depuration process and damages the facilities of waste water treatment plants. Among the methods proposed to control sulfide production, addition of nitrate is considered one of the most suitable, although the mechanism is scarcely understood. Nitrate could inhibit sulfate-reducing bacteria or stimulate sulfide oxidation by nitrate-reducing sulfide-oxidizing bacteria. The aims of this work were to confirm the stimulation of nitrate-reducing sulfide-oxidizing bacteria after the addition of nitrate, find the minimum effective nitrate concentration and to study the interaction between the N and S biogeochemical cycles under different nitrate: sulfide ratios. Two experimental bioreactors were set up at Guadalete-WWTP (Jerez, Spain) and fed with the waste water after sand and oil removal. One of the bioreactors was fed with nitrate at four different concentrations (0.12, 0.24, 0.5 and 1mM) for periods of three days. Several physicochemical variables were measured in the water phase (NO_3^- , NO_2^- , SO_4^{2-} , H_2S , pH, etc.) and in the air (H_2S) until initial sulfide concentration was recovered. Activity and presence of microorganisms implicated in the cycles studied were analyzed in the water phase and biofilms by molecular biology techniques. Addition of nitrate resulted in a quick (within 3h) decrease of sulfide in the water phase. Sulfide elimination efficiency increased with nitrate concentrations following MichaelisMenten kinetics ($K_m = 0.7\text{mM NO}_3^-$). Suppression of nitrate addition resulted in a recovery of initial net sulfide production in about 3h. Species of *Sulfurimonas denitrificans* and *Paracoccus denitrificans*, as well as a variety of sulfate-reducing bacteria, were detected during this study. Results confirm that nitrate efficiently stimulates sulfide oxidation in the bacterial community.

Abstract Category

06 Engineered Environments

PS.06.111 AMOUNT AND DIVERSITY OF TETRACYCLINE (*tetC* AND *tetM*) AND MERCURY RESISTANCE GENES (*merA*) IN THE AQUACULTURE SEDIMENTS IN THE BALTIC SEA

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Microbes in sediments below fish farming cages are exposed to a variety of compounds used in aquaculture such as antimicrobials and metals. We assessed the amounts of two different tetracycline (Tc) resistance genes (*tetC* and *tetM*) and mercury resistance gene (*merA*) in the sediment of four fish farms and their surroundings in the Baltic Sea by using quantitative polymerase chain reaction (qPCR). Moreover, the presence of four Tc resistance genes (*tetA*, *tetE*, *tetG* and *tetW*) was studied by normal PCR. The diversity of the resistance genes was analysed by sequencing of the gene libraries. Two of the farms were sampled over four successive summers and two of the farms once. Both bioavailable and total amounts of Tc and mercury were measured. Our results show a substantial increase in the copy numbers of *tetC* and *tetM* (both were present in about 10^7

copies g^{-1} sediment, corresponding to about 10^{-2} copies per a copy of 16S rRNA genes) and the presence of *tetA*, *tetE*, *tetG* and *tetW* genes in the sediments under aquaculture farms at all sampling times. None of the Tc resistance genes were found in samples taken away from any of the farms. The amount of *merA* was consistently higher at fish farm sites (about 10^6 copies g^{-1} sediment) than at pristine sites although the increase was smaller since *merA* was also found in many of the samples taken from outside of the farms. The *merA* phylotypes were different between fish farm and pristine sites and also between the farm sites. The diversity of Tc resistance genes was relatively low and no differences between the sites were found. Tc concentrations were below limit of the detection in all sites and mercury concentrations were very low and did not differ significantly among sites. The copy numbers of *merA*, *tetC* and *tetM* remained elevated even in the absence of any obvious selection pressure. Reasons for that may include stability of the genes or constant introduction of the genes with e.g. fish feed

Abstract Category

06 Engineered Environments

PS.06.112 NADH-DEPENDENT CHROMIUM (VI) REDUCTASE ACTIVITY IN *Klebsiella oxytoca* P2

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Microbial detoxification of Cr(VI) has gained importance lately due to the efficiency of bacterial reduction of the highly toxic Cr(VI) to the comparatively less toxic Cr(III). The details of the reaction mechanism are still unclear. Bioreduction of Cr(VI) can occur directly as a result of microbial metabolism (enzymatic reduction) or indirectly by a bacterial metabolite oxidation. In aerobic conditions, most of the chromate reductases reported at the moment are cytosolic and reduce Cr(VI) to Cr(III) both inside and outside the cytoplasmatic membrane. Some studies showed that the Cr(VI) reducing activity of intact cells of chromium resistant bacteria, isolated from soils contaminated with dichromate, may be useful for Cr(VI) detoxification in polluted environments. The objective of this study is to assess the Cr(VI) reduction by whole cells of the indigenous *Klebsiella oxytoca* P2 and study the effect of electron donors such as NADH in this process. In previous studies performed in this lab, five autochthonous strains were obtained from polluted soil, surface water and sediments of industrial areas of Buenos Aires Metropolitan Area, Argentina. *Klebsiella oxytoca* P2 was selected for this work because it presented the maximum decrease in Cr(VI) concentration (74.32%) in pure cultures. Reduction of Cr(VI) was detected at 32°C using whole cells at different growth stages, resuspended in phosphate buffer 0.05 M (pH= 7.25). The maximum reduction was obtained using cell cultures at exponential growth stage (t=6hs), being the effect significantly increased by the presence of NADH. No significant reduction was detected in the absence of the cells (abiotic reduction) in spite of the presence of the electron donor. Reduction experiments using buffer TRIS-HCl 100mM, pH= 7.0 were also performed to compare reduction yields. A deeper understanding of the enzymatic role of

reductases would allow us to establish the best conditions for suitable bioprocess for Cr(VI) bioremediation.

Abstract Category
06 Engineered Environments

PS.06.113 THE DEVELOPMENT OF NOVEL UP-FLOW BIOELECTROCHEMICAL SYSTEM FOR DETOXIFICATION OF NITROAROMATICS

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Bioelectrochemical System (BES) has been involved into the refractory wastewater treatment recently, such as detoxification of nitro-aromatics, decoloration of azo dye and dechlorination of perchlorate. However, the normal system construction with ion exchange membrane (IEM) could cause the acidification of anolyte and increasing the internal resistance. Moreover, the membrane also can limit the scaling-up and application of BES. In this study we developed a novel up-flow BES without IEM for the treatment of nitrobenzene, the model of nitro-aromatics. Two kinds of deployment of electrode were compared. (i) Anode deployed under the cathode, (ii) Cathode deployed under the anode. In the first condition, the anode potential increased from -421mV (vs. SCE) to 31mV with the influent nitrobenzene concentration increasing from 20mg/L to 100mg/L, which implied the electrochemical activity of bioanode was inhibited significantly. Whereas, in the second condition, the anode potential can maintain around -400mV even the influent nitrobenzene concentration getting 500mg/L, which indicated that when cathode was deployed under the anode, the cathode not only reduces nitrobenzene but also protect the exoelectrogens on the anode from the toxicity of nitrobenzene. Further more, the efficiency of nitrobenzene remove was investigated. The nitrobenzene removal rate can achieve $13.52 \pm 1.1 \text{ mol m}^{-3} \text{ ACV d}^{-1}$ (apparent cathode volume ACV) with the nitrobenzene removal efficiency of $99.3 \pm 0.3\%$ and aniline formation efficiency of $93.4 \pm 1.3\%$ at the applied voltage of 0.1V. Additionally, the internal resistance of the BES was measured by impedance spectroscopy. The results were only dozens of ohms under different conditions, lower than that with membrane.

Abstract Category
06 Engineered Environments

PS.06.114 ISOLATION AND CHARACTERIZATION OF SHIGELLA FLEXNERI G3 FOR EFFECTIVE CELLULOSIC SACCHARIFICATION UNDER MESOPHILIC CONDITIONS

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Known cellulolytic microorganisms can be divided into two main categories: aerobic fungi (brown-, white- and soft-rot fungi) and bacteria. Recently, anaerobic bacteria (e.g. *Clostridium thermocellum*, *Zymomonas mobilis*) were proved to be used for biomass conversion, and anaerobic processes have the advantages over aerobic processes in terms of lower cell biomass, easy implementation for simultaneous saccharification and fermentation. However, cellulose hydrolysis under mesophilic anaerobic conditions is generally slow and inefficient. Thus the goal of this study is to isolate bacteria capable of rapid production of high yields of oligosaccharides from lignocellulosic under mesophilic conditions. A novel *Shigella* strain (*Shigella flexneri* str. G3) displaying cellulolytic activity under mesophilic, anaerobic conditions was isolated and characterized in this work. The bacterium is gram-negative, short rod-shaped, nonmotile, and non-spore-forming, with optimal activity of producing glucose, cellobiose, and other oligosaccharides from cellulose (Avicel PH-101) at 37°C and pH 6.5. Approximately 50% of cellulose was hydrolyzed in the modified ATCC 1191 medium containing 0.5% cellulose, and the oligosaccharide production yield and specific production rate reached 252 mg g⁻¹ of Avicel and 5.21 mg g⁻¹ of Avicel h⁻¹ in the 50 hrs incubation, respectively. To our knowledge, this represents the highest reported oligosaccharide yield and specific rate from cellulose for mesophilic bacterial monocultures. *S. flexneri* G3 is thus an attractive candidate for rapid conversion of cellulose to oligosaccharides in biofuels applications under mesophilic conditions.

Abstract Category

06 Engineered Environments

PS.06.115 AN INNOVATIVE INTEGRATED REACTOR SYSTEM FOR SIMULTANEOUS REMOVAL OF CARBON, SULFUR AND NITROGEN BASED ON BIOLOGICAL NICHES

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In the past decades, scholars paid much attention to the effective solution of sulfate-laden organic wastewater discharged from the production of sugar, alcohol, pharmaceutical products and so on. This kind of wastewater usually contains high concentrations of sulfate and ammonia, which can contaminate the receiving waters, endanger the livings and arouse eutrophication in them. The conventional biological treatment methods focus on removing sulfate and ammonia in separate processes where different kinds of bacteria exert their respective functions. This results in the complexity and high cost. An innovative system for simultaneous removal of organic carbon, sulfur and nitrogen was developed to consist of three reactors, i.e. sulfate reduction and organic matter removal (SR-CR), autotrophic and heterotrophic denitrifying sulfide removal (A&H-DSR) and nitrification (AN). The essential operational parameters for SR-CR reactor are sulfate and organic loading rates, hydraulic retention time (HRT), COD/SO₄²⁻ ratio and pH, and for A&H-DSR reactor are sulfide and nitrate loading rates, HRT, pH, S²⁻/NO⁻ ratio and COD/NO₃⁻ ratio. Laboratory tests identified the optimal conditions for SR-CR reactor include HRT ≥ 24h, sulfate loading rate ≤ 7.5 kg SO₄²⁻/m³•d and organic loading rate ≤ 10

kgCOD/m³•d, COD/SO₄²⁻≥2 and pH≥6.5. For the A&H-DSR process, the optimal conditions were found to be sulfide loading rate ≤6.0kg S²⁻/m³•d, nitrate loading rate ≤3.5 kg NO₃⁻/m³•d, S²⁻/NO₃⁻≥1, COD/NO₃⁻≥1.25:1 and pH≥7.5. When operating at so-determined optimal conditions sulfate, ammonia and organic matter removals of 99%, 90% and 99%, respectively, were obtained, so to recover ≥90% elemental sulfur (S₀) from the sulfide-laden wastewater streams. The predominant functional organisms include *Clostridiaceae* sp., *Desulfomicrobium* sp., *Methanosaeta* sp. in the SR-CR reactor, and *Sulfurovum* sp., *Pseudomonas aeruginosa* and *Denitratisoma* sp. in the A&H-DSR reactor.

Abstract Category
06 Engineered Environments

PS.06.116 BIO-COMMUNITY ANALYSIS DURING THE ANODE BIOFILM REFORMATION IN A TWO-CHAMBER MICROBIAL ELECTROLYSIS CELL FOR H₂ PRODUCTION

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Microbial electrolysis cell (MEC) is a recently developed bio-electrochemical system that can produce hydrogen from organic at high hydrogen recoveries, but the composition and structure of the microbial communities in this system have not been extensively studied. To better understand community changes in microbial electrolysis cell (MEC), community reformation of anode biofilm was studied using single strand conformation polymorphism (SSCP) and GeoChip. Biofilm communities, enriched in a long-term running two-chamber MEC reactor (applied 0.6 V) performing coulombic efficiency (CE) 35% and hydrogen yield (Hy) 31%, were used as inoculum to start a new reactor for 3 months till stably producing hydrogen (CE = ~35% and Hy = 30%). SSCP results demonstrated that similar community composition reassembled again with no big structure changes, e.g. disappearance of exoelectrogens, maybe caused by microbial competition. More details by GeoChip indicated that detected gene intensity increased significantly after hydrogen production. Four genera, detected based on cytochrome genes, contributed significantly to electron generation or transport including *Desulfovibrio*, *Rhodospseudomonas*, *Shewanella* and *Geobacter*. These results suggest that electron generation and hydrogen production was enhanced by enrichment of cytochrome genes and exoelectrogens involved, rather than community structure adjustments.

Abstract Category
06 Engineered Environments

PS.06.117 A RAPID SELECTION STRATEGY FOR AN ANODOPHILIC CONSORTIUM FOR MICROBIAL FUEL CELLS (MFCs)

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Current generation in microbial fuel cells (MFCs) is dependent on the presence of exoelectrogenic bacteria that oxidize organic matter and transfer electrons to the anode. Pure exoelectrogenic cultures have been tested as inoculums for MFCs, but the power densities generated were usually lower than those obtained using a mixed culture in the same MFC, although some pure cultures produced power densities equal to or higher than those of mixed culture. Rich sources of microorganisms, such as wastewater, activated sludge, and sediments, are often used as inoculums for MFCs, and analyses of anode biofilms in these systems has shown great bacterial diversity in anodophilic communities, but did not reveal specific trends in dominant members. This finding may be due to the large number of non-exoelectrogens in the anode biofilm. Thus, selective pressure in MFC systems may not be sufficient to exclude non-exoelectrogen. In this study, a rapid selection method was developed to enrich for a stable and efficient anodophilic consortium (AC) for microbial fuel cells (MFCs). A biofilm sample from a microbial electrolysis cell was serially diluted up to 10^{-9} in anaerobic phosphate buffer solution and incubated in an Fe(III)-acetate medium, and an Fe(III)-reducing AC was obtained for dilutions up to 10^{-6} . The activity of MFC inoculated with the enrichment AC was compared with those inoculated with original biofilm or activated sludge. The power densities and coulombic efficiencies of the AC (226 mW/m², 34%) were higher than those of the original biofilm (209 mW/m², 23%) and activated sludge (192 mW/m², 19%). The start-up period of the AC (60 h) was also shorter than those obtained with the other inocula (biofilm, 95 h; activated sludge, 300 h). This indicated that such a strategy is highly efficient for obtaining an anodophilic consortium for improving the performance of an MFC.

Abstract Category
06 Engineered Environments

PS.06.118 MULTIPLE COMMUNITY STATES OF PLANKTONIC COMMUNITIES ON FUNCTIONAL GENE STRUCTURE IN MICROBIAL ELECTROLYSIS CELL (MEC)

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Microbial electrolysis cells (MEC) show promise for efficient recovery of hydrogen and methane from waste using exoelectrogenic microorganisms. However, gas production is variable based on reactor setup states. To understand this variation, 15 MEC reactors (one control reactor in open circuit) were inoculated with a long-term MEC reactor biofilm at a fixed applied voltage of 0.7 V using glucose as the carbon source. Samples were taken every 24-h after gas production had stabilized and analyzed using GeoChip 3.1. Gas production (H₂, CH₄ and CO₂) was analyzed every cycle using a gas chromatogram.

Principle component analysis (PCA) grouped the reactors based on gas production: high-hydrogen (50~60% H₂, 20~30% CH₄), moderate-hydrogen (25~50% H₂, 30~55% CH₄), high-methane (55~75% CH₄, 10~25% H₂), and little gas production. Difference on functional gene structures distanced away reactor 9# (high-hydrogen), 12# and 13# (moderate-hydrogen) and control reactor in detrended correspondence analysis (DCA) based on all genes, and similar community structure presented over the rest of reactors that grouped tightly. Although cytochrome genes did not show a significant change among groups based on gas production, high-hydrogen reactor were detected special community structure composed of only four genera (*Shewanella*, *Pseudomonas*, *Geobacter* and *Bradyrhizobium*) out of the ten detected in all reactors as based upon cytochrome gene results. Subsequent exposure to pH 4 disturbance temporarily inhibited gas production. High-methane and little gas producing communities clustered together tightly in DCA results ordination plots based on all functional genes detected and showed little change during pH disturbance. In contrast, high-hydrogen communities showed a change in the community structure over time and during pH disturbance. These results demonstrate that exoelectrogenic communities with high hydrogen production showed a higher sensitivity to disturbance than the other communities.

Abstract Category
06 Engineered Environments

PS.06.119 IMMIGRATION AS A DRIVER OF MICROBIAL COMMUNITY STRUCTURE: SLOUGHING FROM A BIOFILM REACTOR IS LINKED TO DOWNSTREAM ACTIVATED SLUDGE COMMUNITY DYNAMICS

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Activated sludge bioreactors are widely used to remove organics and nutrients from wastewater. However, the role of immigration in structuring activated sludge microbial communities is little understood. Converging lines of evidence from a year-long series of weekly samples at a full-scale wastewater treatment plant indicated a strong link between aeration basin influent NO₂⁻ and shifts in activated sludge microbial community structure. To further investigate this association, we sampled four locations along a transect within this plant: 1) plant influent; 2) trickling filter biofilm; 3) trickling filter effluent; and 4) the activated sludge bioreactor. Here, we show via a polyphasic approach that influent NO₂⁻ is a signature of microbial immigration from the upstream biofilm-based trickling filter to the activated sludge bioreactor. High-density phylogenetic microarray (PhyloChip) analyses revealed an overabundance of methanogens and sulfate-reducing bacteria in the trickling filter and suggested microbial transport to the activated sludge via the trickling filter effluent. Furthermore, ammonia-oxidizing bacterial (AOB) *amoA* copy number increased by an order of magnitude between plant influent and trickling filter

effluent, indicating accumulation of AOB in the trickling filter and significant immigration to the activated sludge unit. Molecular fingerprinting (T-RFLP) analyses corroborated by clone libraries showed that *Nitrosomonas europaea* dominated the trickling filter, while a '*Nitrosomonas*-like' lineage dominated in activated sludge. *N. europaea* was previously shown to dominate in activated sludge during elevated influent NO₂⁻ events, suggesting that activated sludge AOB community dynamics are driven in part by immigration via sloughing from the upstream trickling filter. Taken together, our results provide compelling evidence that immigration between coupled process units can significantly influence activated sludge microbial community structure.

Abstract Category

06 Engineered Environments

PS.06.120 THE MICROBIAL SIGNATURE OF AEROSOLS PRODUCED DURING THE THERMOPHILIC PHASE OF COMPOSTING

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Bioaerosols generated at composting plants are released during processes that involve vigorous movement of material: fresh waste delivery, shredding, compost pile turning, screening... These bioaerosols are a cause of concern because of their potential impact on the health of workers and residents living close to such facilities. The main goal of this research was to look for phylotypes common to several bioaerosols which would represent a microbial signature of compost aerosols. These phylotypes could be used to evaluate bioaerosol dispersal around composting plants and their associated environmental impacts. The microbial diversity of bioaerosols collected at five open industrial composting plants treating different types of waste (green waste, municipal solid waste, sludge etc.) was characterized by a culture-independent approach. Bioaerosols were collected during the turning of compost piles in thermophilic phase. Bacteria and Eukaryota rDNA libraries were built for each of the five bioaerosols and more than 800 sequences were analysed. Bacterial diversity was represented by eleven different phyla but was largely dominated by *Firmicutes* and *Actinobacteria*. Fungal sequences corresponded mainly to *Thermomyces*, *Aspergillus* and *Penicillium*. The structure of microbial diversity at the phyla level was similar, but differences in the phylotype distribution were observed. Core species of the composting bioaerosols were identified. Two phylotypes, affiliated to *Saccharopolyspora rectivirgula* and *Thermomyces lanuginosus*, were present in the five bioaerosols. Three indicators of compost aerosols were identified and quantified by qPCR to study the dispersal of bioaerosols emitted by different composting plants. In conclusion, defining the microbial signature of composting aerosols gave us access to indicators that can be used for analyzing bioaerosol dispersal.

Abstract Category

06 Engineered Environments

PS.06.121 CHARACTERIZATION OF CULTURABLE THERMOPHILIC MICROORGANISMS IN COMPOST FED-BATCH ENRICHED WITH DIFFERENT CARBON SOURCES

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Background and aims-Nowadays renewable energy is gaining more attention as a mean of addressing climate change and the depletion of fossil fuels. One form of renewable energy is bioethanol which can be produced from agricultural feedstocks. In addition, bioethanol can be derived from lignocellulosic waste, reducing the use of food crops as a source for bioenergy. However, the production costs for ethanol derived from wastes are high. The aim of this study was to identify thermophilic microorganisms which are capable to ferment xylose, glucose and cellulose. These could be used in a fermentation at elevated temperature allowing in situ distillation. Samples were collected from a green waste composting plant and used to inoculate bioreactors containing xylose, glucose or cellulose. In order to create different selective conditions both aerobic and anaerobic environments were established in which an initial temperature of 65°C was gradually raised to 70°C. Daily, samples were taken for chemical analyses and replaced with fresh media. In addition, these samples were microbiologically analyzed weekly by plating on TSA and incubating them under aerobic or anaerobic conditions at 70°C. Isolated colonies were characterized using 16S rDNA sequencing. No major differences in microflora were observed as a function of the carbon sources used. However a clear difference was found between bioreactors incubated aerobically and anaerobically. *Geobacillus* sp. and *Thermoanaerobacter* sp. predominated under aerobic and anaerobic conditions, respectively. The highest ethanol concentration was observed under anaerobic condition with glucose as the carbon source. *Thermoanaerobacter* sp. are well known ethanol producers and will be screened for ethanol production. Community profiling techniques, i.e. terminal restriction fragment length polymorphism and clone library analyses, will be used to characterize and identify non-culturable community members

Abstract Category

06 Engineered Environments

PS.06.122 AMMONIA-OXIDIZING ARCHAEA/BACTERIA COMMUNITY STRUCTURES AND COPETITION IN TWO BIOREACTORS WITH DIFFERENT SALINITY

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The discovery of ammonia-oxidizing archaea changed our understanding of the microbial community involved in nitrification. Salinity was often recognized as an important factor

affecting ammonia-oxidizing community both in natural environments and full-scale bioreactors. Under these environments, salinity, however, was not the only factor in determining ammonia-oxidizing community, and therefore, it is difficult to conclude its effect on competition between ammonia-oxidizing archaea and bacteria. In this study, two bioreactors mimicking the operation of municipal wastewater treatment plant were seeded with the same inoculums and operated under the same conditions with 25 mg-N/L of ammonia in the feed but with different salinity level for more than 300 days. Both bioreactors were able to achieve over 95% of nitrification efficiency. After 60 days of operation, distinct ammonia-oxidizer populations (bacteria and archaea) were enriched under different salinity levels based on results of terminal restriction fragment length polymorphism. Results of cloning and sequencing targeting on *amoA* also confirmed different ammonia oxidizing bacteria and archaea community structures developed in the two bioreactors investigated. Real-time quantitative polymerase chain reaction targeting on *amoA* was performed to determine ammonia-oxidizing archaea and bacteria present in the two bioreactors. The results showed that ammonia-oxidizing archaea presented in a higher copy numbers than ammonia-oxidizing bacteria in both bioreactors. In the high salinity bioreactor (3.5%) the copy number ratio of ammonia-oxidizing archaea to bacteria attained a value as high as 500, while the ratio for the low salinity bioreactor (0.25%) was less than 30 all the time. Our results clearly confirm that high salinity condition present a selecting force for enriching ammonia-oxidizing archaea.

Abstract Category

06 Engineered Environments

PS.06.123 DEVELOPMENT OF A HIERARCHICAL OLIGONUCLEOTIDE PRIMER EXTENSION ASSAY FOR QUALITATIVE AND QUANTITATIVE ANALYSIS OF METHANOGENS IN ANAEROBIC REACTORS

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In this study, a newly developed molecular method, hierarchical oligonucleotide primer extension, was used to qualitatively and qualitatively analyze methanogenic Archaea in the sludge from anaerobic reactors treating industrial wastewaters. In total, 31 primers targeting 16S rRNA sequence were designed with specificity at the levels of domain (1), order (4), genus or species (26) to cover most cultured methanogens. The results showed that 65.9%~100.1% of methanogens in the domain Archaea were detected. The methanogens in order *Methanosarcinales* were predominant in the sludge samples, accounting for relative abundance of 65.9%~91.7% in the domain Archaea. Within order *Methanosarcinales*, three genera, namely *Methanosaeta*, *Methanosarcina* and *Methanomethylovorans* were frequently detected. *Methanosaeta* group that uses acetate for methane production was present in high percentage (49.4%~105.1%) in the sludge from different anaerobic reactors, whereas the other acetate-utilizing methanogen

Methanosarcina (93.7%~105.6%) proliferated in high abundance in the sludge from reactors treating wastewater from liquid crystal display manufacturing. It was found that *Methanomethylovorans* of 3.6%~24.4% in the order *Methanosarcinales* inhabited the sludge from different reactors, and the abundance increased to 93.1% in the sludge from anaerobic sludge bed reactor treating wastewater from liquid crystal display manufacturing. The hydrogen-utilizing methanogens detected were *Methanoculleus* (4.8%~97.3%), *Methanofollis* (1.1%~8.9%), and *Methanospirillum* (1.1%~30.7%) in the order of *Methanomicrobiales* and *Methanothermobacter* (8.4%~20.3%) in the order of *Methanobacteriales*. In conclusion, the assay has been established for quantitative detection of methanogens in the anaerobic sludge from bioreactors. Further increasing numbers of oligonucleotide primers in future, we anticipate that the assay can be extended to detect the methanogens in other anaerobic ecosystems.

Abstract Category
06 Engineered Environments

PS.06.124 NITROGEN REMOVAL PERFORMANCE AND MICROBIAL COMMUNITY CHANGES IN ANAEROBIC BAFFLED REACTOR DURING LANDFILL LEACHATE TREATMENT

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A novel treatment process with anaerobic baffled reactor (ABR) with six chambers and hybrid biofilm reactor (HBR) was introduced to a landfill leachate full-scale treatment in Guangzhou, China. After three-month performance, 34.9% anaerobic ammonium nitrogen removal efficiency was achieved and 6.4×10^7 cells/mL ammonium oxidizing bacteria density was detected in ABR when the concentration of influent ammonium nitrogen ranged from 336.24 to 685.09 mg/L. To elucidate the changes of microbial community structures and their functions during nitrogen removal, a high throughput functional gene array (GeoChip) integrated with 454 sequencing, DGGE and FISH was used to detect the microbial functional communities of active sludge samples collected from the six chambers of ABR. The results showed that anaerobic granule sludge and the ANAMMOX bacteria were formed throughout the reactor. Although different microbial communities were established in different chambers, denitrifying bacteria were detected as the most dominant genus in all of the six chambers. . . The functional gene and microbial OTU number detected from the six chambers were increased along the influent flow, while the gene overlapping was decreased. The nitrate reducing functional genes (narG) were dominant over the other genes involved in denitrification. Canonical correspondence analysis (CCA) results suggested that microbial community function was highly correlated with the concentration of influent ammonium nitrogen concentration, pH, and hydraulic retention time (HRT).

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Abstract Category
06 Engineered Environments

PS.06.125 THE EFFECT OF PULP MILL EFFLUENTS ON THE MICROBIAL PROPERTIES OF ANAEROBIC GRANULES

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In anaerobic wastewater treatment, bacteria hydrolyze and degrade complex organics into simpler compounds, and ultimately methanogens produce CH₄ from these intermediates. A high concentration of biomass is required for efficient removal of organics and fast CH₄ production. In certain high rate anaerobic reactors, microorganisms form granular aggregates, which enhances settling of biomass. Degranulation can cause problematic washout of biomass, which reduces CH₄ production and results in high solid content in the treated wastewater and expensive reseeded of the reactor with sludge from off-site sources. An in-depth understanding of granulation and degranulation is essential to enhance treatment quality, maximize energy production and minimize cost. The overall objective of this project is to understand the cause of degranulation in a full scale high rate reactor in the pulp mill Tembec Temiscaming. It is hypothesized that components of the wastewater impact the microbial composition of granules, which alters the physical properties of granules, leading to degranulation. In the first stage of this study, the effect of specific in-mill effluents on the microbial composition of granules was investigated using sludge collected from Tembec. Clone libraries of the 16S rRNA genes revealed that the archaeal community consisted predominantly of three species: *Methanosaeta*, *Methanobacterium*, and *Methanomethylovorans*. Real-time quantitative PCR results showed that the relative abundance of *Methanomethylovorans* decreased significantly after the sludge treated Tembec in-mill effluents for three months. The bacterial clone library revealed 37 distinct OTUs. DGGE is being utilized to survey the changes in the bacterial community. The correlation of the physical and microbial properties of granules, reactor performance and wastewater compositions will be analyzed to understand the cause of the degranulation problem and to propose strategies to enhance the stability of granules.

Abstract Category
06 Engineered Environments

PS.06.126 CHARACTERISTICS AND DYNAMICS OF MICROBIAL COMMUNITY STRUCTURES IN A WASTEWATER TREATMENT PLANT RECEIVING MULTI-ANTIBIOTICS

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As the concentrations and types of pharmaceutical antibiotics present in wastewater treatment plant inevitably change the microbial community of activated sludge, the purpose of the present study was to investigate the characteristics and dynamics of microbial community structures in a wastewater treatment plant receiving high concentrations of streptomycin sulfate (2044.7-5866.1 ug/L), beta-lactam antibiotics including cephalosporin and penicillin G (3.18-23.13 ug/L in total), etc.. All of the antibiotics decreased significantly after the treatment with four biofilm reactors. The sludge sample was taken from each reactor, and the characteristics and dynamics of the microbial community structures were investigated using techniques including fluorescence in situ hybridization, denaturing gel gradient electrophoresis for bacteria, archaea and eumycota. Fluorescence in situ hybridization analyses showed that, in comparison with the first biofilm reactor, the ratios of archaea, eumycota and bacteria to total microbe counts (using DAPI staining) in the last biofilm reactor changed from 42 to 9%, 7 to 18%, and from 51 to 72%, respectively. Denaturing gel gradient electrophoresis was used to follow the population dynamics of bacteria, archaea and eumycota in the four biofilm reactors. No obvious change of archaeal profiles was observed from reactor 1 to reactor 4, while the diversity of bacterial community showed an increasing trend (Shannon index increase in series from 2.97 to 3.77, similarity indices value from 0.64 to 0.93). The eumycota community, on the other hand, was found to be more dynamic (similarity indices values from 0.41 to 0.86). In general, the above results showed that archaea were less influenced by the antibiotics than bacteria and eumycota. Although antibiotics are reported to be only poisonous to prokaryotic organisms like bacteria, they may influence the composition of eukaryotic organisms through some unknown mechanisms.

Abstract Category
06 Engineered Environments

**PS.06.127 MONITORING THE PHYSIOLOGICAL STATUS OF
GEOBACTER SPECIES BY QUANTIFYING KEY METABOLIC PROTEINS
WITH *GEOBACTER*-SPECIFIC ANTIBODIES**

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Quantifying environmental gene transcript abundance has proven to be an effective strategy for diagnosing the physiological state of microorganisms in natural environments and during engineered bioremediation. However, environmental transcript analysis can be technically challenging, which may limit its widespread application, and it does not account for the impact of translational modifications on protein abundance. An alternative strategy is direct quantification of key proteins whose abundance might be diagnostic of important physiological states. To evaluate this strategy, an antibody-dependent approach for quantifying protein abundance was developed, and initially

applied to the study of *in situ* bioremediation of uranium-contaminated groundwater at the Department of Energy study site in Rifle, Colorado, in which acetate is added to promote microbial reduction of U(VI). The phase of active U(VI) reduction, when *Geobacter* species predominate was investigated. Studies with chemostat-grown cultures of the *Geobacter bemidjensis* demonstrated correspondence between abundance of the TCA-cycle enzyme citrate synthase and rates of metabolism as well as high expression of the nitrogenase, NifD, when ammonium concentrations were limiting. The abundance of these proteins in the subsurface *Geobacter* community was quantified with *Geobacter*-specific antibodies. In proteins extracted from groundwater samples the abundance of *Geobacter* citrate synthase was correlated with the concentration of acetate, and the reduction of U(VI). NifD abundance increased when ammonium concentrations dropped below detection limits. These results suggest that the physiological status of the subsurface *Geobacter* community can be monitored by quantifying key metabolic proteins with *Geobacter*-specific antibodies. This antibody-based approach may be useful for diagnosing the physiological status of other subsurface microbial communities.

Abstract Category

06 Engineered Environments

**PS.07.001 ENZYME ASSAY FOR OXIDO-REDUCTASE ACTIVITY
MEASUREMENT IN SOIL AND PLANT RESIDUE SAMPLES**

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Phenol oxidases (laccase) and peroxidases (Lignin and manganese peroxidase) belonging to class oxido-reductase are the principal enzymes responsible for biodegradation of most recalcitrant compounds i.e. phenols via an oxidative process (Kandeler et al.1999). Although measurement of the enzymatic oxidation of phenols is an important measure of the soil microflora's capacity to degrade potentially recalcitrant organics, the proposed methods to perform this measurement are few and sometime questionable.

In this context, our study is focused on 1) comparing different methods of oxido-redutase activity measurement by using their relevant substrates and 2) to evaluate the effect of certain abiotic factors (temperature, pH) on enzyme activities measured by these methods which will enable us to find optimum conditions for oxido-redutase activity measurement in soil and plant residue along decomposition.

Activity of phenol oxidase and peroxidases were measured on soil, maize residue and incubated soil mixed with maize residue. Within this contrasted media, enzyme activities were determined by taking in account several factors including substrate concentration, temperature, pH and time of enzyme-substrate incubation. The kinetic parameters V_{max} and K_m (Michaelis and Menten, 1913) were studied regarding different substrates of Phenol oxidase and peroxidases. Substrates under these studies are Syringaldazine, 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), L-3,4-dihydroxyphenylalanine (L-DOPA, 3,3,5,5-tetramethylbenzidine (TMB), and pyrogallol. The results will be discussed in relation to soil ecological and enzymatic functions relative to biodegradation in soil.

Abstract Category

07 Recalcitrant Compound Degradation

**PS.07.002 LINKING TRFLP TO QPCR TO IMPROVE QUANTIFICATION OF
POPULATIONS IDENTIFIED BY STABLE ISOTOPE PROBING**

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Stable isotope probing (SIP) is a powerful tool for linking function to specific microbial populations. This technique is of particular interest when examining the degradation of nitrogenous compounds, since radioactive isotopes of nitrogen are not available. However, the buoyant density increase in ^{15}N -labeled DNA is much lower than the increase achieved with ^{13}C -DNA. This makes the complete isolation of the ^{15}N -labeled DNA in SIP gradients, especially for lower G+C microbes, very difficult even when using base-pair selective intercalating dyes and isopycnic separation on density gradients. Therefore, reliably identifying shifts in DNA content across density gradients due to ^{15}N incorporation is crucial. Standard tRFLP (terminal restriction fragment length polymorphism) profiles provide a measure of microbial diversity and can reliably detect large-scale changes in populations, however quantitatively they are limited. To improve the sensitivity, reproducibility and quantification of

tRFLP analyses, the number of initial 16S rRNA gene copies contributing to each peak in the community profiles was determined using real-time qPCR with an intercalating dye and terminating amplification in exponential phase. Accuracy and reproducibility were evaluated by amplifying mixed genomic DNA, cloned 16S rRNA gene standards and by spiking soil DNA with genomic DNA. Effects of initial DNA concentration and extent of amplification beyond the threshold cycle (CT) on quantification were evaluated. Peak heights and areas correlated well with DNA added (R² 0.8 to 1) when amplification was terminated at an appropriate stage in exponential phase. The optimized method was then used in an SIP analysis to identify and quantify populations actively degrading ¹⁵N-compounds.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.003 THE ISOLATION OF CELLULOLYTIC BACTERIA FROM SOILS UNDER HARSH ENVIRONMENTAL CONDITIONS

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The interest in using the cellulose as a resource for the generation of other type of molecules indicated a need in improving the industrial processes and enzymatic activity for the cellulose degradation. Then, exploiting extreme environments as a source for new cellulolytic enzymes residing in adapted microbes constitutes an interesting attempt. Here it is presented the use of environmental-mimicking enrichments in order to recover adapted bacteria from three distinct environments (Antarctic soil, Brazilian caatinga soil and mangrove soil) with the ability to use cellulose as a carbon source. Enrichments were made in flasks containing liquid minimal medium or common used TSB medium, supplemented with cellulose or glucose. Enrichments were made for 24 days under shaking and for each environmental sample the conditions were variable; 4°C for Antarctic soil sample, 60°C for the Brazilian caatinga soil sample, and at 28°C but with media amended with 5% NaCl for mangrove soil. After the enrichments, bacterial isolates were obtained by plating, and characterized for the presence of endo and exoglucanases. A higher occurrence of isolates with the cellulolytic activity was observed in samples from the Brazilian caatinga, followed by the Antarctic soil and then the mangrove soil. Most of the active isolates revealed to produce endoglucanases (153 out of 352) and only 42 were able to produce exoglucanases. In addition, the genome fingerprinting of the isolates by BOX-PCR has indicated the efficiency of the enrichments in selecting distinct bacterial groups from treatments. Furthermore, a trend was observed for the selection of more bacterial groups in enrichments with cellulose than in cultivations with glucose, possibly indicating the need of bacterial consortia to degrade cellulose. These active isolates represent an important collection to be used in future biotechnological program of cellulose processing.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.004 EXPRESSION OF GENES INVOLVED IN TCE DECHLORINATION IN BATCH EXPERIMENTS WITH CLAYEY TILL SEDIMENTS

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Chlorinated solvents such as trichlorethene (TCE) are a major threat against drinking water and fresh water environments many places around the world. Intensive research has been performed the recent years in order to utilize the abilities of microbes such as *Dehalococcoides* sp. to remediated contaminated aquifers on-site. Because *Dehalococcoides* sp. are anaerobic bacteria which are very difficult to culture in a laboratory, they are typically detected and monitored using molecular tools. In the vast majority of cases where microbes have been used for TCE remediation purposes the characteristics of the subsurface environment has been relatively sandy. In this project we did batch experiments with a slurry mixture of clay till sediment, water, three different concentrations of TCE, and with and without the commercially available dechlorinating bacterial culture KB-1. One of the obstacles when doing bioremediation in clayey till sediments with very low biomass is that it is often very difficult to extract nucleic acids from. The aim of the present study was to develop a suitable extraction procedure for DNA and mRNA from clayey sediments, and to apply this in order to investigate microbial dynamics based on mRNA as well as DNA. We did succeed to develop a suitable nucleic acid extraction protocol for these difficult sediments with an increase in extraction yields of >1000-fold. Using this extraction protocol we obtained high resolution expression profiles of the functional genes *vcrA*, *bvcA*, and *tceA* all reported to be involved in TCE dechlorination and found a good correlation between expression of *vcrA* genes and cis-DCE/VC dechlorination in the KB-1 amended batches, while in the non-amended batches we observed expression of both *vcrA* and *bvcA* as a result of TCE dechlorination.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.005 BENZENE-DERIVED CARBON FLUXES REVEALED BY COMBINING STABLE ISOTOPE PROBING OF RNA, FATTY ACIDS AND PROTEINS AND ISOTOPE FRACTIONATION ANALYSIS

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Benzene is a common environmental contaminant which poses a risk to human health. A microbial benzene degrading community was characterized and the benzene-derived carbon flux through a food chain was traced. Coconut fiber colonized by a benzene degrading biofilm was sampled in an aerated groundwater pond system and incubated with ¹²C or ¹³C-benzene in a laboratory scale for 2 days and 8 days. After 8 days, Chironomid larvae were added to the

system. The microbial diversity was analyzed by a clone library of 16S rDNA and metaproteomics. Microbial species involved in benzene degradation were identified by stable isotope probing (SIP) of RNA and T-RFLP analysis. Protein-SIP combined with isotope fractionation analysis offered functional information. The carbon-flux from bacteria to eukaryotes was elucidated by fatty acid-SIP. RNA-SIP and T-RFLP analysis showed the involvement of several species on benzene degradation mainly belonging to Betaproteobacteria similar to *Zoogloea* EMB62, *Zoogloea ramigera*, *Dechloromonas aromatica* RCB, *Ferribacterium limneticum* strain cda-1, *Comamonadaceae* bacterium, *Hydrogenophaga* sp. and *Thauera* sp. Protein-SIP and the observed isotope enrichment factors suggest a dihydroxylation pathway with m-cleavage by catechol 2,3-dioxygenase. Different microbial proteins showed a high ¹³C-incorporation (up to 95 atom%) and the phylogenetic affiliation of these proteins agrees with data obtained from RNA-SIP approach. SIP of fatty acids demonstrated a carbon flux from bacteria to the fatty acids 20:4 ω 6 and 20:5 ω 3 indicative for protists. Fatty acid extracted from larvae also showed ¹³C-incorporation. Combining RNA-, protein- and fatty acid SIP techniques with isotope fractionation analysis allowed identification of microbial key players and degradation pathways involved in benzene degradation as well as elucidation of benzene-derived carbon flux from bacteria to eukaryotes.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.006 SHORT-TERM SPATIAL AND TEMPORAL DYNAMICS OF PHYSICAL-CHEMICAL AND MICROBIAL PATTERNS IN A TAR OIL CONTAMINATED AQUIFER

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The introduction of organic contaminants such as aromatic hydrocarbons into a porous aquifer leads to the formation of a plume with a characteristic redox zonation along its horizontal and vertical axes. Recently, the identification of steep small-scale physical-chemical and microbial gradients by high-resolution multi-level sampling of groundwater and sediment allowed the precise identification and localization of biodegradation processes at a tar oil contaminated site in Duesseldorf. Sulfate reduction was found to be the dominant redox process coupled to BTEX and PAH oxidation. Results from five years of repeated sampling uncovered pronounced spatial and temporal dynamics of physical-chemical and microbial gradients, partly non-correlated to hydraulic conditions, i.e. groundwater table fluctuations. The consequences of hydrogeochemical dynamics for biodegradation processes and associated microorganisms are poorly understood. Such dynamics can be hypothesized to either enhance (via increased mixing) or hamper (by forcing bad conditions on established sessile degraders) the net contaminants removal. Hence, one aspect is to elucidate how attached microorganisms cope with changing redox conditions and how this is related to the overall natural attenuation potential. Recently three short-term sampling campaigns have been conducted to study the timescale of dynamics. We could observe significant shifts for the concentrations of e.g. toluene, sulfate and sulfide along a vertical plume cross section within less than 2 weeks. In contrary, microbial patterns, i.e. total cell numbers and active biomass exhibited a delayed dynamic more in the range of month. It was often been speculated that dynamics increase a site's natural attenuation potential. However, our recent

findings propose that short-term hydrogeochemical dynamics interfere with effective overall biodegradation; reaction time of degraders in aquifers seems to lack behind the physical-chemical changes.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.007 CHARACTERIZATION AND BIODEGRADATION OF THE VISCOSITY COMPONENTS OF HEAVY OIL

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Background: Heavy oil is formed due to hydrocarbon biodegradation along with other physical and chemical processes. Collectively, these mechanisms increase viscosity, decrease oil quality and complicate recovery efforts. While several microbial enhanced oil recovery processes have been investigated, biological approaches to decrease oil viscosity are rare. We investigated some of compounds in oil specifically associated with viscosity to determine if microbes might eventually be used to biodegrade them and enhance recovery operations. Methods: The viscosities of four model crude oils were compared before and after extraction with hexane and dilute alkali. The alkali-extracted material was characterized by ¹H-NMR and GC-MS. The viscosity-altering compounds were tested for their susceptibility to anaerobic decay with several enrichment cultures. Results: Viscosity reductions were observed following the removal of the asphaltene fraction in all oils, but only some were impacted by the dilute alkali treatment. The alkali extracts were composed of naphthenic acids with carbon numbers ranging from C5-C18 and mostly zero to two ringed constituents. Addition of these components back to extracted oil restored the viscosity characteristics. Asphaltenes were more amenable to anaerobic decay than the naphthenic acid constituents. Conclusions: Naphthenic acids are postulated as products formed via the anaerobic oil biodegradation processes. We found that these components can significantly contribute to the viscosity of oil. When compared with several inocula, asphaltic components proved more amenable to anaerobic decay than the naphthenic acid fraction.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.008 IDENTIFICATION OF GENES INVOLVED IN THE CATABOLISM OF THE PHENYL UREA HERBICIDE LINURON IN *VARIOVORAX* SP.

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Phenylurea herbicides such as linuron are commonly used for pre- and post-emergence control of annual grasses and broad-leaved weeds and are frequently detected in groundwater and surface water. Enrichment studies suggest that members of the genus *Variovorax* play an important role in the *in situ* degradation of linuron in agricultural soils. Linuron is degraded by direct hydrolysis

of the amide bond to the metabolites 3,4-dichloroaniline and *N,O*-dimethylhydroxylamine which are further mineralized. Linuron degrading *Variovorax* are responsible for the hydrolysis step and/or further mineralization of 3,4-dichloroaniline. Although the biochemical pathway for linuron mineralization has been, at least partially, established, no information exists on the genetics involved. A fosmid bank of genomic DNA of the linuron/3,4-dichloroaniline mineralising strain *Variovorax* sp. SRS16 was constructed and fosmid clones were selected which contained analogues of the *tdnQ* gene involved in aniline degradation in aniline degrading *Betaproteobacteria*. In addition, complete sequencing of the genome of *Variovorax* sp. SRS16 was performed. This way, a tandem gene cluster consisting of a gene cluster showing significant resemblance with a multicomponent aniline dioxygenase gene cluster and a gene cluster showing significant resemblance with the chlorocatechol degradation cluster involved in 2,4-dichlorophenoxy acetic acid degradation, was identified. We suspect this tandem gene cluster to be responsible for degradation of 3,4-dichloroaniline. Located in front of this cluster, a gene showing significant resemblance with a metal-dependent hydrolase was found, which could be responsible for linuron hydrolysis. Gene expression studies are being performed to confirm the involvement of these gene clusters in linuron degradation in *Variovorax* sp. SRS16.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.009 *IN SITU* BEHAVIOR OF *VARIOVORAX* SP. IN AGRICULTURAL SOILS TREATED WITH THE PHENYLUREA HERBICIDE LINURON

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Different research groups have isolated linuron degrading *Variovorax* strains from diverse European agricultural soils and the genus has been considered as playing a crucial role in linuron degradation in the environment. However, nothing is known about the *in situ* behavior of *Variovorax* in linuron treated soils. The availability of tools to monitor their community size and diversity dynamics is crucial for fully understanding the role of *Variovorax*. Primers targeting specifically the *Variovorax* 16SrRNA gene were designed and used to optimize a PCR-DGGE and a real-time PCR protocol targeting the *Variovorax* diversity and community size, respectively. An agricultural soil with a history of linuron treatment was incubated in soil microcosms, treated with either a single supply of linuron, a discontinuously supply of linuron or no linuron supply. 0, 1, 5, 9, 14, 21 and 70 days after the start of the experiment, soil samples were taken to conduct ¹⁴C-linuron mineralization assays and to extract DNA for *Variovorax* specific real-time PCR and PCR-DGGE analysis. Exposure to linuron resulted in a significant decrease in mineralisation lag time and the dominance in the *Variovorax* community by a phylotype previously associated with linuron degradation through isolation. At day 70, the single treated soil showed a shift back to the initial conditions. Only as a response to discontinuous linuron supply a significant increase in number of *Variovorax* was observed that became visible after 70 days of incubation. These results support the hypothesis that the genus *Variovorax* is involved in *in situ* linuron degradation in linuron treated soils.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.010 LIGHTNING EFFECT ON SOIL MICROBIAL BIOREMEDIATION

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Soils bacteria have a huge metabolic potential for soil bioremediation. Unfortunately, they do not always possess the right metabolic capability for the target pollutant, which might have arrived too recently in the ecosystem for bacterial adaptation. In this research, we used lightning-assisted genetic transformation with the aim of increasing the soil bioremediation potential of indigenous soil bacteria. This approach was studied at the laboratory-scale with artificial lightning generators. A high voltage generator was used to deliver an electric charge of between 4-7 kV to soil after having added plasmids (pBLN) containing a lindane-degrading gene (linA). After the sample was shocked, it was emptied into a UV-sterilized plastic jar. Three mg of lindane was added to each of the jars of soil that were then incubated at 29°C for 14 days with periodic shaking and air intake. The samples were analyzed for lindane degradation by a gas chromatograph coupled with mass spectrophotometer. Soil DNA was extracted to test for the presence and expression rate of the dehalogenase coding gene linA via real-time polymerase chain reactions (qPCR) of both the DNA and the mRNA. These results showed the feasibility of lindane-degradation gene electrotransformation by soil bacteria and the rapid lag time of its expression after transformation. Our results showed that the use of in situ electrotransformation was able to improve pollutant degradation rates by increasing degradation gene copy numbers. After both electrotransformation and electroporation, the bacteria displayed an increase in lindane degradation as measured by increases in chloride concentration and gene copy number, and decrease in lindane concentration. This study confirms that lightning might help non-natural competent bacteria to transform exogenous DNA and illustrates the possibility of lightning assisted bioremediation.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.011 INTERACTIONS BETWEEN THE BACTERIAL COMMUNITY DIVERSITY AND DIFFUSE POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) CONTAMINATIONS IN AGRICULTURAL SOILS

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In France, atmospheric inputs are the main source of PAH contamination in soil. This is a chronic and diffuse contamination, characterized by large area extents, relatively low contaminant concentrations and the lack of identifiable point sources. The main objective of our study was to better understand the interactions between the microbial diversity and diffuse PAH contaminations in agricultural soils. Microcosms of agricultural soils from Yvetot (France) were enriched with PAHs following three conditions: (i) microcosms enriched with phenanthrene, (ii) microcosms enriched with pyrene and (iii) microcosms enriched with a mixture of 8 PAHs (phenanthrene, pyrene and the six PAHs listed by the World Health Organisation). For each microcosm, two concentrations of PAHs were used: 30 and 300 mg.kg⁻¹. Controls without PAH were also established to confirm that differences in the microbial community were caused mainly by the presence of hydrocarbons. Triplicated microcosms were analysed after 8, 30 and 60 days.

PAHs degradation were determined by Gas Chromatography-Mass Spectrophotometry (GC-MS).

The bacterial community diversity was characterized by molecular fingerprint (DGGE) and real-time PCR on the 16S rRNA gene. This global and low-resolution approach was combined with a specific study on the *Pseudomonas* bacterial genus by using the *oprF* gene (Bodilis et al., 2006). The *Pseudomonas* are thus mainly present among cultivable bacteria in the rhizosphere, and are often described for their exceptional ability to degrade xenobiotics (including PAHs) in all compartments of the environment, i.e. soil, sediment and water (Niepceron et al., 2010). We discuss the usefulness of a combined approach, by using global studies and studies of a specific bacterial population, both with molecular and culturable methods.

Bodilis et al., 2006. *Environmental Microbiology*. Vol. 8 (1544-1551)

Niepceron et al., 2010. *FEMS Microbiology Ecology*. Vol. 71 (137-147)

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.012 ISOLATION OF BACTERIA FROM A WASTEWATER TREATMENT PLANT AND THEIR ABILITY TO DEGRADE CELLULOSE

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Cellulose degradation is of high biotechnological importance because the resulting sugars can be used for biofuel production. The process can be carried out by bacteria as well as fungi. We used the diffusion chamber approach to isolate bacteria from the aerobic sludge of the wastewater treatment plant in Hamilton Ohio, which treats a combination of municipal and paper mill wastewater. The diffusion chamber is an in situ incubation device that allows samples to be incubated in their original environment before isolating bacteria into pure culture. We obtained 130 isolates that belonged to 7 different phyla. Most of the bacteria (113) were isolated after one or multiple transfers through the diffusion chamber. Molecular analysis using Denaturing Gradient Gel Electrophoresis (DGGE) of the diffusion chamber- vs. pour plate-derived material showed population shifts between the different diffusion chamber generations as well as between the diffusion chambers and the corresponding pour plates. Most of the isolates were tested for cellulose degradation using the Congo red method. In summary 45 out of the 126 tested isolates were able to degrade cellulose. Interestingly more than half of the isolated Gamma-Proteobacteria and Bacteroidetes degraded cellulose whereas only a small fraction of the Alpha- and Beta-Proteobacteria did. In the final experiment cellulose degraders and non-degraders were grown in pure and mixed cultures in media with and without cellulose. Most bacteria grew better in consortia than in pure cultures, however the growth yield of many consortia decreased in the presence of cellulose. Only a small number of consortia exhibited higher growth yield in the cellulose containing medium. These results indicate that the presence of non-cellulose degrading bacteria could have a stabilizing effect on the bacterial cellulose degradation. This observation will be further investigated on selected consortia.

Abstract Category
07 Recalcitrant Compound Degradation

PS.07.013 INFLUENCE OF ENVIRONMENTAL STRESSES ON THE PHENANTHRENE-DEGRADING ACTIVITY AND COMPOSITION OF SOIL BACTERIAL COMMUNITIES

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Polycyclic aromatic hydrocarbons (PAHs) such as phenanthrene are environmental pollutants of both natural and anthropogenic origin. Although PAH biodegradation is well studied, knowledge on the effects of natural variations of soil conditions on the composition and catalytic activity of PAH-degrading communities is scarce. The goal of this study is to determine the influence of abiotic stress factors on the phenanthrene-mineralizing potential and composition of the endogenous bacterial soil community, with a special focus on the diversity and abundance of Sphingomonads. A historically PAH-contaminated soil and a pristine soil freshly spiked with phenanthrene were subjected to dry/wet cycles. Mineralization of phenanthrene was determined at different time points by means of ¹⁴C-labeled phenanthrene assays. The composition of the bacterial community, as well as the diversity of *Sphingomonas* spp. and *Sphingomonas*-specific PAH-dioxygenases, was monitored using 16S rDNA PCR-DGGE. In addition, the abundance of PAH-dioxygenase gene transcripts was determined with qPCR. Mineralization data showed that the freshly spiked soil quickly developed phenanthrene degradation potential. For both soils, drying to 10 % of their water holding capacity only had a limited effect on the phenanthrene-mineralizing activity of the community. The composition of the bacterial and the Sphingomonad community changed over time but finally no differences in community structure were observed between the different moisture contents. Our results indicate that the phenanthrene-mineralizing activity of the endogenous bacterial community is maintained even after exposure to severe drought. In addition, differences in soil moisture content did not result in different bacterial communities. The results of the qPCR analysis and of ongoing experiments dealing with the influence of freeze/thaw cycles will be presented.

Abstract Category
07 Recalcitrant Compound Degradation

PS.07.014 ASSESSMENT OF BACTERIA POTENTIALLY ASSOCIATED TO BIODEGRADATION OF AROMATIC HYDROCARBONS IN AMAZONIAN DARK EARTH

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Soil ecosystems harbor an immense diversity of bacteria which to a large extent uphold a pool of genes responsible for soil functioning. In this context, Brazilian Amazonian soils rise as a rich habitat for a huge and, until now, no accessed diversity of bacterial genes. The aim of this study was to investigate the potentially *Bacteria* associated to biodegradation of aromatic hydrocarbon in Amazonian Dark Earth (ADE) soil from "Caldeirão Experimental Station" (Iranduba, Manaus-

AM). A specific culture-independent approach was conducted to assess the diversity of genes encoding for the catalytic subunit of aromatic dioxygenases (*bphA*), responsible by the degradation of aromatic pollutants. The *bphA* gene library was constructed yielding a total of 100 clones. Twenty-eight Operational Taxonomic Units (OTUs) (cut-off 0.05) were observed, and one representative sequence per OTU was considered for the phylogenetic analyses. *Jackknife*, *Chao1* and *ACE* richness were determined, as well as *Shannon-Weaver* and *Simpson* diversity indices, which were 3.02 and 0.05, respectively. Furthermore, the 'plateau' was reached for the rarefaction curve. Among a total of a hundred isolates retrieved from soil, twenty unique bacterial isolates determined by 16S rRNA gene sequencing were analysed by a screening related to their biodegradation potential, through a bioassay using a chemical compound (2,6-dichlorophenolindophenol) as redox dye, and eighteen showed degradation activity. Overall, 95%, 78% and 95%, were able to degrade naphthalene, phenanthrene and biphenyl, respectively, compared to the dye decoloration presented in four DSMZ strains used as patterns, showing that most of cultivable bacteria had biodegradation potential. This study contributes to a better understanding of *bphA* gene diversity in ADE soils, and also instigate for further gene bioprospection studies from *Bacteria* capable to degrade aromatic hydrocarbons. Support: FAPESP, CNPq

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.015 IDENTIFICATION OF BOREAL FOREST LIGNOCELLULOSE DEGRADING MICROBES USING STABLE ISOTOPE PROBING

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Boreal ecosystems contain one third of the world's soil organic matter (SOM), a large portion of which is plant-derived lignocellulose. The decomposition of lignocellulose and resulting CO₂ efflux is mediated by microbes, yet the identity, function, and environmental controls of boreal soil microbes are not well understood. Mechanisms of the breakdown of lignocellulose and its constituents are also unknown, but may take place through synergistic interactions between microbes targeting different portions of the molecule. Stable isotope probing (SIP), allows identification of microbes and genes involved in degrading substrates including lignocellulose and its constituents. The aim of this study is to identify degraders of lignocellulose in boreal forest soils and to unravel the synergistic interactions of microbes degrading it.

Soil samples were collected from a mixed white spruce and paper birch stand at Bonanza Creek Long Term Ecological Research station (BNZ-LTER). Microcosms with homogenized soil samples were incubated with ¹³C labeled lignocellulose or one of two of its constituents, cellulose or vanillin. Labeled DNA was separated, cloned, and sequenced to identify bacteria and fungi utilizing carbon from these substrates.

Preliminary results indicate that fungi dominate the degradation of lignocellulose and cellulose, while bacteria dominate vanillin utilization. Preliminary sequence analysis places one fungal lignocellulose utilizer in the order Sebaciales.

Lignocellulose appears to be decomposed synergistically by a consortium of fungi and bacteria in soil. Future work includes SIP using soils from sites subjected to long term climate

manipulation treatments. This ongoing study will provide insights into potential feedbacks to climate change by identifying microbes capable of releasing CO₂ to the atmosphere by decomposing SOM. Identification of these microbes may be applicable to cellulosic biofuel production.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.016 INVOLVEMENT OF BACTERIAL COMMUNITIES IN PAHS BIODEGRADATION AND TOXICITY ELIMINATION IN A CHRONICALLY CONTAMINATED SLUDGE DURING ANOXIC/OXIC SWITCHES

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Oxic/anoxic switches are commonly found in nature as in water/sediment interfaces or in bioturbated sediments. This fluctuating conditions influence the bacterial dynamic and its role on organic compounds degradation as hydrocarbons. Little attention has been dedicated to this phenomenon and therefore the knowledge on how the oxic/anoxic switches may affect the fate of hydrocarbons, their associated toxicity and the native bacterial communities in chronically contaminated environments is still scarce.

The aim of this study was to correlate the bacterial communities dynamics with hydrocarbons removal and toxicity changes in a chronically contaminated sludge during oxic/anoxic switches. These switches conditions (5 days of anoxia/3 days of oxa during 5 consecutive cycles) were compared to a permanent oxic and a permanent anoxic conditions during 42 days of incubation, with bioreactors systems.

Dynamic of bacterial communities was assessed by T-RFLP based on the 16S rRNA and on the RNA of the functional ring hydroxylating dioxygenase (RHD). Under switching and permanent oxic conditions, a first modification of the bacterial community structures was observed due to the oxygen addition. Then, further modifications characterized by a succession of bacterial populations were identified correlated to a sequential utilization of substrates. Interestingly, RHD transcripts were found all along the time irrespective of the condition. At the end of the incubation period, the percentages of PAH removal were similar for all conditions. However, under switches conditions, after an aeration of one day following the anoxic period the percentages of PAH-removal were similar to that observed under permanent oxic condition after 1 day of incubation. Nevertheless, elimination of toxicity was higher under permanent oxic condition probably due to a better elimination of PAH metabolites or other toxic compounds present in the sludge.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.017 LIGNINOLYTIC FUNGI AS EFFICIENT DEGRADERS OF POLYCHLORINATED BIPHENYLS AND THEIR BREAKDOWN PRODUCTS

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Polychlorinated biphenyls (PCB) and their toxic degradation products still represent a serious risk for the environment. There are a lot of studies dealing with various biodegradation techniques, including mainly bacterial transformation. However, PCB transport into the bacterial cells represents a limiting factor for their degradation and the biphenyl upper pathway leads to chlorobenzoic acid (CBA) production as dead-end metabolites. On the other hand, ligninolytic fungi (LF) with their extracellular low-substrate-specificity enzymes represent a perspective alternative for aromatic pollutants biodegradation. The aim of this work was to screen for PCB degradation abilities among 8 selected LF strains in liquid media and consequently in a real contaminated industrial soil. The best results were obtained in low nitrogen-mineral liquid medium, where *Pleurotus ostreatus* 3004 degraded more than 90% of the initial amount of PCB (400 µg of Delor 103 per 20 ml of media) in 42 days and *Irpex lacteus* removed more than 75% of PCBs. The soil degradation experiment revealed that out of the most toxic PCB congeners, *Pleurotus ostreatus* and *Irpex lacteus* were able to remove 51% and 34% of PCBs, respectively. The process was also monitored in a view of ecotoxicity changes and changes in microbial populations using analysis of specific markers (ergosterol, phospholipids fatty-acid analysis). An attention was also paid to identification of PCB metabolites produced during the degradation. These results showed that the fungi were able to hydroxylate and methoxylate PCBs and further to perform a ring fission of the original compounds resulting in production of CBAs. Therefore, another set of degradation experiments in liquid media and soil was performed with CBAs as substrates. The results showed that LF were very efficient in degradation of these compounds, as well.

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Abstract Category

07 Recalcitrant Compound Degradation

PS.07.018 ROOT EXUDATES MODIFY THE DIVERSITY OF ACTIVE PHENANTHRENE-DEGRADERS IN AN AGED PAH-CONTAMINATED SOIL AS REVEALED USING STABLE ISOTOPE PROBING

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Polycyclic aromatic hydrocarbon (PAH) are ubiquitous and persistent contaminants in industrial wasteland soils. Dissipation of PAHs in plant rhizosphere can be significantly improved in

comparison to unplanted soil, although divergent results were reported in the literature, i.e. no or inhibitory effect of the rhizosphere. To better control phytoremediation processes it is thus a challenge to precise the influence of root exudates on PAH bacterial degrader activity and diversity in soils. The aim of our study was to determine whether the diversity of phenanthrene-degrading bacteria in an aged PAH-contaminated soil is affected by the addition of plant root exudates. DNA isotope probing (SIP) was carried out with microcosms of soil with and without addition of natural ryegrass exudates and with ^{13}C -labelled phenanthrene (PHE). PHE degradation was slightly delayed with exudate addition after 4 days of incubation, while after 12 days the rate of PHE-degradation reached 68% with and without exudates. Temporal thermal gradient gel electrophoresis (TTGE) analyses of 16S rRNA genes were performed on recovered ^{13}C -enriched DNA fractions. One major finding was that root exudates modified the PHE-degrading bacterial diversity. With PHE as a unique fresh carbon source, *Pseudoxanthomonas* and *Microbacterium* were the major PHE degraders, while in the presence of exudates, *Pseudomonas* and *Arthrobacter* were favoured. These two different PHE-degrading bacterial populations were also distinguished through functional PAH-ring hydroxylating dioxygenase (PAH-RHD α) gene analyses. We also identified bacteria which were activated by the carbon supply through the exudates and found that *Pseudomonas stutzeri* and *Pseudomonas putida* strains containing PAH-RHD α genes were favoured only in microcosms with exudates.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.019 ENHANCED ANAEROBIC REDUCTIVE DECHLORINATION OF POLYCHLORINATED BIPHENYLS BY ORGANIC ENRICHMENT

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Even if the manufacture and industrial use of polychlorinated biphenyls (PCBs) have been banned for decades, PCB environmental contamination and in particular sediment contamination remains a concern. PCBs represent a family of 209 congeners, formed by a biphenyl backbone chlorinated at 1 to 10 positions. Bacterial communities can completely degrade PCBs by the cooperation of anaerobic and aerobic bacteria. The anaerobic reductive dechlorination is more effective with highly substituted PCBs. This can be enhanced by enrichment with organic substrates. We have studied one soil and two sediments historically polluted with PCBs at different concentrations. Soil and sediment samples were placed in anaerobic microcosms and enriched with three different concentrations of: molasse, lactate and soy bean oil. Microbial community structures and diversity were measured by Ribosomal Intergenic Spacer Analysis and phylogenetic microarrays. Some functional dehalogenase activity were measured by qPCR. In addition, a comparison of metaproteomic profiles was done. PCBs composition and total organic mass were measured and statistically correlated to chemical analysis during PCB biodegradation. The added organic substrate induced active anaerobic conditions which affected both the microbial community and the rate of PCB degradation.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.020 QUANTITATIVE PROTEOME ANALYSIS OF A *MYCOBACTERIUM VANBAALENII* TRANSPOSON MUTANT DEFECTIVE IN THE *NidA* RING-HYDROXYLATING OXYGENASE

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The Rieske non-heme iron aromatic ring-hydroxylating oxygenase (RHO) NidAB from *Mycobacterium vanbaalenii* PYR-1 has been proposed to be involved in the initial oxidation of pyrene, one of the polycyclic aromatic hydrocarbons (PAHs). Using proteomics approaches we investigated a *M. vanbaalenii* PYR-1 transposon mutant, T8F7, that is defective in the gene *nidA* to see how the metabolism of PAHs including pyrene is affected.

The mutant cells were grown in a media with the presence of 50 μ M of phenanthrene, pyrene, and fluoranthene. A control was grown with the absence of PAHs. Whole-cell proteome analysis of the mutant T8F7 was conducted by the combination of 1-dimensional gel electrophoresis and nano-liquid chromatography tandem mass spectrometry.

In the proteome analysis, a total of 2,390 proteins were identified with 1,345 proteins being abundant more than 2-fold. Among them 843 proteins were commonly more than 2-fold abundant in all three treatments. The majority of proteins were classified as involved in cell metabolism, which include those related to PAH degradation. Among 194 genes associated with aromatic hydrocarbon degradation, about 95 proteins, including 6 aromatic ring-hydroxylating oxygenases were expressed. The *nidA* gene product with the other downstream proteins was not detected. It indicated that prior study of the reduced pyrene transformation efficiency of this mutant strain T8F7 was most likely resulted from the absence of NidA protein. We compared the protein expression profile of T8F7 with that of wild-type strain PYR-1.

From the results of proteome analysis and the previous pyrene metabolism study, it is demonstrated that 1) *nidA* is mainly involved in pyrene degradation and 2) there is alternative catabolic proteins(s) which PYR-1 could rely on for the degradation of pyrene. These results demonstrate the functional redundancy and robustness of PAH metabolic pathways in PYR-1.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.021 BIODEGRADATION OF 4,4'-DIBROMODIPHENYL ETHER IN RIVER SEDIMENT

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Polybrominated diphenyl ethers (PBDEs) are a class of widely used flame retardants. These compounds have been detected in the river sediment and may influence the ecosystem. In this study, we investigated the biodegradation of 4,4'-dibromodiphenyl ether (BDE-15) and assessed the change of microbial community in river sediment from southern Taiwan. BDE-15

concentration was measured by GC-ECD. The microbial community change was clarified by denaturing gradient gel electrophoresis (DGGE) analysis of the PCR amplified 16S rDNA fragments. BDE-15 aerobic and anaerobic degradation remaining percentage of three sampling sites ranged from 7 to 16 % within 56 d incubation, and 20 to 99 % within 77 d incubations, respectively. Under aerobic conditions, BDE-15 degradation was enhanced by the addition of yeast extract, rhamnolipid, surfactin, and inhibited by addition of brij 30, brij 35. Under anaerobic conditions, BDE-15 degradation was enhanced by the addition of vitamine B12, and inhibited by addition of zero-valent iron, brij 30, brij 35, acetate, lactate, pyruvate. Of the microorganism strains isolated from the sediment, we found that strains H1, H2, H3, H4 and H5 expressed the best biodegrading ability. BDE-15 degradation was enhanced when the five strains were added individually to the sediment. However, BDE-15 degradation had no significant effect when the five strains were added simultaneously. Our results also show the addition of various substrates changed the microbial community in river sediment. These results could offer the feasibility for removal of PBDEs in river sediment by microbial degradation.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.022 CHARACTERIZATION OF A CHITINASE FROM AUTOLYSING MUSHROOM OF *COPRINELLUS CONGREGATUS*

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Unlike bacterial cells, fungal cell contains chitin as the cell wall component. Fungal cells must have chitinase to enlarge their cell walls during their growth phase. An inky cap, *Coprinellus congregatus*, generates mushrooms which become black ink droplets during their maturation. When the autolysed tissue and the liquid droplet were examined by the light and electron microscopy, the cell walls of basidia disintegrated and no cell wall was observed in the liquid. In order to get large amount of the chitinase which shows high degrading enzyme activity against fungal cell walls, chitinase expression system is required. We have isolated 2 chitinase cDNAs (*chi1* and *chi2*) from the autolysing mushroom tissues, and confirmed their expression at the mushroom-autolysing phase by the real time polymerase chain reaction. We have constructed the chitinase expression system using our new expression vector. The promoter used in this system was induced by external pH adjustment in *C. congregatus*, and Chi1 was produced from the genetically transformed cells. We have determined the biochemical characteristics of the chitinase. In conclusion, a chitinase from an autolysing mushroom was successfully expressed and characterized using new expression system.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.023 EXAMINING SULFUR UTILIZING MICROBIAL BIOREMEDIATION OF PERCHLORATE: BENCH-SCALE AND PILOT-PLANT SCALE

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Perchlorate (ClO_4^-) is a common water and soil contaminant, hazardous to human health if ingested and regulated in the US. A packed-bed, up-flow bioreactor system to reduce ClO_4^- to innocuous byproducts was established at bench- (200 ml and 1 L) and pilot- (200 L) scale. Packing material combined sulfur pellets, as sole electron donor, and crushed oyster shell, to maintain pH. ClO_4^- , the electron acceptor, was fed to bench reactors at 5 mg/L and the pilot reactor at 0.1 mg/L. The goal was to investigate the microbial community established and processes that occurred in the reactors. An enriched bacterial consortium that consistently reduced ClO_4^- to below detection limits was inoculated into the reactors. Function was measured by ClO_4^- disappearance and coupled with PCR and qPCR detection of the ClO_4^- reducing genes, *pcrA* and *clt*. The bacterial community completing the function was examined using molecular techniques. ClO_4^- reduction occurred with *pcrA* and *clt* detection and approximately 10^5 copies/ml of *pcrA* were detected. Throughout the reactor, in zones where ClO_4^- reduction did or did not occur, DGGE showed that the ClO_4^- reducing community remained diverse within each zone, yet all zones exhibited similar richness. Further bacterial community analyses from different reactor zones suggested other functions, sulfur-oxidation and sulfate-reduction, could occur throughout the zones regardless of the presence of ClO_4^- . The bacteria completing these functions varied in abundance depending on the level of ClO_4^- in the reactor. From a process approach, only function was significant. However, the microbiology of this system has not previously been studied and was of interest, particularly if function fails. The novel function of ClO_4^- reduction with sulfur-utilization appeared to be achieved by a consortium of microorganisms but ClO_4^- reducing bacteria needed to be present in certain numbers to maintain function at the low concentration of available ClO_4^- .

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.024 ANALYSIS OF PHENANTHRENE CATABOLIC PATHWAY IN *SPHINGOMONAS PAUCIMOBILIS* 20006FA USING A PROTEOMIC APPROACH

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In bioremediation applications, cellular physiology is an important issue which provides essential biological knowledge for the design and optimization of biodegradation processes. In order to study the phenanthrene catabolic pathway, the *Sphingomonas paucimobilis* 20006FA proteome was analyzed under two different growth substrate conditions, using glucose or phenanthrene as sole carbon source. Kinetics of growth, phenanthrene degradation, and 1-hydroxy-2-naphthoic acid production, a key metabolite of phenanthrene degradation, were monitored. Cells were harvested at the stationary phase (96 h). The protein analysis was performed by two dimensional gel electrophoresis and mass spectrometry (MALDI-TOF/TOF MS). At the harvest time, a 32 % of phenanthrene was biodegraded, with the concomitant accumulation of 147 ppm of 1-hydroxy-2-naphthoic acid. Soluble cellular protein fractions were analyzed using immobilized pH gradient (IPG) strips within the window of isoelectric point (pI) 4-7 and a molecular mass range of 6.5 to 200 kDa. Ten PAH-induced proteins were identified, including enzymes from the upper

catabolic pathway of phenanthrene (extradiol dioxygenase, benzene dioxygenase, dihydrodiol dehydrogenase, 2-hydroxy-benzylpyruvate aldolase); and from the lower catabolic pathway of phenanthrene, all belonging to the meta cleavage pathway, (catechol 2,3 dioxygenase, 4-oxalocrotonate decarboxylase, 2-hydroxymuconic semialdehyde hydrolase, 2-hydroxypent-2,4-dienoate hydratase, 4-hydroxy-2-oxovalerate aldolase). The glutathione S-transferase, a multifunctional enzyme involved in the cellular detoxification and excretion of a large variety of compounds, was also identified. This proteomic approach enabled the full description of the phenanthrene degradation pathway we have previously proposed by means of key intermediate metabolites identification.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.025 CHARACTERIZATION OF MICROBIAL CULTURES IN TWO IMMISCIBLE PHASES FOR EXTRACTION, CONCENTRATION AND BIODEGRADATION OF ENDOCRINE-DISRUPTING COMPOUNDS

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The endocrine-disrupting compounds (EDCs) can mimic or alter the action of endogenous hormones. They have been detected in sewage treatment effluents and surface waters, follow incomplete removal from sewage treatment facilities. Abnormalities linked to EDCs exposure has been observed in aquatic environment. Furthermore, they had been detected at lower levels, down to ng/L which contributes for the currently inability of water treatment facilities to eliminate them effectively from wastewater. In this study, we used two-portioning phase microbial system to concentrate and biodegrade EDCs. The portioning phases were silicone oil (liquid) and Hytrel (a solid polyester-polyether copolymer), where EDCs were dissolved or absorbed. These two-portioning phase systems were used to enrich microbial consortia adapted to grow on estrogens (estrone, estradiol, estriol, ethinylestradiol) and/or alkylphenols (bisphenol-A, nonylphenol) as the sole source of carbon. Genetics profiling by PCR-DGGE showed that each enrichment was composed of a distinct bacterial population. The degradation of estradiol, estriol and estrone was better with Hytrel (91% to 99%) than with silicone oil (67% to 95%). At the opposite, the degradation of alkylphenols and ethinylestradiol, was more pronounced in cultures with silicone oil (89% to 99% for alkylphenols and 25% to 40% for ethinylestradiol) than with Hytrel (35% to 99% for alkylphenols and 1% to 10% for ethinylestradiol). This is the first report in which a two-portioning phase system was used to concentrate EDCs in a water-immiscible phase to improve the biodegradation of EDCs. The process has showed to be effective and the introduction of an immiscible phase into a tertiary treatment in the sewage treatment facilities could open a promising pathway for its future instatement.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.026 COMPARISON BETWEEN TOTAL AND ACTIVE BACTERIAL COMMUNITIES IN SOIL CONTAMINATED BY CHLORINATED COMPOUNDS, USING PYROSEQUENCING AND MICROARRAYS.

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Over 100 tons of the solvent tetrachloroethylene (PCE) were released into the environment (air, soil, water) during the year 2004. Unfortunately, PCE is degraded to metabolites, which are more toxic than the PCE. In some cases, these metabolites are further degraded to relatively harmless products such as ethylene. This study focused on the bacterial community structure during PCE degradation in order to evaluate the relationship between the microbial community and the accumulation of these toxic metabolites. Multiple microcosms supplied with different organic substrates were artificially contaminated with PCE. A thymine analog, bromodeoxyuridine was added to the microcosms and incorporated into the DNA of replicating cells. We compared the total and the active bacterial communities at different times by using phylogenetic microarrays and pyrosequencing in order to identify microorganisms and functional genes associated with the complete PCE degradation to ethylene. In addition, the differential effects of the organic substrate addition on bacterial communities were analyzed by using phylogenetic microarray. This integrative approach highlighted both the key bioindicators and the ecological functions correlated with complete degradation of PCE. The correlation between the chemical analyses and the phylochip data identified several potential bacteria that can oxidize hydrogen, in addition to known PCE degraders. These results suggest that PCE degradation can be a competitive process in soil and that it can occur in competition with other processes at low redox potential.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.027 ROLE OF PH BUFFER ON TCE REDUCTION AND COMPOSITION OF DECHLORINATING CONSORTIA

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Biological reductive dechlorination of trichloroethene (TCE) is an attractive and successful method for TCE removal from contaminated field sites, however, its success and efficiency can be greatly influenced by pH buffering. DehaloR² is a novel mixed dechlorinating consortia developed for field applications; it is able to reduce TCE to ethene at fast rates using various fermentable and non-fermentable substrates as electron donors. The objective of our work was to test the effect of pH buffers and concentrations (NaHCO₃ or HEPES; 2.5-30 mM) on reductive dechlorination and microbial community structure. In NaHCO₃-buffered batch reactors fed with fermentable substrates, we noticed diminished dechlorination rates due to a pH decrease (down to 6.2), mainly caused by proton and CO₂-producing fermentation reactions. Removal of CO₂ from these reactors resulted in pH increases and faster dechlorination rates. As HEPES is not carbonate-contributing, the reactors containing this buffer did not exhibit a pH problem and completed dechlorination efficiently at a pH value of 7.1. For the purposes of reductive dechlorination, HEPES was a better buffer choice than NaHCO₃. When we fed hydrogen to the batch reactors as the sole electron donor, dechlorination rates decreased at low buffer

concentrations due to a pH drop caused by proton-producing dechlorination reactions. At high NaHCO₃ concentrations, TCE dechlorination stalled at vinyl chloride due to proton-consuming homoacetogenesis reactions that were driving the pH up to 8.3. HPLC data revealed little net acetate production at low NaHCO₃ concentrations and a doubling of acetate production at high NaHCO₃ concentrations. Additionally, molecular data supports an increase in the number of populations relevant for TCE dechlorination with increasing buffer concentrations. Our findings point out the potential need for pH buffering at contaminated sites in order to achieve fast and efficient chloroethene degradation.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.028 EFFECT OF DIFFERENT BIOSTIMULATION STRATEGIES ON BACTERIAL COMMUNITY STRUCTURE OF A CHRONICALLY HYDROCARBON-CONTAMINATED SOIL IN ANTARCTICA

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Biostimulation seems to be the best bioremediation strategy to enhance the natural biodegradation process in chronically contaminated soils from cold climate. Therefore, selection of the most efficient nutrients is an essential step. Also the presence of surface-active compounds has proved to enhance the removal of hydrocarbon. The aim of this study was to evaluate the effect of different nutrients sources, in the presence or absence of a surfactant Brij®700, on the bioremediation of chronically hydrocarbon-contaminated soils from Jubany Station, Antarctica. Inorganic salts (NSS) and fish meal (FM), alone and with Brij®700 (NSSB and FFB), were analyzed. Additionally, a commercial product (CP, mixture of enzymes, nutrients and surfactants) and an untreated control (CC) were evaluated. The systems were monitored during 45 days by counting heterotrophic (HB) and hydrocarbon-degrading bacteria (HDB) and measuring total hydrocarbons concentration (TH). Changes in the soil bacterial community structure were studied by PCR-DGGE. All evaluated nutrients sources, with or without B, increased HDB more than three orders of magnitude except CP, which only increased one order compared with CC. However, at the end of the assay, only CP showed a significant hydrocarbon removal (70%) compared with the CC (40%). DGGE profiles from FM and FMB showed that fish meal produced a shift in bacterial community structure compared with the others systems. CC, CP, NSS and NSSB stimulated similar communities. The CP bacterial community at the end of the assay showed a similarity of 80% compared with those observed in CC at the beginning of the assay. The results showed that the CP represents an attractive option because it exhibited the best degradation efficiency combined with the minimal changes in soil bacterial community. This fact suggests that CP would stimulate mainly the most efficient hydrocarbon-degrading bacterial present in the natural soil community.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.029 RESPONSE OF SOIL MICROBIAL COMMUNITY STRUCTURE TO POLYCYCLIC AROMATIC HYDROCARBON CONTAMINATION

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Polycyclic Aromatic Hydrocarbons (PAHs) are a class of hazardous organic chemicals that consist of at least two fused benzene rings in cluster, linear or angular arrangements. These compounds are of concern both from a public health and environmental perspective due to their toxicity and recalcitrance. PAHs are carcinogenic and are recognised as priority pollutants by the European Union and United States Environmental protection agency. The aim of this study was to examine the effect of PAHs on soil microbial community structure.

Soil from a wood treatment plant was amended with a 2-ring PAH (naphthalene), two 3-ring PAHs (fluorene and phenanthrene), two 4-ring PAHs (fluoranthene and pyrene) and a 5-ring PAH (benzo(*a*)pyrene) and incubated at 22 °C for 50 days. Microcosms were harvested over time. PAHs were extracted using a solvent-based extraction method and quantified by gas chromatography. The effect of PAHs on microbial community structure was assessed using both culture dependent and culture independent methods. Numbers of culturable bacteria were determined using a most probable number method and PAH degrading bacteria were enumerated on Bushnell-Haas medium amended with PAHs. Dehydrogenase activity was used as an indication of microbial activity in soil and molecular fingerprinting of bacterial communities was carried out by terminal restriction fragment length polymorphism.

Soil microbial communities readily removed 2, 3, and 4-ring PAHs, with rates of degradation decreasing with increasing number of rings present in the PAH. However, little degradation of the 5-ring PAH, benzo(*a*)pyrene was observed. All PAHs had an initial toxic effect on soil microbial activity but numbers of culturable bacteria and bacteria capable of growing on PAHs as the sole carbon source increased over time. Molecular fingerprinting indicated that PAH type had an effect on soil microbial community structure.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.030 INFLUENCE OF NICKEL CONTAMINATION ON THE BACTERIAL COMMUNITY AND THE DEGRADATION OF CRUDE OIL IN NATURAL SOILS

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The petrochemical industry is responsible for some of the major releases of organic and inorganic pollutants. Vanadium, arsenic, chrome and nickel are some of these inorganic pollutants, which shows long persistence in the environment leading to their accumulation. Hence, the aim of this study was to assess the influence of nickel contamination on the degradation of crude oil in natural soils and the effect of the co-contamination on the bacterial

community structure. The experimental set up was defined by Complete Factorial Analysis, using three parameters (oil, nickel and humidity). The response parameter used was total petroleum hydrocarbon (TPH) quantification by infrared spectrophotometry. The microbial community was monitored using plate count, and most probable number (MPN) oil degrader count and bacterial 16S rRNA gene polymerase chain reaction - denaturing gradient gel electrophoresis (PCR-DGGE). DGGE analysis have shown that the effect of contamination of over the microbial community was not significant since most of the bands showed no change over the course of the treatment. However, TPH quantification indicated that the presence of nickel has set the degradation of oil back during the first 15 days of the experiment. Besides, the level of humidity was the most influential parameter measured aiding the degradation even with high levels of nickel, while high levels of oil had a negative effect on the degradation. A similar pattern was observed for the MPN counts, however the counts were higher in soils with higher amounts of hydrocarbons. These results indicate that the presence of nickel have a negative effect on the degradation of oil although the humidity was the most influential factor analyzed.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.031 MICROBIAL REDUCTIVE DEHALOGENATION OF POLYCHLORINATED BIPHENYLS

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Our study is focused on biotic degradation of polychlorinated biphenyls (PCBs) under strictly anaerobic conditions. During the process well-known as reductive dehalogenation highly chlorinated PCBs are transformed to the less chlorinated forms. Reductive dehalogenation is the only known biological way of transformation nona as well as deka chlorinated biphenyls to the lower chlorinated forms.

Dehalogenating consortia were isolated under different conditions from sediment of Strazsky Sewer located in the Slovak Republic. The locality around Strazsky is long term highly contaminated dumpsite (from near by plant CHEMKO Strazske — manufacturer of PCBs) with high probability of dechlorinating microflora prevalence. Various potential acceptors and donors of electrons (Aroclor 1248 or 1260; Delor 103 or 106; acetate with hydrogen, lactate or pyruvate) were used for obtaining of sediment-free anaerobic consortia.

We tested the possible role of yeast extract in the growth medium for sustaining dechlorination activity in consortia originally primed with 4-4 dibromobiphenyl. No dechlorination was observed in batch cultivation treated with acetate plus hydrogen without yeast extract addition. However dechlorination was remarkable if yeasts extract was added. But in this case, the lag phase was extended to 400 days. When the acetate was replaced by pyruvate or lactate, dechlorination of Aroclor 1260 was faster.

We obtained a set of different consortia which were able to dechlorinate PCBs. Congener-specific gas chromatography revealed that flanked *meta*- as well as flanked and unflanked *para*-chlorines are dechlorinated by our consortia with different efficiency.

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Abstract Category

07 Recalcitrant Compound Degradation

PS.07.032 CHARACTERIZATION OF DECHLORINATING POPULATIONS IN THE WBC-2 CONSORTIUM

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Anaerobic degradation of chlorinated contaminants in the environment using bioremediation is gaining support within industry as a viable mechanism for remediation. This is primarily due to advances in molecular biology techniques, which allow unprecedented levels of characterization and control. In this study, the microbial composition of the WBC-2 microbial consortium earlier described, was investigated. WBC-2 dechlorinates 1,1,2,2-tetrachloroethane to the non-toxic end product ethene. A 16S gene clone library identified the dominant bacteria in WBC-2 as members of the genera *Dehalococcoides*, *Dehalobacter*, *Veillonaceae* and *Acetobacterium*. From this clone library, one clone out of 94 corresponded to a 16S rRNA sequence sharing 91% similarity to *Dehalococcoides*, and was dubbed Oddball chloroflexi. Approximately three years ago, the original WBC-2 consortium, maintained on 1,1,2,2-tetrachloroethane, 1,1,2-trichloroethane, and cis-dichloroethene, was used to inoculate three daughter bottles, each fed only one of the original substrates. Experiments done on these daughter cultures indicate the versatility of the WBC-2 microbial community. Substrate range experiments show that the enrichment culture fed 1,1,2,2-tetrachloroethane can degrade both cis and trans-dichloroethene with comparable rates. The WBC-2 microbial community has shifted under enrichment on different substrates. Notably, the Oddball chloroflexi is predominant in the bottles that have been exposed to 1,1,2,2-tetrachloroethane, indicating that this novel organism may play a role within the 1,1,2,2-tetrachloroethane degradation pathway. Further research to classify the reductive dehalogenase genes present in the four WBC-2 enrichment cultures is on going.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.033 IDENTIFICATION OF ACTIVE BACTERIAL & FUNGAL CELLULOLYTIC COMMUNITIES USING STABLE ISOTOPE PROBING & IN-DEPTH RDNA SEQUENCING ACROSS PINE FOREST SOILS

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Cellulose is a dominant form of carbon in plant litter feeding the soil microbial populations below ground. The relative contribution and identities of cellulolytic bacteria and fungi are poorly understood. To illuminate general patterns, we sought to compare active cellulolytic communities in pine forest soils obtained from 4 geographic regions with different soil properties. Soil microcosms were established by addition of ¹³C-UL-maize cellulose. Total

carbon dioxide concentrations in ^{13}C -maize cellulose amended and unamended treatments were measured over a period of ca. 30 days via gas chromatography and appeared to correlate with initial organic matter and nitrogen concentrations. The active microbial communities were identified using DNA-SIP combined with rDNA sequencing of the enriched and nonenriched fractions. A total of 1939 high quality, LSU fungal rRNA gene sequences and 3408 SSU bacterial rRNA gene sequences were generated from time zero, enriched fractions, and non-enriched fractions of the microcosms using Sanger sequencing. Previously unknown cellulolytic proteobacteria were consistently found in the enriched fractions across the pine soils. Quantitative PCR using group-specific primers targeting these groups confirmed that their biomass increased as a result of incubation with ^{13}C -maize cellulose. Known and previously unknown cellulolytic ascomycota were present in the enriched fractions. An in-depth assessment of these cellulolytic microbial communities was generated using pyrotag sequencing with adapted primers for the bacterial SSU 16S rRNA (V3/V4 region) and the fungal LSU 28S rRNA genes. Even though these soils differed in many physical and chemical properties, the active cellulolytic communities associated with pine species were similar across these soils based on β -diversity. This illustrates that the substrate is more important than soil chemistry (over the range we examined) in shaping the cellulose-degrading guild

Abstract Category

07 Recalcitrant Compound Degradation

**PS.07.034 ADVANCED METAGENOMIC INSIGHT OF A
HEXACHLOROCYCLOHEXANE-DECHLORINATING COCULTURE OF
DEHALOBACTER SP. AND *SEDIMENTIBACTER* SP.**

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The importance of organohalide-respiring *Dehalobacter* species in the bioremediation of chlorinated organics has been well recognized as is their limited range of metabolic modes. However, little is known about the full genomic repertoires of these dedicated degraders. Here we report detailed insight into the genome sequence of *Dehalobacter* sp. E1 that grows in strict coculture with *Sedimentibacter* sp. B4. The coculture metagenome and the genome of strain B4 were sequenced using 454 GS-FLX technology. Using the genome of strain B4 as a reference, all contigs belonging to the genome of strain E1 were filtered, binned separately and annotated. The resulting draft genome of strain E1 is approximately 2.6 Mbp in size and harbors 10 putative reductive dehalogenase (Rdh)-encoding gene clusters. Seven of these are most closely related to chlorophenol Rdh genes and 3 to tetrachloroethene Rdh genes, including the *pceABCT* operon in *D. restrictus*. This predicts that the dehalogenating potential of strain E1 is greater than previously anticipated. This was confirmed in degradation assays showing effective dechlorination of trichloroethene by the coculture, which was coupled to growth. Nearly all Rdh genes are located adjacent to genes encoding transcription regulators of the Crp/Fnr family or the MarR-type. Transcriptional analysis by RT-qPCR showed specific induction of several Rdh genes during growth on β -hexachlorocyclohexane.. Advanced genome annotation indicated that *Dehalobacter* sp. is highly evolved to utilize chlorinated compounds. The strict dependence of strain E1 on *Sedimentibacter* sp. B4 is likely due to the fact that the larger *Sedimentibacter*

genome of approximately 4.2 Mbp encodes a wide range of metabolic capabilities, including the catabolism of a large spectrum of carbohydrates, the biosynthesis of amino acids, and the production of several vitamins.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.035 DECHLORINATION OF DIOXINS AND PCBs BY CHLOROETHENE-RESPIRING DEHALOCOCCOIDES SPP.

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Polychlorinated dioxins (PCDDs) and polychlorinated biphenyls (PCBs) are ubiquitous, toxic pollutants. Greater understanding of pathways of dechlorination by dechlorinating bacteria is needed. This project investigated PCDD and PCB dechlorination by mixed cultures containing either *Dehalococcoides ethenogenes* strain 195 or a TCE-contaminated groundwater aquifer strain designated *Dehalococcoides* PCE-RU11. Cultures were grown on tetrachloroethene. Subcultures were spiked with 1,2,3,7,8-PeCDD, 1,2,3,4-TeCDD or PCB 114. The mixed culture with strain 195 was tested with Aroclors 1242, 1254 and 1260. Dechlorination was examined at 25 and 35°C and in the presence or absence of co-amended 1,2,3,4-trichlorobenzene (1,2,3,4-TeCB). PCDD and PCB congener specific analysis was by gas chromatography-mass spectrometry. The mixed culture with strain 195 dechlorinated 1,2,3,7,8-PeCDD, PCB114 and the three Aroclors. The 1,2,3,7,8-PeCDD was dechlorinated to 1,3,7,8-TeCDD and further to a trichloro-dioxin - a detoxification pathway since no 2,3,7,8-TeCDD was formed. The rate of dechlorination of 1,2,3,7,8-PeCDD at 35°C (the temperature at which strain 195 was originally enriched) was greater than that observed at 25°C. The mixed culture with strain 195 dechlorinated PCB114 and the three Aroclors mainly by removing doubly flanked chlorines. The rates of dechlorination of PCDDs and PCBs were stimulated by the addition of 1,2,3,4-TeCB. The PCE-RU11 enrichment dechlorinated 1,2,3,4-TeCDD to 1,2,4-TriCDD, while PCB 114 was dechlorinated to PCB63 and PCB74 via removal of a doubly flanked chlorine. Cultures with strain 195 and PCE-RU11 dechlorinated PCDDs and PCBs, often to less toxic daughter products. The use of chloroethene-respiring strains for bioaugmentation in remediation of PCDD- and PCB-contaminated sites warrants further investigation. In particular, investigation of whether the individual PCDD or PCB congeners can support growth of these bacteria is on-going.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.036 ATRAZINE DEGRADATION BY BACTERIAL CONSORTIA FROM POTABILIZATION TREATMENT PLANTS

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Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) has been used as a herbicide from the 1950s to date to control a variety of upland weeds. In recent years, concern about the persistence, mobility and toxicity of triazines is expanding around the world. Intensive

agriculture in developing countries and climate change that cause strong rains increase herbicide leaching from soil. As a consequence, superficial water courses are threatened. To date atrazine-degrading-microbes have been isolated mainly from soils with high or prolonged exposition to the herbicide. The metabolic pathways and the genes involved are well known as well their ability to exchange and transfer among different species. The degradation potential of bacteria transiently or not exposed previously to atrazine has not longer explored. In this work, we report the atrazine degradation by consortia obtained from different potabilization plants treating freshwater in which atrazine has not been detected or has been transiently detected in low concentrations. Enrichments from the incoming freshwater, flocculated sludge, and sand filter showed atrazine degradation in 20 days. Media supplemented with organic and energy carbon source and different atrazine concentrations as nitrogen source were used to select different consortia. The molecular and physiological characterization of stable enrichments shows that the ability to degrade atrazine is present in bacterial communities exposed and not exposed to the herbicide. These consortia are potential tools for bioremediation of water sources used in water supplying plants.

Abstract Category

07 Recalcitrant Compound Degradation

**PS.07.037 DEGRADATION OF FLUORINATED ORGANIC COMPOUNDS BY
ECTOMYCORRHIZAL FUNGI**

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Due to the high persistence of pollutant organic compounds in soil and natural environments, enhancement of their remediation has gained interest over the last decades. Fluorinated compounds are important soil and water pollutants, since they are mainly used in the manufacture of pharmaceuticals and agrochemicals products. The increasing restriction of the disposal of contaminated soils and the need to clean up potentially valuable agricultural sites helped developing rhizosphere remediation. Although the degradation of pollutant compounds by bacteria and some fungal groups has been addressed, little is known on the degradation of such compounds by ectomycorrhizal fungi. The aim of this study was to assess ectomycorrhizal fungi potential for degrading fluorinated organic compounds, namely 3-fluorophenol and sodium trifluoroacetate. Fungal growth and resistance to different levels of those compounds was evaluated in solid media supplemented with glucose to promote fungal growth. A total of 15 ectomycorrhizal fungal isolates were used on the screening. The compound 3-fluorophenol inhibited up to 50% of fungal growth. However, sodium trifluoroacetate was able to promote up to 20% fungal mycelium growth, suggesting that ectomycorrhizal fungi may be able to use sodium fluoroacetate as a carbon source. To determine if the degradation of sodium fluoroacetate and 3-fluorophenol by ectomycorrhizal fungi occurs, batch liquid growing cultures were established. The disappearance of target compounds and formation of other metabolites was assessed. Attempts are under way to identify the metabolites formed during degradation. The present study will contribute to evaluating the role ectomycorrhizal fungi may play on the degradation of fluorinated organic compounds on polluted soils.

Abstract Category
07 Recalcitrant Compound Degradation

PS.07.038 BACTERIA INVOLVED IN BENZENE BIODEGRADATION UNDER NITRATE-REDUCING CONDITIONS

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Petroleum products frequently contaminate ground water and soil. Benzene, one of the most toxic petroleum products, can be mineralized under anoxic conditions by microorganisms. Despite significant efforts over the past decade, little is known about the bacteria capable of and the pathways responsible for anoxic benzene degradation. Our objectives were to identify bacteria and mechanisms involved in anoxic benzene degradation. Bacterial consortia that degrade benzene under nitrate-reducing conditions have been enriched and maintained in our laboratory for several years. Using 16S rRNA gene clone libraries, we established the presence of several bacterial genera that are likely involved in benzene mineralization. Among the Operational Taxonomic Units (OTUs) we identified, some are closely related to other benzene-degrading *Dechloromonas* and *Azoarcus* species. Our clone libraries also revealed sequences that are related to *Pelotomaculum* and *Chlorobi* sequences. We employed quantitative PCR to monitor the abundance of these phylotypes in the mixed consortia during benzene degradation. This enabled us to correlate the presence of these species with benzene degradation. A significant increase in the abundance of *Pelotomaculum* species was observed during benzene degradation, suggesting their involvement in benzene degradation. While *Dechloromonas* 16S rRNA gene copies increased during benzene degradation, their overall numbers were too low to invoke a significant metabolic role. The changes in the abundance of *Azoarcus* and *Chlorobi* were not correlated with benzene degradation. These microorganisms may use another electron donor substrate, such as hydrogen. In conclusion, multiple bacterial genera are maintained over many years and successive transfers in these cultures, however *Pelotomaculum* appears to be the major benzene degrader.

Abstract Category
07 Recalcitrant Compound Degradation

PS.07.039 BIOLOGIC DEGRADATION OF THE PERSONAL CARE PRODUCT CONSTITUENT, TRICLOSAN, BY A SPHINGOMONAS SP. ISOLATED FROM WASTEWATER ACTIVATED SLUDGE

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Trace-level contaminants originating from personal care products have recently been found to cause substantial ecological impacts. Municipal wastewater effluent is an identified source of these contaminants to aquatic environments. Though wastewater treatment facilities were not

designed to remove these compounds, removal efficiencies of up to 90% have been reported. However, this removal level may still be insufficient to ensure ecological health, and alternative treatment options are needed. This study investigated biologic removal of triclosan by activated sludge enrichments with the intention of identifying microbes capable of biologic triclosan removal during wastewater treatment. Triclosan is a common additive to personal care products, including toothpastes, soaps, and lotions. Beginning with activated sludge from a municipal treatment facility, we have isolated a *Sphingomonas* species (designated MC1) capable of rapid degradation of triclosan. The degradation capability of MC1 has been maintained through multiple growth cycles without the selective presence of triclosan. Simultaneous triclosan degradation and MC1 growth have been demonstrated in nutrient rich media (LB and R2 media) and in fully defined media where triclosan where trace-level vitamins are the sole carbon source. In batch experiments starting with either 1mg/L or 5µg/L, final triclosan concentrations of less than 3ng/L have been achieved, often in less than 18 hours. MC1 holds strong potential for bioaugmentation to the activated sludge process during wastewater treatment - an environment characterized by complex organic materials and µg/L triclosan contamination levels - thus, providing a biologic means to mitigate triclosan pollution associated with wastewater effluent. On-going work includes further characterization of the isolate, including the substrate range, and development of molecular methods for monitoring MC1 following augmentation to activated sludge.

Abstract Category

07 Recalcitrant Compound Degradation

**PS.07.040 GENOME ASSEMBLY OF THE 1,4-DIOXANE-DEGRADING
PSEUDONOCARDIA DIOXANIVORANS STRAIN CB1190**

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The actinomycete genus *Pseudonocardia* represents organisms with interesting ecological niches, including associations with ant-fungal gardens and degradation of xenobiotic compounds. Here we present the first genome of a free-living *Pseudonocardia* species, *Pseudonocardia dioxanivorans* strain CB1190, which uses the groundwater contaminant and potential carcinogen 1,4-dioxane as a carbon and energy source and is thus of interest for its application in the bioremediation of 1,4-dioxane-polluted groundwater. Genomic DNA was sequenced with next-generation technology, including unpaired and mate-paired (10 kb) Roche/454 FLX and FLX Titanium libraries (~ 2 million reads in total) and a 36 bp unpaired Illumina library (~33 million reads). 454 sequences were de novo assembled in parallel with Newbler and Celera assemblers, while Illumina sequences were de novo assembled with the edena assembler. These assemblies were combined using minimus2 and the scaffolds were annotated with the Oak Ridge National Laboratories annotation pipeline. The draft genome consists of 8 scaffolds totaling 7.2 Mb, with 73% G+C and 6866 open reading frames. A full set of tRNAs is present, as are pathways encoding twenty amino acids. Several putative monooxygenases were identified and we are now investigating their role in 1,4-dioxane degradation with quantitative RT-PCR and biochemical activity assays. Although strain CB1190 can fix nitrogen and is thus of interest for its potential

for bioremediation in fixed-nitrogen-depleted groundwater, no nitrogenase genes were identified in the draft genome or using degenerate PCR targeting *nifH* or *nifD*. We are designing an expression microarray to identify regulated genes that permit growth on 1,4-dioxane and in the absence of fixed-nitrogen. The combination of genome sequencing and microarray experiments will allow us to improve our understanding of *Pseudonocardia* gene regulation and metabolic systems relating to 1,4-dioxane biodegradation and N₂ fixation.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.041 BIOLOGICAL DEGRADATION OF PYRENE IN SOIL: THE COMBINED ROLE OF SOIL MICROORGANISMS AND *LUPINUS* PLANT SPECIES.

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Polycyclic aromatic hydrocarbons are common contaminants of soil and are recalcitrant to degradation. Current remediation methods are expensive and environmentally unsustainable. This study explored the phytoremediation of pyrene by native soil microflora in the presence of *Lupinus albus* and *L. angustifolius*.

Soil was artificially contaminated with 80 mg kg⁻¹ pyrene. Unvegetated pyrene controls and planted pyrene and uncontaminated mesocosms were maintained in quadruplicate under greenhouse conditions for 9 weeks. Soil was sampled over time and pyrene determined by gas chromatography. Most probable number analysis was performed to selectively enumerate eukaryotic or prokaryotic pyrene degraders.

Unvegetated pyrene controls had consistently lower numbers of all pyrene degrading microbes than any planted treatment. Planted, pyrene contaminated treatments had the highest number of pyrene degrading microorganisms. Removal of pyrene from artificially contaminated soils was significantly higher ($P < 0.05$) with lupins compared with unvegetated controls. After 8 weeks of plant growth, 86%, 91% and 48% of pyrene was removed for *L. albus*, *L. angustifolius* and unvegetated treatments respectively. Removal of pyrene was positively correlated with numbers of pyrene degrading prokaryotes ($P < 0.05$).

Lupins appeared to select for pyrene degrading microbes, as indicated by increased numbers in the planted treatments compared with unvegetated controls. Specific root exudates may be involved in this process. Increased numbers of pyrene degrading prokaryotes led to increased pyrene degradation under lupins, indicating that loss of pyrene is likely via biological degradation. This result is supported by the lack of pyrene in leachate or plants. This study showed enhanced degradation compared with some research using grass species. In some cases, such as under low nutrient conditions, lupins may be superior phytoremediation candidates.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.042 EFFECTS OF VINYL CHLORIDE ON THE DIFFERENTIAL GENE EXPRESSION AND CARBON ISOTOPE FRACTIONATION OF *D. ETHENOGENES* STRAIN 195.

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Little is known about the physiological response of *Dehalococcoides* bacteria to conditions that limit their growth and dechlorination activity. Because an accumulation of vinyl chloride corresponds to a stationary phase growth transition in *Dehalococcoides ethenogenes* strain 195, it may serve as one such growth-limiting factor. To investigate the effects of vinyl chloride (VC) on the physiology of strain 195, a whole-genome microarray was applied to the transcriptome of the organism when grown on trichloroethene (TCE) while subjected to elevated concentrations of VC. Additionally, the stable carbon isotope fractionation of TCE was quantified under similarly-elevated VC concentrations. The presence of excess vinyl chloride did not result in a statistically-significant difference in the isotopic enrichment factor of the TCE fractionation. The observed enrichments were -12.5 and -12.1‰ for the VC and control conditions, respectively. The difference between these values is within the analytical error (0.5‰) of the GC-C-IRMS. Increased concentrations of vinyl chloride resulted in the differential expression of two putative reductive dehalogenase genes (DET0180 and DET1545) in the microarray analysis. DET0180 was up-regulated 2-fold under increased concentrations of vinyl chloride, while DET1545 was down-regulated 4-fold. Although the absolute hybridization signals of the RDases in the control were both lower than that of the *tceA* gene, the upregulation of DET0180 in the vinyl chloride condition produced a signal intensity nearly the same as the *tceA* gene (which was not differentially expressed). These results suggest that vinyl chloride has an effect on the genomic regulation of *D. ethenogenes* strain 195, and may contribute to physiological differences observed in a stationary growth phase. However, this effect is not detected by the stable isotopic fractionation of its electron acceptor TCE.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.043 SERVICES OF MICROBIAL DIVERSITY: WHAT ABOUT DIVERSITY OF FUNCTIONAL GENES AND XENOBIOTIC DEGRADATION?

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Divers studies dealing with the role of diversity in ecosystems functioning suggest that great diversity is associated with higher performances on ecosystems processes. Most of these studies have been focused on the study of phylogenetical diversity targeting the 16S rRNA genes. However, although important functions in the ecosystems, such as pollutant removal, can be related to the presence of specific functional genes, the role of diversity of functional genes on ecosystem services has not been evaluated. Hence, it is well known that the first step of degradation of pollutants such as polyaromatic hydrocarbons (PAH) is performed by dioxygenases which are encoded by functional genes presenting a large diversity. However, the implications of this functional diversity have not been assessed. The objective of this study was

to evaluate the impact of the diversity of PAH ring-hydroxylating dioxygenase-encoding genes (RHD), on the capacity to degrade wide range of PAH by sludge microbial communities. The methodology consisted on the evaluation of the degradation capacity of different microbial communities to remove 14 PAH compounds. These microbial communities displayed contrasted RHD and 16S rRNA diversity as assessed by, respectively, clone library sequencing and single strand conformation polymorphism (SSCP). Pollutant removal was assessed by respirometry and measuring PAH concentrations by chemical analysis (HPLC-Fluo) in bioreactor experiments. Our results showed that independently of the phylogenetical diversity, the microbial communities with high functional diversity displayed higher capabilities to remove a wide range of polyaromatic hydrocarbons. In conclusion, this study showed that functional diversity strongly affects ecosystem functions; thus, functional diversity is a more important parameter than phylogenetical diversity when considering ecosystem services such as xenobiotic removal.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.044 ANAEROBIC BIODEGRADATION OF CHLORINATED BENZENES IN DREDGING SLUDGE

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Chlorinated benzenes are widely used as solvents, insecticides, fungicides, and intermediates in the chemical industry and are ubiquitous environmental contaminants in dredging sludge. Due to their toxicity, persistence and biomagnification in the environment they are of great concern. Reductive dechlorination of hexa- and tetrachlorobenzene isomers was investigated in sludge from the canal Ieper-Ijzer, Belgium. All microcosms, inoculated with sludge and amended with chlorinated benzenes as electron acceptor and lactate as electron donor and carbon source, were active. Dechlorination in the microcosms proceeded up to monochlorobenzene. The microcosms were transferred during two stages in order to enrich the dechlorinating species. The sediment free culture growing on 1,2,4,5-tetrachlorobenzene was transferred on ten different chlorinated benzenes in order to investigate its substrate specificity. The culture showed activity for hexa-, penta-, the three isomers both of tetra- and of tri-, and 1,2-dichlorobenzene. Quantitative PCR with genus specific primers revealed that *Dehalococcoides* spp. were present in all and growing in most of these cultures, indicating their role in dechlorination. Dechlorination depending on cometabolic processes of syntrophic fermenters occurring in the methanogenic mixed culture was excluded by inhibition tests using 2-bromoethanesulfonic acid as inhibitor of methanogenic bacteria. This study reveals that dredging sludge offers a niche for bacteria that are able to link growth and reductive dechlorination of a wide spectrum of chlorinated benzenes.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.045 ALPHAPROTEOBACTERIA DOMINATE ACTIVE 2-METHYL-4-CHLOROPHENOXYACETIC ACID HERBICIDE-DEGRADING BACTERIA IN SOIL AND DRILOSPHERE

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2-Methyl-4-chlorophenoxyacetic acid (MCPA) is a widely used herbicide and subject to aerobic microbial degradation. Betaproteobacteria are assumed to degrade MCPA in soil based on quantitation of *tfdA* encoding oxygenases initiating MCPA degradation. Earthworms stimulate growth and activity of MCPA-degrading bacteria in soil. To resolve active MCPA degraders in soil and drilosphere (i.e., worm burrow walls and cast), 16S rRNA stable isotope probing (SIP) was performed in soil columns under conditions designed to minimize incubation biases. Agriculturally relevant, low concentrations of [U-¹³C]MCPA (20 µg g_{DW}⁻¹) were degraded within 27 days in the presence of earthworms. Total 16S rRNA analysis revealed 90 active family-level taxa, 33 of which were not affiliated with known families, indicating phylogenetic novelty in bulk soil and drilosphere. 21 and 19 major active taxa occurred in the drilosphere and bulk soil, respectively. 12 of those taxa assimilated MCPA-¹³C and were affiliated with Alpha-, Beta-, Gammaproteobacteria, Actinobacteria, and Firmicutes. *Sphingomonadaceae* and *Bradyrhizobiaceae* of the Alphaproteobacteria dominated MCPA-assimilating bacteria, indicating that those taxa were major MCPA degraders in the drilosphere and soil. In oxic microcosms of bulk soil and burrow wall material supplemented with high concentrations of [U-¹³C]MCPA (300 µg g_{DW}⁻¹), *Sphingomonadaceae*-related taxa dominated MCPA consumers, while Betaproteobacteria (*Burkholderiaceae*-, *Comamonadaceae*-, and *Oxalobacteraceae*-related taxa) dominated MCPA consumers in cast microcosms. Microcosms indicated that fast-growing copiotrophs like *Sphingomonadaceae* and *Comamonadaceae* outgrew slow-growing oligotrophs like *Bradyrhizobiaceae* under high substrate conditions, highlighting the importance of using in situ near substrate concentrations and incubation conditions for SIP. The collective results indicate that Alpha- rather than Betaproteobacteria are major MCPA degraders in soil and drilosphere.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.046 ANALYSIS OF THE LIGNOCELLULYTIC ENZYMES FROM AGRICULTURAL WASTE COMPOST BY MASS SPECTROMETRY

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There are 23 million tons of agricultural wastes produced every year in Taiwan. The typical agricultural wastes in Taiwan are rice husk, rice bran, sugar cane debris and mushroom residua, which are often used in composting. The enzymes released by microbes during composting play a key role in the biodegradation of the organic matters. The temperatures during the composting can reached 60-80°C. Cellulases, for example, secreted by microbes during composting are considered to be potential in applications on biofuel production. However, they are difficult to be isolated due to humic substances in the environmental samples. This study aims to improve the extration of the extracellular proteins from compost samples and the identification of active

cellulase by mass spectrometry. The compost sample (30% rice husk, 10% rice bran, 40% mushroom residua and 20% coconut fiber) was collected when the total cellulase activities reach peak period during composting. After the compost debris, microbes and humic substance were removed, the extracellular protein was extracted and precipitated by TCA followed by separation in SDS-PAGE. The gel was then stained by cellulase enzyme activity. The active bands were cut and digested by trypsin. The peptides were sequenced by ESI-MS/MS. Seventy-four proteins, including one hemicellulases (*Streptomyces sviveus*), and two novel lignin-degrading enzymes, laccases (*Spongipellis* sp.), were identified. Our result demonstrated the metaproteomic analysis of the expressed extracellular proteins in compost. Particularly, potentially useful proteins expressed from uncultivated microbes can be investigated.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.047 DIVERSITY AND PROPERTY OF BACTERIAL STRAINS IN THE DEGRADATION OF OCTYLPHENOL POLYETHOXYLATES

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Octylphenol polyethoxylates (OPEOn), also known as Triton X-100®, a nonionic surfactant, was widely used in industrial, agricultural, and domestic applications. The product is a mixture consisting of OPEOn (n = 4~15) with an average unit of ethoxylate at 9.5. In this study, forty three bacterial strains were isolated from soil samples of pesticide factory, rice field, and drainage of the university dormitory in northern Taiwan. They can grow on OPEOn as sole carbon source and energy. Except species from *Pseudomonas*, other strains such as *Moraxella osloensis*, *Aquaspirillum peregrinum* ss *integrum*, *Photobacterium logei*, *Ochrobactrum* sp., *Cupriavidus* sp., *Ensifer adhaerens*, *Achromobacter xylosoxidans*, and *Brevbacterium iodinum* have not been reported to be capable in the degradation of the surfactants. Among them, *B. iodinum* was the only Gram-positive bacterium. The diversity of the isolated OPEOn-degraders from the green house of a pesticide factory is higher than the rice field. The drainage of dormitory only contained species from *Pseudomonas*. Comparison between these three environments indicates that the variety and quantity of contaminants could select different types of microorganisms in the individual environment. HPLC-MS analysis demonstrated the cleavage of ethoxylate chain in the degradation mechanism in *Pseudomonas* sp. TX1 and *B. iodinum*. Comparison of the kinetics of OPEOn degradation of *Pseudomonas* sp. TX1 and *B. iodinum* showed the ethoxylate chain degradation by *B. iodinum* is faster than the Gram-negative bacterium, TX1. However, the growth rate and the oxygen consumption activity of *B. iodinum* are lower than strain TX1. The two bacteria serve nice models in the mechanism study of biodegradation of OPEOn and its estrogenic recalcitrant metabolites.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.048 COMPARATIVE METAGENOMICS OF TWO *DEHALOCOCCOIDES*-CONTAINING DECHLORINATING MICROBIAL CONSORTIA

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KB-1 and DONNA2 are *Dehalococcoides* (Dhc)-containing enrichment cultures capable of the complete reductive dehalogenation of chlorinated ethenes. These enrichment cultures contain different strains of Dhc (strains KB-1 TCE/KB1-VC and strain 195 respectively), and are maintained with different electron donor, medium, and growth conditions. The KB-1 and DONNA2 enrichment cultures were subjects of metagenome sequencing at the Joint Genomes Institute (JGI). This sequence data allows in-depth examination of the community structure and metabolic interactions within two distinct Dhc-containing communities, including prediction of critical metabolic pathways beyond reductive dehalogenation. In order to accurately determine community structure from the metagenome sequences, we developed a custom binning heuristic, and implemented novel methods to allow scaffolding information and organismal abundance to inform the binning process. We tested the performance of the heuristic on simulated metagenome datasets available from the JGI (Mavromatis *et al.*, 2007, Nature Methods 4:495-500), and applied it to the Dhc-containing enrichment culture metagenomes. Using a custom pipeline, we predicted the metabolic profile of each "binned" community member as a means of further resolving metabolic interactions and community-wide potential. Our binning heuristic, in combination with the metabolic profiling pipeline, represent a novel and cohesive method for examining enrichment culture metagenomes, which take advantage of the lower community diversity in these datasets compared with soil, environmental, or gut metagenomes.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.049 CHARACTERIZATION OF A NOVEL FUNGI *PENICILLIUM SP.* YT02 WITH HIGH LIGNOCELLULOSIC-SACCHARIFICATION ACTIVITY

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Penicillium spp. have been identified as potential cellulase producers for bioconversion of lignocellulosic material but their cellulase systems have not been investigated in detail. In this study, the enzyme activity and saccharides production profiles of a newly isolated *Penicillium* strain (*Penicillium sp.* str. YT02) was characterized. The optimal culture conditions for the strain based on the highest cellulase activity and saccharification ratio, were 30 °C, pH 5.5, and an agitation speed of 130 rpm. Compared to the most commonly used fungal strain *Trichoderma reesei* (ATCC 24449), higher cellulolytic activity and saccharification capacity were observed in YT02 with Avicel and various insoluble lignocellulosic substrates. Similar total filter paper and CMCase activities (0.5-0.6 U mg protein⁻¹) but much higher β-glucosidase (0.78 U mg protein⁻¹) and xylanase (9.0 U mg protein⁻¹) activities were detected in YT02, which is consistent with the results of the enzyme activities staining for glucanases, β-glucosidase, and xylanase. The volumetric activities in relation to the amount of protein of *P. sp.* YT02 cellulases were

determined with a model substrate, Avicel, a microcrystalline cellulose. In addition, the theoretical saccharification ratio of YT02 reached up to 70% within 100 hrs with pretreated cellulosic materials. The protein content in the supernatant of the YT02 culture was less, but more stable over time than that of *T. reesei*, which may provide an advantage for cellulose degradation. These results suggest that *P. sp.* YT02 is a potential strain for industrial bioprocessings of cellulosic plant biomass.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.050 DEVELOPMENT OF PRIMERS TARGETING RIESKE DIOXYGENASES WITH ACTIVITY TOWARD DIBENZO-*P*-DIOXIN

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Polychlorinated dibenzo-*p*-dioxins are among the most recalcitrant and toxic chemicals found in the environment. We developed primer sets that target nucleic acids coding amino acid conserved sequences from all known angular dioxygenases with known activity toward dibenzo-*p*-dioxin (DD). These genes form three clusters; representatives from the clusters include: (Group 1) DD dioxygenase, (Group 2) dibenzofuran (DF) 4,4a dioxygenase, and (Group 3) carbazole 1,9a dioxygenase. Hidden Markov Models (HMMs) were constructed using sequences from each cluster to harvest all similar sequences from the DDBJ/EMBL/GenBank non-redundant protein database (nr). In total, ten or fewer sequences composed each group after this search. Degenerate primers were designed from amino acid consensus regions of the sequences to target not only known sequences, but also novel sequences with the same function. PCR conditions were optimized and the primer sets were validated with an average of 4 positive and 10 negative controls. With few exceptions, the primer sets were specific to only the gene cluster for which they were designed, and not closely neighboring clusters. The primer sets were then used to produce amplicons from a PCB contaminated rhizosphere sample. Amplicons of the correct length were observed from Group 1 and 2, and a clone library was made. Clones from Group 1 showed 91-99% similarity to DD dioxygenase, PAH dioxygenases, DF dioxygenase. These clones contained well-known conserved amino acids for Rieske dioxygenases in positions previously described for the Fe-binding site. Group 2 amplicons showed 95-99% similarity to DF dioxygenases and also contained the conserved amino acids. These results show the primer sets were specific to the desired genes in pure culture and environmental samples. The primers will be used for gene-targeted pyrosequencing in efforts to recover greater diversity of these genes and to determine if different groups are selected in different environments.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.051 DECOLORIZATION OF REACTIVE BLUE 13 BY ASPERGILLUS NIVEUS AND CITOTOXICITY OF BIOTREATED DYE SOLUTION

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Reactive Blue 13 was decolorized by *Aspergillus niveus*. In this study, various physicochemical parameters such as, pH, temperature, agitation speed, color concentration, dead pellets decolorization of fungi and repeated-batch study are investigated and over 90% decolorizations were obtained at pH 7, 30°C and 100 rpm. Chinese Hamster Lung V79 379A fibroblasts which obtained from Institute of Fermentation, Osaka (IFO, Japan) were used to assess the cytotoxicity of samples obtained from water treated with *Aspergillus niveus* that include 100 mg/ml dye. Cytotoxicity was determined by the MTT method. 20%, 40% and 80% doses of treated water samples that include 100 mg/ml dye showed cytotoxicity after three days. It may conclude that 100 mg/ml initial dye concentration can be treated by *Aspergillus niveus* with minimum 3 days period. Toxicity was increased from 20% to 80%. Over eighty percent of viability was observed at 20% doses and over forty percent of viability was observed at 40% doses. 80% was very toxic. Results showed that 100mg/l dye solution treated with *Aspergillus niveus* can be discharge receiving water with 20% and 40% doses.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.052 PLANT-MICROBE-POLLUTANT INTERACTIONS CONTROL MICROBIAL COMMUNITY STRUCTURE IN RHIZOREMEDIATION OF POLYCYCLIC AROMATIC HYDROCARBON-CONTAMINATED SOIL

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Biodegradation of organic contaminants such as polycyclic aromatic hydrocarbons (PAH) is accelerated in the presence of plants, due to the enrichment of microbial communities in the rhizosphere. Various studies have demonstrated that the efficiency of this rhizoremediation process depends strongly on which plant species are used, but the mechanisms that influence this dependence are not yet fully understood. It has been proposed that PAH catabolic activity is enhanced in the rhizosphere merely because higher microbe numbers are present compared to bulk soil (the "rhizosphere effect"), but it is also possible that specific microbial groups are enriched in rhizospheres under pollutant stress. In this study, plant effects on the development of rhizosphere microbial communities in contaminated soils were assessed for two grasses (*Festuca rubra*, *Holcus lanatus*) and for white clover (*Trifolium repens*), using a novel split-root model. Plants were cultivated with their roots divided between two independent pots containing either uncontaminated soil or PAH-contaminated soil (500-1000 µg pyrene or phenanthrene per g soil). Microbial community development in the two halves of the rhizosphere were assessed by T-RFLP analysis of the 16S rRNA gene for bacteria and the ITS region for fungi. PCA analysis showed that the fungal and bacterial communities in the grass rhizospheres were grouped entirely by the presence or absence of PAH in the respective rhizosphere, and no plant effects were observed. However, in *Trifolium* rhizospheres, the microbial community structure in the uncontaminated part of the split-root model was significantly influenced by the presence of PAH-contamination in the other part of the root system. The results suggest that *Trifolium* may respond to PAH-contamination stress in a systemic manner, releasing signal molecules that help determine the rhizosphere community composition under these conditions.

Abstract Category
07 Recalcitrant Compound Degradation

PS.07.053 THE IMPACT OF SONOLYTIC TREATMENT ON ATRAZINE IN SOIL-WATER SLURRY

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Background and aims: Sorption on soil particles can limit degradation of pesticides. We studied whether the bioavailability and subsequent microbial degradation of atrazine could be enhanced by sonication.

Methods: The experiments were done in triplicate in flasks containing 50 ml of water and 15 g of dry soil from 11.3 - 14.6 m below soil surface, while the water table was at 14 m. Soil was from the groundwater area having atrazine in water, and contained microbes able to degrade atrazine. The concentration of atrazine was 100 mg/l. Sterilized controls were autoclaved on three successive days for 1 h at 121°C, 101 kPa. Sonication times were 0, 20 and 30 minutes twice a day for seven days, after six days adaptation period without sonication. Samples were taken from the water phase, and atrazine concentration was analyzed using high performance liquid chromatography equipped with an ultraviolet detector (225 nm).

Results: The atrazine concentrations in the water phase dropped during the six days adaptation period in autoclaved controls to 70.8 ± 5.7 mg/l, and in non-sterilized flasks to 49.2 ± 5.3 mg/l. Sonication for ten times increased the measured atrazine concentration in flasks with indigenous soil microbes to 66.7 ± 2.8 mg/l, while the amount was 44.0 ± 3.1 mg/l without sonication. In sterilized controls, changes in atrazine concentrations due to the sonication were smaller than in non-sterilized soils.

Conclusions: The initial drop in atrazine concentrations was at least partly due to sorption on soil particles. Microbes may have had an impact on lower concentrations measured in non-autoclaved slurries, and autoclaving may also have affected soil structure and thus atrazine amount in the water phase. Sonication increased atrazine concentrations in water phase of slurries with microbes, but whether sonication increased bioavailability and thus biodegradation, remains unclear.

Abstract Category
07 Recalcitrant Compound Degradation

PS.07.054 DEHALOCOCCOIDES SPECIES IN UNCONTAMINATED ENVIRONMENTS

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Members of the *Dehalococcoides* genus of bacteria have been found to dechlorinate a wide range of persistent organic pollutants. Because chlorinated anthropogenic pollutants have only been produced during the last century, the organisms' ability to dechlorinate these compounds is curious. Our previous research suggested that these species evolved to use natural chlorinated compounds, thus having a natural ecological niche. The goals of this research were to determine if *Dehalococcoides*-like organisms found in uncontaminated and contaminated environments had

different dehalogenase genes and if *Dehalococcoides*-like organisms could be enriched from uncontaminated soils with a natural chlorinated compound.

Samples were collected from uncontaminated and contaminated sites. Quantitative polymerase chain reaction was used to measure the quantities of *Dehalococcoides*-like organisms based on 16S rRNA gene sequence. Terminal restriction fragment length polymorphism was used to analyze the diversity of dehalogenase genes and cloning and sequencing was used to identify putative dehalogenases. Batch reactors were used to determine whether chloroanthones could be dechlorinated by and enrich for *Dehalococcoides*-like organisms from an uncontaminated soil sample.

Numbers of *Dehalococcoides*-like organisms increased over time in reactors amended with a chloroanthone, compared to un-amended controls. In addition, the number of *Dehalococcoides*-like organisms was greater on the surface of chloroanthone-soaked solids compared to control solids. Finally, a diversity of dehalogenases has been found present in uncontaminated environments.

Dehalococcoides-like organisms are part of natural, uncontaminated ecosystems and may be integral in the cycling of chlorine and carbon. Putative dehalogenase genes are also present in uncontaminated systems. *Dehalococcoides*-like organisms appear to be able to grow on a natural chlorinated compound.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.055 KINETICS, DIVERSITY & ROLE OF HEXACHLOROCYCLOHEXANE (HCH) DEHYDROCHLORINASE (LinA) & HALOALKANE DEHALOGENASE (LinB) IN ENZYMATIC BIOREMEDIATION OF HCH

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India is one of the leading producers of HCH (Hexachlorocyclohexane) in the world. Among its eight isomeric forms only the γ isomer possesses the insecticidal property. For each ton of γ -HCH purified, 10-12 tons of waste α -, β - and δ isomers are generated and discarded creating dangerous dumpsites all over the world. Owing to the recalcitrant and toxic nature of HCH isomers, there has been a strong emphasis on developing technologies for its clean-up. Bioaugmentation and biostimulation have proven effective but are extremely slow thus shifting focus to enzymatic bioremediation. The pathways of degradation of each HCH isomer are different. LinA (HCH dehydrochlorinase) and LinB (haloalkane dehalogenase) catalyse the primary steps in degradation of all HCH isomers. Here, we aim to explore the possibility of enzymatic bioremediation of HCH contaminated sites by analysing the kinetics of the enzyme variants of LinA and LinB.

For comparative kinetic analysis, LinA and LinB were heterologously expressed and purified by Ni-NTA column chromatography by FPLC. Enzyme activity in terms of rate min⁻¹ was determined by assaying substrate depletion using Gas Chromatography.

Kinetic analysis marked out two variants; LinA γ 1-7 and LinB SSO4-3 as the potential enzymes to be used as a starting resource for the enzymatic bioremediation of HCH. LinA γ 1-7 exhibits significant turnover numbers of ~12200, 7600 and 270 molecules min⁻¹ on α -, γ - and δ -HCH respectively and no activity on β -HCH. LinB SSO4-3 converted ~83 and 10 molecules min⁻¹ of β - and δ -HCH respectively. None of the LinB's showed any activity over α - and γ isomers.

Enzymatic bioremediation for HCH has never been attempted, but seems feasible using LinA and LinB. LinA γ 1-7 can be directly taken to field while is a starting point for protein engineering studies aimed at improving the catalytic performance of LinB SSO4-3, such that the entire gamut of HCH isomers can be tackled by these two by these two enzymes.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.056 METATRANSCRIPTOMIC EXAMINATION OF EUKARYOTIC GENES INVOLVED IN MAIZE LITTER

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Soil fungi, especially members of the Basidiomycota, are important players in the degradation of soil-borne recalcitrant organic matter, and their activities are expected to become increasingly important as more sustainable agriculture regimes are adopted. A number of fungal enzymes have been identified that can carry out some key steps in the initial breakdown of complex organic molecules, such as lignin, but we still have relatively limited knowledge of the suite of genes involved in the in situ degradation of recalcitrant organic matter in soils. We therefore adopted a metatranscriptomic approach to examine the eukaryotic genes involved in organic matter decomposition. Mesh bags were filled with plant litter from dried maize leaves and buried in mesocosms containing organic farm soil, with mesocosms lacking litter bags serving as controls. After 70 days, soil adhering to the mesh bags was collected for analysis. Total RNA was extracted from recovered soil and controls and polyA cDNA synthesized. Resulting cDNAs were then subjected to pyrosequencing via the 454 GS FLX platform. Similarly, rRNA genes were examined after cDNA synthesis using an rRNA-targeting primer. Approximately 200,000 mRNA-derived reads were obtained, with 70% of reads being longer than 400 nucleotides. DNA sequences were assigned to ORFs and ORFs to Pfam protein family, Gene Ontology (GO), and Enzyme Commission number (EC). Approximately 40% of all reads could be assigned to the Pfam level. We subsequently examined the genes and gene families overrepresented in litter bag treatment for the identification of genes potentially involved in litter degradation. The taxonomical binning and putative functions of these differentially expressed transcripts allowed for the identification of putative gene markers that may be useful in assessing the capabilities of soils to degrade complex organic matter.

Abstract Category
07 Recalcitrant Compound Degradation

**PS.07.057 METHANOGENIC BENZENE DEGRADATION PATHWAYS
EXAMINED BY STABLE ISOTOPE TRACERS**

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Anaerobic benzene degradation is a challenging process. Especially under methanogenic condition, benzene degrading bacteria and the pathway have not been identified. It is necessary to clarify mechanisms of the degradation for further development of bioremediation techniques. We conducted stable isotope tracer experiments to identify the degradation products and to determine the pathways. A methanogenic benzene degrading consortium had been obtained from lotus field sediment. Isotope-labeled benzene, toluene, phenol and benzoate, of which carbon atoms in the benzene ring were labeled, were added to the consortia in vials together with unlabeled benzene. The consortium degraded benzene and all benzene derivatives. An unknown compound was detected in the liquid phase only in the presence of phenol. It was identified as 4-hydroxycoumarin (4-HC) by mass spectrometry and an authentic chemical. When ¹²C-Benzene and ¹³C-Phenol was added, only ¹³C-labeled 4-HC was detected. Thus, benzene degradation was not through phenol in this culture. About 90% of benzene was converted to the final product, CH₄ (41%) and CO₂ (49%). Carbon atoms in the benzene ring of the benzene derivatives were also turned into CH₄ and CO₂ in 1:1 ratio. It was stoichiometrically same as the conversion of benzoate by *Syntrophus aciditrophicus*, which is one of the phylogenetically closest isolated bacteria to a clone of putative benzene degrading bacteria, Hasda-A (Sakai *et al.*, 2009). The number of Hasda-A increased only in the culture fed with benzene. Phenol, toluene and benzoate addition did not affect the number of Hasda-A. Hasda-A grew by benzene degradation, but other benzene derivatives were utilized by other bacteria. The methanogenic benzene degrading consortium can mineralize benzene, toluene, phenol and benzoate to CH₄ and CO₂ almost completely. The formation of 4-HC only from phenol indicated that benzene degradation was not through phenol. (Sakai *et al.*, J Biosci Bioeng 108, 501, 2009)

Abstract Category
07 Recalcitrant Compound Degradation

**PS.07.058 BIOREMEDIATING HEXACHLOROCYCLOHEXANE (HCH):
PLAUSIBLE SOLUTIONS & CHALLENGES AHEAD**

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Lindane, the γ -isomer of hexachlorocyclohexane (HCH) is a hazardous insecticide used in the past to protect crops. Its purification leaves behind non-insecticidal HCH isomers which are generally discarded in the open creating dumpsites. HCH residues from these dumpsites percolate into soil and groundwater contaminating all components of the environment. Such highly contaminated sites exist in many parts of the world and require immediate contamination. Our group is

working to find possible solution(s) for the decontamination of HCH contaminated sites using bioaugmentation, biostimulation and enzyme-based approaches. The role of *lin* genes in degradation of HCH isomers and their metabolites was studied in *Sphingobium indicum* B90A using routine molecular biology methods. Bioaugmentation of HCH contaminated soil was attempted using corn-corb immobilized cells of B90A. *Ex-situ* biostimulation of contaminated soil was performed by tilling and providing nutrients and water intermittently. Cloning, expression and kinetics of enzymes LinA and LinB was performed using routine protocols. For all, HCH degradation studies were done by gas chromatography. Corn-corb immobilized B90A cells applied repeatedly to HCH contaminated soil degraded 85-95% HCH in 24 days. HCH degrading sphingomonads were isolated from HCH dumpsite and stimulation of indigenous microbial population in dumpsite showed 70 to 90% HCH degradation in 24 and 240 days respectively. We cloned, sequenced and performed kinetic analysis of 16 LinA and LinB variants in order to determine the best enzyme variants which can be taken up and/ further improved for conducting field trials. Our study describes the advantages and pitfalls associated with three approaches, bioaugmentation, biostimulation and enzymatic bioremediation for decontamination of HCH contaminated sites. This understanding of the Lin/Sphingomonad system gained over the years can be used to develop an economically viable bioremediation technology for HCH.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.059 FATE OF DICOFOL IN MICROCOSM UNDER AEROBIC AND ANAEROBIC CONDITIONS

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The fate of organic micropollutants that can be found in sewage was studied in treatment processes. Simulations of wastewater aerobic and sludge anaerobic treatments with radioactive molecules allow tracing the mineralization and distribution of these compounds in these systems. Direct data obtained in this work was compared with mathematical models or chemical measures in the wastewater treatment plants. This study focused on the biodegradation of ¹⁴C-dicofol organochlorine by wastewater aerobic treatment and sludge anaerobic post-treatment in microcosm. The aerobic process occurred within 7 days during which wastewater were withdrawn daily and biological sludge was reused during 7 days. Later this sludge was submitted to anaerobic digestion treatment for different times. The performance by physical-chemical parameters was satisfactory compared with lab-scale processes. Radioactivity was monitored in both treatments. High pressure liquid chromatography provided with UV detector was used to measure dicofol and dichlorobenzophenone in the different phases of the process. Dicofol reduced the efficiency of the aerobic process. 0.1% of dicofol was mineralized by aerobic treatment and major part of radioactivity was found as dichlorobenzophenone as sorbed in biological sludge. The biodigestion process was not affected by residues found in sludge. The mass balance showed that 12% radioactivity was found in the gas phase and small amounts of residues (dicofol and dichlorobenzophenone) were found in the biodigested sludge. These results suggest that biodegradation process is running with formation of other metabolites.

Financial support: Faperj and Pronex.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.060 CONSTRAINS TO PERSISTENCE CHARACTERIZATION

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The persistence of xenobiotics results from the lack of or reduced degradation and biodegradation capacity under natural conditions. Bioaccumulation allows increase in amounts up to poisoning concentrations on non target living organisms such as animals, plants and microorganisms. Persistence measured by half life in the environment does not discriminate between the fate by biodegradation from disappearance by leaching, volatilization, non-extractable bound or runoff. To measure mineralization, radiolabeled molecules need to be used under the confined simulated environmental conditions of a microcosm. In this work mineralization of ¹⁴C-atrazine was measured under different simulation conditions such as: 30g soil in Erlenmeyer with 80% and 20% field capacity at room temperature; with tillage and non tillage soils; soils previously treated (1 year before) with atrazine; microcosms with 7Kg of undisturbed soils with or without plants. Mineralization ranged from 45% down to 0.002% of the applied amount. Half life data of soils under different climate conditions obtained in our laboratory together with literature data shows a broad range of values. In cases where mineralization was lacking or high conclusions could be drawn easily, but this was not every time the case as with atrazine in which mineralization was shown to be strongly influenced by simulation conditions. Interpretation needs to select the most environmental representative experiment for atrazine considering the worst soils. Environmental residue data showing atrazine as the most polluting compound in the ground water is an important argument in favor to ban this pollutant. Persistence characterization with many substances is not easy to characterize but is of key importance to ban some compounds toward a more sustainable environment.

Financial support: FAPERJ, PRONEX and CNPQ

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.061 BIODEGRADATION AND BIOREMEDIATION OF CHLORINATED POLLUTANTS USING *MYCOBACTERIUM* SP. NBB4

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Mycobacterium species are well-known for causing infectious diseases such as tuberculosis. However, the majority are non-pathogenic saprophytes, found in soils, sediments, and water. Environmental mycobacteria are capable of biodegrading many important pollutants, and such strains (either indigenous or inoculated) are useful for clean-up of contaminated sites (bioremediation). We previously isolated *Mycobacterium* strain NBB4 from estuarine sediment

using ethene as carbon and energy source. Here, we show that NBB4 can biodegrade the important pollutants *cis*-dichloroethene, 1,2-dichloroethane and vinyl chloride.

Pollutant degradation was first studied in dense suspensions (4 ml) of ethene- or ethane-grown cells in phosphate buffer, using gas chromatography and chloride assay. Ethene-grown cells degraded vinyl chloride and *cis*-dichloroethene, while ethane-grown cells degraded 1,2-dichloroethane. All pollutants were degraded to below detection limits after 24 hours. Stoichiometry of chloride release was 0.84 mol/mol for vinyl chloride, 1.05 mol/mol for *cis*-dichloroethene, and 1.00 mol/mol for 1,2-dichloroethane, indicating vinyl chloride is nearly completely dechlorinated, while organochlorine metabolites are likely to be accumulating from *cis*-dichloroethene and 1,2-dichloroethane.

The effectiveness of using NBB4 for bioremediation was tested in microcosms (4 ml) containing aquifer sediment from a contaminated site. Both *cis*-dichloroethene and 1,2-dichloroethane were biodegraded in microcosms containing NBB4 (>90% loss after 24 h), while no significant pollutant loss was seen in uninoculated microcosms. Dilution-plating revealed that viable NBB4 cells persisted in the site material in high numbers for 1 month.

We have shown that NBB4 is a promising agent for bioremediation of sites contaminated with chlorinated pollutants.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.062 QUERYING THE GENOMES OF UNSEQUENCED *DEHALOCOCCOIDES* STRAINS VIA A GENUS-WIDE MICROARRAY

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Dehalococcoides spp. are important for bioremediation because they can convert the carcinogenic chlorinated ethenes to the benign product ethene. The genomes of four *Dehalococcoides* strains (195, CBDB1, BAV1, VS) have recently been sequenced. Using this sequence information, a genus-wide microarray was constructed via the Affymetrix platform. The microarray consists of >4,300 probe sets to target the ~6,000 genes within the four strains. Genes in each strain are targeted by as many unique probe sets as possible so that the respective strains can be differentiated. The constructed microarrays were validated by hybridizing genomic DNA from two of the sequenced strains to the microarrays and the results were as expected, with the probe sets targeting the respective strains responding positively.

Subsequently, the microarrays were used to query the genomes of two unsequenced *Dehalococcoides* strains, ANAS1 and ANAS2. Physiologically, the two isolated strains are different from each other with strain ANAS1 able to dechlorinate trichloroethene (TCE) only to vinyl chloride, while strain ANAS2 can dechlorinate TCE to ethene. Despite the physiological

differences, surprisingly, the genomes of the two strains are similar to each other and to strain 195 except for genes that are within the high plasticity regions or integrated elements. The genomic differences that can be correlated to the different chlorinated ethene usages include the presence and absence of the reductive-dehalogenase (RDase) genes with assigned dechlorination functions. That is, the *tceA* gene is present in ANAS1 while the *vcrA* gene is detected in ANAS2. Overall, the combined genomic and physiological results suggest that the core genome phylogeny of *Dehalococcoides* populations may not be as accurate an indicator of dechlorinating function as the presence of characterized RDases. These results suggest that horizontal gene transfer of RDases is a major driver in shaping the physiology of *Dehalococcoides*.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.063 RAPID AND COMPLETE MICROBIAL REDUCTIVE DECHLORINATION OF CHLOROFORM.

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Bioremediation of organochlorine polluted environments is a well established technique which utilises dehalogenating bacteria which reductively dechlorinate organochlorines to their hydrocarbon counterpart. One draw back of bioremediation is that some organochlorines are inhibitory to microbial processes e.g. Chloroform (trichloromethane). A key to the advancement of the bioremediation industry is the discovery of dehalogenating bacteria capable of chloroform respiration. Only one such discovery exists to date where a *Dehalobacter* population was demonstrated to be capable of transforming chloroform to dichloromethane. Here we report the discovery of a microbial population capable of rapidly mineralising high concentrations of chloroform. Cultures were established with sediment sampled 4.5 m below ground surface from an aquifer polluted for over 40 years with a mixture of organochlorines. Cultures in mineral media were amended with emulsified vegetable oil, 1,2-dichloroethane (500 μM) and chloroform (170 μM). A lag of 90 days was observed before complete removal of both organochlorines occurred over a further 14 days. Subsequent re-administration of chloroform showed consistent improvement in dechlorination rates to a point where 420 μM was mineralized within 3 days. Transferred cultures (10%) fed with hydrogen and acetate showed conversion of chloroform (420 μM) to dichloromethane over 15 days. This finding suggests that this conversion is due to dehalo-respiration. Subsequent depletion of dichloromethane was much slower than in parent cultures suggesting more than one micro-organism is responsible for the complete mineralisation of chloroform. Microbial community analysis has revealed the presence of *Dehalobacter* species in the culture at abundances too low to explain the observed chloroform removal rate. This consortium is of great fundamental and commercial interest for both its chloroform respiring properties and its tolerance to high solvent concentrations.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.064 IN SITU DETECTION OF LOW COPY GENES IN THE RADIONUCLIDE TRANSFORMING AND REDUCTIVELY DECHLORINATING GENUS

ANAEROMYXOBACTER WITH RING-FISH

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Anaeromyxobacter spp. show great potential for bioremediation of heavy metals, radionuclides and chloro-organic substances. A variety of strains have been isolated that exhibit different degradative capabilities. Unfortunately, these strains cannot be distinguished solely based on 16S rRNA gene similarities. To explore a focused, intelligent, bioremediation-based design that encompasses the full range of complex microbial ecological interactions in a community, reliable and rapid analytical tools for real time in situ detection of target cells and their functions and activities are needed. To achieve this goal, we compared an array of in situ detection tools for targeting: i) alternative ribosomal genes with standard FISH protocols, and ii) functional genes encoding cytochrome C oxidase, nitrous oxide reductase, and reductive dehalogenase by RING (Recognition of INdividual single Genes)-FISH. In contrast to mRNA CARD-FISH, RING-FISH has not been explored in great depth. To date, confounding experiences have been observed when using RING-FISH, suggesting that this protocol has not been fully optimized. To explore the potential applications and define the actual limits of RING-FISH detection in mixed communities, we applied RING-FISH to individual strains and mixed consortia containing Anaeromyxobacter and other dechlorinating and metal-reducing bacteria. Depending on the organisms and the specific target genes, RING-FISH yielded reproducible results with some of the probes and samples, while data generated with other probes and certain samples were highly variable. Our current understanding of the optimal design of RING-FISH probes and the physical-molecular mechanisms leading to the probe signals is thus incomplete. However, based on current results, new guidelines for the optimal design of RING-FISH probes and modified hybridization protocols could be identified to improve specificity and reproducibility.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.065 SCREENING ALASKAN TREES FOR RHIZOREMEDIATION POTENTIAL USING THE GEOCHIP AND 454-PYROSEQUENCING

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Rhizoremediation relies on plants to biostimulate microbial degradation of pollutants, such as polychlorinated biphenyls, aromatic hydrocarbons and pesticides. A hypothesized mechanism for this plant-microbe interaction is via the release of plant secondary metabolites into the root zone. Alaskan tree species are particularly promising candidates for rhizoremediation because of their

high quantity and diversity of secondary compounds. However, little is known about the capacity for Alaskan tree species to biostimulate pollutant degradation or the biodegradative potential of Alaskan forest soils. The GeoChip microarray enables the study of bacterial, fungal and archaeal functional genes involved in biogeochemical and ecological processes in a rapid, high throughput and semi-quantitative fashion. The GeoChip was used to screen the microbial functional potential of root zone soils associated with four vegetation types, which were dominated with either black spruce, white spruce, paper birch or aspen, located within the Bonanza Creek Long Term Ecological Research Site (BNZ LTER). Microbial community composition and structure was also investigated using 454-pyrosequencing. Preliminary results indicate that microbes with the genetic potential to degrade chlorinated solvents, hydrocarbons, herbicides, pesticides, and other aromatic compounds are present in varying amounts in the rhizosphere of each tree species. Our research suggests that genes for aromatic pollutant degradation are present in measurable amounts in a pristine forest ecosystem and vary in abundance and diversity with dominant tree species.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.066 INVESTIGATING CARBON FLOW THROUGH A PCB-DEGRADING SOIL COMMUNITY USING STABLE ISOTOPE PROBING

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Stable isotope probing (SIP) is an elegant technique which involves tracking isotopically-labeled substrates into microbial biomarkers that provide phylogenetic information. In addition to enabling the identification of substrate-utilizers, SIP also has the potential to investigate synergistic biodegradation and carbon flow through microbial ecosystems during extended incubations. In this study, we identify microbes responsible for degradation of biphenyl, which is an analogue of polychlorinated biphenyls (PCBs), and benzoate, a product/intermediate of biphenyl degradation and analogue of chlorobenzoates. By performing incubation time-courses, we aim to also track carbon flow through the biphenyl-degrading consortium. ¹³C-labeled biphenyl or ¹³C-benzoate were added to microcosms containing PCB contaminated soils from Drury Gulch, Kodiak, Alaska. ¹³CO₂ respiration was assessed at days 1, 4, and 14 using GC-IRMS and confirmed the degradation of both ¹³C-labeled biphenyl and benzoate in the microcosms. ¹³C-labeled DNA was isolated by density-gradient centrifugation and T-RFLP analyses revealed distinct community structures for biphenyl and benzoate utilizers. Community sequence analysis of bacterial 16S rRNA genes are underway using 454-Pyrosequencing. SIP performed in parallel with multiple substrates is being applied to reveal the microbes performing the upper (biphenyl) and lower (benzoate) degradation pathways, revealing information concerning synergistic biodegradation and carbon flow in weathered PCB contaminated soil systems.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.067 BIODEGRADATION OF POLYISOPRENE (RUBBER) BY NOVEL STRAINS OF ACTINOMYCETES

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Rubber waste (i.e used tires and latex gloves) is becoming a worldwide waste disposal problem. Current procedures are primarily based on land-filling or recycling don't provide satisfactory solutions. Hence, we have focused on development of microbial biodegradation process of waste rubber. During this study we used enrichment techniques to isolate microorganisms capable utilizing rubber as sole carbon source. This enrichment yielded two actinomycetes strains (*Actinomadura* sp. and *Streptomyces* sp.) which were isolated from disposed tire piles. this strains were found capable of degrading rubber (cis-1,4 polyisoprene - latex). Initial extracellular biodegradation-activity was evaluated by measuring the radial clear zones on latex agar plates. This was followed by H-NMR and FTIR analyses of synthetic cis- 1,4 polyisoprene in culture containing the tested actinomycete. FTIR analysis revealed changes in polymer structure. Polyisoprene exposed to *Actinomadura* sp. FTIR spectra showed a new carbonyl peak (1717 cm⁻¹) followed incubation time of 10 days. H-NMR analysis determined the carbonyl peak as an aldehyde group (peak at 9.5 ppm). On the other hand FTIR and H-NMR spectra of polyisoprene exposed to *Streptomyces* sp. showed structural changes but no new peak at the carbonyl area. These results suggest biodegradation of polyisoprene by oxidative cleavage mechanism by *Actinomadura* sp.. Conversely, the absence of oxidative effect in *Streptomyces* sp. in spite of the production of clear zones might be a result of a different mechanism of biodegradation which need further study.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.068 POPULATION DYNAMICS IN BIOAUGMENTED SEQUENCING BATCH REACTORS FOR TREATMENT OF BIPHENYL WASTEWATER

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Dyella ginsengisoli LA-4 with the ability to degrade biphenyl was previously isolated from activated sludge. The bioaugmentation of biphenyl removal by strain LA-4 in sequencing batch reactors (SBRs) was studied in this study. The results showed that the cells of strain LA-4 can shorten the start-up time of the system. Different inoculums had the significant impacts on bioaugmentation, and the results demonstrated that the system with 3.96% inoculums had the strongest removal efficiency, which had the relatively stable function when the biphenyl concentration of influent was 500-1000 mg/L. The PCR-DGGE was used to reveal the structural changes of microbial community in the treatment system. The results showed that the microbial diversity was decreased in the activated sludge system and 1.98% inoculums system. As the biphenyl concentration was increasing, strain LA-4 was disappeared in 1.98% inoculums system. However, the introduced strain LA-4 was persistent in the augmented systems inoculated 3.96% strain.

Abstract Category
07 Recalcitrant Compound Degradation

PS.07.069 BIOLOGICAL REDUCTIVE DECHLORINATION OF PARA-CHLOROANILINE AND DEGRADATION OF ANILINE IN SEDIMENTS FROM AN INDUSTRIAL SITE

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Aniline and para-chloroaniline (PCA) are high volume production chemicals that are often found as environmental contaminants. We studied aniline and PCA degradation and PCA dechlorination under anaerobic conditions in sediments from an industrial site contaminated with aniline and PCA, in addition to other organic and halo-organic pollutants. Sediment microcosms spiked with aniline or PCA were established for groundwater aquifer and canal sediments under nitrate-reducing, Fe (III)-reducing, sulfate-reducing and methanogenic conditions using 20% sediment slurry in site water. Two locations were studied within the site-contaminated and background (lightly contaminated). Aniline produced via dechlorination of PCA was confirmed by gas chromatography-mass spectrometry analysis following derivatization. Bacterial community analysis was performed using polymerase chain reaction coupled to denaturing gradient gel electrophoresis (PCR-DGGE). Aquifer and canal sediment microcosms showed loss of aniline under nitrate-reducing, Fe (III)-reducing and sulfidogenic conditions, and PCA loss under nitrate-reducing conditions. In methanogenic microcosms of background canal sediment we observed loss of PCA concomitant with aniline production when lactate was added as an electron donor/hydrogen source. PCA loss was not observed under methanogenic conditions in microcosms prepared using background or contaminated aquifer sediments, or contaminated canal sediment. Aniline loss was also observed in separate microcosms prepared under methanogenic conditions using the background canal sediments. The terminal daughter products of dechlorination of highly chlorinated anilines have previously been reported to be di- or monochloroanilines. Results from this study support the possibility for complete dechlorination of the monochloroaniline, PCA, to aniline and subsequent degradation of aniline under anaerobic conditions.

Abstract Category
07 Recalcitrant Compound Degradation

PS.07.070 SCREENING FOR FUNCTIONAL GENES CONFERRING RDX ACTIVITY IN GENOMIC DNA LIBRARIES.

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RDX or hexahydro-1,3,5-trinitro-1,3,5-triazine is an explosive commonly used in military training. It is of particular concern due to its carcinogenic effects and recalcitrant nature. RDX is also highly motile in soil and thus pollution of water is a major concern. A number of microorganisms have been found degrade RDX *ex-situ*; however RDX degradation *in-situ* is still relatively uncertain. Pure culture studies have provided insight into enzymes involved in RDX

degradation, in particular one only found in RDX degrading microorganisms, a cytochrome P450 enzyme, *xplA*. *xplA* has been found to remove the nitro groups from RDX, the initial step in RDX breakdown. The genes required for this enzyme have only been detected in areas with previous RDX exposure.

We have made use of a high throughput assay for the screening of recombinant libraries with the ability of detecting functional genes involved in RDX degradation, in particular the *xplA* gene. This assay utilizes the Griess assay under the assumption that RDX degradation is initiated by the release of a nitro group from RDX as nitrite, which can subsequently be detected. It has been successfully applied to the detection of the *xplA* gene in a *Rhodococcus* 11Y genome library. The work presented here illustrates the findings when this assay was used in conjunction with Stable Isotope Probing, whereby soils showing active RDX degradation were exposed to ¹⁵N-RDX. Once degradation had occurred ¹⁵N labeled DNA was extracted via ultracentrifugation, with the ¹⁵N-DNA used to create a genomic library. This library was then screened for functional RDX degrading genes using the Griess assay. A second screen of a gene library from an RDX enrichment culture was also carried out.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.071 BIOSTIMULATION DISRUPTS AQUIFER EUKARYOTE COMMUNITIES

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Enhanced anaerobic bioremediation of pollutants in aquifers via electron donor amendment is an attractive strategy for site clean-up because it is often cheaper than physical/chemical alternatives and can be performed in situ with naturally occurring microorganisms. Diametrically, the long term consequences of the bioremediation approach are rarely investigated and poorly understood. Our objective was to determine if electron donor amendment is a disturbance to aquifer microbial communities. The aquifer under investigation is in southeast Iowa near the Iowa Army Ammunition Plant. It is contaminated with the explosive 1,3,5-trinitro-1,3,5-triazine (RDX) at concentrations of 150 µg/l or less. Acetate was periodically injected into the groundwater from October 2007- May 2009 with the aim of creating sustained RDX reducing conditions. Organisms were collected during this time and community rRNA genes (from eubacteria and eukarya) were PCR-amplified from whole community DNA extract. Denaturing gradient gel electrophoresis was performed to identify proportions of taxa in the groundwater samples. Differences in profiles were visualized using detrended correspondence analysis (DCA) Eukaryotic Shannon diversity significantly decreased as dissolved oxygen concentrations decreased ($R^2 = 0.72$, $p < 0.001$) during biostimulation consistent with observations in disturbed surface waters. Additionally, DCA indicated eukaryotic community structure irreversibly shifted from its native condition in contrast to prokaryotic community structure observed to recover within 7 weeks. The eukaryote shift was manifest as replacement of the nine most abundant taxa following biostimulation. Our data indicates adding carbon to this aquifer disturbed the eukaryotic community. These results expose aquifer eukaryote sensitivity to pollution and will

guide efforts to preserve novel subterranean taxa. Reactivity of eukaryotes to environmental change may also prove useful as an indicator of aquifer state

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.072 ECOLOGY AND DISTRIBUTION OF BACTERIA CAPABLE OF ANAEROBIC TRANSFORMATION OF CHLORINATED METHANES

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Chloromethane (CM), dichloromethane (DCM), chloroform (CF), and carbon tetrachloride (CT), industrially produced at a scale of several million tons per year, are common groundwater contaminants. Because chlorinated methanes are also formed via natural processes, exposed microorganisms may exploit these compounds as sources of energy or carbon. Only three bacterial cultures are known to grow at the expense of CF, DCM, or CM transformation, yet the distribution of these organisms in the environment is unclear. Our ability to predict and control the environmental fate of chlorinated methanes is inhibited by a lack of knowledge regarding the microorganisms, pathways, and transformation mechanisms. To address these shortcomings, sediment samples from 45 geographically distinct locations (3 sites with and 42 sites without reported contamination) were used to establish sediment slurry microcosms amended with 5 mM lactate or 0.3 mM carbon dioxide and up to 0.3 mM CT, CF, or DCM. Microcosms incubated at room temperature were monitored for chlorinated methane consumption. In microcosms established with materials from 5 sites, CT transformation occurred in live incubations but not in killed controls. In contrast, CT reductive dechlorination to non-stoichiometric amounts of CF occurred in 38 live microcosms and corresponding killed controls. CF transformation was not observed. DCM concentrations were reduced to below detection after 5 weeks of incubation in 13 of 45 carbon dioxide amended microcosms, and consumption rates increased upon consecutive amendments. Interestingly, only 6 lactate-amended microcosms exhibited DCM transformation, suggesting that carbon dioxide enhanced DCM consumption. Supernatants from microcosms showing robust DCM transformation activity were transferred to fresh medium to establish sediment-free enrichment cultures, obtain mass balances, and elucidate dominant DCM transformation pathway(s) at both contaminated and "pristine" sites.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.073 CHARACTERIZATION OF TRICLOSAN TOLERANT AND TRICLOSAN DEGRADING BACTERIA IN WASTEWATER TREATMENT PLANTS

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Triclosan is an antimicrobial agent widely used in many household and personal care products. Widespread use of this compound has led to the accumulation of triclosan in surface waters, fish and sediments; hence, becoming an emerging and persistent environmental pollutant. The aim of this study has been to determine the identity of triclosan tolerant and triclosan degrading microorganisms and to investigate their identities and their proteomic profiles.

Biodegradation of triclosan by activated sludge biota occurs at approximately 70ng/gSS/day, by first order kinetics. The active triclosan-degrading communities in full-scale wastewater treatment plants and in the enriched reactor samples were identified with stable-isotope probing using ^{13}C -labeled triclosan, and the identities further confirmed by ^{14}C -labeled triclosan microautoradiography in combination with fluorescence in situ hybridization.

The identities of triclosan tolerant population were determined by applying a stable-isotope probing approach using broad mixtures of ^{13}C -substrates with and without triclosan. The triclosan tolerant organisms were sequenced and compared to the overall diversity. The proteomic analysis of isolates was carried out by *in vivo* metabolic labeling (^{15}N and ^{13}C substrates) followed by multi TOF-MS (Protein-SIP).

Results revealed that the amount of most identified proteins (>99%) was unaffected by the presence of triclosan. Nevertheless, abundance of a small number of proteins was significantly increased (>80x) or decreased (>70x). Among these were a membrane Porin-type efflux transporter and a TonB-family receptor protein as well as several metabolic enzymes. The metabolic pathways thus putatively involved in tolerance and degradation of triclosan are being identified and primers designed for these key biomarkers. Expression of the biomarker genes is also being measured using qPCR in wastewater treatment plants and receiving habitats in order to determine regulatory parameters.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.074 GROWTH CHARACTERISTICS OF A PCB DECHLORINATING ULTRAMICROBACTERIUM

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Due to their physicochemical properties, polychlorinated biphenyls (PCBs) have been widely used for industrial application, resulting in the accumulation and persistence of these highly stable and toxic compounds in the sediment of aquatic environments. Some microorganisms are able to transform PCBs by a process of anaerobic dehalorespiration. However, these microorganisms are difficult to culture in sediment free media and few have been isolated in pure culture. The recent isolation of the strain DF-1, which is capable of anaerobically dechlorinating PCBs, offers the chance to better understand the anaerobic dechlorination process and to identify the physiological factors that influence the transformation of PCBs in anaerobic conditions. This objective requires accurate tools to monitor in parallel the growth and the physiology of this slow growing ultramicrobacterium. A qPCR approach combined with dechlorination activity assays

enabled us to determine the growth characteristics of DF-1 and identify factors that accelerate the dechlorination rate of PCB congeners. The results of these studies are presented.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.075 MICROBIAL REDUCTIVE DEBROMINATION BY MARINE ASSOCIATED BACTERIA RESULTS IN STABLE ISOTOPE FRACTIONATION

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The distribution of organobrominated compounds is ubiquitous and in recent years there has been a rising concern regarding their possible impact on the environment. Microbial degradation is one of the key factors determining the ultimate fate of the organohalides. One approach of monitoring biodegradation of organobromines is to measure the stable isotopic shifts in carbon bonds during the initial steps of microbial transformation. We have previously been able to enrich debrominating bacterial communities from different sediment samples and sponge species, including *Aplysina aerophoba*. In this study, we determined whether the debromination sustained by sponge and sediment cultures resulted in stable isotope fractionation. These cultures were supplemented with 2,6-dibromophenol as a model organobromine compound. Samples taken over a two week period were analyzed by high performance liquid chromatography for debromination. Organobromines were then extracted for stable isotope fractionation analysis by gas chromatography combustion isotope ratio mass spectroscopy. Findings indicated that *Desulfoluna spongiiphila*, a debrominating bacterium isolated from *A. aerophoba*, displayed a carbon isotope fractionation of $\alpha_C = 1.00309$. Likewise, the enrichment culture from a Peruvian sponge, also demonstrated a similar fractionation ($\alpha_C = 1.00248$). Furthermore, a sediment enrichment culture, collected from the New Jersey coast exhibited a slightly lower, but not significantly different isotope fractionation with $\alpha_C = 1.00195$. Interestingly, in vivo sponge experiment resulted in a carbon fractionation that was 10x lower than enrichment cultures tested. Overall the debromination of the bromophenolic resulted in a stable isotopic fractionation that indicates highly similar enzymes or metabolic pathways were involved from the various cultures tested. These results will be important for interpreting any stable isotope signal observed for organobromine compounds in a marine environment

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.076 INTEGRATED PROTEOMIC, GENOMIC AND METABOLOMIC ANALYSES OF PYRENE DEGRADATION PATHWAY IN *NOVOSPHINGBIUM PENTAROMATIVORANS* US6-1

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous organic pollutants which have contaminated the environment through improper disposal of materials such as creosote, coal tar, and hydrocarbon fuels. The high molecular weight (HMW) PAHs (four or more fused rings) are of particular environmental concern because of their potential mutagenicity and carcinogenicity in mammalian test organisms and in a number of critical aquatic species. Degradation of Pyrene in *Novosphingobium pentaromativorans* US6-1 was investigated using a combination of metabolomic, genomic, and proteomic technologies. Total proteins were separated by one- and two-dimensional gel electrophoresis after pyrene exposure, and then identified by liquid chromatography/tandem mass spectrometry (LC-MS/MS). Genome sequence data were obtained and aromatic catabolic genes and tricarboxylic acid (TCA) cycle genes that are responsible for the complete degradation of PAHs have been particularly focused on. Pyrene metabolites were isolated from the culture medium and analyzed by high-pressure liquid chromatography (HPLC) and nuclear magnetic resonance (NMR). By integrating genomic annotation information with identified metabolites and proteins, we proposed a pyrene degradation pathway for *N. pentaromativorans* US6-1. Degradation of pyrene in *N. pentaromativorans* US6-1 proceeds via multiple metabolic routes initiated by mono-(C-1,2 and C-4,5) and dioxygenation (C-4,5) reactions, further degradation via either *o*-phthalate pathway or salicylate pathway, both pathways were subsequently entered TCA cycle. In monooxygenation reaction, 1-Pyrenylsulfate and 1,6-Dimethoxypyrene were not previously reported as intermediates in the degradation of pyrene by bacterial strains, demonstrate new branches in the pathway. Results in this study establish a basis for the understanding of metabolisms of microbial degradation of HMW PAHs, which is necessary in order to design efficient and predictable bioremediation procedures.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.077 ANAEROBIC BIODETERIORATION OF DIESEL FUEL AND BIOCORROSION

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The desulfurization of diesel may have an impact on the fuel infrastructure. We hypothesize that the degree of sulfur removal increases both anaerobic fuel biodegradation and metal biocorrosion. To test this, diesel cuts with various sulfur amounts were incubated with a marine inoculum from a seawater compensated ballast tank obtained from a US Navy ship. We also evaluated the inhibitory effect of selected organosulfur compounds on a known hydrocarbon (HC)-degrading methanogenic consortium. Lastly we tested *Desulfoglaeba alkanexedens*, a known alkane-degrading sulfate reducing bacterium (SRB), for its ability to biocorrode carbon steel.

Inoculum was collected from the USS Gettysburg and different diesel formulations were obtained from a refinery. Sulfate loss was monitored by ion chromatography and methane production by gas chromatography. DNA was extracted from the ballast water and assayed for the presence of alkylsuccinate synthase (ass) genes. Biocorrosion in resting cell suspensions of

D. alkanexedens were electrochemically monitored and analyzed by light and electron microscopy.

Sulfate loss varied as a function of the fuel sulfur content with rates of $17 \pm 2.6 \mu\text{M/d}$ in high sulfur incubations to $48 \pm 4.8 \mu\text{M/d}$ with ultra low sulfur diesel incubations. The *ass* gene was found in the ballast water suggesting that sulfate loss might be coupled with anaerobic HC decay. The effect of organosulfur compounds on HC methanogenesis was inconclusive. However, a corrosion rate of $3.09 \pm 2.2 \text{ mils/y}$ and localized corrosion was observed with the pure culture, while an abiotic control (20mM NaS) exhibited uniform corrosion and only $1.5 \pm 0.6 \text{ mils/y}$.

The removal of sulfur from fuels makes them more susceptible to microbial decay and a model HC-degrading SRB differentially caused pitting corrosion. These results suggest that new formulations can be associated with increased fuel deterioration and metal loss.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.078 THE FORMATION MECHANISM OF BIOLOGICALLY IMMOBILIZED CERAMIC PARTICLES AND THE DISTRIBUTION OF MICROORGANISMS

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Immobilized microorganism technology is an effective method of removing water pollutant in source waters. The effect of biologically immobilized ceramic particles is better than simple filter or engineering bacteria liquid in removing the pollutant, due to the two factors including the adsorption and entrapment function of the filter materials in the biofilter and the biodegradation of the microorganisms. This study analyzed the formation mechanism of biologically immobilized ceramic particles: the large pore on the surface of ceramic particles entrapped the microorganisms, with its surface positively charged to adsorb the microorganisms; then, the amorphous matter secreted by the microorganisms made them immobilized on the ceramic particles. Finally, the variation of microorganism layers in the immobilized bioreactor was observed and investigated to reveal the succession regulations of microorganisms and the characteristics of different layers of microorganisms.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.079 INFLUENCE OF CELL SURFACE ENGINEERING ON PHYSIOLOGICAL PROPERTIES OF YEAST HEAVY METAL BIOSORBENT

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The yeast *Saccharomyces cerevisiae* has good ability to sorption wide range of heavy metals; its cultivation is well managed. The number of high—affinity covalent ligand—metal interactions in *S. cerevisiae* W303 was increased by using carboxyl—terminal part of sexual adhesion

glycoprotein α -agglutinin (AGa1Cp) as an anchoring domain for surface display of short metal binding peptides in the cell wall. The aim of the study was to assess the influence of the genetic modifications on technological properties of prepared strains and the possibility of their application in biofilm based processes as metal sorbents.

The yeast strains were cultivated in media containing variable C— and/or N—sources. Growth rate and yield coefficient were calculated. Cell surface hydrophobicity was assayed by method of microbial adhesion to hydrocarbons. X—ray photoelectron spectroscopy was used for characterization of cell surfaces. The morphological parameters of cells and the biofilm formation were monitored using image analysis.

Cell surface engineered strains reached higher growth rate and yield coefficients on the all used C—sources. Enrichment of the surface of *S. cerevisiae* with the C—terminal part of mannoprotein α -agglutinin resulted in a significant increasing of cell surface hydrophobicity. The strain had also significantly higher ability to colonize both hydrophobic (silicon) and hydrophilic (glass) carriers. Statistical analysis of results showed strong (95%) correlation between the presence of C=O / O—C—O groups of the cell surface and its hydrophobicity.

The cell surface engineered strain enriched with the C—terminal part of mannoprotein α -agglutinin, possessing increased heavy metal sorption capacity, showed excellent growth characteristics and good ability biofilm formation. It is thus a good candidate for development of biofilm—based heavy metals sorption processes.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.080 PHYLOGENETIC ANALYSIS OF BACTERIAL COMMUNITIES IN SOIL CONTAMINATED WITH PHENANTHRENE AND DIFFERENT CONCENTRATIONS OF CHROMIUM(VI)

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The objective of this study was to investigate the impact of Cr(VI) concentration on the diversity of soil bacterial community and phenanthrene degrading activity, during bioremediation process of phenanthrene and Cr(VI) co-contaminated soil. Soil microcosms contaminated with 2000 mg of phenanthrene and different Cr(VI) concentrations (0, 25, 50, 100, 200 and 500 mg) per Kg of dry soil, were studied. Temperature and soil moisture were controlled to support bioremediation. Periodically phenanthrene concentration (GC-FID) and the water exchangeable Cr(VI) fraction (WEF) (diphenylcarbazide assay) were determined. In order to investigate the changes in the genetic diversity of the soil bacterial communities, DGGE analysis was performed at different treatment times and dominant bands were excised and reamplified. The purified amplicons were cloned and the inserts were sequenced. Nucleotide sequences were compared to sequences in the GenBank database and phylogenetic tree was performed. Although all contaminated microcosms showed a complete phenanthrene elimination, the presence of Cr(VI) caused a initial lag phase with a low phenanthrene elimination rate, this lag phase was significantly higher (35 days) in the microcosm contaminated with 500 mg kg⁻¹ of Cr(VI). Also, a major persistence of Cr(VI) in the

WEF was observed in this microcosm. Phylogenetic analysis showed that the highest Cr(VI) concentration produced a selective shift on the predominant bacterial populations of the phenanthrene degrading community. In the soil microcosms contaminated with up to 200 mg kg⁻¹ of Cr(VI), the sequences of predominant DGGE bands were affiliated with the Sphingomonadaceae family. The DGGE profiles of the microcosm contaminated with 500 mg kg⁻¹ of Cr(VI) presented a predominant band, that appears only after 38 days of treatments, and which sequence showed a phylogenetic relationship with the Micrococcaceae family, most closely matched that *Arthrobacter* sp.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.081 MOLECULAR AND PHYSIOLOGICAL CHARACTERIZATION OF A HIGHLY ENRICHED *DEHALOCOCCOIDES* CONTAINING CULTURE

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A *Dehalococcoides* dominated culture coupling growth to the reductive dechlorination of tetrachloroethene to ethene was enriched from groundwater of the contaminated field site in Bitterfeld, Germany. *Dehalococcoides* spp. are known to be capable of the reductive dehalogenation of a wide variety of halogenated organic compounds. Therefore, a molecular and physiological characterization of the enrichment culture was performed. The overall microbial diversity in the culture was assessed, putative reductive dehalogenases were identified and the reductive dehalogenation of diverse halogenated organic compounds by the culture was tested. The PCE grown enrichment culture was transferred to test for the dehalogenation of various organic contaminants including chlorobenzenes, pentachlorophenol, vinyl bromide and dichloroethane. Growth was assessed by applying qPCR targeting *Dehalococcoides* spp. The presence and diversity of putative reductive dehalogenases were tested by PCR using degenerate primers. Additionally, a 16S rRNA gene clone library was prepared to assess the overall diversity of the microbial community in the enrichment culture.

Growth coupled to dehalogenation could be proven for several halogenated substrates including vinyl bromide and 1,2-dichloroethane dehalogenation to ethene or hexa-, penta- and tetrachlorobenzene dehalogenation to trichlorobenzenes. In addition, pentachlorophenol and γ -hexachlorocyclohexane were dehalogenated by the cultures as well. Nine novel putative reductive dehalogenases were identified. The 16S rRNA gene clone library confirmed that >80% of clones were affiliated with *Dehalococcoides* spp.

The highly enriched *Dehalococcoides* culture was capable of growth coupled to dehalogenation of a wide variety of halogenated substrates. Further research should allow identification of the function of the at least 9 novel putative reductive dehalogenases present in this culture.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.082 ANOXIC BIOTRANSFORMATION OF MONO- AND DICHLOROBENZENE

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Monochlorobenzene (MCB) and dichlorobenzenes (DCB) form large plumes at contaminated field sites indicating slow anaerobic degradation. MCB and DCB are formed during microbial degradation of hexachlorocyclohexanes and higher chlorinated benzenes or enter the aquifer as chemicals. Thus far, information related to anaerobic biodegradation of MCB and the DCB is very limited.

Two model sand columns were provided with anoxic, sulfate rich, contaminated groundwater containing MCB and, in one parallel, 1,2-DCB. Laboratory microcosms were prepared under strict anoxic conditions from the MCB contaminated groundwater and amendments of electron donor or electron acceptor were made to test anaerobic oxidation or reductive biotransformation, respectively.

In both sand columns, reductive dehalogenation of 1,2-DCB to benzene was indicated by the change in concentration and carbon isotope compositions. MCB concentrations did not significantly change comparing the inflow to the outflow. In the columns, mobilization of Fe²⁺ was observed, indicating the presence of iron reducing processes. Microbial community analysis using DGGE showed no significant differences between inflow and outflow of the columns. Sequences derived from the major bands were related to *Desulfobacterales*. ¹³CO₂ production in laboratory microcosms amended with ¹³C-labeled MCB or 1,2-DCB suggested the presence of microorganisms capable of mineralization of MCB and DCB. Nitrate or Fe³⁺ addition to microcosms stimulated the mineralization of MCB compared to the controls.

The potential for anoxic oxidation and reductive dehalogenation of mono- and dichlorobenzene was present in the groundwater and column systems in Bitterfeld but further research is required to confirm the activity and to investigate the degradation pathway and the responsible microbial community.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.083 ASSESSMENT OF QUANTITATIVE DETECTION OF ATRAZINE DEGRADATION GENES IN BOREAL AND TROPICAL SOILS

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Atrazine and its degradation products are globally among the most commonly found pesticides in groundwater. Its aerobic degradation pathway and genes for complete mineralization and the corresponding genes are well described in *Pseudomonas ADP*. The initial degradation reaction is coded by *atzA* and *atzB* genes. The aim of this work is to assess whether the presence and abundance of these genes correspond to the atrazine mineralization potential of soils in different climates. Soil was collected in Finland at a site where low amounts of atrazine and its

degradation products had been observed in the groundwater, and agricultural fields in India with different atrazine application histories. DNA was extracted from these soils and *atzA* and *atzB* genes were quantified with quantitative PCR. The atrazine mineralization of the soils was tested by microcosm studies using ^{14}C -ring-labelled atrazine at 10°C and 30°C. In the Finnish soil samples less than 3 % mineralization was observed during 25 days at 30 °C. The *atzA* gene was detected in amounts of 10^3 gene copies g^{-1} soil (dw) by qPCR. In this soil the amplified gene fragment and melting peak profiles were similar to those of the positive control, *atzA* and *atzB* of *Pseudomonas ADP*. The Indian soils showed mineralization up to 52% at 30 °C, and the *atzA* gene abundance was up to 10^5 gene copies g^{-1} soil (dw). Melting peaks of the PCR products were similar to that of the control, but the size observed on agarose gel was larger, and its sequence needs to be analyzed. These preliminary results indicate that the amount of *atz* genes in soil reflects the degradation potential of soils. The *atz* genes were more abundant in soils with recent atrazine addition, but also soils with no recent atrazine application some genes were found. The applicability of the qPCR method for *atzA* and *atzB* genes in environmental samples requires a thorough validation.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.084 ANALYSIS OF MOLECULAR WEIGHT DISTRIBUTION DEGRADED LIQUID POLYSTYRENE BY *Bacillus thuringiensis* STR-Y-O

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Expanded polystyrene (EPS) is widely used in various applications. Due to its lightness, it is commonly used as a buffer in packaging, such as for the cushioning of consumer electronics. EPS material that cannot be recycled, due to concerns with smell or other contaminants that have become attached, has been disposed of in landfills and where possible by incineration. These methods of disposal cause various environmental problems. Considering these matters, the aim of this article is to construct a bio-recycling strategy by elucidating a mechanism for polystyrene (PS) degradation via use of *Bacillus thuringiensis* STR-Y-O. STR-Y-O appears to be capable of degrading polystyrene to the styrene monomer, and further decomposing the styrene monomer to both H₂O and CO₂. The polystyrene degrading process can be activated by the addition of limonene as a solvent. We analyzed the molecular weight distribution of degraded liquid polystyrene by STR-Y-O.

STR-Y-O was cultured in a 100ml flask containing 20ml of M9 medium and cultured at 35 °C with a rotary shaker set to 140rpm until reaching the logarithmic phase (OD₆₀₀ is 0.2). When OD₆₀₀ was reached at 0.2, the EPS was dissolved in 200 μ l (0.3mg/ml) of limonene, which was then added to the STR-Y-O cultured medium. The PS/STR-Y-O medium was cultured for eight days. The degraded PS in this cultivated medium was extracted using dichloromethane. The molecular weight distribution analysis of degraded liquid polystyrene was carried out with a gel permeation chromatograph (GPC).

As a result of the GPC analysis, no significant peaks were detected. The degraded material registered a low molecular weight of about 1,000 from an initial mean molecular weight of 11,672. The results reveal that most of the polystyrene was degraded through the STR-Y-O reaction from polymers to monomers, eventually leading to inorganic matter such as CO₂ and H₂O.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.085 EXPRESSION OF MULTIPLE α -KETOGLUTARATE-DEPENDENT DIOXYGENASE GENES DURING DEGRADATION OF THE HERBICIDE (R,S)-DICHLORPROP IN AGRICULTURAL SOIL

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The *tfdA* gene encodes an α -ketoglutarate-dependent (α -KG) dioxygenase that initiates the degradation pathway of many phenoxyalkanoic acid (PAA) herbicides. Recently, two novel α -KG dioxygenases encoded by *rdpA* and *sdpA* genes have been described and linked to stereospecific degradation of chiral PAA herbicides. It is still unclear whether multiple α -KG dioxygenase-encoding genes are actively expressed during the degradation of PAA herbicides in soil. The present study focused on the presence and activity of multiple indigenous α -KG dioxygenase-encoding genes (*rdpA*, *sdpA*, and *tfdA* group I) during degradation of the chiral herbicide (R,S)-dichlorprop. Agricultural soil was amended twice with 20 mg kg⁻¹ of (R,S)-dichlorprop and trace amounts of (R,S)-[Ring-U-¹⁴C]-dichlorprop. Soil samples were taken at appropriate time intervals over two cycles of biodegradation. Presence and activity of multiple dioxygenase-encoding genes were followed by quantitative real-time PCR of DNA and mRNA while DGGE and cloning was used to analyze the diversity of indigenous genes and transcripts. The soil microbial community responded to (R,S)-dichlorprop exposure with high growth of potential degraders as measured by the presence of dioxygenase-encoding genes. *rdpA* DNA reached over 10⁶ copies g⁻¹ of soil while *sdpA* and *tfdA* both reached over 10⁵ copies g⁻¹ of soil. All three target genes were transcribed during both cycles of herbicide degradation but a time shift existed between maximum expression of each gene. DGGE gel analysis indicated that the *tfdA* genes present prior to herbicide exposure were not the same as those proliferating over time nor were they dominantly expressed. These findings indicate that multiple α -KG dioxygenase-encoding genes co-exist in soil and can be actively expressed independently of each other to initiate chiral herbicide degradation pathways. This is also the first report describing patterns of *rdpA* and *sdpA* gene expression from an indigenous soil community.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.086 A THREE-LAYER DIFFUSION-CELL TO INVESTIGATE the BIO-ENHANCED DISSOLUTION OF TCE DNAPL

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Reductively dechlorinating bacteria can result in a dissolution enhancement of chloroethene Dense Non-Aqueous Phase Liquids (DNAPL). Nevertheless, different factors are likely to limit this bio-enhanced dissolution. We developed and tested a new lab-scale set-up to investigate the factors limiting the bio-enhanced dissolution of a trichloroethene (TCE) DNAPL. This new set-up is a 12 cm diffusion-cell consisting of three layers, i.e. a 5.5 cm central sand layer, a 3 cm top water layer and a 3.5 cm lower DNAPL layer. The water layer is frequently refreshed to remove TCE and its degradation products and to provide electron donor, while the DNAPL layer maintains the saturated TCE concentration. The resulting concentration gradients induce diffusion of TCE and electron donor through the porous layer in opposite directions. A first experiment tested two abiotic and two biotic diffusion-cells. The sand layer of the biotic cells was inoculated with a dechlorinating culture, while 4 mM formate was added as electron donor to the top water layer. In the abiotic cells, a linear concentration profile evolved. In the biotic cells, TCE had a steeper concentration profile and was completely converted to cis-dichloroethene at 2.5 cm distance of the DNAPL. Dechlorination in the sand layer was likely inhibited up to a distance of 1.5 cm from the DNAPL, as in this part the TCE concentration exceeded the toxic threshold of 2.5 mM. Biotic dissolution fluxes were a factor 2.4 +/- 0.2 enhanced in comparison to abiotic dissolution fluxes. This study showed that a diffusion-cell is a simple lab-scale set-up that allows the measurement of the DNAPL dissolution flux. Future experiments will use the diffusion-cell set-up to investigate the factors limiting the bio-enhanced dissolution of TCE DNAPL.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.087 HOW DYNAMIC IS STABLE? UNEXPECTED REACTIVITY OF KEY CONTAMINANT DEGRADERS TO HYDRAULIC FLUCTUATIONS IN A TAR-OIL CONTAMINATED AQUIFER

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Aquifers harbour mankind's most important drinking water resources, but beyond being regarded as abiotic resource, we are only beginning to grasp their functioning as ecosystems. Especially, aquifers are classically perceived as extremely stable ecosystems, where dynamics occur only over decades, or centuries. Also after anthropogenic pollution, this may require up to centuries for being naturally attenuated. The ecological principles governing natural attenuation are not well understood. However, it has been shown in recent years that microbial contaminant degraders and their activities establish especially in biogeochemical gradient zones surrounding contaminant plumes, where transverse dispersive mixing provides a constant supply of electron donors and acceptors. Here, for the first time, we provide clues on how such specialized degrader populations and their degradation activities are interwoven with on-site hydrodynamics (i.e. shifts in the groundwater table). Such dynamics can be hypothesized to either increase (by increasing the mixing) or decrease (by imposing unfavorable conditions on locally established degraders) net contaminant removal. We combined fine scale depth-resolved monitoring of geochemical parameters as well as toluene degrading microbial communities in a tar-oil contaminated aquifer over almost five years of sampling. The plume zones characterized by highest degradation activities were congruent to the appearance of distinct bacterial community

patterns, as well as high degradation gene abundances. After a pronounced transient oscillation of the water table, however, established degrader populations collapsed, convergent to a marked increase in maximum contaminant concentrations. Our results reveal an unrecognized negative correlation between short-term habitat dynamics and natural attenuation in aquifers. This is an important step towards a better understanding of the ecological controls of contaminant degradation in groundwater ecosystems.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.088 PLANKTONIC VERSUS BIOFILM LIFE-STYLES IN HYDROCARBONS REMEDIATION BY POLYHYDROXYALKANOATES ACCUMULATING *PSEUDOMONAS*

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Oil's contaminated sites are stressful environments for the microorganisms. We hypothesized that some features that increase microbial survival in these environments such as biofilms formation capability or physiological characteristics like reserve substances accumulation (i.e. polyhydroxyalkanoates (PHAs)), could provide to the bacteria some advantages to cope with the environmental stress. We used three polyhydroxyalkanoate producers: *Pseudomonas* sp. KA-08, isolated from a contaminated sludge, and the Antarctic strains *P. extremaustralis* and *P. extremaustralis*/ pGEc4 (transformed with a plasmid containing *alk* genes) to analyze the effect of PHA accumulation in the hydrocarbon degradation under both planktonic and biofilm growth condition. Two types of bacterial inocula- with or without PHA accumulation- were used. Static biofilm in microtiter plates or planktonic cultures in shaken flasks were assayed. Diesel was selected as pollutant to be tested due to its ubiquity in natural environments. One of the hydrocarbon bioremediation problems is the hydrophobic nature of this pollutant that can be solved by the biosurfactant production ability of microorganisms. To analyze the presence of biosurfactants, free cell supernatants were tested by the "drop collapse" assay. The putative surfactants were pre-purified and the chemical nature of the compounds was analyzed by TLC. Diesel degradation was analyzed by GC. Surfactant production and diesel degradation were considerably higher in all strains when PHA was accumulated. *Pseudomonas* sp. KA-08 showed enhanced oil degradation compared with *P. extremaustralis* in both biofilm and planktonic state. Bacteria attached to solid surface (biofilms) were more efficient in diesel degradation than planktonic ones. These results could be useful for environmental biotechnology purposes by analyzing relevant bacterial characteristics to optimize remediation of hydrocarbon contaminated sites.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.089 TRANSCRIPTOMIC ANALYSIS OF BENZOATE AND PHENOL METABOLISM BY HYPERTHERMOPHILIC ARCHEON *FERROGLOBUS PLACIDUS*

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Ferroglobus placidus, a hyperthermophile isolated from a marine vent, constitutes the first report of an archaeon able to couple oxidation of aromatic compounds with Fe(III) oxide reduction. Preliminary analysis of the genome sequence of *F. placidus* revealed genes for the metabolism of benzoate that are homologous to genes found in bacteria that degrade benzoate via CoA ligation, including multiple copies of benzoyl-CoA ligase (*bclA*) and the benzoyl-CoA reductase operon (*bcrABCD*). Sequence analyses indicates that the benzoate degradation pathway of *Rhodospseudomonas palustris* is the most closely related to that of *F. placidus*. For instance, both organisms contain genes that code for all 4 subunits of benzoyl-CoA reductase (*bcrABCD*), whereas not all of them are present in *Geobacter*, *Thauera* or *Azoarcus* species. In addition, both *F. placidus* and *R. palustris* lack the gene that codes for 6-hydroxycyclohex-1-ene-1-carboxyl-CoA dehydrogenase, which appears in all of the benzoate-degrading *Geobacter*, *Thauera*, and *Azoarcus* genomes. The *F. placidus* genome also has genes coding for enzymes that are associated with the initial steps of phenol degradation, phenylphosphate synthase and phenylphosphate carboxylase. These genes are associated with a specific gene cluster, as previously described in *Thauera* and *Geobacter* species. Transcriptomic analyses were conducted in order to elucidate the proteins involved in the oxidation of benzoate and phenol during growth on Fe(III) oxide. Quantitative RT-PCR revealed that genes from the CoA ligation pathway (*bclA*, *bcrC*, *badH*, *dch*, and *oah*) are significantly up-regulated during growth with benzoate or phenol as the sole electron source compared to acetate. These results suggest that, despite its growth at high temperature and the fact that it is a member of the Archaea, *F. placidus* may metabolize aromatic compounds via pathways not significantly different than those of mesophilic bacteria.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.090 AROMATE-DEGRADING ANAEROBES IN A LANDFILL LEACHATE PLUME WITH LOW CONCENTRATIONS OF BTEX

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Leachate of old landfills poses world-wide a serious treat to the environment. We examined the microbiology and biogeochemistry of the aquifer polluted by the Banisveld landfill, Boxtel, the Netherlands, in order to understand and monitor the natural attenuation of landfill leachate better. Benzene, toluene, ethylbenzene and xylene (BTEX; <180 microgram/l) comprised a small part of the Dissolved Organic Carbon (~100 mg/l). BTEX was degraded under iron-reducing conditions. Microbial communities were dominated by anaerobic, iron-reducing *Geobacteraceae*, a family that also contains species able to degrade mono-aromates. Our expectation was that mono-aromate degradation would be performed by *Geobacteraceae*. We examined the presence of anaerobe aromate degraders by culturing and by culture-independent amplification of the genes encoding benzylsuccinate synthase (*bssA*), the enzyme performing the first step in the degradation of alkylbenzenes, and 6-oxocyclohex-1-ene-1-carboxyl-CoA hydrolase (*bamA*), an enzyme in the anaerobic central metabolism of benzoyl-coA. *bssA* sequences closest related to

Georgfuchsia toluolica were dominantly present throughout the aquifer. The iron-reducing Betaproteobacterium *G. toluolica* was isolated from the Banisveld aquifer and is only capable of mono-aromate degradation. In contrast, only a small fraction of *bamA* sequences was closest related to *Georgfuchsia* while over 40% was closest related to *Geobacteraceae*. *bamA* genes were found at about tenfold higher copy numbers than *bssA* genes. This indicates the presence of a considerable pool of microorganisms that possess the benzoyl-coA pathway and degrade other aromates or are involved in syntrophic interactions with BTEX degraders. We suggest that due to its specialization on mono-aromates, *Georgfuchsia* competes successfully with *Geobacter* species for mono-aromates. *Geobacter* species are more generalists and thrive on other carbon sources in landfill leachate.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.091 2,6-DICHLOROBENZAMIDE (BAM) MINERALIZATION BY AMINOBACTER SP. MSH1 DURING STARVATION DEPENDS ON CELL POPULATION MAINTAINING VITAL MEMBRANE FUNCTIONS

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Denmark relies on clean groundwater as its sole, natural source of drinking water; hence, groundwater wells are carefully monitored for residual contaminants such as the important pesticide metabolite 2,6-dichlorobenzamide (BAM). Nevertheless, a future scenario may include efficient water remediation relying on degrader bacteria in sand filters characterized by very low nutrient levels and limited bacterial growth. Aim of this study was to verify maintenance of mineralization capability and physiological state in BAM-degrading *Aminobacter* sp. MSH1 under growth-arrested conditions in mineral salts (MS) solution and natural groundwater. Amendments of ¹⁴C-labelled BAM (0.1mM) were made after up to 8 weeks of growth arrest (C and N starvation) to monitor BAM mineralization; cell survival was monitored by flow cytometry using a panel of vitality stains to differentiate intact, depolarised and dead cells. After up to 3 weeks of starvation in MS solution still showed an immediate burst of growth-linked mineralization after BAM amendment, but a long lag phase (approx. 40 days) for resumption of growth-linked mineralization was seen after 8 weeks of starvation. In contrast, cells in natural groundwater always maintained a low, constant mineralization, and never showed a growth-linked increment of mineralization after BAM amendment. From flow cytometry data we deduced that mineralization took place by a subpopulation of intact cells maintaining vital membrane functions. For comparable BAM levels, the cell-specific mineralisation rate calculated per intact cell was similar in all experiments (10-13 moles BAM-C intact cell⁻¹ day⁻¹) independent of medium (MS solution or groundwater) or starvation period. *Aminobacter* sp. MSH1 seems a suitable candidate for sand-filter remediation of drinking water; our results further suggest that remediation should be optimized towards obtaining a maximum size of the intact cell fraction of degrader bacteria.

Abstract Category
07 Recalcitrant Compound Degradation

PS.07.092 BIODEGRADATION AND STRESS IN THE PHYLLOSHERE

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In soil, aquatic and marine environments, bacterial degradation of organic pollutants has been well studied. However, via deposition or volatile absorption, these pollutants can also accumulate on plant leaves, where they become accessible for degradation by bacteria that colonise the leaf surface, or phyllosphere. The phyllosphere is an environment that evokes high levels of bacterial stress due to rapid changes in humidity, temperature and solar radiation. In this project, we investigated how bacteria cope with phyllosphere stress in relation to their catabolic activity towards pollutants. For this, we used two different approaches. Firstly, we tested the phyllosphere performance of several model strains with different catabolic capabilities. Of these, only 4-chlorophenol degrading strain *Arthrobacter chlorophenolicus* A6 exhibited a phyllosphere fitness that was comparable to our control bacterium, leaf coloniser *Erwinia herbicola* 299R. Secondly, we isolated pollutant-degrading bacteria from two phyllosphere environments: an apple orchard that was treated with the foliar pesticide triadimenol, and a vegetated shoulder along a major Dutch highway. Enrichment cultures were checked for growth on 4-chlorophenol, a degradation product of triadimenol, and toluene, a common traffic-related pollutant. Several 4-chlorophenol degrading strains were recovered from the apple orchard phyllosphere and were all confirmed to belong to the genus *Arthrobacter*. Toluene degrading phyllosphere bacteria from near the highway were identified as *Rhodococcus* sp. Phyllosphere fitness and stress resistance were variable between isolates. One of the *Arthrobacter* strains was an excellent leaf coloniser, superior to A6 and 299R. Ongoing studies are exploiting the available genomic resources for *A. chlorophenolicus* A6 to obtain a transcriptional profile of this strain under phyllosphere conditions and expose any hardwiring between genes involved in stress resistance and pollutant degradation.

Abstract Category
07 Recalcitrant Compound Degradation

PS.07.093 AN ENZYME MATRIX FOR HEMICELLULOSE BREAKDOWN

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The need for renewable and sustainable raw materials for industry and society has become one of the most pressing issues for the 21st century. This has rekindled interest in the use of plant products as industrial raw materials for the production of liquid fuels and other products such as biocomposite materials. It is mostly comprised of cell walls that are composed of energy rich polymers including cellulose, various hemicelluloses and the polyphenol lignin and thus sometimes termed lignocellulosics. Hemicellulose is the second most abundant biopolymer component of plant cell walls. Hemicellulose composition is a complex mix of linear polymers

containing xylose (xylan), arabinan, galactomannan branched xylans as well as glucose oligomers branched with xylose all decorated with acetyl, methyl esters and ferulic acid side chains. The main pentose constituted backbone is broken down by xylanases cross-linking side chains constituted of xyloglucan, arabinan and galactomannan are broken down by xyloglucanases, arabinanases, galactanases and mannanases and finally decorating side chains, which severely interfere with enzyme activity, are removed from the main polymers by acetyl esterases, methyl esterases and ferulic acid esterases. Thus, when xylanases, arabinases, arabinofuranosidases, glucuronidases and ferulic acid esterases and acetyl esterases work together, large quantities of sugars; glucose, xylose, arabinose, galacturonic acid and galactose are generated which are direct substrates for ethanol (biofuel) production. Here we report on high-yield expression and secretion of a comprehensive set of 13 hemicellulases, including xylanases, xylosidase, xylo-endoglucanase, arabinase, arabinofuranosidases, mannanases, glucuronidases, ferulic acid esterase and acetyl esterase. We demonstrate strong synergistic effects on xylans, arabinoxylan and sorghum substrates as well as enhanced fermentable sugar production with addition of ferulic acid esterases and acetyl esterases.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.094 COMPARISON BETWEEN MONITORED NATURAL ATTENUATION AND BIOAUGMENTATION PROCESSES USING A TROPICAL SOIL CONTAMINATED WITH OIL

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Oil spills represent a big threat to the environment. Many times they happen during the petroleum extraction process and the concomitant spill of produced water used in secondary recovery, which usually contains high concentration of salts, can enhance the environmental impact. Bioremediation is an efficient strategy to clean-up oil contaminated sites and different techniques, such as monitored natural attenuation (MNA) and bioaugmentation, are suggested to be applied in impacted areas. In this study, three bacterial strains able to degrade crude oil as the sole carbon source in presence of 7% of NaCl were isolated from a soil sample located in the Northeast of Brazil previously contaminated with crude oil and produced water. These strains were identified as *Gordonia alkanivorans*, *G. rubripertincta* and *Rhodococcus equi* based on their 16S rRNA gene sequences and showed degradation rates, in vitro, of 64.4, 70.4 and 67.7% of TPH and 70.9, 79.6 and 60.1% of total n-alkanes, respectively. To study the potential of these strains in bioremediation processes, microcosms were constructed using soil amended or not with 7% of NaCl and crude oil. Moreover, the present study also aimed to compare bioaugmentation and MNA processes using the microcosms during 90 days, and to evaluate the impact of the selected strains in the native bacterial community. For that, molecular approaches, such as PCR-denaturing gel gradient electrophoresis (DGGE), were used. After 90 days-incubation period, the DGGE profiles showed that while the intensity of the band corresponding to *G. alkanivorans* increased in bioaugmented microcosms samples (with or without NaCl), the bands corresponding to the other two introduced strains disappeared after 30 days. No changes in

the structure of bacterial community were observed in the DGGE profiles when *G. alkanivorans* was added to the microcosms. These results suggest its application for bioaugmentation in this tropical soil.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.095 BIODEGRADATION OF TEXTILE EFFLUENT BY INDIGENOUS FUNGAL CONSORTIA BASED ON BOX-BEHNKEN DESIGN OF RESPONSE SURFACE METHODOLOGY

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The highly contaminated textile effluents has become a matter of concern over the last few decades as it pose severe health problems and solemn impacts on natural water bodies & land in the adjoining areas. In the present study isolated fungal consortia *Penicillium decumbens* 8194 & *Aspergillus foetidus* 8796 are used for the degradation of color and removal of chemical oxygen demand (COD) from textile industry effluent. Numerous factors decide the technical & economic viability of dye removal technique therefore; experiments were designed according to Box-Behnken design matrix of response surface methodology (RSM). Experiments for colour degradation & COD reduction were performed with untreated textile effluent. Various essential nutrients for finest growth of microbial cultures were added in the form of Potato dextrose broth. Experiments were performed for the pre- determined incubation period of 6 days under shaking aerobic conditions. Liquid aliquots were withdrawn; filtered, centrifuged & clear supernatant was scanned spectrophotometrically at lambda max for percent colour removal. Standard dichromate reflux method was used to analyse COD. The influence of process variables, temperature & pH on reduction of COD & decolonization were evaluated using second-order polynomial multiple regression model. All the experiments were performed in triplicates towards the construction of a quadratic model. Maximum decolorization rate of 78.15% & COD reduction up to 62.38% were observed in the incubation period of 6 days. The value of R² & adjusted R² were closer to 1.0 which advocates a high correlation between the observed values & the predicted values. Regression model applied here provides an excellent explanation of the relationship between the independent variables & the responses. The use of an experimental design with RSM permits the rapid screening of a large experimental domain for optimization of biodegradation process of recalcitrant compounds like textile dyes.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.096 DELFTIA TSURUHATENSIS - A PROMISSING DECOMPOSER OF PHTHALIC ACID ISOMERS

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Plastic industry uses significant amount of phthalic acid derivatives as plasticizers. These compounds are known as potential hazardous waste for human and the environments. Phthalates are animal carcinogens, can cause fetal death, malformations, and reproductive toxicity in laboratory animals. Toxicity profiles and potency vary by specific phthalates. During a routine examination, we have isolated several clones of unfamiliar microorganism from imported and locally manufactured mineral water supplied in polyethylene-terephthalate (PET) plastic bottles. Using 16s rDNA sequencing we have identified these clones as *Delftia tsuruhatensis*, first isolated in 2003 from activated sludge collected from a domestic wastewater treatment plant in Japan. *D. tsuruhatensis* is a gram negative, motile rod shape bacterium which belongs to the *Betaproteobacteria* lineage. In this research, we accomplished a comprehensive morphological, physiological and biochemical characterization of investigated strains. *D. tsuruhatensis* multiplies and survived for almost a year in plastic mineral bottles while *E. coli* strains survived for not more than 15 weeks. We have compared the ability of the different *D. tsuruhatensis* clones to degrade about 90 different metabolites including several phthalate isomers. The original *D. tsuruhatensis* degrades terephthalic acid but can not degrade phthalic acid; our isolates degrade phthalic acids but are unable to degrade terephthalic acid. High levels of phthalic acid or terephthalic acid inhibit bacterial growth but do not kill the bacteria. While phthalic acid inhibits the growth of the original clone when present in a mixture with terephthalic acid, terephthalic acid does not inhibit growth of our isolates when supplemented simultaneously with phthalic acid. Interestingly iso-phthalic acid is inert to all tested strains. We assume that *D. tsuruhatensis* is a most interesting and promising candidate for purifying phthalate contaminated discharge water.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.097 UNDERSTANDING LOW TEMPERATURE ANAEROBIC DIGESTION OF TRICHLOROETHYLENE: A 16S rRNA AND METAPROTEOMIC APPROACH

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Low temperature anaerobic digestion (LTAD) is a successful, cost-effective and energy efficient wastewater treatment option, with potential applications in bioremediation. Here, a novel approach combining traditional molecular analysis of community dynamics by 16S rRNA gene-based techniques (DGGE and qPCR) with metaproteomics, was used to gain an insight into the microbial processes involved in trichloroethylene (TCE) biodegradation. Four expanded granular sludge bed (EGSB) bioreactors (R1 and R2-operated at 37°C, R3 and R4-operated at 15°C) were employed for the treatment of a volatile fatty acid based wastewater. In addition, R1 and R3 were supplemented with increasing concentrations of TCE (10-60 mg l⁻¹), a potentially carcinogenic chlorinated aliphatic compound commonly found in industrial wastewater. Bioreactor performance was monitored by COD removal (%), biogas production (l/day) and biogas methane content (%). Effluent levels of TCE and its primary degradation derivatives, 1,1 dichloroethylene (DCE), cis 1,2 DCE and trans 1,2 DCE, were monitored by gas chromatography-mass

spectrophotometry (GC-MS). Total genomic DNA was extracted from the granular biomass at significant time points during the trial. The subsequent development of the archaeal community structure was monitored by qPCR, and it remained stable despite TCE addition. DGGE was employed to monitor the dynamic changes in the bacterial populations. To investigate the functional significance of this dynamism, biomass samples collected at the trial conclusion were analysed by 2D-gel electrophoresis reproducible shifts in metaproteomic patterns were identified in response to TCE addition. This data indicates the feasibility of utilising a lower temperature range for treatment of TCE, as the reactors displayed the ability to convert TCE to one or more DCE isomers, while maintaining an efficient and stable reactor performance, on a par with traditional higher operational temperatures.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.098 THE ROLE OF THE COPPER-BINDING ENZYME - LACCASE - IN THE BIODEGRADATION OF POLYETHYLENE BY THE ACTINOMYCETE RHODOCOCCUS RUBER

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Polyethylene is considered one of the most durable plastic polymers. Practically non-biodegradable polyethylene and accumulates in the environment posing an ecological threat to man and wildlife . We have previously isolated a strain of the actinomycete *Rhodococcus ruber* (designated C208; EC 1.10.3.2.) that was capable of utilizing and degrading polyethylene. Here, we report on the role of the bacterial copper-binding enzyme, laccase in the oxidation and degradation of polyethylene by this strain. Copper markedly affected the induction and activity of laccase, resulting in polyethylene degradation. RT-PCR, facilitating quantification of mRNA induction, showed a 13-fold increase in laccase mRNA levels as compared with the untreated control. Addition of copper to C208 cultures containing polyethylene enhanced the biodegradation of polyethylene by 75%, as compared with the non-amended control. Furthermore, when an extracellular isoform of laccase collected from the media of copper-induced cells was incubated with polyethylene, reductions of 20% and 15% were obtained in the Average Molecular Weight and Average Molecular Number with the polymer, respectively. Fourier Transformed Infra Red analysis of similar polyethylene films incubated with the extracellular laccase exhibited an increase in the carbonyl peak, indicating that enzymatic oxidation by laccase plays a major in polyethylene biodegradation

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.099 IMPROVED LINURON DEGRADATION IN LAB-SCALE ON-FARM BIOREMEDIATION SYSTEMS BY SUPPLEMENTING LINURON-PRIMED SOIL CONTAINING LINURON-DEGRADING VARIOVORAX

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Lab-scale microcosms were used to examine whether pesticide primed soils are preferentially used for bioaugmentation to improve and ensure pesticide mineralization in on-farm biopurification systems (BPS). Microcosms containing a mixture of peat straw and soil, inoculated with either linuron-primed soil or non-primed soil, were irrigated with clean or linuron containing tap water. The lag time of linuron mineralization, recorded for samples regularly taken from the microcosms, was indicative for the dynamics of the linuron mineralizing biomass within the samples. Bioaugmentation with linuron-primed soil immediately resulted into the establishment of a linuron mineralizing population in the system, which increased within two weeks when fed with the pesticide or remained its size when irrigated with tap water for a period of at least 12 weeks. Unexpectedly, also in microcosms inoculated with non-primed soil a linuron mineralizing population developed, but only after an extended period of linuron feeding. Additional experiments, in which non-primed soils from different origins were used for bioaugmentation, showed that linuron-mineralization only developed with some soils. Concomitant with the increase of the linuron degradation capacity in BMs bioaugmented with linuron-primed soil upon linuron feeding a specific *Variovorax* phylotype, closely related to the linuron-degrading *Variovorax* sp. SRS16, proliferated in the matrix, suggesting that *Variovorax* is involved in linuron degradation in the biomix in both set-ups. Our data indicate that supplementation of pesticide-primed soil to the matrix present in a BPS will result with more certainty and more rapidly into the establishment of a pesticide mineralization population and activity in a BPS.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.100 META-PLASMID: *IN SILICO* RECONSTRUCTION OF A PLASMID-LIKE CIRCULAR DNA MOLECULE AND ITS POSSIBLE ROLE IN THE RETRIEVED ENVIRONMENT

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Various catabolic plasmids for aromatic compounds have been identified in bacteria. They are relatively large (50 - 500 kb) and contain complete sets of genes for compound utilization. However, these findings are based entirely on bacteria that are easy to culture in the laboratory. Previously, we constructed a metagenomic library using an activated sludge used to treat industrial wastewater containing various aromatic compounds. We then screened the library for extradiol dioxygenase, a key enzyme in the aerobic degradation of aromatic compounds, and identified a total of 91 positive fosmid clones. In the present study, we analyzed 38 of the

fosmids to gain insight into the presence and organization of genes involved in aromatic degradation. Through *in silico* assembly of the inserts from several fosmid clones, we reconstructed a circular DNA form. This "meta-plasmid", designated pSKYE1, was relatively small (36.7 kb) and possessed genes for DNA replication and stable maintenance. What is striking is that pSKYE1 contained only a subset of genes for aromatic compound degradation; the encoded enzymes, phenol hydroxylase and extradiol dioxygenase, are capable of aromatic compound detoxification. Thus, pSKYE1 appears to be a "detoxification apparatus" that may confer a survival advantage on its bacterial host in the microbial community.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.101 DECHLORINATION OF MONOCHLOROPHENOLS BY ANAEROBIC MICROBIAL COMMUNITIES ENRICHED FROM A RIVER SEDIMENT CONTAMINATED WITH CHLORINATED COMPOUNDS

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Various dechlorinators utilizing chlorophenols have been isolated from a wide range of anoxic environments such as paddy soils, fresh or marine sediments and sewage sludges. However, most of these dechlorinators are unable to utilize monochlorophenols (MCPs). In this study, we enriched cultures to dechlorinate MCPs from a contaminated river sediment for obtaining novel MCP-dechlorinators.

A sediment sample was collected as microbial source from a river sediment that had been contaminated with benzene and volatile chlorinated compounds for years. A synthetic liquid medium containing MCP mixtures (2-MCP, 3-MCP and 4-MCP) and lactate was inoculated with the sediment sample and anaerobically incubated at 30°C. The degradation of MCPs and lactate and production of gases during incubation of cultures were measured to illustrate the biotic reactions.

An original culture containing the sediment was able to dechlorinate all MCPs regardless of substitutional positions of chlorine and did not accumulate phenol and benzoate during dechlorination of MCPs. This culture was transferred to fresh sediment free-liquid medium. Second enrichment culture more preferably dechlorinated 2-MCP and 3-MCP than 4-MCP and degraded phenol and benzoate produced during dechlorination of MCPs. Enrichment cultures after second times of transfers showed similar features to second enrichment culture. Dechlorinating activity in each culture continued after refeeding of MCPs. All the cultures produced methane.

Enrichment cultures containing MCP-dechlorinating activity were obtained. After a few transfers, dechlorinators utilizing *ortho*- or *meta*- substituted MCPs were predominantly enriched. Dechlorinator utilizing *para*-substituted MCP was enriched in an original culture with effect of sediment. Anaerobic microbial community enriched also contained degraders of phenol and benzoate and methane-producing *Archaea* with MCP-dechlorinator

Abstract Category
07 Recalcitrant Compound Degradation

PS.07.102 MICROBIAL COMMUNITY STRUCTURE IN ATRAZINE CONTAMINATED ENVIRONMENTS

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The indigenous microbial community structure in soil may be altered due to the pesticide contamination, and pesticides have impacts on microbial activity. The biodegradation is the major process to remove most pesticides in environment, including atrazine (6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine). We examined pesticide effects on microbial communities in subsurface groundwater deposits and soil layers with increasing depth within the boreal region in Finland.

The subsurface deposits were collected from groundwater well and sampling pipes, and in drillings. Pesticides were extracted and analyzed by high pressure liquid chromatography. Phospholipid fatty acid analyses combined with the statistical principal component analysis were used to characterize microbial community structures.

The proportions of Gram-negative bacteria with 18:1 ω 7c, or tuberculostearic acid containing actinomycetes increased in the deposits with the atrazine contamination. In the pristine deposits, the amount of 18:2, 18:1 ω 9c, and cyclopropane fatty acids was high, indicating the highest proportion of fungi and, on the other hand, slow microbial growth and stationary phase. In surface soils, the monounsaturated fatty acids, and iso- and anteiso branched fatty acids were abundant in PLFAs, but their proportions declined with depth while straight-chain saturated fatty acids increased. The differences in microbial communities between top soils were greater than between subsurface soils.

Atrazine affected microbial community structures in subsurface deposits. However, the depth of the deposit in comparison to the surface soil had stronger impacts on microbial community structures than pesticide contamination, indicating the significance of organic matter content of the soils and deposits for the number and amount of phospholipid fatty acids. The high diversity found in surface soil may provide more possibilities for atrazine dissipation.

Abstract Category
07 Recalcitrant Compound Degradation

PS.07.103 CHARACTERIZATION OF *DEHALOBACTER* CULTURES THAT REDUCTIVELY DECHLORINATE CHLORINATED ETHANES AND CHLOROFORM

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We have enriched *Dehalobacter*-containing cultures that reductively dechlorinate chlorinated ethanes and chloroform. Three subcultures derived from the same parent enrichment have been maintained separately on 1,1,1-trichloroethane, 1,1-dichloroethane and chloroform. Their maximum dechlorination rates are all over 100 $\mu\text{mol/L/day}$. Previous studies have shown that *Dehalobacter* is responsible for dechlorination in these cultures. Current efforts are devoted to

the isolation of *Dehalobacter*. Intensive serial transfers, with the chlorinated compounds and hydrogen as substrates, have led to daughter cultures highly enriched in *Dehalobacter*. However, the fact that these daughter cultures tend to grow slowly places challenges on the complete isolation of *Dehalobacter*. The supplementation of the sterile supernatant from the mixed parent cultures was found to be able to restore the cell growth in these daughter cultures. Such supplementation will be used to facilitate further isolation work. qPCR is being used to track *Dehalobacter* and other organisms during dechlorination to identify important microbial partners. Identification of the reductive dehalogenases (RDases) that catalyze these dechlorination reactions is on-going. Several putative sequences have been identified using degenerate PCR primers. Dechlorination activity is readily observed in cell-free extracts of these cultures. The use of native polyacrylamide gel electrophoresis as a separation technique to isolate active RDases from cell-free extracts is currently being attempted. RDases will be assayed for dechlorinating activity and identified using mass spectrometry, to ascribe putative sequences to function.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.104 STUDYING THE 'UNKNOWN' WITH METABOLIC DESIGN, A NEW PROBE DESIGN SOFTWARE FOR EXPLORATIVE FUNCTIONAL MICROARRAYS DEVELOPMENT IN MICROBIAL ECOLOGY

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The world of microorganisms comprises a vast diversity of living organisms, each with its individual set of genes, cellular components and metabolic reactions. In order to explore these unlimited and unexploited metabolic potentialities in various ecosystems, it is important to use high throughput tools like functional microarrays which allow the simultaneous analysis of thousands of genes. However, most classical functional microarrays used specific probes able to monitor only known sequences and could not then appreciate the complete microbial gene diversity of complex environments. We then developed a new algorithm, implemented in the user-friendly program named Metabolic Design, to ensure the design of efficient and explorative probes. Validation of our approach have been performed by studying eight enzymes involved in the degradation of polycyclic aromatic hydrocarbons from the model strain *Sphingomonas paucimobilis* sp. EPA505 using a first designed microarray of 8,048 probes. The gene sequences encoding enzymes involved in such pollutants degradation are not yet available for this bacterial strain. As expected, during the biodegradation kinetics of phenanthrene, naphthalene and a mixture of these both pollutants, we identified with the DNA microarray approach the targeted set of genes induced by the pollutants. We validated the identification of these new genes by sequencing and confirmed the quantitative discrimination of our microarrays by real time PCR. A second microarray of 39,216 probes, targeting forty genes involved in the biodegradation of various aromatic hydrocarbons have been developed to assess metabolic capacities of microbial communities in an aromatic hydrocarbons contaminated soil. Results highlight the potential microbial metabolic pathways involved in the pollutants degradation for this ecosystem. In

conclusion, Metabolic Design is a powerful and efficient tool to design explorative probes to monitor metabolic pathways in complex environments.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.105 NOVEL THERMOPHILIC CELLULOSE-DEGRADING BACTERIA ISOLATED FROM SOIL AND PRUNING TREE COMPOST

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Cellulose is a homopolysaccharide composed of glucose units, and the cellulose chains are joined by hydrogen bonds and Van Der Waal's force. As a result, crystalline structure is formed, leading low accessibility to enzymes. To make cellulose to economically competitive, efficient enzymatic processes that hydrolyze the cellulosic polysaccharides to fermentable sugars are needed. Thermostable cellulases have advantages in industrial applications because higher processing temperature make mixing easier and reduce biological contamination. The aims of this study were to isolate and characterize novel thermophilic cellulose-degrading bacteria.

Soil and pruning tree compost were suspended in saline. The supernatants were inoculated into enrichment liquid medium and incubated at 50°C. Cellulose-degrading bacteria were screened and purified by using cellulose containing plates and identified based on the 16S rDNA sequences. The representative strains, SCA3a and TC22-2b, were examined the growth properties, catalase and oxidase activities, substrate utilization, and fatty acid composition.

The strain SCA3a and TC22-2b degraded microcrystalline cellulose and carboxymethylcellulose, respectively. 16S rDNA sequence analysis indicated that they are novel species of the genus *Paenibacillus*. Both SCA3a and TC22-2b were aerobic, spore-forming, grew optimally at 55°C and pH 7-8, catalase and oxidase positive, and utilized cellobiose, fructose, galactose, glucose, maltose, mannose, melibiose, lactose, sorbitol, sucrose, trehalose and xylose. The major fatty acid of SCA3a and TC22-2b were n-C16:0 (36%) and iso-C16:0 (29%), respectively.

The results indicated that the strain SCA3a and TC22-2b were novel cellulose-degrading bacteria of the genus *Paenibacillus*. Biochemical characterization of their cellulases is now in progress.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.106 INVESTIGATION OF BACTERIAL METABOLISM OF AROMATICS IN CONTAMINATED SOIL THROUGH STABLE ISOTOPE PROBING

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Due to their diverse metabolic capabilities, some microorganisms are able to degrade xenobiotic compounds and thereby help to remediate some environmental problems. One reason for the wide range of metabolic capabilities is a rapid evolution of metabolic pathways in bacteria as well as structural similarity of xenobiotics to some of the natural substrates. Stable isotope probing (SIP), in combination with metagenomic analyses, is currently the most effective tool for investigating the diversity of bacteria potentially responsible for ecologically relevant processes. DNA-SIP involves the analysis of ¹³C-labeled metagenome after stable isotope labeled compounds have been pulsed into a microbial community and the biomass of active cells utilizing the substrate become labeled. This microcosm-based study employed DNA-SIP to investigate bacteria that actively utilize aromatic pollutants and/or their analogues biphenyl, 4-chlorobiphenyl, naphthalene, and benzoate in soil contaminated by polychlorinated biphenyls and polycyclic aromatic hydrocarbons, whether it be bulk soil, soil enriched with selected plant secondary metabolites or natural products rich in these metabolites. Our results show that altering the soil environment highly influences the diversity of populations actively participating in the metabolism of aromatics. In general, most aromatics-utilizing bacteria detected were members of *Proteobacteria*. Functional gene analyses resulted in the detection of dioxygenases that clustered with proteobacterial dioxygenases as well as those that were rather distant from dioxygenases of well-studied strains. In conclusion, our research suggests that ecologically relevant microbial populations coupled with more effective removal of contaminants are rather diverse and can be fostered by the presence of selected plant species and/or natural products.

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Abstract Category

07 Recalcitrant Compound Degradation

PS.07.107 MOLECULAR AND GENETIC CHARACTERIZATION OF WILD STRAINS OF *Trichoderma* spp. AND EVALUATION OF THEIR PHENOL-OXIDASE EXTRACELLULAR ACTIVITY

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The genus *Trichoderma* includes different species of filamentous fungi that can produce extracellular enzymes that degrade plant cell walls. The extracellular lacase activity has been reported for some *Trichoderma* species; however, more studies are needed to evaluate the presence of these kinds of enzymes or other phenol-oxidases from wild strains. The aim of this

work was to isolate wild strains of *Trichoderma*, to conduct a molecular-genetic characterization and to evaluate their production of extracellular phenol-oxidases. Twenty five strains of wild filamentous fungi were collected from different forest ecosystems from the State of Michoacan, Mexico. They all have the conidiation pattern which is characteristic of *Trichoderma* genus. The sequences of the ITS region from the Nuclear Ribosomal Unit of all the strains were obtained. Sequences were analyzed by a Blast comparison with the sequences present in the GenBank and it was found an identity greater than 90% with the ITS region of different taxa from the *Trichoderma* genus that included the species *viride*, *atroviride*, *harzianum*, *koningii* and *inhamatum*. Identity with *Trichoderma* related teleomorphs from the *Hypocrea* genus were also found, mainly the species *lixii*, *rufa*, *koningii*, *viridescens* and *vinosa*. Most isolates were grown at 28 °C in Vogel solid medium and reached the log phase after 84 h of incubation. The wild strains ability to produce extracellular phenol-oxidases was evaluated in solid Vogel medium using seven different dyes (250mg/mL) along with guaiacol (0.01%) and tannic acid (0.5%) as substrates. It was found that strains CMU1, CMU8, CMU196 and CMU221 were able to discolorate 5 of the dyes and produced an oxidation halo in the presence of guaiacol and tannic acid. The rest of the strains could only discolorate 4 or fewer dyes. The results presented here suggest that some of the strains that were evaluated produce extracellular phenol-oxidases.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.108 STRAIN SELECTION OF WILD FILAMENTOUS FUNGI WITH EXTRACELLULAR PHENOL-OXIDASE ACTIVITY

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Discoloration and oxidation assays of phenolic substrates in solid and liquid media represent a useful tool to select fungal strains with biotechnological potential to degrade phenolic substances that are produced in several industrial processes. Such discoloration and oxidation ability has been associated to the production of extracellular phenol-oxidases. This type of studies is seldom performed in wild filamentous fungi isolated from forest ecosystems in Mexico so their potential biotechnological utility is not known. In this work, 13 wild strains were selected from a fungal collection isolated from the State of Michoacan, Mexico and their ability to discolorate a solid medium containing phenolic colorants was tested. The strains selected were *Neurospora sitophila* (26CMU), *Pestalotiopsis paeonicola* (29CMU), *Lasiodiplodia theobromae* (39CMU), *Diaphorte ambigua* (48CMU), *Glonium pusillum* (59CMU), *Umbelopsis ramaniana* (81CMU), *Didymella bryoniae* (89CMU), *Mucor hiemalis* (96CMU), *Pleurotus djamour* (149CMU), *Fusarium incarnatum* (219CMU), *Infundibulicybe gibba* (234CMU), *Trametes versicolor* (TA1-CMU), and *Trametes cervina* (TC1-CMU). The strains were grown in Malt Extract Agar (MEA) and 9 phenolic substrates were added in concentration of 250 mg/mL. Guaiacol (0.01%) and tannic acid (0.5%) were also added. The strains were incubated at 28°C. Their growth as well as their discoloration or oxidation ability was evaluated. The strain with the faster growth was

59CMU whereas the 89CMU presented the slowest growth. The strains 149CMU, TA1-CMU and TC1CMU showed the greater discoloration activity. The strains 29CMU, 39CMU, TA1-CMU had the greater oxidative activity over guaiacol and tannic acid. The results reveal that strains 149CMU, TA-CMU, TC-CMU and 29CMU possess biotechnological potential to degrade phenolic substances. It is therefore, important to continue studying these strains in liquid medium in order to evaluate their production of extracellular phenol-oxidases

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.109 DIVERSITY OF HYDROCARBON-RELATED CATABOLIC GENES IN OIL SAMPLES FROM POTIGUAR BASIN (RN, BRAZIL)

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Biodegradation in petroleum reservoirs may result in physicochemical changes in crude oil and natural gas properties, being responsible for decrease of saturated hydrocarbons and yielding heavy oil with low economic value. The knowledge of the diversity of catabolic genes in petroleum-associated microbial communities is still scarce. Studies focusing on the diversity, abundance and distribution of hydrocarbon degradation genes will allow us to gain a better understanding about the role of microorganisms in the biodegradation processes that take place in petroleum reservoirs. The aim of this study is to evaluate the diversity of genes involved in hydrocarbon degradation of oil samples from Brazilian petroleum fields by using molecular approaches based on the construction of catabolic gene libraries (alkane monooxygenase - alk and dioxygenase - ARHDs). DNA was extracted from biodegraded (GMR75) and non-biodegraded (PTS01) oil samples and used for PCR amplification of alk and ARHD catabolic genes. Two libraries were constructed for each oil sample. After sequencing, BLASTX analyses revealed that Alk sequences were highly related to known sequences found in distinct genera encompassing *Xanthomonas*, *Acinetobacter*, *Marinobacter* (*Gammaproteobacteria*), *Paracoccus* (*Alphaproteobacteria*) and *Streptomyces* (*Actinobacteria*). ARHD sequences were related to sequences found in different strains belonging to the genera *Pseudomonas*, *Acinetobacter* (*Gammaproteobacteria*), *Burkholderia*, *Ralstonia* (*Betaproteobacteria*) and *Rhodococcus* (*Actinobacteria*). The diversity of the catabolic genes was shown to be similar between GMR75 and PTS01 oil samples, suggesting that although present in both oils these genes may not be the only responsible for *in situ* hydrocarbon degradation or that they may not be active in the microbial communities under study. Additional sample effort is being conducted in order to obtain a high coverage of the catabolic gene diversity and thus yield conclusive results.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.110 APPLICATION OF CHLORATE REDUCTION AS A NOVEL CONCEPT FOR THE BIOREMEDIATION OF HEAVILY POLLUTED ANAEROBIC SOILS

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It has been estimated that around 430.000 soil sites in the Netherlands are heavily contaminated with xenobiotic organic compounds or heavy metals. About 60.000 of these sites need to be remediated, but this remediation will be very expensive.

In situ bioremediation by using naturally occurring microorganisms or introduced microorganisms is a very attractive option. For in situ bioremediation the appropriate conditions for the microorganisms involved need to be created. Bioremediation of soils polluted with oil components requires the introduction of oxygen via aeration.

However, aeration of anaerobic soils is difficult to achieve and very costly. In this project the possible use of chlorate as terminal electron acceptor and oxygen source will be investigated. Unlike oxygen, chlorate is highly soluble and easy to introduce in soil via the groundwater. Biological (per)chlorate reduction is a unique type of anaerobic respiration, because in the conversion of the intermediate chlorite, molecular oxygen is produced. This light-independent in situ oxygen production is exceptional and offers innovative possibilities for bioremediation of anaerobic soils polluted with hydrocarbons. Biochemical routes that require mono and di-oxygenases cannot be employed under anaerobic conditions, but they can be employed when (per)chlorate is the electron acceptor, as oxygen is produced in situ. This research project aims to get insight into chlorate dependent anaerobic biotransformation of a range of organic pollutants as BTEX, fuel oxygenates as MTBE, and mineral oil and to develop strategies to apply chlorate reduction as a source of oxygen for bioremediation of anaerobic soils.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.111 MICROBIAL TETRACHLOROMETHANE DEGRADATION IN A CONTAMINATED AQUIFER: STRUCTURAL COMMUNITY PROFILING AND CHARACTERIZATION OF DECHLORINATING BACTERIA

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Tetrachloromethane (carbon tetrachloride, CCl₄) is an industrial solvent whose use has now largely been discontinued due to its toxicity. This work aimed at characterising the microbial community of an Alsace groundwater aquifer exposed to CCl₄ for several decades following an accidental spill in 1970, and at isolating strains from the contaminated site that enhance degradation of tetrachloromethane.

16S rDNA genotyping using an optimized protocol for terminal restriction fragment length polymorphism (Penny *et al.*, 2010) was performed, and enrichment cultures under a variety of anoxic conditions were set-up from inocula sampled at the contamination source.

Terminal restriction fragment length polymorphism profiles of the microbial community showed no significant change over a 15 month period during which clean-up of the aquifer using physico-chemical methods took place. The microbial community was predominantly constituted of Bacteria, with Archaea representing less than 0.5% of detected procaryotes. Strains capable of degrading tetrachloromethane (TM) at 10 mg/l were enriched and then isolated from

concentrated contaminated groundwater from the site, and included *Klebsiella* sp. strain TM2 and *Clostridium* sp. strain TM5 related to previously reported CCl₄-degrading bacteria, as well as strain TM1, a strictly anaerobic Gram-positive strain related to *Sporotalea*, a phylum for which dehalogenation of CCl₄ has not yet been described. Strain TM1 displayed an atypical 120 bp insertion in one of its 16S rDNA copies, produced a thermostable metabolite also found in the culture medium and associated with transformation of CCl₄, withstood oxygen exposure without significant loss in viability, and maintained its CCl₄ degradation efficiency when cultivated under simulated groundwater conditions.

Sporotalea strains may be well-suited for following *in situ* degradation of CCl₄ in aquifers contaminated by this compound.

Abstract Category

07 Recalcitrant Compound Degradation

PS.07.112 DETECTION OF GENOME-WIDE GENE EXPRESSION IN PSEUDOMONAS PUTIDA KT2440 GROWING IN SOIL BY MICROARRAY ANALYSIS

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To obtain knowledge of bacterial response to various factors, such as toxic compounds, in their soil environment, it is necessary to detect bacterial gene expression in the soil. Microarray techniques are powerful tools to monitor gene expression and have been extensively applied in many aspects of biological studies. However, due to the difficulties involved in RNA extraction from soil, microarray techniques have not yet been used to survey genome-wide bacterial gene expression in soil. By using a recently developed strategy to extract high quality RNA from soil, we successfully performed a genome-wide survey of bacterial gene expression in soil. Total RNA was extracted from multiple sterilized soil microcosms inoculated with *Pseudomonas putida* KT2440 containing a chloroaromatic degrading plasmid in the presence or absence of 3-chlorobenzoic acid. Custom-designed NimbleGen *Pseudomonas putida* KT2440/pSL1 microarrays were used for gene expression analysis. Of the 5346 genes on the array which included both genomic and plasmid-encoded ones, 5% and 4.5% of genes showed up- and down-regulation, respectively. Biochemical pathway analysis suggested that the benzoate degradation via hydroxylation pathway underwent the most significant changes after treatment with 3-chlorobenzoic acid. The 3-chlorobenzoic acid-induced expression of 3-chlorobenzoic acid degradation genes located in genome and plasmid was confirmed by real-time reverse transcription-polymerase chain reaction. Further analysis of cellular responses to 3-chlorobenzoic acid revealed repression of carbon, nitrogen, and sulfur metabolism. Microarray analysis using RNA extracted from soil could be a powerful tool to study the function of uncharacterized genes in the genome of soil bacteria, and has the potential to be applicable to raw soil. To our knowledge, this is the first report of the microarray analysis of the genome-wide bacterial gene expression in soil.

Abstract Category
07 Recalcitrant Compound Degradation

**PS.07.113 MICROBIAL COMMUNITY DEVELOPMENT DURING CONCURRENT
FE(III) REDUCTION AND CHLORINATED ETHENE DECHLORINATION**

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Reductive dechlorination of chlorinated ethenes in the subsurface environment is usually influenced by alternative terminal electron accepting processes (TEAPs). Previous studies of the effect of alternative TEAPs on dechlorination mostly focused on nitrate, sulfur oxyanions or carbon dioxide. Very limited information is available for the effect of Fe(III) on reductive dechlorination, though Fe(III) is the dominant electron acceptor in many aquifer sediments. The objectives of our study were to determine the effect of Fe(III) on chlorinated ethene biodegradation and to characterize microbial community compositions and dynamics during concurrent microbial dechlorination and dissimilatory Fe(III) reduction. In microcosm experiments with contaminated aquifer sediment and groundwater, the effect of different forms of Fe(III) on trichloroethene (TCE) dechlorination was tested; the rates and extent of dechlorination and Fe(III) reduction were quantified. Sediments amended with TCE and poorly crystalline Fe(III) (hydr)oxides showed higher TCE degradation rates than sediments with only TCE but no Fe(III), and the former had higher extent of ethene production with little accumulation of *cis*-1,2-dichloroethene (*cis*-DCE) or vinyl chloride (VC). In the sediments amended with TCE and ferric nitrilotriacetate (Fe-NTA), TCE was transformed primarily to *cis*-DCE and then gradually to VC and ethene. 16S rRNA gene clone library and sequencing were performed on the microcosm samples, in which *Dehalococcoides* and Geobacteraceae were identified as the major dechlorinating and Fe(III)-reducing populations respectively. Quantitative PCR targeting 16S rRNA genes of the two groups and RDase genes (*tceA*, *bvca*, *vcrA*) further revealed the microbial community development along the processes. Batch experiment with enrichment cultures are being performed to elucidate the potential interaction of dechlorinating bacteria and Fe(III)-reducing bacteria in different steps of reductive dechlorination.

Abstract Category
07 Recalcitrant Compound Degradation

**PS.07.114 IDENTIFICATION OF TOLUENE DEGRADING MICROORGANISMS
IN A CONTAMINATED SOIL USING H₂¹⁸O STABLE ISOTOPE PROBING**

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Stable isotope probing (SIP) with ¹³C-labeled substrates, such as toluene, has been an important new advance in identifying microorganisms that degrade and assimilate pollutants. A similar approach using H₂¹⁸O can be used to label microbial DNA in SIP experiments and to characterize all growing microorganisms in soil. By comparing the growing communities in soils exposed to toluene versus soils without toluene, we characterized toluene degrading organisms

from an auto salvage yard. Before SIP experiments were initiated we enriched for toluene degraders, and a toluene degrading organism was isolated and identified as *Rhodococcus jostii* RHA1 through complete 16S rRNA sequencing. We tested the ability of the soil and the isolate to degrade toluene and found that 48ppm of toluene was depleted in 8.5 days in soil, and 24 ppm toluene was degraded in 13 hours for RHA1 MSM broth cultures. In SIP experiments, DNA was extracted from two soil treatments: soil incubated for two weeks with H₂¹⁸O, and soil incubated for two weeks with H₂¹⁸O and 48ppm toluene. In soils to which only H₂¹⁸O was added, two distinct bands formed after isopycnic centrifugation. These two distinct bands also formed with DNA from soil incubated with both H₂¹⁸O and toluene, but in addition, a third, lower band was present. To identify the organisms associated with the DNA present in the third band, the complete 16S rRNA PCR products were sequenced from 30 clones. Five of the thirty clones represented an Arthrobacter closely related to *Arthrobacter phenanthrovorans*. Three of the clones represented *R. jostii* RHA1, and were identical to the 16S rRNA gene of our isolate. Additionally, clusters of bacilli, and acidobacteria were identified. We suggest that H₂¹⁸O SIP may offer additional insights into pollutant degradation experiments by characterizing microorganisms that grow after pollutant exposure and may especially be valuable in studying the impact of complex cocktails of pollutants in soil.

Abstract Category

07 Recalcitrant Compound Degradation

**PS.07.115 DECOLORIZATION OF AZO DYES WITH A NEW ISOLATED YEAST
ISSATCHENKIA ORIENTALIS**

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An yeast strain (YP-1) for degrading azo dyes was isolated on selective media containing Reactive Brilliant Red K-2BP, and identified as *Issatchenkia orientalis*. This yeast strain could effectively decolorize Reactive Brilliant Red K-2BP not more than 400mg/L. It gave a maximal decolorization (99%) for Reactive Brilliant Red K-2BP(100mg/L) after incubation for 12h, based on the optimal culture conditions with the inoculation volume 10%(V/V), the pH within the range from 3 to 9, and the concentrations of (NH₄)₂SO₄ and glucose not lower than 0.02%, and 0.2%, respectively. The research results on the mechanism of decolorization showed that *Issatchenkia orientalis* could absorb Reactive Brilliant Red K-2BP and subsequently biodegrade it. Besides, *Issatchenkia orientalis* could also decolorize another azo dye Reactive Black 5(200mg/L) with 99.5% decolorization rate.

Abstract Category

07 Recalcitrant Compound Degradation

**PS.07.116 MOLECULAR-BIOLOGICAL CHARACTERIZATION OF A NOVEL,
SEDIMENT-FREE MIXED CULTURE SHOWING EXCEPTIONALLY RAPID
DECHLORINATION OF TRICHLOROETHENE TO ETHENE**

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Trichloroethylene (TCE) contamination of groundwater at over 400 U.S. EPA National Priority sites emphasizes the need for efficient and cost-effective remediation strategies for this probable human carcinogen. The bacteria genus *Dehalococcoides* has been studied for its unique ability to metabolically convert TCE to harmless ethene through reductive dechlorination. A handful of sediment-free, TCE-dechlorinating microbial communities have been described, but the quest for very rapidly dechlorinating organisms continues. Here we report on a novel, TCE-reducing, sediment-free, stable consortium enriched from an estuarine sediment. We have maintained the consortium for over a year with reductive dechlorination rates twice as fast as any other characterized culture grown in a defined medium at similar temperatures. Use of a proprietary mixture of simultaneously fermentable substrates serving as both electron donors and carbon sources minimized the loss of reduction equivalents to methanogenesis. In an electron flow analysis, production of volatile fatty acids (VFAs) accounted for ~80% of the electron consumption, biomass for ~19%, and reductive dechlorination for ~1%. Using quantitative PCR (qPCR), we monitored the dominant microbial groups during conversion of TCE to ethene. General bacteria and *Dehalococcoides* increased throughout the experiment, whereas members of the *Geobacteraceae* family increased primarily during the reduction period of TCE to the intermediate cis-Dichloroethylene (cis-DCE). Homoacetogen copy number increased until the VFAs stabilized. We also observed an abundance of previously characterized reductive dehalogenases. Clone library data suggest the presence of new *Dehalococcoides* and homoacetogen strains. Due to its exceptionally fast reductive dechlorination rates, this consortium shows significant promise for application in bioremediation.

Abstract Category

07 Recalcitrant Compound Degradation

PS.08.001 ACCURATE DETERMINATION OF HORIZONTAL GENE TRANSFER FREQUENCY BY THE USE OF MOLECULAR SIGNATURE TAGGED RECIPIENT STRAIN

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Analysis and comparison of numerous complete prokaryotic genome sequences led to assess that a significant proportion of bacterial genes could have been acquired from other bacterial species by horizontal gene transfer (HGT). HGT is recognized as the main mechanism that has contributed to microbial evolution and has led to bacterial adaptation to various environments. However, numerous questions remain about the role that natural environments play in maintenance and dispersion of bacterial genes and about the frequency with which these genes are exchanged among indigenous bacteria. For example, there is a strong discrepancy between HGT frequencies deduced from in silico analysis and those resulting from in situ experiments in which transduction, conjugation, and natural genetic transformation were studied. When considering complex and heterogeneous environments such as soil, detection and quantification of HGT events occurring in situ are limited by the lack of sensitivity and specificity of cultivation based methods that are, in addition biased by difficulties in discriminating between independent transfer events and clonal multiplication of recombinants. Our objectives were to develop a new approach to provide a better assessment of the actual frequency of gene transfer. This was done by tagging every cell of a recipient *Acinetobacter baylyi* strain by a specific molecular signature that would detect independent transfer events as colonies exhibiting different molecular signatures. We will present the tagging technology and preliminary in vitro and in situ (soil) conjugation results based on the use of donor strains containing a conjugative plasmid and the tagged *A. baylyi* strain as recipient.

Abstract Category

08 Horizontal Gene Transfer

PS.08.002 METHYLOBACTERIUM EVOLUTION TOWARDS HALOGENATED METHANE UTILIZATION: THE 380 KB CHLOROMETHANE UTILIZATION CMU PLASMID

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Chloromethane (CM), the most abundant halocarbon in the atmosphere, is responsible for at least 15% of chlorine-catalysed ozone destruction in the stratosphere. Some methylotrophic bacteria are able to use CM as the sole carbon and energy source and represent a potential sink for CM. The only known pathway for CM catabolism by aerobic bacteria was investigated for strain *Methylobacterium extorquens* CM4 (Studer *et al.*, 2002). Essential genes for growth on CM were termed *cmu* for chloromuthane utilization. This work aimed to elucidate acquisition of halomethane utilization and adaptive response upon CM degradation. Whole genome sequencing and proteomics of the CM-utilization reference strain CM4 and comparison with the genome

sequence of other *M. extorquens* strains unable to degrade CM (Vuilleumier *et al.*, 2009) were performed.

In strain CM4, known genes for CM utilization are found in a different organisation than in other CM-degrading microorganisms and associated with a unique 380 kb plasmid. This designated *cmu* plasmid harbors putative transfer-promoting genes, uncharacterized genes related to C1 metabolism, as well as genes involved in the biosynthesis, transport, and metabolism of cobalamin and tetrahydrofolate, the two essential cofactors for CM utilization by the *cmu* pathway. Proteins encoded by genes located on the plasmid (5) and the core genome (28) were more abundant in CM-grown than methanol-grown cells.

The genes involved in bacterial metabolism of CM are present on the *cmu* plasmid in strain CM4 and have been acquired by horizontal gene transfer. Paralogs of these genes are found in the *Methylobacterium* core genome. Their role in carbon assimilation and adaptive response to toxicants remain to be clarified. Whether plasmid *cmu* alone may confer the ability to grow on CM remains to be investigated.

Abstract Category

08 Horizontal Gene Transfer

PS.08.003 INFERRING THE BREADTH OF PLASMID HOST RANGE BASED ON GENOMIC SIGNATURE

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Despite the important contribution of self-transmissible plasmids to horizontal gene transfer among bacteria, very little is understood about the range of hosts in which these plasmids have evolved (designated as 'evolutionary host range'). Earlier studies have suggested that plasmids acquire the host's oligonucleotide composition (so-called 'genomic signature') over evolutionary time due to amelioration and/or acquisition of chromosomal segments. It should therefore be possible to infer the host range of plasmids by comparing their genomic signatures with those of all available bacterial chromosomes. The goal of this paper was to predict the evolutionary host range of self-transmissible plasmids belonging to six incompatibility (Inc) groups, whose host ranges are generally thought to be narrow (IncF, IncH, and IncI) or broad (IncN, IncP, and IncW). The level of diversity among candidate evolutionary hosts for each plasmid was determined using taxonomic classification at different ranks and using genetic distance between 16S rRNA sequences or between genomic signatures. Our results suggest that the evolutionary host range is the broadest for IncP plasmids, narrowest for IncF and IncI plasmids and intermediate for IncH and IncN plasmids, which is in general agreement with their known replication range. The host range of IncW plasmids could not be adequately evaluated because hosts with similar genomic signatures were not available. However, this finding raises the hypothesis that IncW plasmids have not ameliorated to any host due to their promiscuous life style. In this era of high-throughput sequencing and metagenomics, this genomic signature comparison method is a useful tool for predicting the host range of novel plasmids.

Abstract Category
08 Horizontal Gene Transfer

PS.08.004 GENETIC VARIATION AND EVOLUTION OF ENZYMES INVOLVED IN MICROBIAL DISSIMILATORY (PER)CHLORATE REDUCTION; PERCHLORATE REDUCTASE AND CHLORITE DISMUTASE

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Perchlorate (ClO_4^-) is a world-wide toxic environmental contaminant, posing a significant health threat. Numerous microorganisms from across the Proteobacteria have been isolated, which reduce perchlorate to benign chloride. The two proteins essential for this metabolism are perchlorate reductase (Pcr: *pcrABCD*) and chlorite dismutase (Cld: *cld*). Despite their metabolic interdependency these have separate regulatory systems for expression. Nothing is known about the evolution and acquisition of these genes in the environment. DNA and amino acid sequence data for the PCR subunits, Cld (using Pfam (PF06778) "cld-like gene"), as well as the 16S rRNA has been collected from a wide number of environmental isolates including *Azospirillum*, and *Dechloromonas* species, as well as sequenced bacterial and archaeal genomes from species not experimentally confirmed to perform this metabolism. Interestingly, Pcr were not found in many of these same genomes as Cld hinting at a potential multi-functionality of the Cld-like proteins identified. Phylogenetic trees for each subunit were used to compare gene disparity across different organisms and their evolutionary relationship relative to the 16S rRNA. Sequence similarity did not mirror the evolutionary relationship established by the 16S rRNA gene indicating gene acquisition through horizontal transfer with each of the separate subunits taking divergent evolutionary paths. This was also supported by phylogenetic trees where known (per)chlorate reducing bacteria ((P)CRB) claded independently from non/untested-(P)CRB bacteria. Studying Pcr and Cld in environmental isolates as well as environmental samples creates a framework for investigating horizontal transfer of these genes, whether different types of the subunits exist in certain niches and whether genes are lost, gained or swapped. Furthermore, these studies may indicate how these proteins evolved and their origins may be established.

Abstract Category
08 Horizontal Gene Transfer

PS.08.005 HORIZONTAL GENE TRANSFER IN AN EXTREME ENVIRONMENT

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Although the Antarctic continent experiences severe environmental conditions there is a high microbial diversity within soils and water samples. These environments constitute a suitable place for the isolation of bacteria that produce cold-active enzymes. As part of a project aimed at the selection of cold-adapted bacteria expressing biotechnological relevant enzymes, a *Janthinobacterium* sp. strain (identified by 16S sequencing) that produce a

cold-active extracellular protease was isolated from a water sample collected in the Uruguay Lake, King George Island, South Shetland. Zymographic analysis performed in presence of inhibitors revealed that this protease belongs to the group of serine-peptidases. To identify the protease gene, degenerated primers for the amplification of subtilisin-like serine proteases were used in PCR reactions. A unique amplification product of approximately 600 bp was obtained. By sequence analysis and PSI-BLASTP search in MEROPS database we established that this protein belongs to S8A subfamily. Similar homologous were found in species from a variety of orders among the phylum Cyanobacteria. A close inspection of S8A subfamily protein members in 36 completely sequenced species belonging to Burkholderiales order mainly discards a phylogenetic close related origin.

Cyanobacterial mats are major representatives of microbiota in Antarctic and are frequently co-isolated with eubacteria. In this context, our results support the transfer of genetic material from cyanobacteria to eubacteria. We thank IAU (Instituto Antártico Uruguayo) and PEDECIBA for partial financial support.

Abstract Category

08 Horizontal Gene Transfer

PS.08.006 PREVALENCE OF INCP-1 PLASMIDS AND IS1071 ELEMENTS IN CONTAMINATED AND NON-CONTAMINATED ECOSYSTEMS

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Horizontal transfer of mobile genetic elements (MGEs) is considered to play a major role in the adaptation of bacterial communities to xenobiotic compounds. Many of these catabolic gene functions are found on broad-host spectrum IncP-1 plasmids and/or bordering IS1071 elements. To examine whether these MGEs are correlated with specific ecosystem pollutions, their prevalence was analyzed in samples taken from ongoing microcosm experiments assessing the effect of pesticide application on evolution of pesticide degradation capacity, from long-term polluted (metals, pesticides, PAHs) environments and from pristine soils, using PCR targeting IncP-1 and IS1071. Samples from soil microcosms treated with the phenylurea herbicide linuron showed stronger PCR signals for both IncP-1 and IS1071 than samples with no linuron treatment and there was a clear correlation with the cumulative amount of linuron added to the microcosms. Strong PCR signals for both IncP-1 and IS1071 MGE were also obtained with samples taken from pilot biofilter systems used for on farm treatment of pesticide-contaminated waste water. Furthermore, IncP-1 was predominately found in pesticide and PAH contaminated ecosystems and clearly less abundant in metal containing soils and pristine soils. IS1071 was present in all other polluted environments, including the metal polluted soils and the pristine soils, indicating that IS1071 elements are more widespread than formerly presumed. Real-time PCR methods are being developed to quantify the IncP-1 and IS1071 content and experiments are going on to examine whether in polluted systems those IncP-1 and IS1071 elements are preferentially associated with xenobiotic catabolic genes relevant for the examined ecosystem.

Abstract Category
08 Horizontal Gene Transfer

**PS.08.007 EVIDENCE FOR HORIZONTAL GENE TRANSFER BETWEEN
STENOTROPHOMONAS AND *CHRYSEOBACTERIUM* IN SOIL**

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Tetracycline resistance (TET-r) belongs to the widespread antibiotic-resistance in environment, since TET has a long application history, represents important global anthropogenic loading for the environment and belongs to the most frequent antimicrobial agent in livestock industry. Despite of these facts there is still lack of information about dissemination of TET-r among soil microflora. We screened TET-r genes in selected 12 TET-r bacterial strains isolated from both agricultural soil 30 years fertilized by liquid hog manure (LHM) containing TET residues and from control unfertilized soil. Both soils were exposed to selective TET pressure for 14 days. 22 primers were tested: 12 designed for effluxes, 8 for ribosomal protection proteins and 2 for inactivators. Only one previously characterized determinant was detected - *tet(K)* in two phylogenetically distinct *Staphylococcus* sp. Furthermore, the *tet(D)*-specific primers yielded PCR products in 3 isolates of *Stenotrophomonas* sp. and 5 isolates of *Chryseobacterium* sp. The fragment sequence revealed gene coding sodium/solute symporter-histidine kinase hybrid protein specific for *Stenotrophomonas* species which was not yet described as resistant determinant. The sequence was characterized by high G/C content and 100% bp sequence similarity in all 9 isolates (consisting from 2 phylotypes of *Stenotrophomonas* and 2 phylotypes of *Chryseobacterium*). All the described TET-r bacteria were isolated from the both soils, however, occurrence of TET-r *Chryseobacterium* and *Stenotrophomonas* were significantly more frequent in control soil treated by TET in contrast to the LHM fertilized soil. The presence of the genes encoding the protein typical for *Stenotrophomonas* sp. detected also in *Chryseobacterium* indicated recent horizontal transfer between both evolutionary divergent genera in the investigated soils. To find out if the concerned hybrid protein is responsible for TET-r is the topic of our future work.

Abstract Category
08 Horizontal Gene Transfer

**PS.08.008 INCREASE OF TETRACYCLINE RESISTANT BACTERIAL
POPULATIONS IN SOILS AMENDED WITH EXCREMENTS OF AN ANTIBIOTIC-
TREATED DAIRY COW**

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Antibiotics (ATBs) are often administered to farm animals to prevent or treat disease. These medications are not completely metabolized, however, by the treated animals, and may thus be excreted along with ATB-resistant fecal microflora. Given that the excrements of treated farm animals are often used as fertilizers, it is important to study their effects on ATB-resistance of indigenous soil microflora. We report on a microcosm study where we compared the number of cultivable tetracycline resistant (TET-r) bacteria in soils amended with excrements from a dairy cow treated with oxytetracycline, soils amended with excrements from a non-treated dairy cow, and non-amended soils. The soils were collected on 3 farms that had never been fertilized with manure, each of the 9 treatments (3 soils x 3 amendments) were replicated 4 times, and microcosms were incubated for 2 months. The relative abundance of TET-r bacteria was estimated by plating cultivation on complex as well as tetracycline-selective media. The TET-r isolates were identified based on their 16S rRNA gene sequences. Relative to the non-amended soil, TET-r isolates (mainly *γ-Proteobacteria* and *Actinobacteria*) increased in soils amended with both ATB-treated and non-treated cow excrements. Soils amended with the 2 excrements did not differ in total TET-r isolates, but differences were found among certain bacterial groups. The main differences consisted in frequent occurrence of *Escherichia*, *Shigella*, *Bacteroides*, *Arthrobacter*, *Comamonas*, *Pseudomonas*, *Streptomyces* in soil amended by ATB-treated cow excrement in contrast to the soil amended with excrement of non-treated cow, which was characteristic by frequent occurrence of *Microbacterium*, *Pectobacterium*, *Flavobacterium* and *Shewanella*. The excrements from the ATB-treated dairy cow increased TET-r fecal microflora as well as TET-r soil indigenous bacteria. It remains to assess whether the horizontal transfer of TET-r genes works between the both microbial groups.

Abstract Category

08 Horizontal Gene Transfer

PS.08.009 ANTHROPOGENIC IMPACTS ON CLASS 1 INTEGRON PREVALENCE AND DIVERSITY IN ENVIRONMENTAL BACTERIA - NOVEL METHODS FOR CULTURE INDEPENDENT CHARACTERISATION

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The importance of environmental bacteria as reservoirs of antibiotic resistance genes is increasingly being recognised as novel genes are discovered that can deactivate current antibiotics. The impact of human activity on selection for antibiotic resistance in the environment is largely unknown, although considerable amounts of antibiotics are introduced via domestic waste water and farm animal waste. Selection for resistance may occur by exposure to sub-lethal levels of antibiotic residues or indirectly by co-selection for genetic elements which carry genes of varying activity. Class 1 integrons are genetic elements that can integrate important antibiotic and quaternary ammonium compound (QAC) resistance genes that confer resistance to certain disinfectants and detergents. It is likely that exposure to detergents may co-select for antibiotic resistance by selecting for integrons. This study aimed to investigate selection for class 1 integrons and resistance genes in environmental bacteria by assessing prevalence using real-time PCR and diversity by novel culture independent methods. Class 1 integron prevalence was

consistently low in pristine soils and control agricultural soils which had not received amendment with sewage sludge or animal slurries, whereas amended soils showed significantly higher class 1 integron prevalence. Characterising integrons and resistance gene diversity in the soil metagenome is still problematic, novel molecular approaches to elucidate the class 1 integron metagenome and the resistome will be discussed. A vectorette - long range PCR approach gives amplification of class 1 integron sequences independent of 3' conserved sequences, allowing an unbiased study of integrated cassette gene diversity and characterisation of ISCR elements or downstream resistance genes.

Abstract Category
08 Horizontal Gene Transfer

PS.08.010 ANTIBIOTIC RESISTANCE IN BOVINE STAPHYLOCOCCI: A RESERVOIR FOR HUMAN INFECTION?

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Coagulase negative staphylococcal infections are a common cause of bacteraemia and post-operative wound infection, and often have decreased susceptibility to antibiotics. Heavy antibiotic use in agriculture has been shown to contribute to antibiotic resistance in human pathogens. Isoxazolyl penicillins and third generation cephalosporins are used in dairy farming, both of which can select for methicillin resistance conferred by the horizontally mobile staphylococcal cassette chromosome *mec*, carrying the *mecA* gene. Unpasteurised bulk tank milk samples from 50 organic and 50 conventional dairy herds were screened for methicillin resistant staphylococci to investigate whether decreased antibiotic use in organic farming results in a reduction in the occurrence and diversity of methicillin resistant staphylococci. Both farming practices harboured methicillin resistant staphylococci, but these were more frequently isolated from conventional farms than organic (56% and 30% of farms, respectively). Methicillin resistant *S. epidermidis*, an important human pathogen, was isolated from more conventional farms than organic (20% versus 2% of farms), and several human pathogenic strains were identified. Resistance to erythromycin, tobramycin and tetracycline was exhibited more frequently by staphylococci isolated from conventionally farmed herds, whereas fusidic acid resistance was more common in staphylococci from organic farms. Nevertheless, isolates resistant to multiple antibiotics were common from both farm types, and quantitative PCR data suggests there is a substantial reservoir of *mecA* in the UK dairy herd as a whole. Methicillin resistant coagulase negative staphylococci are increasingly reported as a cause of human infection in the health care environment, and antibiotic selection in agriculture may provide a reservoir of these which can be disseminated to the human population.

Abstract Category
08 Horizontal Gene Transfer

PS.08.011 THE IMPACT OF WASTE WATER TREATMENT PLANT EFFLUENT ON RESERVOIRS OF ANTIBIOTIC RESISTANT BACTERIA IN AQUATIC

SEDIMENTS

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Waste water treatment plants receive complex mixtures of chemicals and bacteria from multiple origins, creating hotspots for selection and transfer of adaptive genes. Studies have demonstrated the presence of antibiotic residues and resistance genes in sewage sludge applied to land. Less is known about the impacts of liquid effluent released into rivers. Class 1 integrons are genetic elements associated with antibiotic resistance in the clinic, causing simultaneous resistance to a broad range of antibiotics. In this study sediment core samples were taken from multiple points upstream and downstream of a large urban waste water treatment plant outfall to analyse the impact on the microbial resistome. Isolation was performed using selective plates amended with various antibiotics and isolates were screened by PCR for class 1 integrons and gene cassettes were characterised. A higher number of coliforms were isolated downstream with a higher proportion of isolates displaying resistance to gentamicin, erythromycin, chloramphenicol, sulfachloropyridazine and cefotaxime. Real-time PCR revealed a higher integron prevalence in downstream metagenomic DNA compared to upstream, with significantly higher *qacEΔ1* prevalence indicative of the introduction of clinical class 1 integrons. A novel application of a vectorette PCR method allowed amplification of integron variable regions from metagenomic DNA. Class 1 integron amplicons were cloned into expression vectors allowing screening for resistance phenotypes. Waste water treatment plant effluent produced significant impacts on class 1 integron prevalence and increased numbers of sediment bacteria resistant to clinically important antibiotics such as 3rd generation cephalosporins. This data suggests that liquid effluent disseminates clinically important resistance determinants to the wider environment, where there is a risk of transfer to the human population through the food chain or direct contact with the environment.

Abstract Category

08 Horizontal Gene Transfer

PS.08.012 EFFECT OF CHANGES IN ENVIRONMENTAL CONDITIONS ON THE HORIZONTAL GENE TRANSFER AND FUNCTIONALITY OF THE TOL PLASMID

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Horizontal gene transfer (HGT) is a widespread phenomenon in the prokaryotic kingdom that occurs readily under harsh environments where genetic adaptation is required for the survival of microorganisms. HGT could be useful for bioremediation to shift microbial communities to promote the degradation of xenobiotics and emerging contaminants. However, to successfully utilize this naturally-occurring phenomenon in engineered settings, the mechanisms and effects of HGT must first be elucidated. The goal of this project was to determine the environmental conditions required for successful HGT events which result in functional phenotypes. The transfer of the TOL plasmid, which includes genes for the degradation of toluene and related compounds, was used as the model system. The presence of transconjugants was verified using a

green fluorescent protein expression system on the TOL plasmid. Data indicate that *Escherichia coli* DH5 α transconjugants harboring the TOL plasmid do not have the ability to degrade and utilize toluene as the sole carbon source except in the presence of a secondary substrate. The results imply that availability of alternate carbon sources may affect the functionality of the transferred plasmid. The lack of functional phenotype in the absence of alternate substrates may be due to differences in guanine-cytosine (GC) content between the TOL plasmid (~60% GC) and the recipient *E. coli* genome (~50% GC). To determine how such differences in environmental and biological conditions affect the horizontal gene transfer of the TOL plasmid, several bacterial strains from various phyla with a range of genomic GC content are being tested as recipients. Other conditions being investigated include alternative nitrogen and carbon sources as well as pH. The results from this study will be helpful for elucidating the necessary conditions to promote successful HGT events that result in the desired functional phenotype.

Abstract Category

08 Horizontal Gene Transfer

PS.08.013 CONTRIBUTION OF HORIZONTAL GENE TRANSFER TO THE ENVIRONMENTAL RESERVOIR OF DISEASE-ASSOCIATED *VIBRIO PARAHAEMOLYTICUS*

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V. parahaemolyticus is an aquatic bacterium that occupies coastal environments worldwide and causes seafoodborne gastroenteritis. Several virulence-associated genes have been identified for *V. parahaemolyticus*; however, the distribution of these genes relative to the phylogenetic relationship of strains from clinical and environmental sources has not been studied. We analyzed seven housekeeping genes for select *V. parahaemolyticus* clinical and environmental strains. We determined the distribution of the main virulence genes, the thermostable direct hemolysins and the type III secretion systems among strains in this study. In addition, we investigated the diversity of plasmids associated with clinical and environmental strains. Phylogenetic analysis of housekeeping genes divided the clinical and environmental strains into separate lineages. Although few of the environmental strains had virulence genes, a surprising number of strains were phylogenetically similar to clinical strains. We identified 12 clinical strains that had no known virulence genes. Among these were several strains isolated from wound infections or blood rather than stool samples. Plasmid analysis revealed a 28.8-kb replicon from an environmental strain that had two genes similar to those of a genomic island of the O3:K6 pandemic clone. Also, we detected 86-kb plasmids that had identical restriction profiles in a clinical strain and three environmental strains. *V. parahaemolyticus* strains in the United States exhibit genetic diversity and variable virulence gene content. Identification of environmental strains with phylogenetic similarity to clinical strains, and clinical strains that did not have virulence-associated genes suggests additional uncharacterized pathogenic mechanisms may exist for *V. parahaemolyticus*. In addition, *V. parahaemolyticus* strains may undergo frequent plasmid-mediated horizontal gene transfer that allows them to adapt to their pathogenic or environmental niche.

Abstract Category
08 Horizontal Gene Transfer

PS.08.014 DIVERSITY AND EXPRESSION OF INTEGRASE GENES OF INTEGRON IN A BACTERIAL COMMUNITY SUBMITTED TO OIL POLLUTION

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Integrations are mobile genetic elements known to be involved in bacterial adaptation and evolution. They all share a common feature: a gene encoding for an integrase. The integrase gene is followed by genes cassettes, which are mobilizable by the integrase activity. Integrations are considered as gene cassette reservoirs and expression systems for the integrated genes. They are ubiquitous in the environment, and spread genes that can confer a selective advantage to bacteria. Although several studies have reported gene cassettes diversity according to different stresses, the integrase genes diversity in bacterial communities submitted to a stress has never been investigated, nor their expression in such context. We present here a study devoted to provide new insights on integrase diversity and expression in bacterial communities submitted to a pollution event.

A coastal mud sediment contaminated with oil was maintained in microcosms during nine months with a tide simulation system. The integrase diversity in the metagenome of samples from these microcosms was investigated by integrase gene libraries analysis at different times along the incubation period. In parallel the integrase transcription was determined by dot-blotting.

Integrase diversity was drastically modified in polluted microcosms compared to the control. One particular integrase sequence was found largely dominant in the metagenome after nine months of incubation after pollutant addition. Integrase expression was successfully demonstrated for microcosms initially contaminated with oil while it was found very weak or absent for control microcosms.

Our results show clearly that the stress due to oil contamination affects both the diversity and the expression level of integrase genes in metagenome. Since these genes are responsible for gene cassettes acquisition our results suggest that integrations play a key role in the adaptive response of bacterial communities to environmental stresses.

Abstract Category
08 Horizontal Gene Transfer

PS.08.015 THE ROLE OF COEVOLUTION IN PLASMID-HOST ADAPTATION

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The spread of antibiotic resistance through pathogenic bacterial populations is an ever-increasing threat, and yet little is known about the rates and evolutionary mechanisms pertinent to this medical crisis. The plasmid-mediated process of conjugation allows phenotypic traits such as

antibiotic resistance to be passed not only among bacteria of the same species, but also between phylogenetically distinct species. We do not know, however, if long-term association with a given host often leads to specialization, and what the underlying evolutionary mechanisms of this process are. The goal of this study is to determine if and how plasmids and bacterial hosts adapt to each other, and to identify the molecular changes involved. To do this, we experimentally evolved the broad-host-range mini-replicon pMS0506 in three different hosts (*Shewanella oneidensis* MR-1, *Pseudomonas putida* H2, and *Pseudomonas koreensis* R28) in serial batch cultures. Changes in plasmid stability in each host were then used as a measure of plasmid-host adaptation. Comparison of the stability of evolved and ancestral plasmids in co-evolved and ancestral hosts demonstrated that, depending on the host, a plasmid can become more stable over evolutionary time through genetic changes in the host (H2), the plasmid (MR-1), or both (R28). The latter strongly suggests that coevolution between a host and its plasmid, *i.e.*, the occurrence of reciprocal, interacting mutations on both genomes, can result in increased plasmid stability in a novel host. A gradual improvement of plasmid stability in coevolving hosts was observed over time, suggesting that multiple adaptive mutations were selected. Ongoing whole genome sequence analysis of evolved and ancestral hosts in combination with stability assays will allow us to identify the interacting mutations that are responsible for plasmid-host specialization through coevolution.

Abstract Category

08 Horizontal Gene Transfer

PS.08.016 ENHANCED PLASMID TRANSFER IN *E. COLI* AND *P. PUTIDA* BIOFILMS AT THE LIQUID-AIR INTERFACE.

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Gene transfer mediated by plasmids is an important mechanism in the adaptive evolution of bacteria. However, there is limited information on the population dynamics of these plasmids in spatially structured populations such as biofilms - a common bacterial life style in various habitats. Although the close proximity of cells in biofilms is expected to promote plasmid transfer by conjugation, published studies show inconsistent results. The objective of this study was to gain insight into factors that promote transfer of the promiscuous highly self-transferable multidrug resistance IncP-1 plasmid pB10 in biofilms. Four different types of bioreactors were used to grow *E. coli* K12 and *P. putida* biofilms. Transfer of plasmid pB10::*rfp* to a recipient *gfp*-marked strain was monitored *in situ* using confocal laser scanning microscopy. Oxygen profiles were measured using a custom-made oxygen microelectrode. In contrast to the submerged biofilm in closed flow cells, where plasmid transfer was negligible, high plasmid transfer efficiency was observed in biofilms floating at the liquid-air interface formed in an open flow cell. A vertical flow cell and a sequential batch culture bioreactor were used to analyze biofilms at different depths and found that extensive plasmid transfer only occurred in a narrow zone near the air-liquid interface. The much lower transfer frequency observed in the lower

zones beneath that interface coincided with rapidly decreasing oxygen concentrations affecting biofilm structure. The effect of anoxic conditions on the transfer of pB10 in *E. coli* in standard plate mating is being investigated. In conclusion, conjugative transfer of pB10 in biofilms only occurred with high efficiency at the liquid-air interface. Further research to determine why dissemination of the promiscuous IncP-1 plasmids is promoted in this particular zone is needed, and possible explanations will be discussed.

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Abstract Category

08 Horizontal Gene Transfer

PS.08.017 HORIZONTAL TRANSFER OF GREEN FLUORESCENT PROTEIN GENE FROM MESORHIZOBIUM CICERI

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Horizontal gene transfer has played a large part in microbial evolution. The ability of microorganisms to exchange genetic information with related or nonrelated bacteria increases the uncertainty of the fate of GEMs. Transfer of the introduced genes to indigenous microbial population may result in unintended effect on non target microorganisms. Therefore, the present study was carried out to assess horizontal transfer of introduced green fluorescent protein (gfp) gene from *Mesorhizobium ciceri*. For this, *M. ciceri*, a symbiont of chickpea was marked with *gfp* gene using suicidal plasmid pAG308. Plasmid pAG308 contains a Tn5-derived mini transposon, carrying a promoterless *gfp* gene and a kanamycin resistance gene. Unmarked *M. ciceri* MC-1 as control and *gfp* marked *M. ciceri* MC-1GFP (107 cells ml⁻¹) were inoculated in bulk soil and rhizosphere soil of chickpea, pea and wheat under contained pot house conditions. Soil samples were taken after 0, 15, 30, 60 and 90 days interval. To confirm whether there were any transfer at the genetic level PCR amplification with *gfp* gene specific primers was carried out. Genomic DNA of soil bacteria growing on LB, Malate and Jensen was amplified by polymerase chain reaction using *gfp* gene specific primers. *M. ciceri* MC-1GFP was taken as a positive control and MC-1 (parent was taken as a negative control) in PCR. Presence of introduced gene was checked in more than 600 bacterial isolates at different sampling time from bulk soil, chickpea, pea and wheat rhizosphere soil. None of the LB, Malate and Jensen media isolate was amplified with *gfp* primers. Some bacterial isolates on LB medium were giving pale green fluorescence under UV light, were not amplified with *gfp* primers. Therefore, fluorescence in the LB medium isolate was not due to the introduced *gfp* gene. Hence, introduced *gfp* gene was not horizontally transferred from MC-1GFP to other soil isolates as confirmed by PCR.

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Abstract Category

08 Horizontal Gene Transfer

PS.08.018 EFFECT OF GREEN FLUORESCENT PROTEIN TAGGED MESORHIZOBIUM CICERI ON SOIL BACTERIAL COMMUNITY STRUCTURE

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The ecological risks associated with use of genetically tagged microorganisms need to be evaluated by determining their effects on the indigenous microbial community. Community profiling by PCR-denaturing gradient gel electrophoresis (DGGE) has been widely used to generate valuable information on microbial diversity and community structure in soil, taking into account both culturable and non-culturable fractions of microorganisms. Effect of green fluorescent protein (GFP) marked *Mesorhizobium ciceri* MC-1GFP on total bacterial community was studied using DGGE in bulk soil, chickpea, pea and wheat rhizosphere soil. For this total soil community DNA was isolated and amplified by using universal 16S rDNA gene primers. The PCR products were analyzed by DGGE at 0, 15, 30, 60 and 90 days of inoculation. The DGGE patterns of all the treatments, at beginning of inoculation were 100 % similar, indicating that the soil populations were homogeneous. The DGGE fingerprints of the bacterial communities from chickpea, pea and wheat rhizosphere soil showed plant-dependent shifts in the bacterial community structure. Fifteen days onwards time dependent effects were also detected in the rhizosphere bacterial communities. However, no significant differences were observed in DGGE pattern due to inoculation of gfp marked *M. ciceri* MC-1GFP on bacterial population as compared to the DGGE pattern of unmarked parent *M. ciceri* MC-1. Therefore, release of gfp marked *Mesorhizobium ciceri* in soil did not show any adverse effect and introduced gfp gene was not horizontally transferred to indigenous soil bacterial community structure under controlled pot house conditions. Acknowledgement: 1. Dr. P.K. Sharma, Dept. of Microbiology, CCS HAU, India. 2. Department of Science and Technology, Govt. of India.

Abstract Category

08 Horizontal Gene Transfer

PS.08.019 OPTIMAL SURVIVAL STRATEGIES FOR PLASMIDS IN CHEMOSTATS AND BIOFILMS

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Some bacterial plasmids have a narrow host-range (NHR) while others have a broad host-range (BHR). We assume that the BHR strategy increases the fitness burden for the host as frequent swapping between hosts limits time for co-evolution between plasmid and host. So what are the advantages of having a broad host range?

An individual-based model of plasmid transfer has been used to identify the optimal host range of a plasmid that ensures its persistence in a multispecies community in structured (e.g. biofilms) and/or unstructured (e.g. chemostat) environments.

As expected due to higher fitness costs, model simulations show that BHR plasmids are out-competed by NHR plasmids in chemostats. However, our preliminary results suggest that the

BHR strategy is competitive in structured communities, where the longer residence time experienced by the cells allows continuous plasmid infection throughout the biofilm layers.

In the chemostat scenario, fitness cost is the critical factor governing the persistence of the plasmid in the population. In contrast, in a biofilm the ability to cross boundaries between patches occupied by different hosts and transfer proficiency become more important factors in determining the fate of a plasmid.

Abstract Category

08 Horizontal Gene Transfer

PS.08.020 METAMOBILOMICS - EXPANDING THE KNOWN ENVIRONMENTAL MOBILOME WITH HIGH THROUGHPUT SEQUENCING

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Our current knowledge of mobile genetic elements (MGEs) has traditionally been obtained through strain cultivation or isolation procedures like exogenous plasmid isolation. These methods, almost universally, involve the introduction of chemical selection biases geared towards antimicrobial resistance or xenobiotic degradation and rarely produce sufficient data to assess mobilome structure on a community-wide scale. Although efforts in genome sequencing have contributed more than 2,000 plasmid genomes, the combined pool of MGEs in nature thus remains largely unexplored. In this study, we conducted a focused investigation, through pyrosequencing, of a metagenome of mostly smaller (1 - 30 kb) circular genetic elements (metamobilome) derived from a wastewater sludge sample. Procurement of sufficient amounts of metamobilome DNA for sequencing involves an isolation procedure enriching for plasmid-size genomes, while ridding samples of chromosomal contamination. This method therefore avoids most selection biases while at the same time providing an overall sample diversity unachievable through traditional isolation methods. Sequencing on the 454 Genome Sequencer FLX (using the Titanium kit) yielded in excess of 190,000 high-quality reads at an average of >300 bp (~50Mbases). From these reads ~7,000 contigs were assembled (mean size 671 bp, largest contig >5 kb). Several contigs were identified as fully sequenced smaller plasmids (most likely high-copy), although only 5% of the total metamobilome reads were mapped to known plasmid genomes. More than 8,000 unique putative protein-sequences were derived with gene finding software and compared against known protein families. Although proteins were shown to be highly diverse, by far the largest proportion of proteins matched functions involved in plasmid or phage replication, or other proteins normally associated with MGEs. Through these methods, the known mobilome has therefore been expanded considerably.

Abstract Category

08 Horizontal Gene Transfer

PS.08.021 PHENOTYPIC AND PHYSIOLOGICAL ALTERATIONS BY ECTOPIC ACYL-HOMOSERINE LACTONE SYNTHASE EXPRESSION BY PSEUDOMONAS PUTIDA

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Many bacteria harbor an incomplete quorum sensing system, wherein they possess only LuxR homologues without the quorum sensing acyl-homoserine lactone (AHL) synthase, which is encoded by a luxI. We tested our hypothesis using *Pseudomonas putida* KT2440 during horizontal gene transfer in the environment the luxI gene could be introduced into a non-AHL producing bacterium whose phenotypes and physiology could be altered.

An artificial AHL-producing broad-host-range plasmid was constructed using a *cviI* gene encoding for C6-AHL (HHL) synthase from *Chromobacterium violaceum* and was introduced successfully into both wild-type and the *ppoR* (a luxR homologue) mutant.

Our data provides evidence to suggest that the PpoR-HHL complex, but neither PpoR nor HHL alone, could attenuate growth rate, antibiotic resistance, and biofilm formation ability. However, swimming motility, siderophore production, and indole degradation could be enhanced by PpoR-HHL. The addition of exogenous indole could increase biofilm formation and reduce swimming motility. Interestingly, indole proved ineffective in the presence of PpoR-HHL, thereby suggesting that the PpoR-HHL complex masks the effects of indole. Our data was supported by transcriptome analyses showing that the possession of the plasmid-encoded AHL synthase altered the expression of many genes on that chromosome.

Our results showed that ectopic luxI expression occurring via horizontal gene transfer can regulate a broad range of specific target genes, resulting in alterations of the phenotype and physiology of host cells.

Abstract Category

08 Horizontal Gene Transfer

PS.08.022 GENOMIC ISLAND-MEDIATED KILLING OF TRANSFER-PROFICIENT BACTERIAL CELLS

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The *clc* element (ICE_{clc}) is a mobile genetic element in the bacterium *Pseudomonas knackmussii* B13. ICE_{clc} normally resides in the host's chromosome but is capable of excising itself and forming a circularly closed DNA molecule, a process that happens during stationary phase in only 5% of all cells in a population. When a suitable recipient cell is present, the excised form of ICE_{clc} can self-transfer from the donor into that recipient and reintegrate in one or more copies of the gene for tRNA^{Gly} (1). ICE_{clc} also confers the ability to metabolize 3-chlorobenzoate (3CBA) on its host by means of the *clcABDE* genes. Several factors were shown to be critical for excision, transfer and integration. One of those is the integrase IntB13, the gene for which lays encoded near one end of ICE_{clc}. A second factor is the presumed integrase activator protein InrR, which is needed for bistable expression of IntB13 in the population (2). Here we use single-cell gene expression studies on growing bacterial microcolonies to examine the question

when ICEclc becomes active in a population of cells and which factors control bistable behaviour. Time-lapse imaging of cells expressing the *intB13* or *inrR* promoters in stationary phase indicated that they are severely inhibited in cell division compared to those cells which do not express *intB13*. IntB13-active cells that donate ICEclc to neighbouring recipients were not able to further divide as well and cell lysis could be observed in several instances. This suggests that ICEclc excision and transfer may lead to induction of a killing system for the donating host cell. The 5% bistable fraction of ICEclc donating cells may be an ecologically trade-off between the proportion of cells subject to killing and an active transfer system.

References

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Abstract Category

08 Horizontal Gene Transfer

PS.08.023 DISTRIBUTION OF PUTATIVE INTEGRON GENE CASSETTES IN THE GENUS *SHEWANELLA*

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Shewanellae inhabit a diverse range of environments that vary in salinity, temperature, and pressure. To date, 19 finished *Shewanella* genomes have been made publicly available, providing a unique opportunity to explore the genetic basis for their ability to thrive in such diverse environments and the role that lateral gene transfer has in acquiring adaptive functions. To differentiate the core versus niche-specific functions, we constructed protein ortholog tables by conducting bi-directional best hit analyses. We found that out of a total 81,215 predicted proteins, nearly half are present in all 19 strains; only 11 percent of the total are unique to a single strain. We hypothesized that the strain-specific genes were either incorrectly annotated as protein coding sequences or had been acquired by horizontal transfer. We were able to map 40 percent of the strain-specific genes to self-mobilizing DNA elements. Ten strains encode at least one integron integrase (IntI) which mediates the excision/integration of gene cassettes from circularized DNA via site-specific recombination between an adjacent AttI site and a homologous AttC recombination site in the target DNA. As predicted, we were able to identify a small number of gene cassettes adjacent to these integron integrases, most encoding proteins that are strain-specific. Upon further analysis of the genomes, we found numerous additional candidate gene cassettes in 1) mapped mobile elements, 2) sites lacking any obvious mobilization genes, and 3) genomes lacking integron integrases. In addition, we identified several insertion elements that appear to target AttC sites and are thus useful for finding additional gene cassettes that are not adjacent to *intI*. We predict that there are over 500 gene cassettes present in these genomes and are conducting experiments to test these predictions. These findings suggest that integron integrases play an important role in lateral gene transfer in *Shewanella*.

Abstract Category

08 Horizontal Gene Transfer

PS.08.024 TOL PLASMID INVASION IS CONTIGENT ON CELL GROWTH AND TEMPORARY CONJUGATION DEREPRESSION

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In situ observations by confocal microscopy have shown that biofilms may be hotspots for horizontal gene transfer (HGT). HGT could therefore be used to bring new degradation activities to resident biofilms, but conditions enhancing invasion of a biofilm by a plasmid and its maintenance are still poorly understood. Our work aims to determine both computationally and experimentally which are the main factors controlling HGT dynamics at the individual cell level.

This study has been implemented using iDynoMiCS, a software platform dedicated to the individual-based modelling of microbial communities. Simulation outputs are compared to cellular-scale observations in perfusion chambers using confocal microscopy techniques.

We first present a sensitivity analysis of the different plasmid dynamic parameters: the maximal distance between two mating cells, the transfer proficiency, recovery and lag times, and the loss probability. We also explore how different growth rates affect plasmid transfer and the resultant ratio of plasmid-free versus plasmid harbouring cells. In addition, we have conducted experimental analysis in order to estimate the most important parameters describing and/or affecting plasmid transfer and persistence (actually time lags and transfer proficiency).

Both experimental and simulation results showed that plasmid invasion is contingent on cell growth and temporary conjugation derepression. This last phenomenon has a great impact on plasmid ability to invade a bacterial population because it reduces significantly the time lag needed for conjugation to happen. However, plasmid epidemic spread stops even under highly nutrients availability conditions when cell division becomes impossible, e.g. due to the lack of space in the cell neighborhood.

Abstract Category

08 Horizontal Gene Transfer

PS.08.025 EFFECT OF VETERINARY MEDICINES ENTERING THE SOIL VIA MANURE ON THE ABUNDANCE AND DIVERSITY OF ANTIBIOTIC RESISTANCE GENES AND MOBILE GENETIC ELEMENTS

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Considerable amounts of antibiotics reach soils through manure fertilization. Manure was shown to be a reservoir of bacteria carrying antibiotic resistance genes and broad host-range plasmids. Thus spreading manure might stimulate the dissemination of antibiotic resistance in soil. Previously soil microcosm experiments performed with two different soils showed that adding sulfadiazine (SDZ) to manure increased not only the abundance of the sulfadiazine resistance genes *sul1* and *sul2* but also their transferability. The effect of repeated manure application and

of the rhizosphere on the abundance and transferability of *sul* genes is presently investigated under field conditions. The qPCR data indicated that repeated application of manure originating from animals treated with SDZ caused a significant increase of *sul2* gene abundance and an enhanced transferability. Again, the most frequently captured plasmids from manure-treated soils belonged to the previously discovered novel group of low G+C plasmids and to the promiscuous IncP-1 ϵ plasmids. The results of the field test thus confirm the data of previous microcosm experiments. The results highlight the importance of both plasmid groups for dissemination of *sul* genes.

Abstract Category
08 Horizontal Gene Transfer

PS.08.026 RESISTANCE TO ANTIBIOTICS AND PRESENCE OF CLASS I INTEGRONS IN *Salmonella enterica* ISOLATES FROM ANIMAL SOURCE FOODS IN MICHOACAN, MEXICO

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The subspecies and serotypes of the genus *Salmonella* represent a serious public health problem in Mexico, because they are commonly found contaminating external surfaces of animal source foods such as meat and dairy products. *Salmonella* serovars are important reservoirs of antimicrobial resistance. Genes conferring antimicrobial resistance in *Salmonella* are often carried in integrons, mobile DNA elements that have likely played an important role in transmission and dissemination of antimicrobial resistance determinants among *Salmonella enterica* strains. Class I integrons are the more frequently found in clinical isolates of enterobacteria. We investigated antimicrobial resistance by disk diffusion assay and integron class I carriage by PCR in 42 isolates of *S. enterica* from animal source foods from several municipalities in the state of Michoacan, Mexico. Resistance to carbenicillin, chloramphenicol, trimethoprim, ampicillin, gentamicin and cephalothin was found in 50%, 33%, 30% and 21% of the isolates, respectively. Resistance to cefotaxime, amikacin, netilmicin, nitrofurantoin, pefloxacin and ceftriaxone was also detected (11% to 2% of the isolates). 33% of the isolates were multiresistant, resistant to at least three different classes of antibiotics. In the screening of class I integrons, different amplification patterns were found in 76% of the isolates, each one with several amplicons in a range from 400 bp to 4000 bp, while no integrons were present in 24% of the isolates. The results suggest that multiresistance of studied isolates is associated with the presence of class I integrons.

Abstract Category
08 Horizontal Gene Transfer

PS.08.027 IDENTIFICATION OF RESERVOIRS OF STREPTOMYCIN RESISTANCE IN THE APPLE PHYLLOPLANE USING CULTURE-DEPENDENT AND

CULTURE-INDEPENDENT APPROACHES

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In the USA and some other countries, the antibiotic streptomycin is used to control *Erwinia amylovora*, a gram-negative bacterium that causes fire blight on various members of the Rosaceae family of plants, including apple and pear. However, streptomycin resistant strains of *E. amylovora* containing streptomycin phosphotransferases, *strA-strB*, within the mobile genetic element Tn5393 have been isolated from some of the treated orchards. The aim of our study was to identify reservoirs of streptomycin resistance genes within the bacterial community of the apple phylloplane (leaf surface) using both culture-dependent and -independent methods. From the bacteria that were directly extracted off the apple phylloplane, a metagenomic approach using functional screening was used to identify the reservoirs of streptomycin resistance genes. A 16S rRNA gene clone library was also constructed. On cultured phylloplane isolates, a PCR-based screening was done to identify each isolate by its 16S rRNA gene and to identify the presence of *strA-strB* and Tn5393. The bacterial community was largely dominated by members of the Proteobacteria, followed by the Bacteroidetes and Actinobacteria. Fourteen streptomycin-resistant clones from the metagenomic library were characterized. All 14 clones contained *strA-strB*. In 13 clones, regions flanking the resistance genes seemed of proteobacterial origin according to matches in GenBank. In one clone, the flanking region had no similarity with known sequences. Excluding *Sphingomonas* species, 95% of resistant proteobacterial isolates contained *strA-strB*. Two of 91 and one of 39 isolates from *Sphingomonas* and Actinobacteria, respectively, contained *strA-strB*. We conclude that the Proteobacteria predominate in the apple phylloplane and are the predominant reservoir of streptomycin resistance genes. Many but not all were in the form of *strA-strB* harbored on a mobile genetic element, Tn5393.

Abstract Category

08 Horizontal Gene Transfer

PS.09.001 GENOMIC COEVOLUTION: INSIGHTS FROM STUDIES OF BACTERIA AND BACTERIOPHAGE

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Coevolution - the process by which interacting species undergo reciprocal evolutionary change - is an important driving force underlying the generation and maintenance biodiversity. Recent advances in sequencing technology have enabled us to study the genomic dynamics of coevolutionary interactions at an unprecedented level of detail.

We used Illumina resequencing technology to sequence whole genomes of bacteria (*Escherichia coli*) and bacteriophage (T3) that coevolved in the laboratory for approximately 200 bacterial generations. We sequenced the ancestral bacterial clone as well as two bacteria that had evolved resistance to the ancestral phage. We also sequenced the ancestral phage and a derived phage type that is able to infect both the ancestral bacterial genotype and the resistant clones.

We found that the two resistant bacterial clones evolved resistance to ancestral phage in different ways -- one through a single nucleotide substitution and the other through a large genomic deletion. Unlike the derived bacterial genomes which each showed a single mutational event distinguishing derived strains from the ancestor, the host-range phage accumulated twenty-seven substitutions by day nineteen of the experiment. Several of these substitutions lie in genes previously shown to be involved in the bacteria-bacteriophage interaction.

Genome re-sequencing in the context of experimental evolution provides new opportunities for studying the dynamics of coevolution. We were able to identify every genetic change that occurred during 200 generations of antagonistic coevolution between bacteria and bacteriophage. Our results highlight the power of new sequencing technologies to answer fundamental questions about how ecological interactions among species shape evolutionary trajectories and genomic change.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.002 INVESTIGATING VIRAL AND MICROBIAL BIOGEOGRAPHY USING GENGIS

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Background and aims Advances in DNA sequencing have provided new insights into the biogeography of organisms and populations. Thousands of georeferenced genome sequences are now available for viral pathogens such as Influenza A, while metagenomic studies such as the Global Ocean Sampling (GOS) expedition are providing microbial biodiversity data from distinct habitats and locations. Our software package GenGIS integrates digital maps with gene sequence, habitat, and hierarchical data, allowing users to visualize and analyze the interplay between these sources of information. Methods Traditional site-by-site information along with 2D and 3D georeferenced trees were used to study the spatiotemporal dynamics of a viral and metagenomic dataset. We introduce an algorithm for calculating, visualizing and testing the significance of the globally optimal fit between hierarchically organized data and a geographic gradient. This approach was used to 1) search for geographic structure in a phylogeny of H1N1pdm genomes and 2) test for significant regional or latitudinal structuring of biodiversity among microbial samples collected in the GOS expedition. Results Visual and statistical investigations of the population structure of H1N1pdm indicated a lack of geographic structure, supporting the wide and rapid dispersal of last year's pandemic strain. An exploration of GOS samples collected from the Northern Atlantic and Caribbean Sea revealed compositional differences between microbial communities from these oceanic habitats, but little evidence of latitudinal structure within either habitat. Compositional differences were largely driven by overrepresentation of *Prochlorococcus* in the Caribbean Sea. Conclusions Biogeographic hypotheses can conflate the effects of dispersal limitation and niche adaptation. Using GenGIS, we have shown that distance alone plays only a minimal role in the phylogenetic structuring of the H1N1pdm and the oceanic microbial populations of the Atlantic seaboard.

Abstract Category
09 Evolutionary Ecology of Microbial Communities

PS.09.003 STRAIN LEVEL DIFFERENCES IN *ESCHERICHIA COLI* TRANSPORT, CELL SURFACE AND ADHESION CHARACTERISTICS

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Given the importance of *E. coli* as an indicator of fecal contamination, it is imperative that genotypic and phenotypic variability among strains of *E. coli* from the same host and/or environmental niche are understood. The goal of this study was to evaluate the effect of strain level differences on the attachment and transport of *E. coli* through porous media. Using selective plating, 339 *E. coli* isolates were obtained from swine lagoon slurry and isolates were characterized by BOX PCR analysis of DNA extracts. Eight isolates, representing 81% of the diversity of *E. coli* isolates, were selected for transport studies and cell surface characterization. Cell properties such as (electrophoretic mobility, hydrophobicity, charge density), the occurrence of surface structures (lipopolysaccharides, autotransporter proteins, and fimbriae) and the transport behavior of each isolate was assessed. Our results show a large diversity in cell properties (hydrophobicity ranging from 6 to 81% and Zeta potential ranging from - 2.0 to - 46 mV) and transport behavior (percent recovery of cells eluted from the columns ranged from 46% to nearly 100%) for the different *E. coli* isolates. Correlation of phenotypic behavior with genotype shows that strains that had high sticking efficiencies to the quartz sand grains were also uniquely possessive of genes for adhesions (*agn43*, *fimH*, *iha*), toxins and siderophores (*hlyA*, *iroN_{E. coli}*). This diversity in genotypic and phenotypic behavior must be taken into account when making assessments of the suitability of using *E. coli* as an indicator organism and when modeling the movement of *E. coli* through the environment.

Abstract Category
09 Evolutionary Ecology of Microbial Communities

PS.09.004 DIFFERENT SALINITIES INFLUENCE MICROBIAL COMMUNITY COMPOSITION AND EVOLUTION IN COASTAL ANTARCTIC LAKES: A NATURAL ECO-EVOLUTIONARY EXPERIMENT

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Mounting evidence indicates that salt concentration affects the evolution and composition of aquatic microbial communities. To advance our understanding in this subject, we have studied the microbial communities inhabiting a group of coastal Antarctic lakes that range from freshwater (0) to hypersaline (>250) using Sanger/454 rDNA sequencing and experiments. Most of these lakes are marine-derived closed basins, containing microbial communities that were exposed to drastic salinity changes since the formation of the lakes (<10,000 years). Other lakes are freshwater and were formed by melt-water from the Antarctic ice-plateau. Our results indicated that long-term salinity shifts had a strong influence on the richness, evenness and possibly evolution of the microbial communities living in marine-derived lakes. In particular, diversity decreased at salinity extremes (i.e. freshwater or hypersaline lakes) suggesting strong environmental filtering and local extinctions. Marine-derived microbes were present in saline lakes, but their richness and evenness differed from ancestral marine-coastal communities. In particular, lakes that became hypersaline appeared to be exclusively dominated by Archaea. Experimental results indicated that ancestrally marine dinoflagellate populations have adapted to diverse lake salinities, suggesting that adaptive evolution may have played an important role in shaping the present microbial communities in the lakes. Typical freshwater bacteria were predominant in freshwater lakes, instead of marine-derived groups adapted to low salinity. Freshwater bacteria clustered phylogenetically with lineages present in ice (European glaciers and perennial ice cover of distant Antarctic lakes) and north temperate lakes, suggesting intra- and intercontinental colonisations. Altogether, our results contribute to

understand how microbial communities react to environmental change and give insight on microbial long-range colonization capabilities.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.005 PROTEOGENOMIC ANALYSIS OF GEOCHEMICAL AND VIRAL CONTROLS ON ACIDOPHILIC MICROBIAL COMMUNITY FUNCTIONING

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Insights into molecular underpinnings of ecological processes will assist ecosystem modeling. We studied how acidophilic biofilm communities function across a range of geochemical conditions (one location sampled five times over two years; pH: 0.7 - 1.2, FeT: 300 - 131 mM). We evaluated changes in species and strain composition as well as changed activity levels and function partitioning. Community composition was assessed using fluorescent in situ hybridization and 454 pyrosequencing datasets (~70 Mbp / sample after replicate read removal) that genomically resolved dominant bacterial (two) and archaeal (seven) populations. Functional analyses relied upon 2D LC tandem mass spectrometry (>4000 protein identifications / sample).

While macroscopically similar, major changes in community composition occurred, e.g., archaeal representation decreased from 65 to 5% at higher pH. Metagenomic data confirmed inferences from proteomic data that the main primary producer, *Leptospirillum* Group II, had a similar genotype across all samples, which resulted from recombination involving genomes of two previously characterized populations. However, data indicate significant gene loss and acquisition relative to those genomes as well as temporal shifts in strain variants that differ subtly in terms of gene sequence and content, particularly in their plasmid regions. A viral bloom (~35% of all reads from one sample) corresponded with the decline of its dominant archaeal host, but did not cause a strong population bottleneck. We infer that host population diversity was maintained due to very high CRISPR locus diversity (anti-viral immune system). Protein profile shifts were moderate relative to community structure shifts.

Species composition adjustment is the main factor in community level adaptation to both bottom-up (geochemistry) and top-down (viral predation) controls. Strain variant dynamics and modest proteomic shifts appear to facilitate persistence of the main primary producer.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.006 VERTICAL DISTRIBUTION OF PROKARYOTES AND INTERACTIONS WITH THEIR ENVIRONMENT IN HONGHU LAKE SEDIMENTS

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Honghu Lake lies the middle reaches of the Yangtze river, has become eutrophic over the past decades. A sediment core sample had been investigated for the interaction and co-evolution between microbes and environmental factors. This 0-70 cm sediment core corresponds to 117 years based on the magnetic susceptibility and isotopic geochronology. Total organic C and organochlorine significantly increased upper 35 cm and 20 cm, respectively. According to this, six bacterial and archeal 16S rDNA clone libraries have been constructed at 10-12 cm, 22-24 cm and 52-57 cm layers. We also analysed the RFLP patterns, sequencing and phyletic evolution. These results showed that there is evident variance of microbial community structure along the depths. The bacteria communities of these layers are related to α -, β -, γ -, δ -proteobacteria, Nitrospiraceae, Chloroflexi, Acidobacterium, Firmicutes, Planctomyces, Verrucomicrobia, Chlorobi, Actinobacteria. However, more Bacteroides (77% and 88%, respectively) had been found in the up and lower layers. Except a higher proportion of δ -proteobacteria (19%), the rest groups presented an uniform

distribution at middle layer. The archeal communities were composed of Crenarchaeota and Euryarchaeota in all three depths, but their community structure was different. Desulfurococcales was detected only in the middle layer, Thermococci and Halobacteria were unique groups in lower layer. The proportion of Thermoplasmata was similar at surface and middle layers (31% and 30%, respectively), but reduced to 13.9% at lower layer; Methanomicrobiales had a similar tendency in these depths comparison with Thermoplasmata. This implies both groups had the same nutritional requirement or ecological niche. From up to lower layers, the proportion of Crenarchaeota was 55%, 48%, and 63%, respectively. In conclusion, microbial composition and diversity has been influenced by organic perturbation and pollutant in different depth of sediment.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.007 POPULATION DYNAMICS OF A DENITRIFYING COMMUNITY FROM A TIDAL FLAT UNDER STABLE CONDITIONS

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Denitrifiers convert nitrate into nitrogen gas in the absence of oxygen and so play an important role in the global nitrogen cycle. Many evolutionary unrelated microorganisms are capable of denitrification and so far the identification of environmentally relevant denitrifiers has been difficult. In this study we have sampled an active denitrifying community from a tidal flat in the Wadden Sea. This community was incubated in a laboratory bioreactor under constant conditions with constant supply of fresh substrates for 6 weeks. A defined mixture of organic compounds was used as the energy and carbon source. To mimic the natural dispersal of microorganisms, fresh inoculum was added daily. The end products of denitrification (e.g. nitrogen, nitrous oxide) were monitored with a mass spectrometer and the efficiency of microbial growth was monitored by heat balancing. Population dynamics was monitored by ARISA and metagenomic sequencing of extracted DNA (454, Illumina) of selected samples. The resulting metagenomes were inspected for functional genes of denitrification and phylogenetic identification of dominant community members. The results were interpreted in the context of neutral and niche models of microbial evolution and the environmental relevance of the enriched denitrifiers was analysed by comparing the detected functional genes with a reference metagenome obtained directly from the flat.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.008 THE DIVERSE ARRANGEMENT AND EVOLUTION OF PHOTOSYNTHETIC SUPEROPERONS IN AEROBIC ANOXYGENIC PHOTOTROPHIC BACTERIA

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Aerobic anoxygenic phototrophic bacteria containing bacteriochlorophyll-a represent an important functional bacterial group in marine ecosystems. The known marine AAPB species are mainly classified to three clades, Roseobacter clade, Erythrobacter-Citromicrobium clade and NOR5/OM60 clade. Ten genome sequences of aerobic anoxygenic phototrophic bacteria and two more genomes with no existence of photosynthetic genes were analyzed in this study (two of them were finished by our lab). According to the arrangement of photosynthetic genes, we discovered that the structure of photosynthetic superoperons could be grouped into four types, based on permutations and combinations of the two conserved subclusters, bchFNBHLM-LhaA-puh and crtF-bchCXYZ-puf. Homologous gene recombination occurred between the two subclusters. The genes (RPA) related to gene rearrangement were also found between the two subclusters in Roseobacter litoralis Och 149. In order to check the effect of the two inserted genes (RPA) on photosynthetic capacity, we measure photosynthetic ability and absorbance spectra of several strains. Interestingly, an abnormal high value of photosynthetic functional cross-section and strange absorbance spectra (very low expression of RC-LHI complex compared LHI) were obtained in Roseobacter. litoralis Och 149. That hints the two inserted genes (RPA) suppress the expression of RC-LHI complex. Two

different photosynthetic superoperons, for the first time, were found in a single strain, *Citromicrobium bathyomarimum* JL354. But, comparison of *C. bathyomarimum* JL354 and *Citromicrobium* sp. JL1363, we thought photosynthetic genes trend to miss in environment.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.009 THE pmoA GENE AS A FUNCTIONAL AND PHYLOGENETIC MARKER FOR METHANOTROPHIC BACTERIA: A META-ANALYSIS CORRELATING PHYLOTYPES AND ENVIRONMENTS

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The pmoA gene encoding a subunit of the methane monooxygenase is widely used as a marker for studying methanotrophic bacteria in the environment. Here, we re-evaluated its applicability for phylogenetic inferences of methanotrophic bacteria and investigated distribution patterns of pmoA phylotypes retrieved from various habitats. We analyzed the correlation of the 16S rRNA gene sequence distance and the corresponding pmoA sequence distance of cultivated methanotrophic bacteria, including the recently described pmoA sequences of *Crenothrix polyspora* and *Verrucomicrobia* species. Furthermore, approximately 3400 pmoA sequences from uncultivated bacteria were amended with data about the respective environments. Comparative sequence analysis was combined with statistical approaches to unravel environmental distribution patterns. The pmoA of *Burkholderia* sp. TS2 and *C. polyspora* showed no correlation to 16S rRNA suggesting earlier lateral gene transfer events. All other distance comparisons revealed a good correlation indicating that pmoA is an overall suitable functional and phylogenetic marker gene. Based on this, we analyzed pmoA phylotypes from various environments showing a distinct clustering of upland soil sequences, and of sequences retrieved from halophilic environments. Furthermore, wetland rice and freshwater environments were characterized by different type Ib clusters showing only a distant relationship to any cultivated methanotrophic bacteria. We conclude that in general, the pmoA gene can be used as a reliable phylogenetic marker to investigate methanotrophs in the environment. Furthermore, our analysis showed that methanotroph diversity seems to be strongly influenced by available methane concentrations and salinity. Aquatic environments in particular are inhabited by specific phylotypes lacking cultivated representatives. Hence, increased cultivation efforts are necessary and should be focused on freshwater and wetland rice.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.010 GENOTYPING OF ENTEROCOCCUS FAECALIS USING EIGHT SINGLE NUCLEOTIDE POLYMORPHISMS TO STUDY THE POPULATION STRUCTURE IN DIFFERENT MICROBIAL COMMUNITIES.

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Enterococcus faecalis plays a significant role in different microbial communities. The aim of this study was to develop a rapid, robust, highly discriminative genotyping method to study the population structure of *E. faecalis* isolates sourced from water and clinical samples, using highly informative sets of single-nucleotide polymorphisms (SNPs).

Informative SNP sets were determined using "Minimum SNPs" which selects for polymorphisms that provides the resolution of a bacterial population based on the Simpson's index of diversity (D-value). Primers were designed using the Primer Express 2.0 (Applied BioSystems). High D SNPs were interrogated using Allele-specific Real-Time PCR to compare the population structure of *E. faecalis* in water and clinical samples from South East Queensland. SNP profiles for all isolates from these communities were obtained. SNP Validation was done by gene sequencing. "Based Upon Related Sequence Types" (eBURST) analysis was done using the online algorithm available at www.mlst.net.

Results: 1. Eight Informative SNP sets provided a cumulative D-value of 0.99 compared to the E. faecalis MLST database, based on 424 sequence types. 2. 20 SNP profiles were found for 49 clinical isolates and 14 SNP profiles were obtained from 32 water isolates. Amongst these, only four SNP profiles were common for both communities. 3. Gene sequencing confirmed the SNP profiles of the isolates tested. 4. eBURST analysis revealed the relationship between the MLST E. faecalis isolates and the SNP profiles of E. faecalis isolates from South East Queensland. Conclusions: An eight SNP typing method for genotyping E. faecalis isolates was developed. This method is rapid, robust and ideally suited to study the population structure and relationships between E. faecalis isolates originating from water and humans.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.011 DIVERSITY OF ENDOPHYTIC ACTINOBACTERIA IN RICE PLANTS GROWING IN AUSTRALIA

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Australian rice is grown under a unique management system which allows the use of plant rotation. The system has enabled Australian rice to be considered a disease-free crop. It also enriches the diversity of the microorganisms in the soil. The aim of this research is to determine the diversity of endophytic actinobacteria from rice plants growing in Australia using culture dependent methods. Actinobacteria were also isolated from rice field soils for comparative purposes. Rice samples were collected from two growing season at two different growing ages. Rice plants were cleaned to remove attached soil and then surfaced sterilized. Plants were sectioned into roots, stems, leaf sheaths and leaf blades. Then cut into smaller fragments and put onto four different isolation media. The plates were incubated at 27°C and 37°C. Actinobacteria isolation from soil was conducted using the serial dilution method with the same media and incubation conditions. Isolates were characterized based on colony morphology and identified using 16S rRNA gene sequencing. The isolation result from season I showed that most endophytic actinobacteria found from rice plants were belonged to the genus Microbispora. Surprisingly, only few Streptomyces strains were found. This result was reproduced in the isolation from rice growing in season II. These findings are in contrast with other studies of endophytic actinobacteria from rice, in which Streptomyces spp. were the most commonly found culturable actinobacteria. The differences in populations may be due to a number of factors including environmental conditions, soil type, rice cultivars or the differences in isolation protocols. When compared to the actinobacteria isolated from rice field soil, endophytic actinobacteria from rice plants were less diverse. This suggests that rice plants preferentially select their resident populations that are expected to provide a beneficial effect.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.012 PROTISTAN MICROBIAL OBSERVATORY IN CARIACO BASIN PART 2; RICHNESS ACROSS GEOCHEMICAL GRADIENTS AND NOVEL ACTIVE DEEP-SEA ANAEROBIC LINEAGES

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We describe here the results of the Cariaco Basin Microbial Observatory (Caribbean Sea, Venezuela). The Basin contains the largest body of marine anoxic water, and is characterized by steep biogeochemical gradients. We surveyed 18S rRNA gene sequence diversity using both Sanger and pyrosequencing-based approaches, multiple PCR primers, and state-of-the-art statistical analyses. We also enumerated eukaryotic cells in different samples using SEM and FISH with

universal eukaryotic probes and utilized a cDNA approach to confirm the presence of viable protists from the deepest anoxic samples. Sampling the Basin at two stations, two seasons, and four depths with distinct biogeochemical regimes, we obtained the largest, and arguably the least biased collection of over 6,000 nearly full-length marine protistan rRNA gene sequences to date, and over 80,000 pyrosequencing tags. This large dataset provided the first statistically sound prediction of total protistan richness in a large and diverse environment: over 36,000 species, defined as almost full-length 18S rRNA gene sequence clusters sharing over 99% sequence homology. This is a small fraction of the grand total of described protists (over 100,000-500,000 species). Therefore, the overwhelming number of described species must be absent from the Basin, evidence for endemic distribution of the majority of protists. The cell numbers enumerated from the oxygenated water column by FISH and SEM are largely congruent. The ratio of FISH to SEM counts decreases to 25% within the redoxcline, 10% just below it, and drops to 8% in the deepest, most sulfidic areas of the basin. This suggests that over 90% of cells in the deep anoxic layers of the Cariaco Basin water column have no close relatives in existing databases. cDNA sequences obtained from this depth confirm their viability. These findings make this habitat exceptionally promising for microbial discovery.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.013 PROTISTAN MICROBIAL OBSERVATORY IN THE ANOXIC CARIACO BASIN, PART I; DIVERSITY, ENDEMICITY, AND DISTRIBUTION ACROSS GEOCHEMICAL GRADIENTS.

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We applied a combination of in-depth Sanger and 454 pyrosequencing to interrogate eukaryotic microbial diversity in the Cariaco Basin, the world's largest truly marine anoxic basin, characterized by steep chemical gradients that create a wide range of microbial habitats. A survey of this magnitude allows a unique investigation into the extent of unknown diversity and the degree of endemism of marine protists. Our dataset was generated from a total of 16 samples from 3 locations across the Basin and 4 depths along the basin's geochemical gradients. For all samples we applied a multiple-primer approach for PCR amplification of 18S rDNA gene sequences. Phylogenetic analyses of Sanger sequences identify new protistan clades from species to class levels within the Radiolaria, group I Syndiniales, MAST Stramenopiles, and Euglenozoa. Our analyses increase the resolution of many underrepresented groups of marine protists and reveal an increased ecological and geographic distribution of several uncultured environmental sequence clades. Several of the novel clades were detected only in samples from the anoxic portion of the basin, suggesting they correspond to endemic lineages. This is supported by canonical correspondence analyses and parametric richness estimations that show a clear division between protistan communities present in the deep anoxic portion of the Basin, those along the oxycline and those higher in the water column.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.014 DETERMINATION OF ANTIBIOTIC RESISTANCE IN THE BACTERIAL COMMUNITY OF BOILING SPRINGS LAKE IN LASSEN VOLCANIC NATIONAL PARK

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We investigated the presence, levels, and patterns of antibiotic resistance in bacterial isolates from Boiling Springs Lake, a high temperature, low pH, low-metal content lake located in Lassen Volcanic National Park in northern California. We screened 41 isolates against 14 antibiotics and bacteriocins

covering 11 classes of antimicrobials. We attempted to determine pH and temperature stability of antimicrobials where resistance was pervasive under a given set of growth conditions. Minimum inhibitory concentrations were determined for some strains showing multi-drug resistance (MDR). PCR primers were designed to screen isolates for MDR efflux pumps from the MFS, RND, and the ABC families. The identity of most of the isolates was determined by 16S rRNA gene sequencing. 54% of the isolates were resistant to 5 or more antimicrobials with 20% being resistant to 8. High-level resistance of up to 200 µg/ml was observed for some strains for some antimicrobial agents. Isolates with nearly identical 16S rRNA gene sequences often displayed different resistance patterns. Many of the isolates grown at pH 2.9 and 50°C demonstrated resistance to nisin, while all isolates grown under these conditions appeared resistant to trimethoprim and ciprofloxacin. Some sensitivity to these drugs was found in isolates grown at pH 5 and 50°C, though the efficacy of trimethoprim and ciprofloxacin at pH 2.9 is questionable. Most isolates were within the *Firmicute* and *Actinobacteria* phyla with members of the *Alicyclobacillaceae* family being the most common. The paucity of resistance to tetracycline, ampicillin, vancomycin, and chloramphenicol, suggests the evolutionary pressures selecting for and maintaining resistance in this extreme habitat are different than have been found in other investigations of antibiotic resistance in pristine environments. The observed resistance may be related to the physiological adaptations of organisms living in low pH, high temperature habitats.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.015 EVOLUTIONARY HISTORY OF BACILLUS SP FROM CUATRO CIENEGAS BASIN

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Population genetics studies have focused mainly on pathogenic and symbiotic organisms; however there are few works that explore the population structure of free living bacteria. Cuatro Ciénegas Valley, located in Coahuila, Mexico, is a place with a great diversity of aquatic environments in which there are several bacterial communities including some genera like *Bacillus*. By studying the *Bacillus* from Cuatro Ciénegas we are beginning to understand the evolutionary history of this group and we also have been able to determine some aspects of the adaptation to the particular environmental conditions of this place. The aim of the present work is to look at the population structure of *Bacillus* isolates from Cuatro Ciénegas in order to understand the evolutionary forces that have been shaping such populations. We used the 16s ribosomal sequences to identify and classify the *Bacillus* group that we are studying and we were able to notice that it is a group widely distributed across the valley and that seems to be different from other *Bacillus* species reported to date. We also amplified and sequenced four other genes (*hsp70*, *recA*, *gltX*, *idh*) to perform a multilocus analysis. This analysis shows that the population studied has a clonal structure and that its distribution is not correlated with environmental factors.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.016 UNDERSTANDING BACTERIAL EVOLUTION AND SPECIATION THROUGH THE STUDY OF EXIGUOBACTERIUM AT CUATRO CIÉNEGAS, COAHUILA, MÉXICO

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To understand the adaptation and speciation processes in prokaryotes is one of the most challenging subjects in evolutionary biology. The Cuatro Ciénegas Basin (CCB) is an excellent ecosystem to study the evolutionary processes that shape bacterial populations and lead to new species formation. The different aquatic systems in CCB allow us to explore the role of ecology and geography in the diversification and adaptation of prokaryotes to different environments and the molecular mechanisms that allow population divergence and differentiation. In this project we studied the molecular evolution of one of the most abundant bacterial groups at CCB called *Exiguobacterium*, a genus of gram-positive non-sporulating bacteria related to *Bacillus*. We obtained the bayesian phylogeny of the 16srRNA of 198 isolates collected along four contrasting aquatic systems at CCB. We have analyzed the genetic structure of one clade ("species") found in the

different aquatic systems using several genetic markers (*citC*, *rpoB*, *recA* and *hsp70*). With these same markers we obtained the concatenated phylogeny of the isolates. The phylogeny obtained shows a clear genetic divergence associated with the habitat of the isolates (sediment versus column water) and found no relation with the environmental conditions nor with the geography of the different aquatic systems. In this work we discuss our main results and advances in order to understand the ecological and genetic processes involved in bacterial speciation.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.017 MOLECULAR POPULATION GENETICS OF A SPATIALLY STRUCTURED POPULATION OF THE THERMOPHILIC CYANOBACTERIUM *MASTIGOCLADUS LAMINOSUS*

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The theory of adaptive radiation is predicated upon divergent natural selection driving phenotypic diversification of populations. A preliminary analysis of a population of the cyanobacterium *Mastigocladus laminosus* that is found along a temperature gradient at White Creek, Yellowstone N.P., suggests that ecologically specialized population members are diverging due to restricted gene flow in certain regions of the genome. Here, we further investigated the distribution of genetic variation in this population for more than 150 laboratory strains isolated from five sampling sites spanning a thermal gradient with mean annual temperatures ranging from 39 to 54°C.

Twenty-four strains were randomly selected from each of the five sites along White Creek and sequenced at 46 loci. In addition, the total strain collection was genotyped by endonuclease restriction analysis for a subset of the variable loci to identify regions of the genome that exhibit extreme genetic differentiation between study sites along the White Creek thermal gradient.

Twenty-one of these loci have nucleotide polymorphisms. Of these variable loci, several have the statistical signature of selection and/or extreme genetic differentiation with regard to population structure. Of particular interest is a locus, *rfbC*, characterized by both extreme genetic differentiation (i.e., a high F_{ST} value), indicating very strong segregation along the temperature gradient, and a statistically significant signature of deviation from selective neutrality.

Our bacterial population in White Creek offers us a unique opportunity to reveal the genetic underpinnings of adaptive radiation, a central goal of evolutionary biology. Future investigation will focus on a finer-scale analysis of candidate regions of the genome, including *rfbC*, to identify the genetic basis of ecological variation in the population.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.018 STABILITY, GENOTYPIC AND PHENOTYPIC DIVERSITY OF SHEWANELLA BALTICA POPULATION IN THE BALTIC SEA

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Studying how bacterial strains diverge over time and how this correlates with specialization to new environmental niches is important for understanding the dynamics of environmental communities. We are studying these questions using collections of *Shewanella baltica* strains isolated from a region of the central Baltic Sea which is characterized by the presence of stable redox gradients and sampled nearly two decades apart. We used multi-locus sequence typing (MLST) to analyze phylogenetic relationship of these strains and correlated the phylogeny to locations in the water column and the years of isolation. We also employed comparative genomic hybridization (CGH) to provide additional details of the genetic diversity among these strains. Furthermore, we screened for the utilization of 95 different carbon sources using the Biolog-GN system. We observed a similar

phylogeny as seen in MLST from analysis of the CGH profile using a presence/absence matrix approach and identified a subset of genes that are potentially important to adaptation to different redox conditions along the water column. We also found significant correlations of the metabolic profiles to the years and methods of isolation. Strains isolated in 1998 displayed higher versatility in carbon source utilization, implying that this characteristic may be a key strategy for their long-term survival. This study effectively characterized genotypic and phenotypic diversity in a population of *Shewanella baltica* strains over a redox gradient and over time. These findings will advance the understanding of bacterial specialization to differentiated environmental niches and will drive further investigation into the mechanisms of evolution of the *Shewanella* species.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.019 DESIGN AND EVALUATION OF UNIVERSAL GROUP II CHAPERONIN PCR PRIMERS FOR ARCHAEOAL DETECTION

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The prokaryotic world is divided into the Bacteria and Archaea domains. Although these two groups are evolutionarily and biochemically distinct, both are found in almost every environment investigated. Within the bacterial realm, robust universal PCR primer sets have been well-characterized and utilized to target the bacterial 16S rRNA gene and the group I chaperonin (cpn60) gene. Both have provided a wealth of data towards understanding the bacterial members of complex communities, with the protein-coding chaperonin gene having advantages in terms of phylogenetic resolution. Alternatively, within the archaeal realm, universal PCR primers for the entire domain have remained limited to the archaeal 16S rRNA gene. The group II archaeal chaperonin gene (known as the thermosome, TF55, CCT or TCP-1) was evaluated as a potential non-16S universal archaeal marker. The testing of various primer combinations with a diverse collection of purified archaeal genomic DNA revealed that a one primer set had the ability to detect all archaeal DNA (both crenarchaeota and euryarchaeota) with GC contents of <60%. Phylogenies based on sequences of the ~800 bp region targeted by the JH0175/JH0178 primer pair are congruent with 16S rRNA phylogenies. In addition, the thermosome-based phylogenies maintain longer branch lengths, which facilitates resolution of closely related taxa. To evaluate the performance of this proposed universal thermosome primer set in a field setting, work is currently underway to detect and characterize the archaeal communities present in rumen samples. Clone library profiles are being generated from a well-established universal archaeal 16S rRNA primer set, methanogen-specific primer set and this new thermosome primer set. Comparisons of the archaeal profiles generated from these three primer sets will allow for the critical evaluation of the JH0175/JH0178 thermosome primer set as a tool for universal archaeal PCR detection.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.020 DRIVING FORCES FOR SPECIALIZATION IN MICROBIAL COMMUNITIES

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Specialization is an important aspect of efficiency, not only in human society but also in microbial communities. Previously, in cultures starting with one single organism, strain polymorphism was observed after several hundreds of generations, even in case of constant environments. We hypothesize that the thermodynamic efficiency is the driving force to evolve such new specialized subspecies. In communities with specialized microorganisms each species may benefit by performing partial substrate conversion instead of expressing enzymes for an entire pathway. Not much is known about such "division of labor" in the environment.

The present study addresses specialization among nitrate reducing model organisms. *E. coli* was taken as a model organism for dissimilatory nitrate reduction to ammonia and *P. denitrificans* as a model denitrifier. These pathways consist of 2 and 4 steps respectively and are excellent cases to study specialization.

Chemostats were inoculated with *E. coli* or with *P. denitrificans* and run under constant conditions for a few hundreds of generations. Samples were taken for metagenome, transcriptome and proteome analysis and high resolution calorimetry was applied to monitor changes in the overall thermodynamic efficiency. Exchange of intermediates was analyzed by ¹⁵N/¹³C labeling and mass spectrometry. We observed microbial evolution and changes in heat production. The results give a basic idea to address the general question whether denitrification is rather performed by a group of specialists than by a single generalist. Finally, knowledge about interspecies communication meets fundamental requirements to understand the complex microbial behavior in natural nitrate reducing communities.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.021 NATURALISATION OF *E. COLI* POPULATIONS IN LOW-TEMPERATURE TEMPERATE MARITIME SOILS

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Soils are typically considered to be hostile environments for *E. coli*, with reported naturalization of the organism in soils limited to certain global regions. We report here the first detection of naturalised *E. coli* populations (>9 years survival) in the relatively oligotrophic low-temperature environments of temperate maritime grassland soils. This study also characterized the genetic diversity and physiology of these environmentally persistent *E. coli* isolates, leached from a Luvic Stagnosol soil. Molecular analysis (16S rRNA sequencing, enterobacterial repetitive intergenic consensus PCR, pulsed-field gel electrophoresis, and a multiplex PCR method) determined the genetic diversity of the environmental isolates, while physiological methods (growth rates, phenotypic array) and proteomics aimed at comparing the metabolic capacity and environmental fitness of the isolates to representative laboratory strains under a wide range of conditions. Molecular analysis indicated that a wide diversity of *E. coli* genotypes were persisting in the soil, with at least 3 clonal groups (A, B1, and D) present. The environmental isolates tested grew well across a broad range of temperatures and media, with higher specific growth rates than laboratory strains under all conditions tested, particularly at lower temperatures (10°C, 15°C) on oligotrophic media (2.3-5.4 times greater growth rates). Phenotypic array and proteomic analysis indicated that an environmental persistent isolate tested possessed a wide metabolic flexibility and produced specialised proteins, including cold shock and stress response proteins, which may assist in the survival and growth of the organism at lower temperatures characteristic of temperate maritime soils. These findings suggest that certain *E. coli* strains may have the ability to colonize and adapt to soil conditions. The resulting lack of fecal specificity has implications for the use of *E. coli* as an indicator of fecal pollution in the environment.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.022 MICROBIAL COMMUNITY ANALYSIS OF CHROMIUM CONTAMINATED SOIL FROM AN INDUSTRIAL DISPOSAL SITE IN TULTITLAN, MÉXICO.

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The impact on the soil microbial community of a long-term environmental exposure to Cr(VI) and Cr(III) contamination was investigated. The soil samples were collected in the vicinity of the industrial disposal site of the former plant "Cromatos de México", where 75,000 tons of waste material was confined inadequately many years ago. Soil DNA from 29, 40, 50 and 60 cm depth was extracted and the 16S rRNA genes were amplified by PCR using universal primers. Results indicate that microbial populations are present despite some extreme contaminant levels in this

industrial waste contaminated site. Sequencing results of 16S rDNA of 120 clones represented phylogenetically diverse bacteria belonging to alpha-, beta-, gamma-, and delta-Proteobacteria, the high and low G + C Gram-positive, green nonsulfur, acidobacteria and others that did not group within a described division; many of them represented phylotypes closely related to uncultured bacteria. The community composition changed at different depths. Our study gives a comprehensive insight into the structure of the bacterial community exposed to extreme chromium contamination levels. Remarkably some bacteria as *Truepera radiovictrix* and *Acidobacterium* spp resistant to heavy metals like uranium were found.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.023 ANALYSIS OF THE POPULATION SUBSTRUCTURE OF AQUATIC SPHINGOMONADACEAE BY MLSA

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The role of recombination, adaptation and selection in shaping bacterial diversity was assessed using aquatic members of the *Sphingomonadaceae* (*Alphaproteobacteria*) as a model group. Our multilocus sequence analysis (MLSA) targets a set of 9 housekeeping genes (*atpD*, *dnaK*, *EF-G*, *EF-Tu*, *gap*, *groEL*, *gyrB*, *recA*, *rpoB*) in *Sphingomonadaceae* and was used to elucidate the population structure and the significance of recombination events in this group.

The new MLSA primers were designed based on all available genome sequences of 5 strains of *Sphingomonadaceae* and 2 strains of the closest phylogenetically related genus *Erythrobacter*, 95 strains of *Sphingomonadaceae* were isolated from Starnbergersee and Walchensee, and subjected to the novel MLSA approach. Based on their rRNA gene sequences, these strains fall into 15 different phylogenetic groups. Multiple isolate were available for all major phylotypes. While 16S rRNA gene sequences were identical for certain members of one 16S rRNA group, the tree of the concatenated sequences of 9 housekeeping genes indicated a significant genomic divergence between the different strains. Based on the frequency of sequence divergence the isolated *Sphingomonadaceae* represent a clonal population, Most significantly, MLSA revealed the presence of distinct subclusters among individual 16S rRNA groups, suggesting different selection pressure between subclusters and the existence of distinct evolutionary units despite the identical or very similar 16S rRNA gene sequences.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.024 PHYLOGENETIC DIVERSITY OF BACTERIAL COMMUNITY LIVING IN THE FLUID OF PITCHER PLANT *NEPENTHES GRACILIS* AND *NEPENTHES TRUNCATA*

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Nepenthes sp., is a tropical carnivorous plant that has pitcher-like structure containing fluid as an insect trap. The fluid inside the pitcher has become a suitable micro-environment for bacterial growth because of insect carcass degradation may provide organic compounds. Recently, not many publications describe the role of bacterial community in that degradation. Our study was aimed to assess phylogenetic diversity of bacterial community living in the pitcher's fluid. Culture-independent technique based on 16S rRNA gene was used to assess bacterial community diversity of two pitcher plants, *Nepenthes gracilis*, and *Nepenthes truncata*. DNA was extracted from fluid using direct extraction method and used to amplify its 16S rRNA gene. Those amplified gene were cloned and further characterized using Amplified Ribosomal DNA Restriction Analysis with three different endonuclease restriction enzymes, such as MspI, RsaI, and Bsh1236I. The bacterial diversity and evenness of the samples were calculated using Shannon-Weiner diversity indices. All clones were sequenced and aligned to genbank database. Phylogenetic tree was constructed by using Mega4 software. The tree was made by Neighbour Joining method and bootstrap analysis with 100 replications. Pitcher plant's fluid were collected from plant nursery located in Ciawi, West Java, Indonesia. A total of 87 clones were obtained and generated 24 different phylotypes. The highest

bacterial diversity index (2.3) and evenness (0.8) were found in *N. gracilis* with 17 phylotypes from 42 clones. Based on BLASTN analysis, we found that *N. gracilis* bacterial clones were closely related to uncultured bacteria, genus *Acidocella*, and *Leifsonia*, while *N. truncata* clones were closely related to unculturable bacteria and genus *Acidocella*. Most of clones from both specimens tend to related to uncultured bacteria meaning the high possibility to isolate novel bacteria species.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.025 BACTERIAL SPECIATION — AQUATIC SPHINGOMONADACEAE AS A MODEL SYSTEM

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The role of recombination, adaptation and selection in shaping bacterial diversity was elucidated by searching for different ecotypes within groups of closely related bacterial lineages (up to 100 % 16S rRNA gene sequence identity). Members of the family *Sphingomonadaceae* constituted an abundant fraction of the *Alphaproteobacteria* in the oligotrophic, alpine Walchensee and the mesotrophic, prealpine Starnberger See. Of these, two phylogenetically tight subgroups of *Sphingomonadaceae*, relatives of *Sandarakinorhabdus limnophila* as well as the novel lineage G1A, were identified in a clone library as the dominant *Sphingomonadaceae*. These two dominant groups were found to be physiologically active throughout the year by RT-PCR and DGGE of *rrn* transcripts. In parallel, a large number of *Sphingomonadaceae* could be isolated by a high throughput cultivation approach combined with PCR based *Sphingomonadaceae* screening. Among them were 8 isolates corresponding to the *S. limnophila*-cluster whereas 65 isolates were related to the G1A-cluster. Based on their dominance and year-round activity *in situ* and the availability of several independent pure culture isolates, these two bacterial groups was used as a model system to elucidate the presence of different ecotypes by qPCR and their specific ecological niches by physiological characterisation and *in situ* growth test.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.026 DIVERSITY ANALYSIS OF UNDEFINED GOUDA CHEESE STARTER CULTURE

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Gouda cheese cultures are complex, undefined starters consisting of closely related strains belonging to the species *Lactococcus lactis* and *Leuconostocs mesenteroides*. Several strains with different metabolic characteristics in terms of proteolysis, citrate metabolism as well as phage and salt resistance, contribute to the performance of the starter culture. This diversity at the strain level ensures the success of cheese fermentation involving population shifts in response to several stress conditions. It is expected that the dynamic behaviour of the population determines the eventual quality of the cheese product. For that reason we set out to unravel the strain diversity and explore the forces that are shaping the composition of the undefined starter culture in an industrial setting.

A collection of single colony isolates has been generated from different time points during cheese making by using several types of rich media to recover the entire spectrum of strain diversity. Amplified Fragment length Polymorphism (AFLP) was used for strain fingerprinting. Plasmid profiling was also applied to observe the level of functional diversity and dynamics. A simplified model starter culture will be formulated on basis of the strain diversity. Metagenome microarrays will be used to trace the compositional shifts by molecular markers. Gene activities and regulatory responses will be investigated by transcriptomics and metabolite analysis will be performed to understand the impact on relevant cheese quality parameter.

AFLP has revealed at least 8 major groups in the starter culture and enabled the tracing of these groups by their genomic fingerprints at each time point during cheese making. In addition, plasmid profiles were found to be highly diverse for the isolates within the major AFLP groups, providing

another level of diversity within starter cultures. Representative strains are currently being sequenced and will be used for the construction of metagenome microarrays.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.027 GLOBAL AND LOCAL DIVERSITY OF *FLAVOBACTERIUM PSYCHROPHILUM*, A FISH PATHOGENIC BACTERIUM, IN JAPAN

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Flavobacterium psychrophilum is a causative agent of bacterial cold water disease and rainbow trout fry syndrome. This pathogen gives serious economic damages against culturing salmonid fish such as rainbow trout and coho salmon in the United States and Europe. In Japan, this pathogen kills ayu. We investigated the population structure of *F. psychrophilum* isolated in Japan by using multilocus sequence typing (MLST).

To investigate the population structure of *F. psychrophilum* at a global (all over the country) and a local (a model river in Shiga prefecture) geographical scale, we used 33 strains and 80 strains, respectively. Genomic DNA was extracted and 7 housekeeping genes (*trpB*, *gyrB*, *dnaK*, *fumC*, *murG*, *tuf* and *atpA*) were amplified by polymerase chain reaction (PCR). PCR products were sequenced and used for MLST analysis.

At a global geographical scale, most of isolates in Japan showed different sequence types (STs) from previously published ST from the US and Europe (Nicolas et al, 2008 Appl. Environ. Microbiol.74:3702-09). It was obvious that the Japanese isolates are highly diverse and show clear association with fish host. Our analysis allows the identification of a rainbow trout group, a Coho salmon group, two carp groups, and three ayu groups. Most of groups made own clonal complex (cluster of related ST), with few exceptions (one carp group and one ayu group belonged to the same clonal complex. Japanese isolates from carp were closely linked with a German carp isolate). At a local scale, strains isolated from various fish hosts and from river water also display a high degree of diversity. Co-infections (infection of the same fish by different isolates) were observed in this bacterial collection.

In conclusion, the clonal complexes were associated with defined host fish species. It was observed a large diversity among Japanese strains, and there was no evidence to suspect that *F. psychrophilum* which infected ayu in Japan arose from the US or Europe

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.028 MULTIPLE COMMUNITY STATES OF PLANKTONIC COMMUNITIES USING LOW PH DISTURBANCE IN MICROBIAL ELECTROLYSIS CELL (MEC)

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Microbial electrolysis cells (MEC) show promise for efficient recovery of hydrogen and methane from waste using exoelectrogenic microorganisms. However, gas production is variable based on reactor setup states. To understand this variation, 15 MEC reactors (one control in open circuit) were inoculated with a long-term MEC reactor biofilm at a fixed voltage (0.7 V) using glucose as the carbon source. Samples were taken every 24-h after gas production had stabilized and analyzed using GeoChip 3.1. Gas production (H₂, CH₄ and CO₂) was analyzed every cycle using GC. Principle component analysis (PCA) grouped the reactors based on gas production: high-hydrogen (50~60% H₂, 20~30% CH₄), moderate-hydrogen (25~50% H₂, 30~55% CH₄), high-methane (55~75% CH₄, 10~25% H₂), and little gas production. Difference on functional gene structures distanced away reactor 9# (high-hydrogen), 12# and 13# (moderate-hydrogen) and control reactor in detrended correspondence analysis (DCA) based on all genes, and similar community structure presented over the rest of reactors that grouped tightly. Although cytochrome genes, as indicators of electron transport, did not show a corresponding or significant change among groups based on

gas production, high-hydrogen reactor were detected special community structure composed of only four genera (Shewanella, Pseudomonas, Geobacter and Bradyrhizobium) out of the ten detected in all reactors. Subsequent exposure to pH 4 disturbance temporarily inhibited gas production. High-methane and little gas producing communities clustered together tightly in DCA results ordination plots based on all functional genes detected and showed little change over time or during pH disturbance. In contrast, high-hydrogen communities showed a change in the community structure over time and during pH disturbance. These results demonstrate that exoelectrogenic communities with high hydrogen production showed a higher sensitivity to disturbance than the other communities.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.029 HABISIGN: A 'CLUSTER SIGNATURE' BASED APPROACH FOR COMPARISON OF METAGENOMES AND RAPID IDENTIFICATION HABITAT-SPECIFIC SEQUENCES.

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Environmental factors play a major role in shaping the nucleotide composition of various microbes found in an environment. Consequently, the oligonucleotide composition of metagenomic samples obtained from related habitats is likely to be highly similar. We present a novel approach, called HabiSign, which uses 'cluster signatures' for rapidly analyzing and comparing the compositional characteristics of metagenomes. Additionally, HabiSign can be used to identify habitat specific sequences.

HabiSign first generates a 'cluster signature' by mapping all sequences in a metagenome to predefined oligo-nucleotide frequency vectors. These vectors represent discrete points in feature vector space, and are precomputed to capture the compositional diversity of prokaryotes. Habitat specific sequences are then identified by analyzing the covariation patterns observed within different components constituting the cluster signature. Being alignment free, the execution of HabiSign algorithm is extremely rapid.

HabiSign has been validated using metagenomic data sets originating from varied habitats. Results indicate that cluster signatures clearly distinguish between samples from diverse habitats. Observing the covariation patterns in cluster signatures generated for metagenomics samples obtained from the gut of lean and obese mice have helped in the identification of sequences specific to either phenotypes.

Comparing the cluster signatures of two or more metagenomes helps in quantifying the relative similarity across metagenomes. We demonstrate that 'cluster signatures' obtained by HabiSign are useful not only for comparing metagenomes, but also for rapidly identifying sequences within/across metagenomes that are specific to particular habitats or phenotypes. The present algorithm has an immense value while performing a comparative analysis of metagenomic samples obtained from healthy and diseased individuals.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.030 PHENOTYPIC AND GENOTYPIC DYNAMICS OF HOST PARASITE COEVOLUTION

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Antagonistic coevolution between hosts and parasites, selects for reciprocal adaptive genetic changes in interacting species resulting in the evolution of host defence and parasite counter-defence. This process has far-reaching consequences for the evolution of genetic and ecological diversity, sexual reproduction, mutation rates and population dynamics. Furthermore, the impact of

coevolution on key traits such as varied parasite host range is central to epidemiological studies and models of infectious disease. In this study we exploited the antagonistic interaction between the bacterium *Pseudomonas fluorescens* SBW25 and its obligately lytic viral (phage) parasite Φ 2 to elucidate the phenotypic and genotypic dynamics of host parasite coevolution within and between different experimental populations through time.

6 replicate populations of hosts (bacterium) and parasites (bacteriophage) were coevolved for ~840 bacterial generations by batch culture serial transfer. 10 bacterial hosts and 10 phage parasite were isolated every ~140 bacterial generations (every 10th transfer, twenty days). At every experimental time-point each individual parasite and each individual host were assayed for infectivity/resistance within each population and across populations.

Phenotypic analysis showed directional selection through time for increased parasite host range and increased host resistance. Using the bacteriophage tail fibre gene as a proxy for parasite genetic diversity and divergence (relative to the ancestral sequence) we investigated parasite molecular evolution within populations, across populations and through time. Initial sequence data indicates low genetic diversity within parasite populations but genetic divergence between parasite populations and consistent with phenotypic data where variability in the rate of coevolution through time was demonstrated, variability in the rate of molecular evolution was also evident.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.031 EFFECTS OF DIFFERENT SCENES OF CLIMATE CHANGE ON THE BACTERIAL COMPOSITION IN MICROBIAL MATS OF CUATRO CIÉNEGAS DE CARRANZA (MEXICO)

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Cuatro Ciénegas is an oasis in the Chihuahuan desert that harbors an ample diversity of microbial mats and stromatolites. Many evidences for genomic and metagenomic data point out that these microbes are descendants from ancient marine communities that have evolved to survive the continental extremely oligotrophic waters of the pozas. How resilient are those communities to the rate of change that man is imposing to the planet? In order to test this idea, we studied experimentally the effect of an increase of temperature and UV radiation in the diversity of the plankton of Cuatro Ciénegas in 15 mesocosm systems formed by 5 treatments: a) same temperature (30°C) and UV as the pozas, b) with fluctuating temperature and normal UV c) with temperature at 40°C, d) with UV radiation and f) with UV filter. The experiment was performed in two stages, the first one (40 days) is the one reported in this abstract. Each mesocosm was seeded with a tray with 12 glass sheets that had been put in three blue pozas 2 years prior to the experiment, and had composite water from the same pozas. The mesocosms are auto-sustainable closed systems since the entrance of nutrients depends exclusively on the maintenance of biogeochemical cycles in the community. 16S rDNA clone libraries from water samples at the beginning and at the end of the experiment were carried out to study the immediate response of these communities subjected to different environmental conditions. The phylogenetic analysis shows drastic changes in the diversity and abundance of the original phyla selecting for taxa that could survive the stress. Nevertheless, the trophic cycles adapted to the new biota, cyanobacteria of different lineages survived and most of the mesocosms sustained multi-cellular invertebrates, suggesting that even if complexity changed the ecosystem is resilient. However, data from the changes in C:N:P as well as the long-term experiment (7 months) will be determinant to sustain these conclusions.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.032 QUANTITATIVE RNA FINGERPRINTING, A NOVEL METHOD FOR COMMUNITY PROFILING

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Most microbial fingerprinting methods are constructed from polymerase chain reaction-amplified RNA or DNA. Consequently, accurate information on relative abundance is lost. Our aim was to

develop a polymerase chain reaction-free fingerprinting method to investigate microbial identity and abundance in a simple way. This technique is based on the separation of small rRNA fragments by polyacrylamide gel electrophoresis on the basis of sequence-dependant conformational differences. The rRNA fragments are created by targeted cleavage of the ribosomal small subunit using Ribonuclease H. The method was tested with several artificial and environmental samples. It showed greatest utility when applied to samples harbouring low diversity. It was also applied to follow community structure changes in enrichment cultures. Preliminary trials showed that the method can be coupled to isotope probing for functional studies. This method, called Quantitative RNA Fingerprinting, is a simple, cost-effective, polymerase chain reaction-free alternative for microbial functional community analysis.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.033 SHOTGUN METAPROTEOMICS OF MICROORGANISMS FROM COASTAL SEAWATER

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In order to understand functioning of metabolic networks in marine cyanobacteria, shotgun proteomic approaches were used to identify proteins expressed during exponential growth in two cultured *Synechococcus* species, *Synechococcus* sp. strain CC9311 (clade I) and *Synechococcus* sp. strain CC9902 (clade IV) that are abundant in coastal seawater. These same techniques were then used to identify proteins in coastal sea water itself. We were able to identify about 59-75% of the predicted proteins of *Synechococcus* cultures and about 5-26% of their proteins expressed in a natural seawater sample. Common heterotrophic bacteria were covered at about the 4-7% level. This unprecedented level of coverage allowed us to also find proteins in natural samples over-expressed relative to cultures, and these often involved light metabolism. A comparison of the proteomes of the two *Synechococcus* species highlights differential expression of orthologous and unique proteins that may contribute to their evolutionary adaptation to coastal environments.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.034 CULTURE CLASH: EVOLUTION OF THE GEOTHERMAL SUBSURFACE COMMUNITY OF TRAMWAY RIDGE, MT. EREBUS, ANTARCTICA

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Tramway Ridge, located near the summit of Mt. Erebus, Antarctica, is the most geographically isolated geothermal environment on Earth. A previous study based on bacterial 16S rRNA clone libraries revealed a unique microbial community that is likely of subsurface origin. To examine spatial distribution of the microbial community at Tramway Ridge, PCR amplicon pyrosequencing of the V5 and V6 hypervariable regions of the 16S rRNA gene was conducted. In the resulting dataset, we identified all previously observed clone library sequences, but at different frequencies. Our new approach revealed the presence of a rich, vertically stratified subsurface ecosystem. The relatively diverse shallow subsurface is dominated by *Meiothermus* (57% abundance) thermophilic nitrogen-fixing *Cyanobacteria* (14%), *Acidobacteria*(5%), *Bacteroidetes*(3%), and *Planctomycetes*(2%), which are common constituents of microbial mats at geothermal sites. The less diverse deeper subsurface community is dominated by a unique archaeon(62%) and an OP10-like group(13%). Additional members of the deeper community include diverse heterotrophic *Chloroflexi* and taxa with unknown phylogenetic affiliations that are most similar to deep-branching environmental clones obtained from other subsurface geothermal sites. These findings support our initial hypothesis that much of the microflora at Tramway ridge has a subsurface origin but also suggests that the evolution of this ecosystem has been heavily influenced by a more recent colonization of cosmopolitan microbial mat species. To further examine our hypothesis on the biogeography of geothermal habitats, this study is being expanded to include additional isolated geothermal soil sites in Antarctica and sub-Antarctic islands. with the ultimate goal of elucidating the relative

contributions of biogeography, competition, and niche specialization in promoting microbial endemism and speciation at Tramway Ridge and other geothermal soil sites.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.035 GENETIC DIVERSITY OF THE *MICROCYSTIS AERUGINOSA* BLOOMED IN EXPERIMENTAL PONDS USING 16S TO 23S rDNA INTERNAL TRANSCRIBED SPACER REGION

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Microcystis aeruginosa is a major species of cyanobacterial bloom found in eutrophic lakes. As this species contains many toxic strains, the blooms also threaten public health such as drinking water and agricultural water use. Moreover, recent studies using multi locus sequence typing analysis or based on 16s-23s rDNA ITS (internal transcribed spacer) region revealed that *M. aeruginosa* blooms in natural lakes consisted of multiple genotypes. The present study aimed to clarify relationship between genotypic diversity of *Microcystis* and the process of their bloom. *Microcystis* blooms were artificially brought about in outdoor experimental ponds (10m X 10m width, 1.7m depth, total; 70m³). We added the lake plankton concentrated with a 5 µm mesh size plankton net (about 3.6m³ from Lake Biwa) to a ratio of 5 : 1 : 0 in outdoor Pond A, B, C, respectively. Inorganic nitrogen and phosphorus were supplemented every two weeks. *Microcystis* cell densities in pond A and B increased exponentially until 42 days, while *Microcystis* cell densities in pond C was negligible and detected only at 63 days. The maximum *Microcystis* cell densities in pond A, B and C were 4.7×10^4 cells mL⁻¹, 1.3×10^4 cells mL⁻¹ and 1.4×10^2 cells mL⁻¹, respectively. Any significant relationships between chlorophyll *a* concentration and *Microcystis* cell density, and between nutrient concentration and *Microcystis* cell density were not found. We also estimated genotypic diversity and dynamics of *Microcystis* in Pond A using cloning of 16S-23S rDNA ITS region. Five different genotypes were detected at 1 day. We, thereafter, discriminated 29 genotypes within 30 samples at 60 days, and 16 genotypes within 20 samples were detected at 147 days. Thus, our method successfully detected *Microcystis* genotypic dynamics during our experimental period. It is also suggested that *Microcystis* bloom consists of more diverse genotypes than previously reported.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.036 GLOBAL PHYLOGEOGRAPHY OF CHITINASE GENES IN AQUATIC METAGENOMES

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So far there is little known about factors that influence phylogeography of bacterial functional genes and if these factors are in accordance with drivers shaping the taxonomic composition of the overall bacterial community. We sampled chitinase genes (family 18 glycosyl hydrolase, GH18) and 16S rRNA genes from an aquatic metagenome dataset (Global Ocean Sampling) to map and compared their global distribution. Chitin is a highly abundant biopolymer in aquatic environments and serves as an important C and N source for microbes. Among the sampled chitinase genes, group A chitinases were by far the most dominant subgroup, accounting for 98% of all extracted GH18 proteins. The phylogenetic-based community analysis using the UniFrac metric suggests that salinity is a major driver for the distribution of both, chitinase and 16S rRNA genes paralleled by a clear shift towards more acidic chitinase proteins with increasing salinity. Similarly, an analog habitat separation for both genes was observed according to latitude and proximity to the nearest coastline. However, if genes extracted from different geographic locations were compared, we found little congruence of chitinase and 16S rRNA gene distribution. The concordant patterns observed for 16S rRNA and chitinase genes along a salinity gradient confirm prior findings that salinity generally shapes the distribution of microbial communities and is reflected in their rRNA as well as in protein coding genes. On the other hand, noticeable divergence in the distribution between chitinase and

16S rRNA genes from different geographic locations indicates the existence environmental factors that specifically act as drivers on this functional trait.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.037 EARLY BIOLOGICAL NITRATE PRODUCTION BY ANAMMOX

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In contrast to existing models of the history of the nitrogen cycle relying on aerobic nitrite oxidation (nitrataion) as the only biological nitrate source, this conceptual study for the first time incorporates anabolic nitrate production from anoxic ammonium oxidation (anammox). In view of the evolution of life, the introduction of nitrate in the environment was of great evolutionary significance, creating a functional pressure for many reductive assimilatory and dissimilatory pathways to originate on early Earth. Anammox bacteria phylogenetically branch off early in the Planctomycetes phylum, and probably played a pioneering role in the two existing views on the nitrogen cycle history. A key event in the Archaean eon was the appearance of oxygenic photosynthesis, introducing for the first time free oxygen in the reduced environment on early Earth. In the 'NitOx' theory, substantial abiotic nitrite and nitrate production was present prior to the advent of oxygen, leading to the early development of anoxic microbial processes consuming nitrite and nitrate. Hence, anammox was the first biological source of nitrate on Earth in this view. Alternatively, in the 'OxNit' school of thought, the first nitrite could only be produced biologically in the presence of oxygen, after which anoxic and aerobic microbial processes evolved to oxidize or reduce nitrite. In this view, anammox would have been the first source of nitrate ever, in the likely case it evolved prior to nitrataion. Future genomic characterization should determine which prokaryotes constituted the first biological tandem of nitrate production on Earth.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.038 PHYLOGENOMIC AND METABOLIC CONTRASTS BETWEEN TWO ENHANCED BIOLOGICAL PHOSPHOROUS REMOVAL COMMUNITIES.

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The metagenomes of two Enhanced Biological Phosphorous Removal wastewater treatment communities, initially described in 2006, provide an opportunity to do comparative metagenomics of geographically disparate but functionally similar communities of microorganisms. These communities share many genetic similarities, including the primary phosphorous removing organism, *Candidatus "Accumulibacter phosphatis"*, but differences exist in flanking community composition, and their separate histories may be reflected in metabolic composition and evolutionary relationships. Phylogenetic profiles covering the two metagenomes and 813 reference genomes summarize the genetic and metabolic content of each community. To identify potential lateral gene transfers, phylogenies were constructed under the null hypothesis that *A. phosphatis* genes from the two communities should be closely related to one another. Contrary to the null, potential transfers were identified, for example, for some phosphate transporters, Australian *A. phosphatis* revealed relationships to Gammaproteobacteria and Deltaproteobacteria while American *A. phosphatis* were related to Flavobacteria. To determine how the variable wastewater sludge environment affects an organism's metabolism, metabolic networks were constructed, and metabolic network shapes examined. Network modularity scores revealed that compared to other Betaproteobacteria, *A. phosphatis* have more modular networks, suggesting an ability to quickly adapt to local changing environments. With the volume of new metagenome sequence information, a variety of computational techniques are needed to tease out biologically interesting patterns that exist in microbial communities. Here, potential within community lateral gene transfers are identified, and the ability of *A. phosphatis* to adapt to variable environments could suggest one reason for its prominence in these wastewater treatment communities.

Abstract Category
09 Evolutionary Ecology of Microbial Communities

PS.09.039 EFFECTS OF PH AND DISTURBANCE ON NITROUS OXIDE EMISSION FROM ARABLE SOIL - A ROLE FOR FUNGAL DENITRIFICATION?

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Denitrification is the biological process by which intermediates of the N cycle are reduced to maintain respiration under oxygen limiting conditions. Incomplete denitrification results in the release of the greenhouse gas nitrous oxide (N₂O). Bacteria are considered to play a predominant role in the production of N₂O, but the contribution of fungi remains poorly constrained. Here we distinguish the contributions of fungi and bacteria to N₂O emission from arable soil under different pH and management, and hypothesized that the proportional contribution of fungi would be higher in low pH soil. Soil was sampled from long-term pH plots under crop rotation. The effects of pH and disturbance on the ratio of bacteria-to-fungi, microbial community structure and potential denitrification were investigated. Distinction between bacterial and fungal denitrification was attempted through application of selective biocides. Potential denitrification was strongly influenced by pH and disturbance with the highest N₂O emission from the slightly acidic soil. Emissions were lower from disturbed soil. The ratio of bacterial-to-fungal biomass remained steady over both pH and disturbance gradients, but total biomass increased with reduced disturbance. The bacterial community structure changed significantly with both pH and disturbance whereas the fungal community structure was only influenced by vegetation. Inhibitor experiments showed that the majority of denitrification was bacterial. Despite the clear effects of both pH and disturbance on microbial communities there was no clear link with denitrification. Thus, denitrification may either be conserved in a small group of organisms, resulting in poor resolution, or is contributed to by different organisms under varying conditions. The hypothesis that fungi play a more important role in N₂O emission under low pH was rejected in this study but it was shown that they play a significant role in N₂O production in arable soil.

Abstract Category
09 Evolutionary Ecology of Microbial Communities

PS.09.040 ECOLOGICAL DISTINCTNESS OF SYNECHOCOCCUS ECOTYPES PREDICTED FROM EVOLUTIONARY SIMULATION: DISTRIBUTION, EXPRESSION, AND POPULATION DYNAMICS

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Molecular studies of community composition and structure conducted on cyanobacterial mats inhabiting the effluent channel of Mushroom Spring, Yellowstone National Park, have shown that native *Synechococcus* populations group into ecologically distinct species-like units, or ecotypes. However, concern over the low resolving power of the genetic markers used (16S rRNA and 16S-23S rRNA internal transcribed spacer) directed us to examine more rapidly evolving loci, such as those encoding proteins. We are currently studying *psaA*, which encodes a core protein in Photosystem I, and is highly expressed *in situ*. We PCR amplified, cloned, and sequenced *psaA* variants from 60°C, 63°C, and 65°C mat samples, and vertical subsections through the upper photic layer obtained using a cryotome, and performed an evolutionary simulation analysis (Ecotype Simulation) that predicts and demarcates putative ecotype populations, assuming the existing ecotype diversity was generated through periodic selection, ecotype formation, and genetic drift. Phylogenetic clades demarcated as putative ecotypes each contained a dominant allelic variant, as well as rarer singleton and doubleton variants containing SNP patterns unique to that putative ecotype. Denaturing gradient gel electrophoresis analyses revealed discrete bands with sequences corresponding to dominant allelic variants of different putative ecotypes. Ecotypes were distributed uniquely in samples collected along flow and vertical gradients, where temperature and light are known to vary. Reverse transcriptase PCR was used to show different temporal patterns of *psaA* ecotype expression through the morning dark-to-light transition period. Ti454 pyrosequencing was used to show that putative ecotypes exhibit different population dynamics in response to 90% light

intensity reduction and removal of ultraviolet radiation. These results suggest that putative ecotypes predicted by Ecotype Simulation are ecologically distinct.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.041 UNEXPECTED INCREASES IN MICROBIAL DIVERSITY ALONG A SALINITY GRADIENT IN A SOLAR SALTERN IN BAJA CA, MEXICO

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There is an oft-repeated assertion based on ecological principles that as conditions become more extreme the diversity of organisms that inhabit those extremes will decrease. While this has been supported in some studies, this is still an open question. There is also the assertion that haloarchaea dominate over halobacteria at the highest salinities. The goal of this study was to use both cultivation and direct molecular sequencing (both 16S rRNA and bop gene sequencing) to compare the diversity of planktonic bacterial and archaeal halophiles at increasing salinity (from 15% to saturated brines at >30%) in the world's largest evaporative saltern, the Exportadora de Sal (ESSA), in Guerrero Negro, Baja CA, Mexico. Members of four main lineages of bacteria and three lineages of archaea were cultured from the ponds and crystallizers, with greater broad-scale phylogenetic diversity of bacteria at lower salinities while cultural archaeal diversity was similar across the gradient. As expected, uncultured diversity was much higher for both bacteria and archaea compared to cultured diversity. Archaeal OTU richness was significantly higher at 30% compared to the 15% salt pond, and was dominated by diverse *Haloquadratum*-like lineages at higher salinity. This pattern is mirrored in preliminary bop gene diversity analyses with greater numbers of highly supported phylogenetic clades from the 30% salinity sample, primarily comprised of uncultured haloarchaeal lineages. Interestingly, bacterial richness was as high or higher than archaea in both 15% and 30% samples. These findings suggest that existing paradigms about the effects of salinity on prokaryotic diversity including the balance between bacterial and archaeal domains may need to be adjusted in some settings and further investigation into the ecology of these diverse uncultured halophiles is warranted.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.042 THE MICROBIAL COMMUNITY ASSOCIATED WITH THE SURFACE OF STURGEON EGGS VARIES WITH DEVELOPMENTAL AGE

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The lake sturgeon (*Acipenser fluvescens*) is an ancient and endangered species, threatened by over fishing and habitat deterioration. Egg maturation of oviparous teleosts occurs in an aquatic environment best described as a dilute solution of bacteria. The interactions between developing eggs and the microbial world has evolved over millions of years. Sudden changes in the ecological balance can have a profound effect on egg mortality and is frequently attributed to microbial activities. To better understand the linkage(s) between the microbial communities of the egg and the ecosystem, we have initiated an analysis of the egg-associated community of sturgeon eggs using comparative 16S rRNA sequence analysis. Aseptically harvested sturgeon eggs had a bacterial community of low diversity (6 detected genera) dominated by *Cloacibacterium* (28%), *Diaphorobacter* (8%) and *Blastomonas* (5%). Fertilized and unfertilized eggs with only brief exposure (10 minutes) to stream water supported bacterial communities more diverse (18 genera) with *Flavobacterium* and *Paracoccus* dominating. Healthy eggs of 3-4 days development in the stream had a community diversity similar to the fertilized eggs but substantially different in phylogenetic composition. Finally, moribund eggs had communities with large populations of *Aeromonas* and *Rhodospirillum rubrum*, not detected in healthy eggs. Unifrac and principle component analysis revealed that the communities associated with healthy eggs were significantly different from those detected in the water column. Populations that appeared abundant in the water were, in some

cases, excluded from the egg surface-associated communities, suggesting that specific selection factors partition the bacterial populations. Fluorescent microscopy reveals a robust community strongly associated with the microfibrinous surface of the egg. Staining egg sections with lysozyme-specific antibody revealed that surface-bound lysozyme may be part of the innate immunity of the egg.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.043 MICROBIAL COMMUNITY DYNAMICS AND PERFORMANCE OF A THERMOPHILIC ANAEROBIC DIGESTER FOLLOWING A SWITCH TO CO-DIGESTION

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Anaerobic digesters present an excellent model system for studying how environmental perturbations affect microbial community structure and function. Digesters are used to recover bioenergy from highly concentrated organic wastes and are routinely subjected to mixtures of wastes with variable and sometimes puzzling outcomes. Little is known about how the microbial community in digesters responds to and limits the adaptability of digesters. The goals of this study were to evaluate how digesters stabilized on one feed (poultry litter) performed when the feed composition was changed and relating this to microbial population dynamics. Populations were evaluated with 16S rDNA clone libraries of both replicate treatment digesters and a control. Treatment digesters were fed with 100% poultry litter, 20% stillage and 40% stillage; a control digester received 100% poultry litter. The digesters were operated for two hydraulic retention times (HRT) (15 days each) for each treatment. Standard chemical analyses were performed to evaluate digester performance. Stable performance was observed in the end of each treatments, with high level of methane in the biogas (64%), COD removal (62%) and lowering of volatile acids. The concentrations of volatile fatty acids (propionate and acetate) especially increased in the 20% treatment but decreased drastically along with a drop in pH (7.8 to 7.5) in the 40% treatment. Methanogenic population dynamics correlated with the performance measures. Population fluctuations were observed in methanogens during the accumulation and consumption of volatile fatty acids. Methanothermobacter was the most abundant methanogen in the control digester as well as the treatment digester at 100% poultry litter. However, the abundance of Methanothermobacter decreased while another hydrogenotrophic group Methanoculleus increased during the 40% stillage treatment. In addition, the abundance of Methanosarcina was correlated to acetate concentration.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.044 MOLECULAR ANALYSIS OF PROKARYOTIC DIVERSITY OF A HYDROELECTRIC POWER STATION RESERVOIR IN BRAZILIAN AMAZONIA

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Considering the huge water resources of Amazonia, some Hydroelectric Power Station (HPS), including Tucuruí HPS, have been built. The microbial diversity of reservoirs in Brazilian Amazonia has not yet been well studied. The aim of this study was to estimate the microbial diversity in an area flooded by Tucuruí HPS. Therefore, 5 samples were collected: (i) MR, middle of the reservoir, (ii) ITU, before the dam and out of reservoir, (iii) C1, an anoxic zone; (iv) from the C1 aphotic layer and (v) C1 sediment. In order to determine the 16s rRNA sequence of Bacteria and Archaea, a metagenomic approach was undertaken. Samples were submitted to DNA extraction using a commercial kit. DNA was used as template for two PCR reactions using two different sets of primers: 8F and 1492R for Bacteria and 341F and 1407R for Archaea. Then, amplicons were cloned and used for the construction of 10 metagenomic libraries. About 1500 clones were chosen at random for sequencing of their inserts. Sequence data were clustered on OTUs for phylogenetic and diversity analyses on Mothur software. Results shows that sediment of C1 layer was the point with the higher diversity, followed by ITU, C1 aphotic layer, C1 photic layer and MR. In all the 5 samples, Archaeas were represented by *Euryarchaeota* and *Crenarchaeotas* phyla and Bacteria were

represented by *Planctomycetes*, *Verrucomicrobia*, *Cyanobacteria* e *Actinobacteria* phyla. In the sediment and in the aphotic layer of C1, *methanosarcinales*, *methanomicrobiales* orders of *Euryarchaeota* phylum as well as α -, β - and γ - *methanotrophic proteobacterias* were predominant. In contrast, *Chlorobi*, *Nitrospirae* and *Acidobacterias* phyla were rare. In MR and ITU samples, *Cianobacterias*, *Proteobacterias* and uncultured Archaea were predominant. Tucuruí reservoir can be considered as an environment of a rich microbial diversity with a notable presence of photosynthetic microorganisms involved in organic matter decay and in greenhouse gas production.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.045 INDIGENOUS MICROBIOTA OF MARINE MAMMALS

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Little is known about the indigenous microbial communities of marine mammals, and the relationship of their microbiota to that of the surrounding aquatic environment. To study the microbial communities associated with these marine mammals, we sampled different anatomical sites of 20 Atlantic bottlenose dolphins (*Tursiops truncatus*) and 8 California sea lions (*Zalophus californianus*) from the US Navy Marine Mammal Program at several time points and surveyed bacterial diversity using a culture-independent, phylogenetic approach. Analyzed samples included oral gingival sulcus swabs, gastric fluid and biopsies, rectal swabs, as well as seawater samples from each animal's open-mesh enclosure in San Diego Harbor, and fish used for feeding. DNA was extracted, and a near-complete 16S rRNA gene was amplified using broad-range bacterial primers, cloned and sequenced. A total of over 26,000 ribosomal RNA gene sequences was obtained and analyzed. A wealth of bacterial species from at least 15 different phyla were observed, with the greatest amount of diversity found in the seawater and oral specimens. Many phylotypes (77% using a 99% cutoff) were novel, i.e., not previously observed in other studies. UniFrac principal component analysis showed that the indigenous microbiota of marine mammals displays intra-host habitat and inter-individual specificity. In addition, the marine mammal-associated microbiota was completely different from their surrounding aquatic environment or their food, suggesting that host-associated factors play a strong role in shaping these indigenous communities. These data will help us better understand the role of microbial communities in health and disease of marine mammals.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.046 EXPERIMENTAL AND TAXONOMIC STUDY OF *IN VITRO* BIOLUMINESCENCE VARIATION IN NATURAL VIBRIONACEAE ISOLATES FROM NEW ZEALAND.

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Bioluminescent bacterial cultures grown in rich medium for extended periods of time may give rise to variant cells producing much lower levels of bioluminescence than the parental isolate. General study of these 'dark' variants has been sporadic and inconclusive.

Our work details, updates, and expands the scientific record of this phenomenon through the experimental and taxonomic study of dark variation in eighty natural, luminous isolates of Vibrionaceae collected from New Zealand marine environments. We studied these isolates using medium-term culture experiments involving shaking and static microcosms filled with rich medium or fresh, sterile-filtered seawater. Analyses of these isolates were performed using simple colony enumeration and visual bioluminescence identification. Isolates from these studies were taxonomically studied using both genus-specific growth conditions and phylogenetic reconstruction via 16S rRNA sequencing.

Our results suggest that dark variation is not a uniform phenomenon among different species of bioluminescent *Vibrionaceae*. Furthermore, notable genera-specific differences in the ability of bioluminescent bacteria to grow and produce dark offspring under different *in vitro* experimental

conditions are reported. These results, specifically the adaptive significance of *in vitro* bioluminescence variation, are discussed within an evolutionary framework.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.047 GLOBAL PATTERNS OF 16S rRNA DIVERSITY AT A DEPTH OF MILLIONS OF SEQUENCES PER SAMPLE

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The ongoing revolution in high-throughput sequencing continues to democratize the ability of small groups of investigators to map the microbial component of the biosphere. In particular, the coevolution of new sequencing platforms and new software tools allows data acquisition and analysis on an unprecedented scale. Here we report the next stage in this coevolutionary arms race, using the Illumina Genome Analyzer II platform to sequence a diverse array of 25 environmental samples and 3 known "mock communities" at a depth averaging 3.1 million reads per sample. We demonstrate excellent consistency in taxonomic recovery, and recapture diversity patterns that were previously reported based on meta-analysis of many studies from the literature (notably, the saline/non-saline split in environmental samples and the split between host-associated and free-living communities). We also demonstrate that 2000 Illumina single-end reads are sufficient to recapture the same relationships among samples that we observe with the full dataset. The results thus open up the possibility of conducting large-scale studies analyzing thousands of samples simultaneously to survey microbial communities at an unprecedented spatial and temporal resolution.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.048 DISTRIBUTION OF ENZYME-ENCODING GENES AMONG MICROBIAL TAXA: ALKALINE PHOSPHATASE

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Microbes drive nutrient mineralization and decomposition in all ecosystems. To initiate the breakdown of chemically complex organic matter into usable forms, microbes secrete extracellular enzymes. However, it is unclear which microbes are capable of producing which enzymes. Linking phylogeny to function, such as extracellular enzyme production, will help us better characterize, model, and understand how microbial communities impact ecosystem processes. Therefore, the aim of this project is to determine how extracellular enzyme genes are distributed among microbial taxa and whether microbes can be categorized into functional groups based on the potential to produce specific enzymes. A set of empirically characterized alkaline phosphatase enzyme sequences was used to query a database of sequenced microbial genomes for potential enzyme-encoding genes. The resulting dataset was analyzed for dispersion of enzyme traits across the phylogeny of the database. At a minimum, 35% of the genomes queried contain one or more identified genes for alkaline phosphatase. These gene orthologs are significantly clustered within subsets of most of the major bacterial groups (Actinobacteria, Bacteroides, Chloroflexi, Cyanobacteria, Firmicutes, Planctomyces, Proteobacteria) and even in a few Archaea. Clustering of alkaline phosphatase genes suggests that certain sub-groups of related microbes may be primarily responsible for mineralizing organic phosphorous within the community. However, there may be other genes that encode for the same or similar function but have yet to be identified. Furthermore, the taxonomic depth and breadth of sequenced microbial genomes must increase before we can confidently assign microbes to functional groups based on extracellular enzymes.

Abstract Category
09 Evolutionary Ecology of Microbial Communities

PS.09.049 SEASONAL VARIATION OF BACTERIAL COMMUNITY IN THE SEA WATER OF GWANGYANG BAY

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Seasonal variation of bacterial community in the sea water of Gwangyang Bay was analyzed using pyrosequencing (GS-FLX titanium) from April, 2006 to February, 2007. A total of 134,034 (spring; 45,061, summer; 33,525, autumn; 38,264, winter; 17,184) sequence reads with valid barcode was obtained from four samples. The number of the sequence reads was reduced to 124,382 after processing. The sequences were classified according to their domains, bacteria (spring; 36,633, summer; 33,508, autumn; 38,158, winter; 16,083) and eukaryotes (spring; 8,428, summer; 17, autumn; 106, winter; 1101). The average length of the processed sequence reads was 437 (spring; 434, summer; 443, autumn; 441, winter; 431) bases. Bacterial sequences consisted of 29 phyla and candidate divisions in spring, 19 phyla and candidate divisions in summer, 27 phyla and candidate divisions in autumn and 18 phyla and candidate divisions in winter. Proteobacteria was the most abundant phylum in spring (26,394 sequences, 72.0 %), autumn (30,909, 81.0 %) and winter (13,250, 82.4 %), but summer was phylum Firmicutes (27,794, 82.9 %). Each sequence read was assigned to taxonomic group using Eztaxon-extended database, holding manually annotated 16S rDNA gene sequence information.

Abstract Category
09 Evolutionary Ecology of Microbial Communities

PS.09.050 GENOTYPIC AND PHENOTYPIC VARIATION IN COMMONLY STUDIED ALGAL STRAINS RELATED TO LIPID ACCUMULATION

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Due to environmental concerns with elevated carbon dioxide levels and the economic drivers for alternative fuel sources, interest in algal-based oils has been re-invigorated. Early research was conducted with the CHLOR-1 green alga strain that was shown to accumulate lipids under different growth conditions. The original CHLOR-1 culture was isolated from a drainage ditch in Colorado in the 1970s and was recently obtained from the Hawaii Culture Collection (Honolulu, HI). Upon streaking the culture on Bold's medium, six colony morphotypes were observed and isolated (PC-2 to PC-7). Comparisons based upon SSU rDNA gene sequences indicated all the isolates were most closely related to *Coelastrrella saipanensis*. repPCR-based genomic fingerprinting was used to further differentiate the strains that displayed phenotypic variation with random primers (OPA09, OBB04, and OPF07). Primer OPA09 did not show differentiation between the six isolates. Primer OBB04 showed similarity between PC-2, PC-3, and PC-4, PC-5 and PC-6 could be grouped, and PC-7 was unique. OPF07 showed that PC-2, PC-3, and PC-4 were similar, PC-5 was distinct, and PC-6 and PC-7 had similar banding patterns. Phenotypic variation was based upon colony morphology, growth responses to pH shifts, and lipid accumulation in comparison to the original mixed culture. Both unbuffered and CAPS-buffered media revealed early pH stress induced lipid accumulation in both total Nile Red fluorescence and specific fluorescence. Furthermore, the unbuffered and CAPS buffered systems revealed TAG accumulation response could be attributed to both pH and nitrate depletion conditions. The combined stress response was much stronger than either pH or nitrate depletion as sole stressors. Based upon genomic fingerprinting and physiological responses, the PC-3 strain represented one of three distinct groups within the CHLOR-1 culture that accumulated lipids.

Abstract Category
09 Evolutionary Ecology of Microbial Communities

PS.09.051 ANALYSIS OF THE CULTURABLE ENTEROBACTERIA IN SMALL-SCALE DAIRY FARMS FROM MEXICO

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Dairy farms can be reservoirs of pathogen bacteria that can move within and between farms in a same producing region, thus generating health problems by milk contamination or dispersion in dairy farm environments. One of the objectives of the present work was to identify species of pathogen enterobacteria in farm environments and milk in small-scale dairy farms in the state of Michoacan, Mexico. Another aim was to compare the community structure of culturable enterobacteria among studied dairy farms in order to evaluate possible similarities associated with production management. 310 enterobacteria isolates were obtained from soil (85), bovine feces (75), milk (71) and udders (79) in eight small-scale dairy farms. Partial sequences of the 16S rRNA subunit gene from isolates were obtained. Alignment by BLAST to sequences in the Genbank of obtained sequences identified 185 isolates as *Escherichia coli*, 5 as *Klebsiella oxytoca*, 11 within *Enterobacter* (*E. cloacae*, *E. sakazakii* and *E. hormaechei*), and 9 within *Shigella* (*S. sonnei* and *S. dysenteriae*), the remaining isolates being identified as enterobacteria not associated with human or animal public health problems. Between 2 and 19 Operational Taxonomic Units (OTUs) were detected by farm using the software DOTUR with a differentiation index of 3%, suggesting differences in structure of the bacterial community. The UniFrac test showed that the structure of the bacterial community is significantly different between most studied farms ($P=0.05$). Principal coordinate analysis (PCoA) grouped farms in three clusters, revealing that the first two coordinates can explain 46.38% of the total variance. The fact that similar community structures are associated with the displacement of staff or forage suggests that management has an effect on the structure of bacterial communities in studied farms, possibly in combination with the microclimatic environment.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.052 RHODOPSEUDOMONAS PALUSTRIS SURVIVES LONGER THAN OTHER PURPLE PHOTOTROPHIC BACTERIA UNDER CARBON STARVATION

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Purple phototrophic bacteria are widely found in nature. It is expected that they can use light energy for maintenance of viability under nongrowth conditions. In this study, we compared carbon-starvation response of *Rhodopseudomonas palustris* belonging to Rhizobiales with that of other two species of purple bacteria.

Rhodopseudomonas palustris CGA009, *Rhodobacter sphaeroides* 2.4.1Ga and *Rhodospirillum rubrum* S1 were grown in carbon-limited medium under photoheterotrophic conditions, and starvation conditions were brought on by carbon exhaustion. Viability of starved cells was assayed by colony forming ability. ATP content of cells was determined by luciferin-luciferase assay. Under dark conditions, viability of *R. rubrum* rapidly decreased to 1/1000 of the initial value after 2 days of starvation and reached 1/50,000 on day 12. *R. sphaeroides* and *R. palustris* remained viable for 6 days after which their viabilities gradually decreased to reach 1/10,000 on day 17 and 1/3,000 on day 30, respectively. Illumination prolonged the viability of the three strains; 2 to 25% of the initial cells survived for 30 days. A decrease in the ATP content of *R. palustris* was correlated with reduction of viability. When *R. palustris* cells were harvested at stationary phase after being grown on rich medium and then resuspended into starvation conditions, these cells maintained intracellular ATP and viability in the dark during starvation. This indicates different mechanism for maintaining cellular ATP from that of light-dependent survival.

Starved cells of *R. palustris* survived longer than the other bacteria in the dark. Photosynthetic ability increased the survivability of purple bacteria. High survivability under starvation was caused by production of ATP using light energy or by suppression of ATP consumption/loss.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.053 INTERFERENCE INTERACTIONS SHAPE STRUCTURE AND DIVERSITY IN SEDIMENT COMMUNITIES

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We analyzed antagonistic interactions as possible shaping forces of structure and diversity of bacterial isolates from freshwater sediments at five sites within a lagoon system in Churince, Cuatro Ciénegas. Phylogenetic diversity of thermo-resistant isolates was determined through 16S rRNA sequences and antibiotic resistance profiles. Interference assays were carried out between 109 isolates, mainly *Bacillus*, assessing their ability to impair the growth of conspecifics and to resist impairment. We observed within-site positive correlation between the ability of isolates for interference competition with their ability to resist interference. This indicates that interference competition strongly impacts microbial diversity within sites. Assessing among site interference competition we detected at least 18 different levels. Antagonistic competition was greatest among sites, rather than within, consistent with the hypothesis that interference competition structures microbial communities. The observation of across-site positive correlation between the ability of isolates for interference competition with their ability to resist interference was surprising, in that it suggests a hierarchical microbial community structure, potentially due to occasional dispersal across sites. With more than 10,000 interactions scored we found that antagonism was linked to a few taxonomic groups and varied with the sample site as well as with the sediment depth. We also found that the taxonomic distribution of the samples is heterogeneous along the Churince system. The observations of abundant interference interactions in sediment communities are consistent with the hypothesized importance of antimicrobials in spatially structured environments. Our results also demonstrate that spatially heterogeneity in microbial communities is likely to readily evolve to antagonistic interactions, even over short distances in stream communities, where dispersal is likely to be relatively frequent.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.054 EFFECT OF BACTERICIDAL COPPER NONYLPHENOL SULFONATE ON SOIL AMMONIA-OXIDIZING BACTERIA AND ARCHAEA

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Copper nonylphenol sulfonate (NPS-Cu) is used as a bactericidal agent in agricultural practices. Because the nonylphenol moiety of NPS-Cu is a pseudoestrogen, its fate in the soil and its effect on soil microbial community represent important information on environmental science. This study was aimed to analyze the response of soil microbial community, especially ammonia-oxidizing organisms, to the compound. A soil column containing 25 g of Humic Allpphene soil was percolated with NPS-Cu solution (125 mg L⁻¹) at 1 ml min⁻¹ and at 30°C for 30 days. Soil microbial communities were analyzed by both clone library analysis and terminal-restriction fragment length polymorphisms (T-RFLP) profiling of the PCR-amplified 16S rRNA, betaproteobacterial ammonia monooxygenase large-subunit (*amoA*), and crenarchaeotal *amoA* genes with extracted soil DNAs as the template. Quantitative PCR was performed to estimate the relative amount of the target genes and combined with the T-RFLP analysis to obtain the profile with decreased PCR bias. NPS-Cu was degraded at a rate of 0.03 mg (g soil)⁻¹ day⁻¹ in the percolated soil column. The incubation with NPS-Cu for 30 days resulted in a 9 % and a 45 % decrease in bacterial and archaeal 16S rRNA genes, respectively. A more significant decrease (93 %) was found in the bacterial *amoA* gene although a small decrease (13 %) in the archaeal *amoA* gene. The clone library and T-RFLP analyses showed that the NPS-Cu addition reduced bacterial and archaeal 16S rRNA gene diversities but did not extensively influence bacterial and archaeal *amoA* gene diversities. In the agent-percolated soil, the majority of 16S rRNA gene clones belonged to *Betaproteobacteria* and *Gammaproteobacteria* and the persistent archaeal *amoA* gene clones were related to "*Candidatus Nitrososphaera gargensis*" with sequence identity levels of 78-87 % (about 635 bp). These results suggested the selective inhibition of NPS-Cu to soil bacterial and archaeal communities.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.055 NETWORK STRUCTURE AND PHENOTYPIC VARIATION *MYXOCOCCUS XANTHUS*

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Phenotypic evolution arises from the interplay of allelic variation and the environment. Simple models of these interactions provide false understanding of phenotypic evolution, suggesting a one to one relationship of a gene to an environmental factor ignoring the possible pleiotropic consequences of alleles on phenotypes and the potential for evolutionary change. Genomic information available for many microbial species provides a rich resource for investigating the impact of pleiotropy on evolution. It has been proposed that regulatory changes of developmental program are less likely to have pleiotropic effects and consequently have a higher likelihood to be beneficial. However, debate on the adaptive potential of alterations in development persists, in part, because it is difficult to integrate genomic and phenotypic information for complex traits and entire phenotypes. We use *Myxococcus xanthus* to investigate constraints on developmental evolution, by studying the impact of genetic changes on phenotype. *M. xanthus* undergoes development when resources become scarce and individual cells migrate towards aggregation centers, forming clumps that will develop into fruiting bodies. We constructed deletions of four kinases affecting the expression of a key gene for development, and studied pleiotropic consequences of these mutations in development. Developmental timing was shortened, and phenotypic traits of mutants varied significantly from the wild type, indicating that pleiotropic consequences occurred and correlate with the genotypic information available. This finding demonstrates that pleiotropic consequences may not be rare as a result of alterations in developmental program and that diversity in many traits can be achieved by modest regulatory changes. It also provides a model system that reduces the difficulty in genotype-phenotype mapping, the identification of pleiotropic effects in the phenotype, and the analysis of

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.056 DOMINANT GROUPS OF PROKARYOTES IN THERMAL HABITATS OF UZON CALDERA, KAMCHATKA

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The diversity of thermophilic microorganisms inhabiting Uzon Caldera (Kamchatka, Russia) hot springs has already been studied by cultivation methods as well as by molecular approach, using 16S rRNA genes amplification and analysis. However, relative abundance of different groups of thermophilic prokaryotes in Uzon hot springs has never been under study. The goal of this work was to obtain quantitative characteristics of microbial communities inhabiting Burlyashchy Pool (90°C, pH 6.7) and ground water on Central Thermal Field (49°C, pH 4.0).

DNA was isolated from samples of water and sediment mixture and the V3 variable region of the 16S rRNA gene was amplified with a pair of universal primers. PCR fragments were pyrosequenced on GS FLX (Roche). The data were analyzed employing the Pyrosequencing pipeline (<http://pyro.cme.msu.edu/>) of the RDP Classifier program packages.

The relative abundance of Bacteria and Archaea in Burlyashchy Pool was found to be 60 and 40%, respectively. Among Bacteria, Aquificales were dominating, represented by the genus *Hydrogenobacter* (20% of all Bacteria), and uncultured Aquificales (30%). 80% of Archaea belonged to the genus *Pyrobaculum*. In the ground water of Central Thermal Field, methanogenic *Verrucomicrobia* of VTAB group (37%) and Acidithiobacillaceae (30%) made the two most significant groups of Bacteria. Dominant Archaea in this habitat either belonged to *Thermoplasmatales* (40%), or were close to environmental clone pJP89 found previously in Yellowstone National Park (34%).

Lithoautotrophic Archaea and Bacteria dominate in both habitats studied. pJP89-related organisms make an environmentally relevant group in a moderately thermophilic and moderately acidic environment, which would justify further attempts aimed on their visualization and culturing.

Abstract Category
09 Evolutionary Ecology of Microbial Communities

**PS.09.057 A GLOBAL BIODIVERSITY STUDY OF MARINE AND TERRESTRIAL
HYPERTHERMOPHILIC NANOARCHAEA**

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The proposed archaeal phylum Nanoarchaeota contains a single cultivated species, the marine hyperthermophile *Nanoarchaeum equitans*. Living as an obligate ectosymbiont/parasite on the surface of another archaeon, the crenarchaeote *Ignicoccus hospitalis*, *N. equitans* has a highly reduced genome and no apparent primary metabolism. Based on rRNA sequences, nanoarchaea have been reported from several high temperature locations on five continents and even from two mesophilic, hypersaline environments. Using both specific amplification of nanoarchaeal rRNA genes and archaeal community pyrosequencing we investigated the phylogenetic diversity and abundance of nanoarchaea in multiple high temperature environments. These included terrestrial fresh water sites in Yellowstone National Park, Azores, Russia and four major hydrothermal vent systems (East Pacific Rise, Guaymas, Mid Atlantic Ridge and Eastern Lau). At some locations, nanoarchaea represented a significant fraction of the archeal community, reaching over 10% in samples from Rainbow hydrothermal vent field on the Mid Atlantic Ridge. Phylogenetic analysis of all available nanoarchaeal sequences indicate clades that organize both by geographic location and by type of environment (marine or terrestrial). While it is not known which (if any) nanoarchaeal lineages may be free living, the phylogenetic classification linked to biogeography suggests separate evolution of the terrestrial and marine clades, potentially dependent on distinct types of hosts. Ongoing laboratory enrichments are aimed at isolating novel hyperthermophilic nanoarchaea and their potential hosts from marine and fresh water samples.

Abstract Category
09 Evolutionary Ecology of Microbial Communities

**PS.09.058 THE DIVERSITY OF CULTURED AND UNCULTURED PSEUDOMONAS IN AN
URBAN WASTE FIELD TRIAL**

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The genus *Pseudomonas* is comprised of a vast and diverse group of bacteria. In this study, the diversity of *Pseudomonas* in a Danish long-term urban waste field trial was compared by using selective culturing and high throughput sequencing. Soil samples subjected to different fertilizer treatments were collected: an unfertilized control soil (U), and two soils treated with accelerated levels of sewage sludge (SA) or cattle manure (CMA), respectively. The aim of the study was to investigate the effect of fertilizers on the diversity of *Pseudomonas* species. Three different culture media selective for *Pseudomonas* were used for culturing. A total of 1200 isolates were pooled from each soil sample and DNA was extracted from these pools. Primers 530F and 1061R were used to amplify the V4 to V6 regions of 16S rRNA (562 bp fragments). The samples were sequenced using a modified version of the 454 FLX amplicon sequencing protocol. Approximately 5,000 DNA sequences with length ≥ 220 bp were obtained from each of the three soils. The sequences were sorted, trimmed and subsequently classified using the RDP's pyrosequencing pipeline. Analysis showed that 99.6% of the 16S sequences could be assigned to the genus *Pseudomonas* with high confidence. A total of 1457 operational taxonomic units (OTUs) were identified at 100% similarity, while 71 OTUs were identified at 97%. Rarefaction curves showed OTU numbers at 97% similarity to be 20, 25 and 17 for the samples U, SA and CMA, respectively. By comparing read distribution across samples within individual OTUs, distinct species distributions patterns were found. While *Pseudomonas* from U and CMA were shown to be dominated by only a few species, the *Pseudomonas* population in SA soil samples had a significantly higher diversity. Using *Pseudomonas* populations as a model, this study therefore indicates that fertilizer treatment could have a profound impact on microbial soil diversity.

Abstract Category
09 Evolutionary Ecology of Microbial Communities

PS.09.059 BACTERIAL ECOTYPES ASSOCIATED WITH FREE SOIL AND RHIZOSPHERE NICHES

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Ecotypes are within-species groups with a specific ecological niche. Through the use of the algorithm Ecotype Simulation (EcoSim) we can identify these ecotypes from protein-coding DNA sequences. Previous studies using Ecotype Simulation have found ecotypes within the named species *Bacillus subtilis*. Ecotypes have so far been shown to be differentially adapted to solar exposures and soil textures, and both generalist and specialist ecotypes have been found. Here we explore the possibility that *Bacillus* ecotypes may associate with the rhizosphere, the soil that is in direct contact with a plant's roots. This environment contains both biotic and abiotic factors that differentiate it from a free-soil environment. We hypothesized that the rhizosphere represents a unique niche that bacteria have speciated to inhabit. The rhizosphere of 7 plant species were sampled at sea level and 2500 m above sea level. Free soil was also sampled at both elevations. Strains were isolated and sequences were analyzed by EcoSim. The majority of isolated strains fell into two ecotypes; one previously undescribed ecotype which does not include any named subspecies or previously discovered ecotypes of *B. subtilis*, and a "generalist" ecotype, which includes strains from *B. subtilis* subsp. *inaquosorum*. These two ecotypes have significantly different ecological associations. One ecotype is a free soil "specialist", while the other is a "generalist" ecotype that can associate with the rhizosphere and other niches including sandy soil and low heat adaptation. Thus, the rhizosphere does represent a set of factors which form a niche that bacteria invade during speciation, and individual plant species may offer differing niches to bacteria. In addition, the free-soil environment also represents a unique niche which a previously undiscovered ecotype is associated with.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.060 CULTIVATION-INDEPENDENT, SINGLE- AND MULTI-LOCUS STUDIES OF SYNECHOCOCCUS SPECIES INHABITING A HOT SPRING MICROBIAL MAT COMMUNITY

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Previous research has shown that sequences of 16S rRNA genes and the 16S-23S rRNA internal transcribed spacer regions may not offer enough genetic resolution to define all ecologically distinct *Synechococcus* populations (ecotypes) inhabiting alkaline, siliceous hot spring microbial mats. To achieve higher molecular resolution, we studied sequence variation in several protein-encoding loci, obtained from 60°C and 65°C sites in the Mushroom Spring mat (Yellowstone National Park, WY), either by PCR-amplification or bacterial artificial chromosome (BAC) cloning and oligonucleotide probe sequencing to identify BACs containing 16S rRNA genes. Single-locus and concatenated multiple-locus sequences were analyzed using the Ecotype Simulation algorithm, based on the Stable Ecotype Model of speciation, to identify putative ecotypes. Depending on the molecular resolution of the locus, up to 13-14 putative ecotypes were predicted from variation in individual and concatenated multiple loci within each of the two 16S rRNA-defined *Synechococcus* lineages present at these temperatures. Many putative ecotypes were sample-specific, suggesting different adaptations to temperature or to other parameters that vary between these sites. Due to under-sampling and conservative putative ecotype demarcation these values may underestimate the true species diversity in these lineages. Examination of BAC clone end sequences suggested that 16S rRNA regions of the *Synechococcus* genomes have undergone genomic rearrangement due to inversion. Multiple lines of evidence suggested that recombination has exceeded mutation in the diversification of these organisms, with recombination/mutation estimates of ~5.6-7. While recombination involving individual loci with phylogenetically distant organisms may have displaced some variants (possibly detected by eBURST analysis of concatenated multi-locus data), it has not eroded the phylogenetic coherence of ecotype clades in this taxon.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.061 EXPERIMENTAL EVOLUTION AND RESEQUENCING OF HIGH-SALT TOLERANT MARINE BACTERIA

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Using marine *Vibrio splendidus* strains as models for co-existing sequence diversity in the environment, we investigate to what extent genetic diversity corresponds to the phenotype of salinity tolerance. We examine closely related wild-type strains and also experimentally evolve high-salt mutants from these. Since environmental factors like salinity involve multiple, unknown loci, whole-genome resequencing of mutants reveals the genetic changes accompanying increased tolerance. We first compare the salinity phenotypes and genomes of wild-type strains. Further, to investigate the genomic and phenotypic plasticity of this trait, we experimentally evolve some of these strains and compare the evolved strains with the ancestors. In order to select for mutations that affect salt tolerance, we perform the selection on solid media plates containing a salinity gradient. This favors mutations related to the selection pressure, not just growth rate as in a chemostat. The process is iterative, using the selected strain from the previous round to seed the next selection cycle. The new salt tolerance phenotype of the evolved strains is measured by growth rate at different salinities. We see changes in the habitable niche in both the range of tolerance and the pattern of growth rate vs. salinity level. Evolved strains can survive 1.6M NaCl, versus 1.2M for ancestor, and grow faster at intermediate levels. Plasticity of the niche and the background effect of the ancestral genome can be quantified based on these phenotypes. To date, genomic resequencing has revealed acquired hypermutator phenotypes with more than 1000 SNPs in two of five mutants with the same ancestor, indicating that rapidly accumulating multiple mutations may be advantageous. Further work to characterize the mutator phenotype and its adaptive potential is ongoing. We are also resequencing more of our evolved strains to determine if this is a consistent response to strong selective pressure.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.062 RICHNESS OF ALGAL VIRUSES (PHYCODNAVIRIDAE) IN THE BRAZILIAN AMAZON

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The Amazon basin incorporates the territory of 8 South American countries but has 60 % of its area (5.846.100 Km²) located in Brazil. It encompasses a vast and complex river system, source of 20% of all free-flowing fresh water on the planet. Although the Amazon Basin is one of the world's most important ecosystems in terms of biodiversity and global climate implications, little is known about the Amazonian microbial communities. The present work evaluates the presence and richness of algal viruses in samples from three important and distinct rivers of the Brazilian Amazon basin, the Cuieiras, Negro and Solimões. The goal was to provide new data and insights about a group of virus that is a major player in many important ecological processes such as termination of phytoplankton blooms, nutrient cycling, and ultimately impacts the atmospheric composition. In July 2007, 8 samples were collected (10 L to 100 L), pressure filtered through glass fiber (1.2 µm) and PVDF (0.45 µm) filters connected in series, and the remaining particulate material concentrated ~1000-fold by tangential flow ultracentrifugation. Extracted DNA was amplified with degenerate primers targeting the phycodnavirus' DNA polymerase gene, and cloned into TOPO plasmid vectors for sequencing. Sequences obtained were submitted to phylogenetic analysis. Phycodnaviruses were detected by PCR only in samples from Solimões and Cuieiras rivers, and the answer to that might lay on some unique chemical and physical characteristics of Negro river. Sequences obtained in this study are the largest dataset of these viruses in lotic environments published up to present. Phylogenetic analysis showed that samples from Solimões and Cuieiras rivers clustered separately, and that Amazonian phycodnavirus' sequences are more closely related to other freshwater sequences than to marine ones.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.063 METAGENOMIC ANALYSIS OF AMMONIA OXIDIZING ARCHAEA FROM AN AUSTRIAN THERMAL SPRING

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Even though ammonia-oxidizing archaea have been detected only recently, they are now considered to comprise a significant component of many microbial communities. Initial genome analyses of the first representatives of this group indicate that they do not belong to *Crenarchaeota* as suggested by 16S rRNA phylogenies but rather form a distinct phylum, the *Thaumarchaeota* (Brochier-Armanet *et al.*, 2008, Spang *et al.*, submitted). As only few ammonia-oxidizing archaea have been successfully enriched and sequenced, we took a metagenomic approach in order to extend our knowledge on the genomic diversity of uncultivated Thaumarchaeota. For this purpose, a fosmid library was generated from biofilm samples taken in a radioactive thermal cave (45°C) in the Austrian Central Alps in which ammonia-oxidizing archaea were found to form a major part of the microbial community and in which we have measured considerable gross nitrification rates using a pool dilution technique. The library contained 18 fosmids with either an *amoA* or 16S rRNA gene affiliating with ammonia-oxidizing archaea. Five of those fosmids were chosen for sequencing and further analysis. Four of the fosmids appear to be affiliated with the soil group I.1b of Archaea. The fifth fosmid in contrast probably derived from an archaeon belonging to the marine group I.1a including *Cenarchaeum symbiosum* and *Nitrosopumilus maritimus*. Informational processing genes present on the different fosmids were analysed phylogenetically providing further support for the distinctiveness of the phylum *Thaumarchaeota*. Additionally, several putative genes that do not have homologues in the current databases indicate that ammonia oxidizing archaea of the soil group contain a specific gene set different from their marine counterparts. Thus, this study provides further support for the distinctiveness of *Thaumarchaeota* but also points to genetic diversity within the different lineages of this phylum that awaits further characterization.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.064 INFLUENCE OF FEEDLOT MANURE APPLICATIONS ON SOIL MICROBIAL COMMUNITIES ASSOCIATED WITH SHORT GRASS PRAIRIE AND CONSERVATION RESERVE PROGRAM GRASSLAND

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Available beef cattle manure exceeds the assimilative capacity of farm land in many areas of the High Plains. About half of the agricultural land base of the fed cattle producing region of the southern High Plains is grassland. This study documents the effect of annual (both fall and spring) applications of three rates of N (50, 125, and 200 kg/ha) in the form of feedyard cattle manure on grassland ecosystems and on soil microbial composition. Replicate treatments (n=4) of feedyard cattle manure N applications plus controls were made to native short grass prairie and old world bluestem grass ecosystems starting in the spring of 2000 and continued until the fall of 2006. A significant increase in biomass in response to N application was first observed in 2004 and continued through the last biomass sampling in 2007. Soil samples for microbial community composition at three depth ranges (0-2.5, 5.0-7.5, and 30.0 to 32.5 cm) were obtained in November of 2008. Microbial composition was investigated via DGGE-PCR methods using both general (V3 region) and improve-group specific 16S-PCR primers (Muhling *et al.*, 2008) targeted at the following groups: *Alpha-*, *Beta-* and *Gamma-Proteobacteria*, *Bacteroidetes*, and *Firmicutes*. DNA sequence analysis of 16S rDNA gene libraries was also employed. DGGE-PCR data were analyzed by hierarchical clustering, group separation evaluation, and principal components analysis. Microbial community composition was most strongly influenced in order of importance by: 1) soil depth, 2) seasonal applications of manure over rate of N, and 3) lastly by grassland composition. Manure applications also resulted in significant differences between 16S gene libraries as did soil depth. The influence of grassland type was subtle and complex. However, soil depth and manure application treatments provided a strong influence on overall microbial community composition and tended to obscure or suppress grassland influences on microbial community composition.

Abstract Category
09 Evolutionary Ecology of Microbial Communities

PS.09.065 A LONG BRANCH ATTRACTION REVEALS AN ADAPTIVE RADIATION IN PSEUDOMONAS FLUORESCENS

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Pseudomonas are remarkable for their capacities to colonize almost all terrestrial and aquatic ecological niches. OprF is the major outer-membrane protein of *Pseudomonas* that plays a role as porin, membrane structural protein and root adhesion. In a previous work, we found that the important polymorphism of oprF gene was linked to the ecological niches of *Pseudomonas*. An evolutionary lineage, including the *P. fluorescens* type strain, was particularly adapted to the rhizosphere (Bodilis et al., 2006). Remarkably, this lineage had been highlighted by a strong incongruence between the oprF phylogeny and the 16S rRNA phylogeny. To explain this incongruence we previously hypothesized an artifact of phylogenetic reconstruction because we found no clue of lateral transfer (Bodilis and Barray, 2006). By using Bayesian methods with a rigorous sampling, including most of the type strains in *Pseudomonas*, we found, for the first time, an oprF phylogeny congruent with the organism phylogeny, demonstrating that a long-branch attraction (LBA) occurred in the oprF phylogeny. The LBA artifact is a well-known artifact in some ancient phylogenies but few studies had described such an artifact in recent phylogenies. The artifact of the oprF phylogeny is explained by faster evolution in the lineage adapted to the rhizosphere, as a consequence of an adaptive radiation. This faster-evolved lineage incorrectly moved to the root of the tree during the phylogenetic reconstruction. We think that analysis of such artifacts could be a valuable tool in molecular ecology to highlight cryptic adaptive radiations in microorganisms.

Bodilis, J., and S. Barray (2006) Molecular evolution of the major outer membrane protein gene (oprF) of *Pseudomonas*. *Microbiology*. Vol. 152 (1075-1088).

Bodilis, J., M. Hedde, N. Orange and S. Barray (2006) OprF polymorphism as a marker of ecological niche in *Pseudomonas*. *Environmental Microbiology*. Vol. 8 (1544-1551).

Abstract Category
09 Evolutionary Ecology of Microbial Communities

PS.09.066 SCALING PATTERNS OF DIVERSITY FROM INDIVIDUALS TO COMMUNITIES IN HUMIC LAKES

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The goal of this study is to compare response to disturbance at varied levels of taxonomic resolution from the individual to the community in an attempt to identify functionally cohesive, ecologically relevant units within the total community. We hypothesize that beneath course and stable community-scale dynamics, there is a relatively dramatic compositional turnover that is unseen at the community scale due to functional redundancy among similar genotypes within the biological system. Based on this hypothesis we predict that response to environmental perturbations may vary dramatically between the population and community. Previously, a small humic lake of the North Temperate Lakes LTER located in northern Wisconsin, USA was artificially mixed in order to disrupt chemical and thermal gradients created by thermal stratification during the summer season. The bacterial community composition was followed across the disturbance by ARISA analysis and pyrosequencing the V6 region of the 16S rRNA gene of both bacteria and archaea. We investigated population scale processes using high-resolution protein-encoding genes from the dominant bacterial population polynucleobacter (using *cox1*) and the rare methanogenic population of archaea (using *mcrA*). Over the span of seven time points across the artificial mixing, we obtained 439 and 349 sequences corresponding to polynucleobacter and methanogens, respectively. The bacterial community appeared resilient to the disturbance in terms of phylum-level composition, while the methanogen and polynucleobacter populations do not appear resilient in composition at the same time scale. Resilience in diversity was seen in the bacterial community and polynucleobacter

population, while methanogen diversity increased and remained elevated. Comparisons between these population and community dynamics are used to assess the individual-level response of distantly related microbial assemblages.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.067 MULTI-LOCUS SEQUENCE TYPING OF POTENTIAL ECOTYPES OF ALICYCLOBACILLUS FROM BOILING SPRINGS LAKE IN LASSEN VOLCANIC NATIONAL PARK

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We are studying the microbial ecology of Boiling Springs Lake, a pH 2, 55°C geothermal feature in Lassen Volcanic National Park, California, USA. Analyses of 16S rRNA genes amplified from lake-derived DNA indicate a stable, low species-richness community dominated by novel Archaea and Bacteria. Heterotrophic firmicutes isolated from the lake were identified by 16S rRNA gene sequences as species of *Alicyclobacillus*. Initial studies indicated that some isolates with nearly identical 16S rRNA gene sequences had distinct phenotypes with regard to permissible growth conditions and antibiotic resistance profiles. In an attempt to genetically distinguish possible ecotypes of these species, we PCR-amplified and sequenced the *cpn60* gene, which has been used to discriminate between subspecies in several genera. We also used the published genome sequence of *A. acidocaldarius* to design PCR primers to target genes commonly used in MLST studies, and genes we expected to be conserved in the *Alicyclobacillaceae*. Pairwise distances for 16S rRNA gene sequences were sometimes greater than for protein-coding sequences. Conversely, in some cases (nearly) identical protein-coding genes were found in organisms whose 16S rRNA genes shared only 87% identity. This pattern is consistent with recent lateral transfer of some of the genes. There was also some indication of possible allele selection; for example, there seemed to be two distinct, highly conserved alleles of *cpn60* in the *Alicyclobacillus* isolates. Our results suggest that *cpn60* is of limited value for intra- and inter-species differentiation in this genus. This work reinforces the notion that multilocus sequence analysis can be used to understand ecotype divergence, lateral gene transfer and genome evolution, and other processes of ecological interest. However, the inability of some primers to amplify genes in all *Alicyclobacillus* strains suggests the need for employing multiple primer sets for each gene analyzed in a genus.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.068 ECOLOGY AND INTERACTIONS OF THE AT AND TI PLASMIDS FROM AGROBACTERIUM TUMEFACIENS C58

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Plasmids are autonomously replicating extrachromosomal units of DNA that are ubiquitous in bacteria. Many plasmids are able to successfully proliferate among bacterial populations in the environment by providing benefits to host cells or by self-transmission between hosts. Because of this ability to conjugate, plasmid fitness is determined by a combination of both host fitness and transmissibility factors. Many bacteria contain multiple plasmids, and interactions between co-inhabitant plasmids are important components of an individual plasmid's fitness as well as the population dynamics of host cells. *Agrobacterium tumefaciens* exists as either a saprophyte in the soil or a phytopathogen, inducing a type of plant tumorigenesis called crown gall. As a facultative pathogen, the ecological niche varies due to changes in rhizospheric conditions, colonization and dispersal cycles, in addition to seasonal changes and agricultural practices. Virulence depends on the presence of the Ti plasmid, the gene content of which suggests that it has evolved towards optimal fitness in the tumor environment. Many natural isolates possess a second larger plasmid called the At plasmid of which the functions are less clear. Increased growth of pAt+ strains in nutrient-limiting conditions suggest that it may provide greater benefits to cells that are living saprophytically. The primary aims of this research are to determine the ecology of these two plasmids, their costs and benefits in both the soil and tumor environments, and to define their relationship with one another. We hypothesize that the At and Ti plasmids will each experience

optimal fitness in the soil and tumor environments respectively. However, due to the spatially heterogeneous and dynamic environment, each plasmid will be stabilized by the presence of the other under conditions whereby it is not providing a direct benefit to host cell

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.09.069 GENOMIC HETEROGENEITY AMONG EXTREMELY CLOSE RELATIVES IN *BACILLUS*

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The origins of bacterial species are still not well understood. Nor is it known how long bacterial lineages persist before splitting into separate, irreversible, daughter groups. Recent technology allows a more detailed exploration of the nature of lineage splitting in bacteria through genomic approaches. With whole-genome sequencing, it is possible to test for recent ecological divergence among extremely close relatives. In this study, we chose to sequence the complete genomes of eight strains from the *Bacillus subtilis* subsp. *spizizenii* clade in order to better appreciate the nature and rate of species formation in bacteria. The strains were isolated from a canyon in Death Valley, USA, and determined to belong to a generalist ecotype. To date, four genomes have been sequenced; the sequencing of the remaining four is underway. Trees constructed from genes shared between genomes show clustering of the strains into distinct groups within the ecotype. Genome content analysis of the completed genomes shows a core group of genes shared among all strains, with additional genes shared only within subsets. Following the completion of all eight genomes, we will analyze shared genes more thoroughly. For genes shared between genome subsets, we will look for accelerated divergence indicating ecological specialization. We will analyze the function of unique genes to better understand the ecological divergence between the strains. These full genome comparisons allow us to characterize the ecological heterogeneity of closely-related strains.

Abstract Category

09 Evolutionary Ecology of Microbial Communities

PS.10.001 THE ROLE OF VEGETATION TYPE ON MICROBIAL PROCESSES AND COMMUNITY COMPOSITION IN POST-MINING REFORESTED SOIL

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While the creation of post-mining spoil areas represent a large anthropogenous impact on ecosystem functioning, the revegetation of these sites offers a unique opportunity to study the role of vegetation in shaping soil microbial properties. Response of microbial soil processes proxied as the activity of extracellular enzymes (cellobiohydrolase, beta-glucosidase, beta-xylosidase, chitinase, arylsulfatase and phosphatases) and the effect on microbial community composition was studied in a set of 28 areas recultivated by seven tree species (alder, larch, spruce, pine, oak, lime, spontaneous succession) approximately 25 years after revegetation in the post-mining deposit area near Sokolov (Czech Republic).

Litter and upper soil horizon (5 cm) were sampled in October, April and August and assayed for enzyme activities and microbial community composition based on PLFA, ergosterol content and qPCR.

For most enzymes, activities were significantly higher in the litter horizon in sited reclaimed with alder, while highest laccase activity was found in sites under oak. Influence of both vegetation type and season on PLFA composition was similar in both horizons and these two parameters explained each approximately 25% of the total variability of the content of individual PLFA molecules. Fungal biomass and enzyme activities in the upper soil were more affected by vegetation than by season, while in the litter horizon fungal biomass underwent significant seasonal changes. PC analysis on both PLFA composition and enzyme activity data showed that samples belonging to the same vegetation type clustered together. Our results show that microbial community composition and microbial processes of the C, N, P, and S cycles are largely affected by vegetation and underline the effect of vegetation type and litter quality for the development of soil de novo.

Abstract Category
10 Forest Soil Ecology

PS.10.002 BACTERIA, ARCHAEA AND AMMONIA-OXIDIZING COMMUNITIES IN SEMI-ARID SOILS

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Arid and semi-arid ecosystems cover a significant portion of the Earth's surface, but the microbial communities present in these systems remain relatively uncharacterized. In this study we describe bacterial and archaeal communities in great basin desert, grassland, pinyon-juniper woodland, and ponderosa pine forest soils from northern Arizona. The microbial diversity at each site was characterized by pyrosequencing a portion of bacterial and archaeal 16S rRNA genes. In addition, fragments of the functional gene *amoA* were sequenced to characterize the ammonia-oxidizing community, which plays a key role in the cycling of nitrogen. Abundances of archaeal and bacterial *amoA* genes were also quantified with real-time PCR and TRFLP profiles for archaeal and bacterial *amoA* genes were developed. 16S rRNA gene sequences from Actinobacteria were predominant at the desert, grassland, and pinyon-juniper sites, while Proteobacterial sequences were nearly as abundant as Actinobacteria sequences in the ponderosa pine forest. Among the most common bacterial 16S rRNA gene sequences found at all sites, were those with a high degree of similarity to known thermophiles and rhizobium. Both archaeal and bacterial *amoA* sequences tended to separate by site. Archaeal *amoA* sequences were most closely related to sequences isolated from soil and sediment environments, while bacterial *amoA* sequences clustered with those from a variety of soil types. Our results show that both the overall microbial community and the ammonia oxidizing community vary among these arid and semi-arid soils.

Abstract Category
10 Forest Soil Ecology

PS.10.003 SPATIOTEMPORAL PATTERNS OF STRUCTURE AND FUNCTION OF FOREST SOIL FUNGAL COMMUNITY REVEALED BY PYROSEQUENCING: CELLULOSE DEGRADATION AS A MODEL PROCESS

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Recent results demonstrate differences in the composition of fungal communities along forest soil depth profiles. The aim of this research was (1) to compare the total fungal and bacterial community composition along a soil depth profile and during the process of litter decomposition; (2) to follow the distribution of functional genes active in cellulose decomposition and (3) to specifically identify cellulose-degrading microorganisms. Microbial community composition was analysed using 454-Titanium pyrosequencing of rDNA and rRNA. The same approach was used to identify the genes (DNA) and transcripts (mRNA) encoding for fungal cellulases. SIP with ¹³C-cellulose was used to identify microorganisms deriving their biomass from cellulose. There is a fast succession of fungal communities on decaying litter with many species appearing/disappearing within a few months. Most cellulase genes are present in litter only during a limited period, showing that cellulose decomposing community changes rapidly. Even more profound differences were found when the litter and humus horizon of forest soils were compared showing a very little overlap of microbial communities (both fungal and bacterial) with indications that communities are vertically structured even within horizons. At any time, the active microorganisms likely represent just a small part of the whole community. Analysis of microbes deriving their biomass from cellulose show a clear distinction of their community from the total community and the fact that cellulose degradation is performed by different microorganisms at different soil depths. Vertical stratification of the properties of forest soils is reflected in the fine stratification of soil fungal community. Although cellulose degradation is performed in different stages of litter decomposition and in different soil depths, there is not a universal group of cellulose degraders but rather many distinct communities associated with organic matter of different age.

Abstract Category
10 Forest Soil Ecology

PS.10.004 CHARACTERIZATION OF ANAEROBIC FOREST SOIL MICROCOSM COMMUNITIES ADAPTED TO UTILIZE PLANT MATERIAL

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Forest soil, where layers of leaf litter have accumulated over decades, is an ideal source for anaerobic plant degraders, as anaerobiosis is more prevalent in compacted, waterlogged and poorly drained soils. Despite industrial interest in plant degradation for enzymes and pretreatment strategies for biofuel development, not much is known about the structure and ecology of anaerobic soil communities. The long-term goal of this project is to understand the details of microbial degradation of plant detritus in anaerobic forest soils. Our experimental strategy entails the establishment and characterization of simplified, stable, and therefore tractable communities of natural assemblages of soil microbes adapted to utilize plant litter. Five replicate communities, established from the same forest soil sample, and maintained on minimal media and plant detritus through more than 20 transfers, have adapted over time into simpler assemblages, amenable to interrogation. Community stabilization is demonstrated by a decrease in intercommunity variation in key metabolites. Levels of mixed acid fermentation products (acetate, butyrate, lactate, formate, propionate and ethanol) measured over time using high performance liquid chromatography, levels of hydrogen gas and carbon dioxide detected via gas chromatography, and total supernatant protein measured at time points during adaptation suggest increased stability of the five replicate communities. The phylogenetic diversity of the adapted communities was characterized using 16S clone libraries, revealing novel strains of *Enterobacter*, *Enterococcus*, and *Clostridia* cluster XIVa. Deeper sequencing over several time points is expected to reveal trends in adaptation at the community level, which will be correlated with concurrent changes in metabolic products, providing insight into the functional roles of specific microbes involved in plant degradation.

Abstract Category
10 Forest Soil Ecology

PS.10.005 ECTOMYCORRHIZAL HYPHAE AND BACTERIA INTERACT TO REDUCE ORGANIC PHOSPHORUS MOBILIZATION

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Ectomycorrhizal fungi provide nutrients to their host trees by means of hyphae that extend beyond nutrient-depleted rhizosphere soil. It is unknown whether the fungal hyphae interact synergistically with soil bacteria because the hyphal growth habit of *Streptomyces* may allow them to compete for similar resources as strand-forming fungi. To assess the interactions between hyphae and soil bacteria, two types of small, sand-filled mesh bags were incubated for four months in the organic soil horizons of a 60 year-old Douglas-fir/paper birch forest in British Columbia, Canada. The bags allowed colonization by ectomycorrhizal hyphae (35 µm mesh) or excluded hyphae, but allowed the migration of soil bacteria (0.5 µm mesh). Suspensions of incubated sand were plated onto low nutrient isolation media to enrich for slow-growing, spore-forming bacteria. Isolated bacteria were identified through 16S rDNA sequencing and their functional potential to break down organic phosphorus and nitrogen compounds assayed using phosphatase and N-acetyl glucosaminidase (NAGase) activities, respectively. Isolates were primarily Actinobacteria (74.3%), followed by Proteobacteria (19.6%) and other classes/suborders (3.7%). A clone library obtained from a pooled sample of sand from all bag types consisted of Proteobacteria (68.2%), followed by Actinobacteria (16.4%). Isolates from hyphae-ingrowth bags had significantly lower average phosphatase activity than isolates from hyphae-exclusion bags. Average NAGase activities were not different. *Streptomyces* spp. were more prevalent in ingrowth bags and grew faster than isolates from exclusion bags. The increased number and growth rate of *Streptomyces* from ingrowth bags coupled with lower phosphatase and lack of increased NAGase production indicates that fungal hyphae can stimulate populations of *Streptomyces* in soil while structuring communities of these potentially competitive bacteria for decreased extracellular enzyme production.

Abstract Category

10 Forest Soil Ecology

PS.10.006 THE SELECTION EXERTED BY THE OIL CONTAMINATION ON MANGROVE FUNGAL COMMUNITY

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Mangroves are typical tropical environment characterized by the interface between the land and the sea, where a particular combination of environmental conditions is found (high salinity, anaerobiosis and periodic tidal flooding). Due to the mangroves location such ecosystem is commonly affected by different sort of contamination, as the oil spill. Although the diversity of prokaryotes (archaea and bacteria) is assessed in mangroves, the fungal community constitutes still an open spot in this subject. Here we show a culture-independent survey on fungal communities found in two mangroves located in the coastline of the Sao Paulo State (Brazil); i) an oil spilled mangrove where 35 million liters of oil was spilled in 1983; ii) a nearby mangrove, not affected by the oil contamination. In each mangrove samples were collected in three location over a transect from the sea to the land (near sea, middle of mangrove, near the land). The ITS-based analysis of the fungal communities by denaturing gradient gel electrophoresis (DGGE), coupled with the principal components analysis (PCA) has revealed differences in the fungal communities in each samples mangrove, and also among sampling locations within each mangrove. In addition, it was possible to verify the occurrence of specific bands in distinct area, with a remark for bands only found in samples collected in the oil spilled location. A phylogeny approach, based on the analysis of 50 sequences of cloned ITS regions from the oil spilled area, has indicated the high occurrence of fungi affiliated to the taxa Basidiomycota and Ascomycota, with a remark for sequences belonging to *Epicoccum* and *Cladosporium*. The high occurrence of such group makes it a name to be considered in the bioremediation of oil in mangrove area. In summary, the data has determined the effects of the oil contamination over the fungal communities in mangroves, and also elected a candidate fungal group for future use of recovering of affected-mangrove area.

Abstract Category

10 Forest Soil Ecology

PS.10.007 WATERSHED-SCALE VARIABILITY IN SOIL MICROBIAL DIVERSITY: ASSESSING HOW SOIL DEPTH AND LANDSCAPE POSITION INFLUENCE BELOWGROUND COMMUNITIES

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Understanding how environmental factors influence the diversity and composition of microbial communities has been an important question in microbial ecology. Previous work has shown that, for soil bacteria, pH is often a good predictor of community structure. However, it remains unclear how soil properties structure microbial communities at more local scales. Here, we analyzed soil bacterial and archaeal communities at a high degree of spatial resolution, relating community structure to detailed measurements of soil and site properties in order to understand how these communities are structured within a landscape, both across the watershed surface and vertically through soil profiles. Soils were collected from 10 transects and 9 soil pits in a montane watershed in Colorado, USA. DNA was extracted from the soil samples and bacteria and archaea were pyrosequenced using barcoded prokaryotic primers targeting the 16S rRNA gene. Soil properties and microbial biomass varied over the watershed and differed between north and south facing slopes. In the soil pits, microbial biomass and moisture decreased with depth whereas pH increased with depth. Pyrosequencing yielded an average of 1160 sequences per sample and we saw consistent changes in soil microbial community structure in relation to soil properties throughout the watershed and with depth through the soil profile. These results demonstrate that the local-scale distributions of both bacteria and archaea are often predictable and that we can effectively map the distributions of individual microbial taxa that play an important role in biogeochemical processes within the watershed.

Abstract Category

10 Forest Soil Ecology

PS.10.008 PATTERNS IN FOREST SOIL MICROBIAL COMMUNITY COMPOSITION ACROSS A RANGE OF REGIONAL CLIMATES IN WESTERN CANADA

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Soil microbial communities can be characterized by community structure and function (community composition) across a spectrum of spatial scales, and variation in soil microbial composition has been associated with a number of environmental gradients. This study investigated the structure and function of soil microbial communities under mature, undisturbed forested sites across a range of regional climates in British Columbia and Alberta, and also examines the variation in community composition within sites.

PLFA analysis was used to investigate the structure of soil microbial communities and total microbial biomass at each site. Extra-cellular enzyme assays established the functional potential of the soil microbial community at each site. Multivariate analysis of the data showed soil microbial communities under different forest types significantly separated along the regional climate gradient by both community structure and function, despite high local variation in the communities. Soil moisture content and soil organic matter concentration consistently exhibited the strongest relationship with microbial community characteristics, although the functional and structural responses to the external drivers were different. Microbial community function was related to the regional annual average precipitation gradient. Most of the locations exhibited unique microbial community functional profiles in their soil layers; however the enzyme activities in the samples from the driest (Ponderosa Pine) and wettest (Mountain Hemlock) locations were notably different from each other and from those of the other locations, especially in the organic layers.

The moist maritime-influenced Coastal Western Hemlock (CWH) forest exhibited microbial community structural characteristics which were unique from those of the other forest locations. The higher abundance of bacteria relative to fungi in the CWH forest soils may be related to the significantly higher available N at this site.

Abstract Category
10 Forest Soil Ecology

PS.10.009 RESPONSE OF SOIL ORGANISMS TO RETENTION OF LIVING TREES AFTER FOREST HARVEST

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Variable levels of retention of living trees is increasingly being favored as an alternative to clearcut-harvesting of forests, and preservation of a functioning soil community may be an important advantage of this management practice. The aim of this project is to assess the potential of green-tree retention (GTR) harvesting to maintain soil microbial and faunal diversity and function after harvesting and to determine the optimal size and pattern (aggregated or dispersed) of retention for this purpose. We sampled pre-harvest and 4 months and 5 years following harvesting of a second-growth forest of Douglas-fir and western hemlock on Vancouver Island, Canada. We determined how soil communities change, whether key species are lost, and if GTR of different aggregate sizes (5 - 40 m diameter (1-60 trees)) and distribution (dispersed vs aggregated) cast different size "shadows" of influence. Prior to harvest, 95% of the soil fauna and a distinct microbial community were found in the forest floor, indicating GTR could serve as refugia for colonization of disturbed areas following harvest. Four months after harvest most soil macrofauna and collembola were influenced more by forest floor disturbance than by the presence of living trees. However, mites and pauropods declined from patch centres out into the cut area. Mycorrhizal diversity and enzyme activities decreased 10-15 m from the edge of all but the smallest patch. There was no effect of harvest on fungal richness, but a shift from mycorrhizal to saprophytic fungal dominance. Five years after harvest fungi were more abundant than bacteria in the retention treatments compared to the uncut forest, and dispersed retention was better than aggregated for maintenance of microbial biomass and enzyme activities across the cut block. The results suggest that retention of large patches is not necessary; retention of single trees located within 20 m of each other will ameliorate change in soil organisms and their functions.

Abstract Category
10 Forest Soil Ecology

PS.10.010 HIGHER TREE DIVERSITY SLOWS MICROBIAL COMMUNITY DECOMPOSITION AND ALTERS COMMUNITY COMPOSITION

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Changes in tree diversity under human activity, with associated changes in litter chemistry and nutrient input, likely alter ecosystem nutrient cycling as a result of shifting microbial growth and metabolism. However, few studies have been performed to directly test soil community responses to tree diversity levels. The Kreinitz tree diversity experiment (Kreinitz, Germany), was planted in 2005 with six temperate tree species representing a range of litter chemistry and leaf decomposition rates: Ash (*Fraxinus excelsior*), Lime (*Tilia cordata*), Beech (*Fagus sylvatica*), Oak (*Quercus petraea*), Spruce (*Picea abies*), and Pine (*Pinus sylvestris*). Tree seedlings were planted in combinations of none to all six species per plot. Fall of 2010, after five years of growth, plots were sampled and analyzed for microbial community composition (lipid analysis) and decomposition potential (extra-cellular enzyme assays). We found that the number of tree species, as well as presence of certain tree species, indeed shifted soil community dynamics. Enzyme activities decreased and microbial biomass (particularly fungal indicators) increased with a greater number of tree species, implying that higher tree diversity levels increase nutrient availability, thus lowering the need for microbial enzyme production per unit biomass. Which species were present appeared to be as important as the number of species. Extra-cellular enzyme activities decreased with Beech presence and showed a trend of increase with Lime presence. Microbial community composition also varied with presence or absence of different tree species. In summary, changes in litter chemistry, associated with changing tree diversity, does result in altered soil community composition, biomass,

and functional potential. Thus, through the mediation of microbial communities, it is likely that higher tree diversity slows decomposition and increases retention of soil carbon and other nutrients.

Abstract Category
10 Forest Soil Ecology

PS.10.011 IMPACTS OF ATMOSPHERIC NITROGEN DEPOSITION ON NITROGEN TRANSFORMATION AND NITRIFYING POPULATION IN FOREST SOILS IN SOUTHERN CHINA

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In southern part of China, forest ecosystems adjacent to industrialized and urbanized areas are being saturated with nitrogen (N) due to high atmospheric N deposition, leading to large emission of N₂O and NO from the soil and leaching of nitrate into watershed. In order to estimate the potential risks and possible consequences of high N deposition into forests, we analyzed N transformation and structure of nitrifying microbial population in forest soils receiving long-term N deposition.

Soil samples were collected from broadleaf forests in two study sites, Dinghushan (DHS) and Heishidin (HSD), located near Guangzhou. DHS is N-saturated forest site with high N deposition (52 kg-N/ha/yr), while HSD is non-saturated site with low N deposition (10 kg-N/ha/yr). All the soil samples were acidic (pH 3.6- 4.2). Gross N transformation rates in the soils were determined by using ¹⁵N technique. DNA was extracted from the soils to analyze community structure of nitrifying microorganisms in soils.

Nitrification rate in soil of DHS site was 2.3-2.9 µg/g-soil/day, which was higher than that of HSD site (1.6 µg/g-soil/day). Nitrification was considerably suppressed in the presence of acetylene, suggesting that autotrophic nitrification was dominant in the soils. In the soil DNA analysis of DHS site, neither 16S rRNA gene nor ammonia monooxygenase gene (amoA) of autotrophic ammonia oxidizing bacteria (AOB) was detected by PCR. Quantitative PCR revealed that 10⁹ gene copies/g-soil of amoA of archaea (AOA) was present in soil of DHS. These results suggest that AOA population, predominating over AOB population, might be responsible for nitrification in N-saturated forest soil of DHS. Mean NO₃⁻ residence time of DHS soils (13 to 92 d) was much longer than that of HSD soil (6 d). This result implies the growing risks of accumulation and leaching of NO₃⁻ in the forest soils with continuous N deposition. This must be taken into consideration in managing the forest ecosystems.

Abstract Category
10 Forest Soil Ecology

PS.10.012 RESPONSE OF SOIL MICROBIAL COMMUNITIES DURING CHANGES IN LAND MANAGEMENT

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The response of soil microbial communities to restoration following disturbances is poorly understood. We studied the soil microbial communities in a forest disturbance-restoration series comprising a native deciduous forest (DF), conventionally tilled cropland (CT) and mid-succession forest (SF) restored in 1964 at the W. K. Kellogg Biological Station, Michigan. In addition, the effect of vegetation and historical management was also investigated. Within the disturbance-restoration series, the total microbial phospholipid fatty acids (PLFA) and soil microbial biomass C decreased with disturbance from DF→CT and then increased upon restoration from CT→SF. While there was no change in the bacterial diversity, the microbial communities were significantly different. 16S rRNA

gene based taxa-specific comparisons revealed significant changes in the composition of four of the eight major phyla. In addition, four phyla also showed significant changes in the relative abundance. The association of some of the most abundant operational taxonomic units with specific soils further illustrated the changes in community structure. With different vegetation, the conifer forest (CF) soils had significantly different community composition and lower pH, C and N content, soil microbial biomass, PLFA and 16S rRNA gene diversity than DF soils. Comparison of mowed grassland (MG), which was derived from forest, and an annually burned early-succession (ES) grassland, which was derived from cropland in 1989, revealed significant differences in the composition of microbial communities, soil C, and microbial biomass. This suggests that long-term factors such as management history are more important determinants of the current bacterial community than the recent impacts of land use. In conclusion, disturbance, vegetation and historical management play significant roles in shaping the composition of soil microbial communities.

Abstract Category
10 Forest Soil Ecology

**PS.10.013 SOIL CHEMICAL ENVIRONMENT AND VEGETATION COMPOSITION
CORRESPOND WITH THE STRUCTURE OF ACTINOBACTERIAL COMMUNITIES**

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Community characteristics of soil actinobacteria were investigated together with their relationship to a range of plant- and microbial-derived metabolites. Two pine forests with contrasting pH and two meadows differing in soil chemistry and vegetation richness were studied. Bacterial community structure and diversity of actinobacteria was assessed by terminal restriction fragment length polymorphism analysis and cloning-sequencing of total bacterial and actinomycetes specific 16S rRNA genes. Quantitative PCR was employed in determining proportions of bacteria, fungi and actinobacteria at the sites. HPLC analysis of soil extracts was used to compare the composition of low molecular weight organic compounds. The communities of bacteria and actinobacteria and composition of the erm resistance genes were distinct at the two forest soils but similar in meadow soils. Differences in 16S rRNA gene based community structure were associated with differences in soil pH, while the erm gene diversity did not correspond to any environmental factor but to the percentage of antibiotic producers at the site. Low molecular weight organic compounds were specifically influenced by site pH, bedrock and vegetation. The structural composition and abundance of actinobacteria in soils corresponded strongly to the chemical environment at a site approving a significant role of this group in decomposition processes.

Abstract Category
10 Forest Soil Ecology

**PS.10.014 THE DOMINANCE OF ACIDOBACTERIA IN BRAZILIAN SAVANNA-LIKE
VEGETATION SOILS**

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The Brazilian Cerrado is a savanna-like region composed by different phyto physiognomies. The aim of the present work is to study the bacterial communities from Cerrado soil associated with different types of vegetation through molecular techniques. Composite soil samples were collected from the Cerrado phyto physiognomies and total DNA of the microbial community present in the soil samples was extracted. The intergenic space region was analyzed through Ribosomal RNA (rRNA) intergenic spacer analysis (RISA). The profile of each RISA profile revealed differences in band distribution reflecting a distinct composition in microbial communities. However, there are also several bands in the same position, indicating that there are many bacterial species in common between the areas of the Cerrado. Next, approximately 2000 clones of bacterial libraries were analyzed using the Ribosomal Database Project and were clustered into operational taxonomic units using the DOTUR

program. The phylum Acidobacteria was dominant in all the communities studied, corresponding to more than 50% of the sequences. Gp1 is the predominant genus of Acidobacteria among the Cerrado areas. Proteobacteria were also abundant in all areas studied. The rarefaction curves showed that there is little difference in bacterial diversity of the Cerrado soil between areas. However, the survey presented here of the Cerrado soil bacterial community using molecular techniques represents a significant improvement over the previous samplings efforts. Our ongoing goal is to have a representative description of the microbial community associated with the Cerrado soils to better understand its function and its adaptation to different stresses in the hope of, not only preserving it, but also to explore its incognita microbial biotechnological potential in the future. We also will present the first pyrosequencing survey on Cerrado soil's bacteria community composition. Financial sponsor: FAPDF, FAPERJ and CNPq.

Abstract Category
10 Forest Soil Ecology

PS.10.015 MOLECULAR CHARACTERIZATION OF THE INDIGENOUS MICROBIAL COMMUNITIES LIVING NATURALLY INTO THE RHIZOSPHERE OF ACACIA SENEGAL TREES IN KENYA AND NIGER

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Acacia senegal is a species of major importance for reforestation in arid African zones. Among other benefits, A senegal produces a multipurpose gum arabic, a commodity traded both locally and internationally. Moreover, soil fertility improvements have been realized with its use through its capacity to fix atmospheric nitrogen in symbiosis with rhizobium. Rhizobial inoculation of mature trees also stimulates gum production. This positive effect of rhizobial inoculation on the gum arabic production may be related to N mineralization in the soil, suggesting that microsymbionts and heterotrophic microbial communities involved in the N cycle interact in the rhizosphere of trees. The aim of our study was to assess the diversity of the total microbial communities naturally present in the rhizosphere of Acacia tree species in different Agro-ecological zones in Niger and Kenya for a better understanding of the relationships between both communities and their manipulation which could be a way for a sustainable management of gum arabic production through improvement of soil fertility. Total microbial DNA from the soil samples was subjected to PCR-DGGE technique. The diversity of microbial populations was assessed using Shannon's index (H) which incorporates community richness and evenness (the relative distribution of species). In both Niger and Kenya, our study clearly shows that the Acacia species have no significant effects on the diversity of total soil microbial communities among the sites (except for 2 sites in Kenya). On the other hand, we have noticed that both bacterial and fungal diversity within A. senegal trees specifically vary according to the sites in both countries. The next step will be to run PCR-DGGE with specific primers targeting functional genes such as *nifH*, *amoA* and *nirK* linked with the nitrogen cycle to identify some specific communities which may explain the positive impact of the rhizobial inoculation on the gum arabic yield by A. senegal trees.

Abstract Category
10 Forest Soil Ecology

PS.10.016 UNDERSTANDING THE EFFECTS OF DEFORESTATION ON THE DIVERSITY OF NITROGEN FIXING POPULATIONS IN THE AMAZON FOREST

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The Amazon Rainforest is responsible for absorbing nearly 2 billion metric tons of atmospheric CO₂ yearly. For this important function to be performed, a balanced input of nitrogen through biological nitrogen fixation is required. In order to gain insights into the significant role of nitrogen in the Amazon Rainforest ecosystem, we isolated nitrogen fixing bacteria from soil samples collected from a primary forest, a pasture converted from primary forest 5 years previously, and a secondary forest established following pasture abandonment. Two isolation methods (semi solid agar medium and solid medium under 2% oxygen concentration) were compared. Isolation medium for both methods contained a sole carbon source composed of glucose, acetate, pyruvate, or malate. A total number of 850 isolates, at least 250 from each of the three sites were obtained. The newly devised solid medium method increased the number of putative nitrogen fixers by 37%. The preliminary results of 16S rRNA and *nifH* (dinitrogen reductase) genes sequence analyses of these isolates suggests the differences in the distribution of microorganisms for each of the site with only few populations being ubiquitous. Culture independent analysis of the nitrogen fixing population diversity among the different treatments is currently underway and will be compared to the isolation procedures.

Abstract Category
10 Forest Soil Ecology

PS.10.017 LAND USE ALTERS THE STRUCTURE OF METHANE-OXIDIZING COMMUNITIES IN SOILS OF WESTERN AMAZONIA

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Methane (CH₄) is a greenhouse gas, and its atmospheric concentration has been increasing due to anthropogenic activities such as fossil fuel usage, rice production, large-scale husbandry of ruminants, biomass burning, and landfills. In particular, in Amazonia, the conversion to agriculture has resulted in the loss of over 17% of original rainforest. In the present study we compared the methane-oxidizing bacterial community (MOB) of western Amazon soils (Rondonia, Brazil) under different land uses, including a pristine forest, a secondary forest site, and a pasture. Soil samples were collected in different land use systems and MOB community composition and diversity were investigated by both PCR-DGGE and by clone libraries of *pmoA* gene fragments (coding for a subunit of the particular methane monooxygenase) and of the MOB 16S rRNA gene. Cluster analysis based on the PCR-DGGE banding patterns indicated that the MOB communities of the pristine and secondary forests were similar to each other but different from that for the pasture site despite all sites being adjacent to each other. Additionally, members of the MOB-I cluster within the *Gammaproteobacteria* were dominant in all soils, while members of cluster MOB-II in the *Alphaproteobacteria* were detected only in the pasture soils. Our results showed that land use change altered the community structure of methane-oxidizing bacteria. Such alteration could change the methane oxidation potential of pasture and secondary forest soil.

Abstract Category
10 Forest Soil Ecology

PS.10.018 CHARACTERIZING THE AEROBIC AMMONIA OXIDIZING COMMUNITIES IN AMAZONIAN SOILS UNDER DIFFERENT LAND USES

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The soil of the Amazon rainforest is one of the least studied habitats microbiologically. Central to soil microbiology and to overall forest functioning is an understanding of which microbes contribute to the nitrogen cycle. Of special concern is the generation of inorganic nitrogen for microbial and plant metabolism. Also, understanding how such nitrogen cycling communities are altered, as Amazon rainforest undergoes conversion to pasture and secondary forest, allows insight into recovery processes of microbial communities encountering stress. Thus, the goal of this study was to characterize the structure and diversity of microbial communities involved in aerobic ammonia oxidation in pristine forest, pasture, and secondary forest. Clone libraries of the gene encoding the A subunit of ammonia monooxygenase were generated from pristine forest, pasture, and secondary forest soil samples from Rondônia, Brazil, for both crenarchaea and bacteria. In all three sites similar taxa involved in aerobic ammonia oxidation, including *Nitrosomonas* species and members of the crenarchaeal Group 1.1b, were found. The community diversity of crenarchaeal aerobic ammonia oxidizers decreased when pristine forest was compared to pasture. This diversity increased when pasture was compared to secondary forest. The diversity of the bacterial aerobic ammonia oxidizer community followed an opposite trend, increasing when pristine forest was compared to pasture but decreasing when pasture was compared to secondary forest. To complement this work, data from quantitative PCR experiments will be provided which quantify the functional potential of crenarchaeal and bacterial aerobic ammonia oxidation in the three soil types tested. These changes in aerobic ammonia oxidizing communities, in soil undergoing land use change, could lead to differences in nitrification kinetics. Also, similar community changes potentially could be seen in other tropical regions undergoing comparable land use change.

Abstract Category
10 Forest Soil Ecology

PS.10.019 MICROBIAL DIVERSITY FROM A NORTHEAST BRAZIL MANGROVE SOIL BY PYROSEQUENCING

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Mangrove ecosystems support diversified soil micro and macro organisms. All these soil organisms, especially the microbiota, play a very active role in the detritus food web of coastal areas. Despite the importance of the microbiota for mangrove structure and function, studies on microbial diversity in Brazilian mangroves are scarce. Thus it is extremely important to apply new molecular techniques that allow the access to the indigenous microorganisms seeking to contribute to biodiversity conservation programs. This study presents the use of high throughput DNA pyrosequencing to assess the soil bacterial diversity from a Northeast Brazil mangrove. Soil samples were collected in areas near to the river and inside the rhizospheres of *Rhizophora mangle* and *Avicennia shaueriana*. DNA was extracted from composited samples with a commercial kit and used to construct a metagenomic library that was subjected to pyrosequencing. The sequences were taxonomically analyzed using the software MEGAN 3.8. A total of 122,406 reads with average size of 509 bp were recovered. From the 10,508 classified reads, the most abundant bacterial group among the 13 found taxa was *Proteobacteria* (78.4%) with higher abundance of the classes *Alphaproteobacteria*, *Gamaproteobacteria* and *Deltaproteobacteria*. Less abundant sequences were assigned to *Actinobacteria* (7.8%), *Bacteroidetes* (1.34%) and *Acidobacteria* (1.09%). *Planctomycetes*, *Firmicutes*, *Deinococcus/Thermus*, *Chloroflexi* and *Cyanobacteria* groups were less than 1%. 1,770 sequences could not be classified in any taxa and 109,000 did not show any BLASTn hits. The present study has significantly expanded our knowledge on the bacterial diversity in Brazilian mangroves and can be used as a starting point for further phylogenetic studies and functional analyses. The great number of unknown reads reinforces the urgency to study this untapped microbial biodiversity for Brazilian mangroves conservation purposes.

Abstract Category
10 Forest Soil Ecology

PS.10.020 METAGENOMIC APPROACH FOR BIODIVERSITY ACCESS FROM BRAZILIAN SOIL COMMUNITIES UNDER SUGARCANE CROP

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BIOTA is a Brazilian scientific program which main purpose is to rescue and study São Paulo state biodiversity. Soil biodiversity has important implications regarding agronomical applications, biogeochemical cycles, greenhouse gas emissions, global climate changes, and biotechnological prospectation. Concerning that, metagenome is a promising way to acquire microorganism diversity information facing that only 1% of all life cells present in soils could be isolated and cultivated in vitro. Thus, we have chosen metagenomic approach to be applied in order to take 16S rRNA representatives from two soil systems in São Paulo state: 1) Native forest, and 2) Sugarcane crop. 16S rDNA fragments were cloned in pGEM T-easy system vector, allowing the DNA sequencing and analysis of bacterial and archaeal communities. FASTA sequences obtained were analyzed by comparison with GenBank from National Center for Biotechnology Information, and phylogenetics analysis were performed using Bionumerics (6.0.1 - Applied Maths) based on aligned sequences. Data revealed that in forest soil communities were rich in *Acidobacterium*, *Verrucomicrobium*, *Proteobacterium*, *Firmicutes*, *Actinobacterium*, while in sugarcane crop soil were found *Firmicutes* and *Proteobacterium*. apoio FAPESP.

Abstract Category

10 Forest Soil Ecology

PS.10.021 SAPROTROPHIC POTENTIAL OF ECTOMYCORRHIZAL FUNGI IN SPRUCE FOREST ECOSYSTEMS

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Ectomycorrhizal (EcM) fungi are common denizens of coniferous forests. There is an ongoing discussion as to whether EcM contribute directly to soil organic matter (SOM) turnover in these ecosystems. To address this question, we compared the saprotrophic potential of EcM mycelia to that of community (EcM and other) fungal mycelia. EcM extramatrical hyphae were captured using in-growth mesh bags. These mesh bags (50µm) were filled with fermentation-humus (FH) substrate, surrounded by sand to deter saprotrophs (-ST), and buried in the FH layer of a spruce plantation. Replicate bags with no sand barrier were also buried (+ST). All mesh bags and adjacent FH layers were sampled after one growing season. The enzymatic activity of the FH layer, dominant EcM tips from that layer, and both -ST and +ST substrates were assessed using fluorogenic or colourimetric substrates. Assessed enzymes included those involved in the decomposition of cellulose (β-glucosidase), hemicellulose (xylosidase), lignin (laccase), chitin (β -N-acetylglucosaminidase), and those that release N (aminopeptidase), P (phosphomonoesterase), and S (sulfatase). Fungal community structure was assessed using tag-encoded 454 pyrosequencing of the fungal ITS1 region. Enzymatic activities did not significantly differ among the three substrates. Correlations were observed between the EcM tips and the different substrates for some enzymatic activities. A high correlation ($r=0.805, p<0.01$) between β-glucosidase activity of EcM tips and the FH layer was influenced by activities of medium-distance explorer-type (MDET) *Piloderma* and *Amphinema* spp. This relationship between MDET and substrate activity was present but progressively weaker in the +ST and -ST substrates ($r>0.640, p<0.10$). The substantial enzymatic activities found in -ST substrates indicate that EcM extramatrical hyphae may be important contributors to SOM turnover in forested ecosystems.

Abstract Category

10 Forest Soil Ecology

PS.10.022 BIOGEOCHEMICAL RESPONSES TO POSTFIRE SALVAGE LOGGING IN A MIXED-CONIFER FOREST SOIL IN CENTRAL OREGON

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Soil biogeochemical responses to compaction, subsoiling, and time since disturbances were investigated 1-3 years after postfire logging in a mixed conifer forest in central Oregon. Treatments included burning with no further disturbance, compaction from heavy equipment, and compaction followed by subsoiling in a randomized block design. We hypothesized that treatments would effect soil biological, physical, and chemical properties. Bulk density, plant-available phosphorus, and phosphatase rates were lowest in the subsoiled treatment, and respiration, incubation nitrogen, and net mineralizable nitrogen were highest in the burn only treatment. Fungal and bacterial communities, investigated with PCR-based molecular methods, revealed minimal or short-term differences among treatments. The mean number of fungal species, slightly lower in the subsoiled than in the compacted treatment, exhibited a short-term decline in all treatments. Bacterial response to carbon substrates did not differ significantly among treatments. Decreased plant-available nitrogen and phosphorus in the soil from mechanical disturbance may have long-lasting effects in an already nutrient-limited system, yet soil microbes, critical to mediating decomposition and nutrient cycling, appear tolerant of the occurrence of wildfire and resilient to disturbance. The results of this study, along with the recovery potential of a site, and the impending risk of future fire in stands differing in structure from historic conditions, bear consideration when considering postfire salvage logging.

Abstract Category

10 Forest Soil Ecology

PS.10.023 IMPACT OF AMMONIA DEPOSITION ON THE ACTIVITY AND COMPOSITION OF AMMONIA- AND METHANE-OXIDISING COMMUNITIES IN FOREST SOILS

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Nitrification is important in global N cycling, oxidising NH_4^+ to NO_2^- and NO_3^- . Ammonia oxidation by ammonia-oxidising bacteria (AOB) and ammonia-oxidising archaea (AOA) is catalysed by the enzyme ammonia monooxygenase (AMO), encoded by the *amoA* gene. AMO of AOB is evolutionarily related to pMMO in methane-oxidising bacteria (MOB). Thetford Forest, UK, consists of Pine (*Pinus nigra*) and Beech (*Fagus sylvatica*) species and has a gradient of NO_3^- and NH_4^+ across a 300 m transect, due to the proximity of pig/chicken farms. Using a seasonal sampling strategy, we investigated effects of increased NH_4^+ deposition on AOB, AOA and MOB community structure and activity in mineral and organic soil across this transect. Beech mineral soil had a greater nitrification potential rate (NPR) at 20 m compared to 300 m. Beech organic soil had greater NPR at 20 m compared to 300 m in January and June only. NPR in Pine organic soil was greater at 300 m compared to 20 m, whilst for Pine mineral soils, rates across the transect were similar. Methane oxidation potential (MOP) measurements revealed no methane oxidation was occurring within any soils during the year, with the exception of Pine and Beech mineral and organic soil at 300 m and Beech organic soil at 20 m sampled in October. PCR amplification of the 16S rRNA and *amoA* genes for AOB and *amoA* genes for AOA was performed. AOB *amoA* genes were detected in most of the organic soils throughout the year. Within the mineral soils, *amoA* genes were detected predominantly in January and April. Based on 16S rRNA genes, changes in AOB community diversity were also observed within the forest soils, with greater diversity in January. These results show that methane oxidation potential of the soils throughout the forest sites is limited. Ammonia deposition may increase nitrification rates depending upon the dominant tree species, which has implications for forest management in mitigating the impacts of organic pollution in the future.

Abstract Category

10 Forest Soil Ecology

PS.10.024 TARGETED PCR-INDEPENDENT 454 PYROSEQUENCING SHOWS A HIGH ABUNDANCE OF ACIDOBACTERIA IN THE 16S rRNA POOL OF ARCTIC SOIL

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Acidobacteria is one of the frequently detected bacterial phyla in various soils. Here we compared three different 454 pyrosequencing approaches to study the bacterial diversity and the role of *Acidobacteria* in Arctic soil. The V1-V3 variable region of the 16S rRNA gene was sequenced 1) from PCR-amplified DNA, 2) from RT-PCR amplified RNA, and 3) without gene-specific PCR amplification directly from the reverse transcribed RNA. Soil samples were collected from acidic (pH_{H2O} 4.2-6.3) and neutral (pH_{H2O} 6.6-7.5) tundra fell soil cores and extracted nucleic acids were pooled before the analysis. In the results from pyrosequencing using 454 FLX chemistry, *Proteobacteria* was the most abundant phylum in the PCR-based analyses of DNA and RNA (methods 1 and 2). However, direct targeted PCR-independent RNA analysis (method 3) showed that *Actinobacteria*, *Acidobacteria* and *Proteobacteria* were all represented in roughly equal frequencies (31-25% of templates) in the rRNA pool in the acidic soils. In the neutral soils *Acidobacteria* were relatively less abundant (16%), but still formed an important part of the rRNA pool. The proportion of sequences with no match to Ribosomal Database Project (RDP) sequences was slightly higher in the targeted PCR-independent approach (17%) than in the PCR-based approaches (10%). Comparison of these results shows that targeted PCR-independent template preparation is an efficient and economical approach for pyrosequencing analysis of the physiologically active bacteria, while PCR amplification with gene-specific primers may introduce some bias, depending on the design of the primer pair used for the amplification.

Abstract Category
10 Forest Soil Ecology

PS.10.025 TREE SPECIES-MEDIATED SPATIAL PATCHINESS OF THE SOIL PHYSICOCHEMICAL AND MICROBIAL PROPERTIES IN THE TOP SOILS OF A TROPICAL MONTANE FOREST

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Feedbacks between plants and soil are a potential driver of plant community dynamics and ecosystem processes such as plant successions and nutrient cycling. To evaluate the importance of plant-soil feedbacks in forest ecosystems, it is fundamental to understand the spatial range within which plant species controls soil physicochemical and microbial properties.

We investigated the spatial pattern of soil properties associated with canopy trees in a tropical montane forest on Mt. Kinabalu, Borneo. We analyzed soil physicochemical properties (soil pH, condensed tannins, total phenolics, organic carbon content, and total nitrogen content), microbial community composition (a profile of biomarker lipid abundance), and extracellular enzyme activity (carbon- and phosphorus-degrading enzymes) as a function of soil depth and distance from the tree trunk of a conifer (*Dacrydium gracilis*) or a broadleaf tree (*Lithocarpus clementianus*). The concentration of condensed tannins in *Dacrydium* leaves is significantly higher than that in *Lithocarpus* leaves, which can be expected to make differences in the soil physicochemical and microbial properties among soils beneath these two tree species.

The concentration of condensed tannins and fungi-to-bacteria ratio were higher beneath *Dacrydium* than beneath *Lithocarpus*. Furthermore, carbon-degrading enzyme activities were lower beneath *Dacrydium*. These effects of the tree species were more distinct on soil properties beneath the tree crown than on those outside the tree crown. These effects appeared to be largely due to differences of litter chemistry, and a distinct spatial range of a unique set of the soil properties was formed corresponding to the above canopy crown.

In conclusion, the species-rich forest on the tropical mountain contains spatially distinct units of soil properties associated with canopy trees, and the spatial pattern can influence ecosystem dynamics in the forest through the effects of plant-soil feedbacks

Abstract Category
10 Forest Soil Ecology

PS.10.026 STRUCTURE OF FUNGAL AND ACTINOBACTERIAL COMMUNITIES IN THE SOIL DOMINATED BY *TRICHOLOMA MATSUTAKE*

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Tricholoma matsutake is an ectomycorrhizal fungus and produces commercially valuable mushrooms. Mycorrhizal seedlings have been infected successfully *in vitro*, however, shiro formation has not been observed *in situ* after outplanting the seedlings. We are interested whether microbial 'helpers' are needed to be associated with *T. matsutake* mycelium to induce shiro formation. This study is to provide information on dynamics of shiro formation of *T. matsutake in situ* by analyzing microbial community structure associated to shiro.

We used PCR-DGGE fingerprinting and direct sequencing to analyze the communities of fungi and actinobacteria from both the mineral and organic layers in matsutake shiro (shiro) and non-matsutake spots (control, defined as matsutake specific negative PCR, one meter aside from shiro) in two podzolized, matsutake producing forest sites in Finland.

In the organic soil layer just above the shiro, there were no significant differences in fungal and actinobacterial richness and diversity of DGGE bands between control and shiro. In the mineral soil layer, the respective richness and diversity of DGGE bands were significantly decreased in shiro comparing to control. However, certain actinobacteria species were more abundant in the shiro than control. These were *Streptacidiphilus sp.* and *Thremomonsporaceae* in the mineral soil and *Spirillospora sp.* and *Actinospica sp.* in the organic layer. In fungal community, we found that *Mortierella sp.* and *Cantharellales* were more abundant in the shiro mineral soil layer, and *Piloderma sp.* *Trichoderma sp.*, *Pichia sp.*, *Cantharellales* were more abundant in the respective organic soil layer compared with the controls from the both investigated forest sites.

Overall, our results suggest that *T. matsutake* influences the fungal and actinobacterial community structures in the shiro soil. Whether these community members directly or indirectly contribute on shiro and fruitbody formation needs to be further investigated.

Abstract Category
10 Forest Soil Ecology

PS.10.027 SUCCESSION OF FUNGAL COMMUNITIES AND CELLOBIOHYDROLASE GENES ON DECAYING HARDWOOD FOREST LITTER USING PARALLEL TAG-ENCODED 454 SEQUENCING

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Due to their production of diverse extracellular enzymes, fungi are important decomposers of plant litter in forest ecosystems composed of cellulose, hemicelluloses and lignin as the major carbon sources. In the course of litter decomposition, the quality of litter gradually changes in consequence of microbial activity and simultaneous changes of the microbial community composition is likely to occur. The aim of this study was to use 454-pyrosequencing and qPCR to characterize the structural changes of fungal community and the pool of genes encoding for exocellulases (cellobiohydrolases). Litterbags with *Quercus petraea* litter were incubated for 24 month *in situ*. To evaluate the persistence of foliar endophytes in the community, oak leaves collected two months before abscission and during litterfall were also analysed. The activity of microorganisms was studied by the measurement of enzyme activities. Cellobiohydrolase activity decreased during litter decomposition. The results show increasing diversity of fungal communities and cellobiohydrolase genes - the Schao estimator of fungal diversity increased from 550 in the beginning of decomposition to 2450 after 24 months. Ascomycetes dominated in the first stages of litter degradation, but their abundance decreased during decomposition to be replaced by basidiomycetes. Fast changes of fungal community composition and cellobiohydrolase gene pool

were detected with a minor share of community members surviving in the litter for longer time. Several foliar endophytes producing cellobiohydrolase were among the species surviving long in litter which shows the importance of their early arrival. The comparison of diversity of cellobiohydrolase genes and fungal community shows, that a relatively minor part of the fungal community is likely involved in degradation of cellulose, the major polysaccharide of oak litter.

Abstract Category
10 Forest Soil Ecology

PS.10.028 GROWTH OR MORTALITY INDEXES FOR MICROORGANISMS IN SOIL BASED ON STABLE ISOTOPE PROBING WITH H₂¹⁸O.

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The rate at which microbial populations grow or die is central to the study of environmental microbiology yet little progress has been made to quantify microbial population dynamics in the environment. We combined quantitative realtime PCR analysis with stable isotope probing with heavy ¹⁸O-water to calculate a growth and mortality index for bacteria and fungi in a ponderosa pine forest soil. DNA extracted from soil incubated with ¹⁸O-water for 6 days was separated into two distinct DNA bands along a cesium chloride gradient after isopycnic centrifugation. The indexes were calculated by comparing the abundance of bacterial 16S rRNA or fungal ribosomal intergenic sequences among soil DNA extracts before and after incubating soil with ¹⁸O-water. Bacteria had a growth index of 1.86 while fungi had a growth index of 6.76 suggesting bacteria grew slightly faster and fungi much faster than the rest of the soil community. The bacterial mortality index was 0.50 and the fungal mortality index was 0.06 indicating both bacteria and fungi died at slower rates than the rest of the soil community. The indexes are impacted by inactive DNA, such as DNA present in plant cells, dead microorganisms, or DNA sorbed to soil constituents, that can not replicate regardless of environmental conditions. A model that describes the impact of this inactive DNA on the indexes indicated the growth index would be overestimated while the mortality index would be underestimated. By comparing the growth indexes of bacteria and fungi the impact of inactive DNA on the indexes can be eliminated. This comparison showed that fungi grew 3.71 times faster and died 9.07 times slower than bacteria after the soil was rewet. This provides evidence that fungi compete well against bacteria in a wet soil. We propose a standard set of equations and notations that can be used to compare growth or mortality rates of any microbial population in soil across different stable isotope probing experiments.

Abstract Category
10 Forest Soil Ecology

PS.10.029 ECTOMYCORRHIZAL ROOT TIP ENZYME ACTIVITIES AND MICROSITE EFFECTS IN CLEARCUT AND FOREST PLOTS AT A HIGH ELEVATION SPRUCE FOREST

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The secretion of hydrolytic and oxidative enzymes by ectomycorrhizal fungi contributes to nutrient cycling in northern forests; however, little is known about how this varies with soil microsite. If decayed wood provides habitat for fungi with unique capabilities for degrading organic matter, retention of downed wood after clearcut logging may encourage physiological diversity within the ectomycorrhizal fungal community of regenerating stands. Ectomycorrhizal communities were investigated in clearcuts and undisturbed stands at a high elevation forest. Inside each of three 10-ha clearcut blocks two 1-ha treatment plots were established, with downed wood generated during harvesting either retained or removed. One-ha plots were also established in the forest adjacent to each cutblock. Our objective was to determine if there were differences in root tip physiological activity between (i) seedlings in retention, removal, and forest plots, or (ii) seedlings in microsites of decayed wood, mineral soil or soil adjacent to downed logs. Individual root tips were assayed for eight extracellular enzymes: acid phosphatase, β -glucosidase, glucuronidase, leucine aminopeptidase, xylosidase, cellobiohydrolase, chitinase and laccase. Significant plot-level differences were detected for five enzymes: activity at forest plots was higher than that at retention plots (cellobiohydrolase $p=0.0380$), removal plots (acid phosphatase $p=0.0300$), or both

(glucuronidase $p=0.0250$ and laccase $p=0.0030$), while activity at forest plots was lower than that at removal plots for N-acetylglucosaminidase ($p=0.0280$). No significant microsite effects were detected overall, but significant differences were detected in glucuronidase activity at one forest plot, and in acid phosphatase, leucine aminopeptidase, and laccase activity at one removal plot. Variations in soil moisture, organic matter content, and fungal community structure may explain the differences between the clearcut and forest plots.

Abstract Category
10 Forest Soil Ecology

PS.10.030 ALUMINUM AND IRON-BEARING MINERALS IN SOIL MATRIX EFFECT MICROBIAL COMMUNITY COMPOSITION DURING DECOMPOSITION OF PONDEROSA PINE LITTER

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Aluminum (Al) and Iron (Fe) are the third and fourth most abundant elements, respectively, in soils and interact with microbes during decomposition through the combined processes of mineral dissolution, adsorption, and complexation of nutrients. Fe is essential to metabolism whereas Al is toxic as a soluble, trivalent cation. While abundant in the soil matrix, their effects on microbial community structure have not been explored. We developed three simple mineral media using sand-size quartz as a control, goethite, a common Fe oxide or gibbsite, an Al oxide. These minerals were mixed with Ponderosa Pine forest litter and inoculated with a microbial extract derived from the same litter. Changes in bacterial and fungal communities were tracked over the 154-day incubation by pyrosequencing fragments of the bacterial 16S and fungal 18S rRNA genes. Respiration rates were significantly lower for the gibbsite treatment. Ordination of bacterial communities using nonmetric multidimensional scaling (NMS) indicated that community composition was dynamic over time. Within each time point communities clustered by mineral treatment. Available Al, Fe, and PO_4^- were the best predictors for the gibbsite, goethite, and quartz-only communities, respectively. Individual analysis of pyrosequences revealed that mineral type was important for some populations but not all. For example, *Acidobacteria*, the most abundant group, did not change in response to mineral type or time. However, individual species such as *Terrimonas ferruginea* competed better in presence of goethite and species such as *Aquabacterium* in the presence of gibbsite. These findings suggest that the presence of Al or Fe oxides, such as goethite or gibbsite, effect small but significant changes in microbial community composition.

Abstract Category
10 Forest Soil Ecology

PS.10.031 IMPACT OF FOREST FERTILIZATION ON SOIL NITRIFIER ACTIVITY, ABUNDANCE AND COMMUNITY STRUCTURE

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Due to the massive outbreak of the Mountain Pine Beetle (*Dendroctonus ponderosae*) occurring in British Columbia (Canada), widespread application of fertilizers to forest soils is proposed as a strategy to accelerate tree growth and shorten rotation times, in order to overcome future projected timber shortfalls. However, the addition of high levels of nitrogen fertilizers to forests can disrupt belowground microbial communities and especially nitrifiers. The objective of our study was to determine how forest fertilization affects nitrification and the abundance and diversity of nitrifying communities. Soil samples were collected in control plots (unfertilized) and fertilized plots (annually and every six years since 1994) from three interior spruce sites and two lodgepole pine sites in northern British Columbia. Potential nitrification was quantified in these soils by a colorimetric method. Contributions of autotrophic and heterotrophic microorganisms to nitrification were compared by acetylene inhibition tests. The abundance and community structure of ammonia-oxidizing bacteria (AOB) and archaea (AOA) and *Nitrobacter* like-nitrite oxidizing bacteria (Nb-like NOB) were determined by real-time PCR and denaturing gradient gel electrophoresis (DGGE). In

some sites, nitrification levels were higher in fertilized plots as compared to control plots. In plots exhibiting high nitrification levels, autotrophic nitrification dominated. AOB and Nb-NOB communities were globally more abundant under the fertilization treatments. However AOA abundance did not vary between control and fertilized treatments. Fertilization affected AOB community structure at all sites and Nb-NOB at three sites. By contrast, AOA community composition was affected by fertilization only at one site. The results indicate that the AOA community in these forest sites respond differently than AOB and Nb-NOB communities to fertilization.

Abstract Category
10 Forest Soil Ecology

PS.10.032 HOW LEAF LITTER FROM TRANSGENIC (HIGH-TANNIN) POPLARS (*POPULUS TREMULOIDES*) AFFECTS SOIL MICROBE COMMUNITIES IN MICROCOSMS

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Researchers are producing transgenic trees with desirable qualities such as pest resistance, improved growth, wood quality, etc. Although transgenic trees are not approved for release in Canada, there is still a need to study model systems to better define risks. One model involves transformed poplars containing elevated tannin levels; these tannins are thought to enhance pest resistance. In microcosms containing soil from poplar plantations, we compared the two-month impacts of leaf litter from high-tannin poplars, poplars transformed only with the β -glucuronidase (GUS) reporter gene, and parental (non-transgenic) poplars. Microcosms without litter were also evaluated. Molecular profiles of soil organisms were generated using PCR-DGGE; sequencing of 16S and 18S rDNA libraries isolated from microcosm soils was also performed. In the DGGE profiles, the parental litter produced the largest variation in bacterial diversity, followed by the GUS-only litter and the absence of litter. In the DGGE profiles, diversity of the detectable fungal community increased significantly with time, in all microcosms except those with transformed litter. Analysis of 16S and 18S sequences indicated that high-tannin litter promoted the growth of γ -proteobacteria and inhibited the growth of α -proteobacteria and arthropods. The transformation process also appeared to inhibit the growth of β -proteobacteria and photosynthetic eukaryotes in microcosms containing high-tannin litter or litter with the GUS reporter gene. This change was presumed to be the result of altered leaf composition. Mosses and other bryophytes proliferated in the absence of litter, and colonised soil surfaces to a lesser degree in microcosms with the parental litter or GUS-transformed litter. These organisms were visibly lacking in microcosms with the high-tannin litter. These results are a preliminary step in using more complex microcosms to understand the impacts of transgenic trees.

Abstract Category
10 Forest Soil Ecology

PS.10.033 EXPLORING THE RELATIONSHIPS BETWEEN MICROBIAL COMMUNITIES, PLANT COMMUNITIES, AND SOIL CHARACTERISTICS IN CHINESE SUBTROPICAL FORESTS

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The relationship between microbial communities, plant communities, and soil features is an important key to understand ecosystem function. BEF China (Biodiversity and Ecosystem Functioning) is a large collaborative project on forest diversity in subtropical China. A main function is to understand and avoid soil erosion through diverse plant community establishment. Soil samples were taken from different depths of four replicate comparative study plots representing three forest age classes. Microbial biomass structure and function were determined using Substrate Induced Respiration (SIR) and Phospholipid Fatty Acid analysis (PLFA). The parameters related to soil features and plant communities were determined by other subprojects. Multivariate statistics were used to compare microbial communities with plant communities and soil parameters.

Results suggested soil depth, and corresponding soil characteristics to be major determinants of microbial communities. Altitude and forest age of the plots had no impact. Nonmetric multidimensional scaling (NMS) analysis showed correspondence analyses (CA) of herb and tree species identity were significantly related to microbial communities. Specifically, results showed that tree cover (%), herbal functional group biomass, herbal species identity scores (CAI), and tree species identity scores (CAII) had significant relationships with microbial communities. Woody plants biomass (height < 1m) was also significant ($r^2=0.7043$) and positively correlated with NMS axis 2. Redundancy analysis showed that microbial community structure corresponded to the interaction of shrub layer by herb layer cover. Specifically, when shrub cover was between 10-20%, AM fungal biomass increased with increased herb cover. In summary, this study shows that, through site-specific soil characteristics, there is a correlation between subtropical plant and microbial communities, suggesting a strong effect of plant cover on the soil microbial communities.

Abstract Category
10 Forest Soil Ecology

PS.11.001 RARE AND NOVEL ACTINOMYCETES ISOLATED FROM MANGROVE RHIZOSPHERE SOIL OF BANGLADESH

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The isolation of slow growing rare and novel actinomycetes have become an important part in Biotechnological industry. Therefore, our main purposes are to gain preliminary insight into the generic diversity of rare actinomycetes in the mangrove rhizosphere soil of Bangladesh.

Isolation of actinomycetes in rhizosphere soils were determined by simple dilution and an agar plate technique. Polyphasic taxonomic studies were conducted using several standard methods as described by Ara et al. (2006; 2007; 2008).

Total count and distribution of actinomycetes was varied in each soil samples. In this study, Micromonospora (37.7%) was the dominant genus in almost all the tested soil samples including other genera Actinomadura (17.2%); Microbispora (8.6%); Nocardiopsis (3.7%); Catellatospora (3.7%); Streptosporangium (3.3%); Nonomurea (2.4%); Sphaerisporangium (2.4%); Rhodococcus or Gordonia (1.6%); Nocardia (1.6%); Saccharomonospora (1.2%); Nonomurea or Actinomadura (1.2%); Luedemannella (1.2%); Virgisporangium (0.8%); Catellatospora or Virgisporangium (0.8%); Actinoplanes (0.8%); Longispora (0.4%); Krasilnikovia (0.4%); Pseudosporangium (0.4%) and unknown actinomycetes (10.6%) were observed. Further, based on the polyphasic taxonomic studies of 22 unknown isolates, 15 strains were proposed as new species which belonged to the genera Micromonospora, Catellatospora, Nonomurea, Actinomadura, Microbispora and 4 other novel genera in the family Micromonosporaceae and Streptosporangiaceae.

This study showed successful isolation of actinomycetes and also concluded that the mangrove rhizosphere soil of Bangladesh are an unexploited and good sources for the isolation of diverse and novel genera of rare actinomycetes.

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Abstract Category

11 Rare Microbes

PS.11.002 DISCOVERIES FROM AN IN-DEPTH SOIL METAGENOMIC ANALYSIS

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Soil probably possesses the largest microbial diversity per unit of volume on Earth and remains a black box for scientists, because of cultivation limits and its extensive biodiversity. In order to unlock this biodiversity, we have focused on one unique soil metagenome, which is from the internationally-recognized Park Grass soil (Rothamsted, UK) and have begun sequencing (7 full pyrosequence plates >3Gbp) different DNA extraction pools. However, few of the sequences overlap exists among the seven sequence datasets (>8 million of sequences of 400bp) and less than three percent of this soil metagenome was successfully assembled. Thus an assembly curve was created to estimate the effort to complete assembly the soil metagenome. In spite of the low metagenomic recovery in the dataset generated, the goal of this work was to establish functional and phylogenetic characteristics of this prairie soil at sufficient sequence depth to provide statistically relevant conclusions about a broad range of domains and to attempt to access the soil rare biosphere. Thus we studied the GC ratio distribution, the impact of soil prokaryote communities for carbon and nitrogen cycles, their adaptation capacities (mobilome), the antibiotic resistance genes naturally present, their potential to degrade natural and anthropogenic compounds, and the pathogen species present.

Abstract Category
11 Rare Microbes

PS.11.003 A NOVEL APPROACH USING DNAZYMES TO REMOVE SELECTED PCR TEMPLATES FROM COMPLEX MIXTURES GREATLY FACILITATES THE MOLECULAR DETECTION OF RARE ORGANISMS

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A central approach in microbial ecology is the PCR-amplification, cloning and sequencing of phylogenetic or functional marker genes from complex samples. Such clone libraries are often dominated by genes from abundant organisms, but rare species are underrepresented and their detection requires high sequencing efforts. We developed a novel approach to dramatically deplete selected targets in RNA or DNA extracted from microbial communities. When applied to dominant community members, our method greatly facilitates the detection of rare organisms by conventional cloning and sequencing. We used catalytic deoxyribonucleotides (DNAzymes) that cleave target RNA molecules with high specificity at a defined target site. The cleaved RNA is no template for reverse transcription-PCR and subsequent cDNA amplification. Those templates, which were not targeted and thus not cleaved by the DNAzyme, can be PCR-amplified and cloned with a higher efficiency. When starting from DNA, an initial in vitro transcription step generates the RNA needed for DNAzyme treatment. To demonstrate the use of this method, we identified heterotrophic contaminants in a high enrichment of the nitrite-oxidizing bacterium "*Candidatus Nitrospira defluvii*" by using genomic DNA or RNA as initial template. The contaminants (belonging to six bacterial phyla) represented in total only 13% of the enrichment culture. They were identified by sequencing less than 40 16S rRNA gene clones after treatment of the template RNA with a *Nitrospira* 16S rRNA-specific DNAzyme. The removal of the

Nitrospira 16S rRNA template was documented by cDNA fingerprinting. A key advantage of our approach is the high specificity that allows the simultaneous use of DNazymes cleaving different targets. New DNazymes can be designed from existing probes or primers. They may also target functional gene mRNA, for example to facilitate the detection of rare members of a defined functional group.

Abstract Category
11 Rare Microbes

PS.11.004 BETA DIVERSITY AND PHYLOGENETIC NOVELTY IN RARE VS ABUNDANT MEMBERS OF THE COMMUNITY IN A MESOPHILIC SULFIDIC SPRING

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Pyrosequencing-based diversity surveys allows the detection of rare members within microbial communities. In most studies, datasets reported originated either from DNA extraction and PCR amplification of a single sample, from single amplification of multiple pooled DNA extractions, or from pooled PCR products obtained from a single or multiple DNA extractions. As such, the spatial differences between members of the microbial communities in close proximity in a single ecosystem have rarely been addressed. Here, we examined the level of β -diversity between four adjacent samples (less than 1 mm apart) from a highly diverse ecosystem (Zodletone spring, OK). A total of 292,130 sequences encompassing V1+V2 region of the 16S molecule were obtained. While the 4 datasets had similar estimates of Shannon diversity (8.07 ± 0.12), species richness (Chao; 50541 ± 6255 , ACE; 91405 ± 14170), Simpson index for species evenness (0.003 ± 0.0005), and proportion of rare Vs abundant clones, the abundant members ($n > 10$) were more similar between datasets (Sorensen index = 0.84, % of shared OTUs between the 4 datasets = 36%) compared to the rare members of the community (Sorensen index = 0.13, 0.36, 0.48, 0.57 for $n=1$, $n \leq 2$, $n \leq 5$, and $n \leq 10$, respectively, and % of shared OTUs between the 4 datasets ranging from 0.1 to 4%). Sequences suggestive of the presence of 42 novel phyla were identified, mainly within the rare biosphere. PCR amplification using lineage-specific forward primers paired with universal reverse primers confirmed that some lineages indeed represent novel candidate phyla, while others represent novel lineages within previously described phyla.. These results highlight the level of β -diversity within the rare biosphere, and suggest that the concept of sequencing an entire highly diverse ecosystem (e.g. soil) requires further revision and clarification.

Abstract Category
11 Rare Microbes

PS.11.005 DEEPER UNDERSTANDING OF THE UNCLASSIFIED 16S rRNA READS USING PANGEA 2.0

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PANGEA (Pipeline for Analysis of Next Generation Amplicons) is a series of bioinformatics tools for high throughput 16S rRNA sequencing analysis (Giongo *et al.* 2010, ISME J.). Primarily, PANGEA was designed to study microbial community diversity. Sequences obtained directly from the sequencer are processed quickly to provide the files needed for sequence identification by BLAST and for comparison of microbial communities. PANGEA incorporates useful text manipulation tools that prepare files to be used in UniFrac and take special attention to the unclassified sequences. PANGEA is provided for users as individual scripts for each step in the process or as a single script where all processes are joined into one program called the 'backbone'. Here version 2.0 of PANGEA is described where two rounds of classification with TaxCollector databases are incorporated to extract the maximum of information about the unclassified reads. The usefulness of PANGEA 2.0 is demonstrated using a dataset of bacterial 16S rRNA sequences derived from Florida and Hawaiian soils. PANGEA 2.0 allows more precise clustering of unclassified reads by comparing all reads to two RDP 16S rRNA databases. One of these has 16S rRNA genes only from isolates while the other includes the entire RDP dataset. PANGEA 2.0 is particularly useful for those datasets derived from natural environments with many novel organisms.

Abstract Category

11 Rare Microbes

PS.11.006 DIFFERENTIATING AMONG THE DOMINANT AND RARE MICROBIAL GROUPS FROM SULFIDIC TERRESTRIAL SPRINGS

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The rare biosphere concept has been tested in a variety of habitats, but has not been evaluated fully from terrestrial habitats. If terrestrial aquatic microbial communities are similar to what has been uncovered from marine settings, then most of the microbial diversity in terrestrial springs will be represented by a long tail of low-abundance groups. We evaluated the rare biosphere concept using 16S rDNA pyrosequencing of microbial mats collected from sulfidic springs at Glenwood Hot Springs (GHS) and South Canyon Hot Springs (SCHS), Colorado, and PBS Spring, Wyoming. Operational taxonomic units (OTUs) containing <0.1% of all sequences per sample were considered rare in that community. Of the OTUs at GHS, 82% belonged to the ϵ -*proteobacteria*, but 80% of the rare OTUs were also affiliated with this class. Similarly, at PBS 11% of the OTUs were affiliated with the δ -proteobacterial genus *Desulfocapsa*, but 9% of rare OTUS were also affiliated with this group. OTUs from SCHS were dominated by sequences related to the γ -proteobacterial genus *Halothiobacillus* (18%), and 18% of rare OTUs was also affiliated with this genus. Similar to what has been observed from marine environments, the taxonomic composition of dominant groups from each of the terrestrial sulfidic springs had similar taxonomic representation among the rare OTUs. This diversity pattern indicates that there are two groups of rare OTUs within the terrestrial springs: 1) Authentic rare OTUs that are comprised of the genetic variants of the dominate microorganisms inhabiting the microbial mats; 2) Overlapping rare OTUs derived from the microbial communities that are not rare in

surrounding, but not sampled aquatic and terrestrial environments. Increasing the number of areas sampled will reveal some of these rare OTUs belong to dominate microbes in adjacent microbial communities and are not part of the truly rare biosphere.

Abstract Category
11 Rare Microbes

PS.11.007 ISOLATION AND CHARACTERIZATION OF IRON OXIDIZING BACTERIA FROM SALINE SLUDGE IN JAPAN

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The application of bioleaching for the removal of heavy metals from electroplating wastewater has been prevented by salt intolerance of the bacterial species used. The main aim of this work was to isolate halophilic iron-oxidizing autotrophic strains from Japanese electroplating wastewater sludge containing ferrous ions and a high concentration of chloride ions.

An iron-oxidizing bacteria, strain FO1, was obtained by using colloidal silica media containing 72 mM of FeSO₄•7H₂O. The physiological analyses, such as salt tolerance, optimum temperature, and pH, were conducted with liquid media. The whole-cell fatty acids of strain FO1 was analyzed by protocol of Sherlock Microbial Identification System. 16S rDNA sequence of strain FO1 was used to construct a neighbor-joining phylogenetic tree using the MEGA package. Strain FO1 was acidophilic autotroph capable of oxidizing ferrous ion under saline condition (up to 4% NaCl). The isolate was determined to be 1-2 μm long and 0.2-0.4 μm wide, which contained carboxysomes. The optimum growth temperature was at 30°C for 4 days incubation. The cellular fatty acids of strain FO1 was composed mainly of 18:0 (16.4%), cyclo-17:0 (10.2%), and iso-3-OH 13:0 (13.8%). The phylogenetic analysis of the 16S rRNA gene of the isolated strain from the sludge showed that *Thiobacillus prosperus* DSM5130 (99%) as nearest neighbor.

The isolate obtained from Japanese electroplating wastewater sludge was physiologically and phylogenetically related to *T. prosperus*. However, our results might be valuable to propose a new classification, since "*T. prosperus*" have not yet been assigned to existing or new genera because of insufficient data.

Abstract Category
11 Rare Microbes

PS.11.008 ENRICHMENT OF AMMONIA OXIDIZING THERMOPHILIC CRENARCHAEOTA FROM ALKALI HOT SPRINGS IN CALIFORNIA

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As one of the most world wide microorganisms, Crenarchaeota are recently regarded as important participant in the geochemical processes. Especially, more and more events and diversity of ammonia oxidizing Archaea demonstrates that their contribution to the nitrogen cycle is of global significance. However, most occurrences focus on the temperature environments from low to high. To enlarge the sign of Crenarchaeota and relative functions, we report one enrichment culture of Crenarchaeota, providing the first discovery for a moderately thermophilic and alkaline nitrifier. The specific media were designed to enrich and isolate novel Crenarchaeota. Moreover, the quantitative-PCR, FISH analysis, and ammonium/nitrogen measurement were applied to analyze this isolate. According out data, we successfully enriched a moderately thermophilic Crenarchaeota with pH 9.0 and temperature 45°C. This cultivation could utilize ammonium in the growth and have a integrity nitrification process, in which both nitrite and nitrate are found. These findings greatly extend the upper pH limit of nitrification and document that the capacity for ammonia oxidation is broadly distributed among the Crenarchaeota.

Abstract Category
11 Rare Microbes

PS.11.009 THE PROKARYOTIC, CULTURABLE DIVERSITY OF ALKALINE SPRINGS AROUND A KENYAN SODA LAKE

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The soda lakes of the East African Rift Valley have a high carbonate and salt content and a pH ranging from 8 to 11. Lake Magadi in Kenya is fed by a number of hydrothermal springs which range in temperature from 36.6°C to 82.7°C. There is a temperature gradient with the hottest springs being recorded at the north of the lake and the cooler springs located to the south of the lake. The purpose of the study is to characterise the aerobic organisms that can be cultured on a specific medium designed to mimic the sampled environment. A range of samples have been collected from Lake Magadi and have been divided into four types: (1) a high temperature area with spring waters around 82°C, at the north end of the lake; (2) a mid range temperature area to the north west, in the region of 60°C; (3) a 40°C area to the west; and, (4) a second 40°C area to the east of the southern end of the lake. Temperature and pH were measured at each site and samples for water analysis were also collected to assess the chemistry of the spring waters. Altering the salt content has allowed us to isolate bacteria that can withstand higher salt content: that is, moderately halophilic organisms. Two types of carbon source, peptone and starch have been used. The pH of the media is set at either 10 or 9.3, reflecting the pH sampled at source, using a carbonate buffer system. All samples were incubated at 37°C. Isolates of *Bacillus*, *Halomonas*, *Alkalibacterium*, *Amphibacillus* and *Planococcus* have been identified via PCR amplification and sequencing of the 16S rRNA gene. Some of the isolates may represent novel species. The isolates of the *Planococcus* and *Amphibacillus* genera have been characterized in terms of their pH and salt tolerance. This work will contribute to our understanding of how bacteria survive/tolerate high pH/salt environments and also has implications in the field of astrobiology.

Abstract Category
11 Rare Microbes

PS.11.010 PLUMBING THE DEPTHS OF THE "RARE BIOSPHERE" IN A TEMPERATE ESTUARINE SEDIMENT

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Recent molecular-based surveys on microbial diversity in varied ecosystems have revealed a long tail in the species rank abundance distribution indicating large number of low abundance microbial phylotypes - the so-called "rare biosphere". Microbial ecologists are keen to understand their biogeography and possible roles in ecosystem functions such as biogeochemical cycling. Rare microbes may play an indispensable role in ecosystem functioning, for instance as a "seed bank", and there is a need to understand how their response to environmental changes, including climate change, affects ecosystem functioning. Studies describing rare microbes have often used deep sequencing efforts, such as high-throughput tag sequencing. Although next-generation sequencing (NGS) is a valuable tool in the identification of rare microbes, it involves enormous effort, particularly with bioinformatic analysis. Also it is not amenable to the analysis of large numbers of samples as required in studies involving analysis of spatial and temporal distribution of microbes. Ammonia-oxidizing archaea (AOA) play a major role in nitrification with studies suggesting that they are dominant over their bacterial counterparts in some ecosystems. In this study, we used a novel approach, combined with a functional gene (ammonia monooxygenase; *amoA*) based analysis, to uncover low abundance ammonia oxidizing archaea (AOA) in a temperate estuarine sediment. This method requires minimal sequencing effort and can be combined with traditional molecular tools to study the microbial community composition from environmental samples. Using the retrieved archaeal *amoA* gene sequences, we performed bioinformatic analyses to test whether sequences were ubiquitous and/or endemic in different environments relative to existing sequence databases.

Abstract Category
11 Rare Microbes

PS.11.011 THE DEVELOPMENT OF CANDIDATE DIVISION OP10-SPECIFIC PCR PRIMERS

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Candidate division OP10, a phylum-level bacterial taxonomic group, was first identified in analyses of 16S rRNA gene sequences from Yellowstone National Park [1], and has since been observed in a diverse range of environments [2]. OP10 physiology was virtually unknown until the recent isolation [3] and subsequent characterisation [4] of the first cultivated OP10 species. The under-representation of OP10 phylotypes in conventional environmental surveys hinders studies of its diversity and ecological distribution. To address this issue, here we aimed to evaluate a set of OP10-specific primers which biases for the detection of OP10 in the environment. The SILVA database was used to generate a reference OP10 16S rRNA gene phylogenetic tree, which was used to assign clades. Clades were compared to non-OP10

sequences to generate specific primers with PRIMROSE. Primers were validated against negative controls and environmental DNA samples containing OP10 species. Positive results were used to generate clone libraries, then sequenced to confirm OP10 presence. Sampling coverage of OP10 in these environments was estimated by collector's curves. The OP10-specific primers tested identified known OP10 sequences, which dominated the libraries generated. OP10 sequences previously undiscovered using traditional universal primers were found. Collector's curves of OP10-specific PCR clones confirmed that all OP10 phylotypes were likely sampled. The primers developed here demonstrate the potential to identify OP10 in the environment with increased efficiency compared to universal bacterial 16S rRNA gene sequence primers. Improved detection of OP10 phylotypes will contribute both to better understanding of its phylogeny and to the identification of new OP10 species for future cultivation

1 Hugenholtz, P et al, *J. Bacteriol* 1998.

2 Portillo, MC et al, *World J Microb Biot* 2008.

3 Stott, MB et al, *Environ Microbio* 2008.

4 Lee, CK, MSc thesis, U Waikato. Hamilton, NZ 2010

Abstract Category

11 Rare Microbes

PS.11.012 HOW MANY AND WHAT PICOPLANKTON ARE IN THE ARCTIC OCEAN?

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Microbes, from the three domains of life, Bacteria, Archaea and Eucarya drive global biogeochemical cycling and are the base of marine food webs, including the Arctic Ocean. Although morphological diversity among the smallest microbes is limited, with each new technological advance in sequencing, the estimates of global microbial diversity increase by orders of magnitude. The Arctic is no exception, with most major groups of microbes having been found in arctic marine waters. Here we provide a brief overview of microbial diversity revealed by environmental surveys of the small subunit rRNA gene (SS rRNA). Similar to larger phytoplankton identified morphologically, small heterotrophic and photosynthetic eukaryotes in the Arctic are a mixture of uniquely arctic taxa and more cosmopolitan species. The recent massively parallel sequencing of the SS rRNA gene has revealed that at finer taxonomic scales arctic bacterial and archaeal communities also differ from their temperate counterparts, consistent with endemism and biogeography linked to water masses. The Arctic Ocean and surrounding seas are hydrographically complex with ca. 15 identifiable water masses and we estimate that there may be over 40000 microbial species equivalents in the Arctic Ocean.

Abstract Category

11 Rare Microbes

PS.11.013 PHYSIOLOGICAL CHARACTERIZATION AND GENOMIC SEQUENCING OF STRAIN T49, THE FIRST CULTURED REPRESENTATIVE OF

CANDIDATE DIVISION OP10

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The candidate phylum OP10 was first discovered in Yellowstone National Park[1] and has subsequently been detected in a wide variety of environments, [2], but the first representatives from this phylum have only recently been cultured [3]. This research describes the physiological characterization of the OP10 isolate strain T49. In addition, the T49 genome has recently been sequenced and annotated. The optimal growth temperature, pH, and preferred growth substrates of T49 were experimentally determined. Fatty acid ratios were measured by gas chromatography, and cell structure was visualized by scanning and transmission electron microscopy. The genome was sequenced by 454 paired-end sequencing, assembled using Newbler, and annotated using Glimmer. T49 grows optimally at 68 C and pH 4.3. It strongly tends towards biofilms rather than planktonic growth, and electron microscopy revealed secretion of extracellular polymeric substances (EPS), some of which appear to be digestive enzymes. While T49 utilizes most mono- and disaccharides, polysaccharide use is limited to those of amorphous structure, such as gellan, rather than regular structure. T49 contains novel fatty acids. T49 was isolated from beneath a soil layer rich in cellulose-degrading bacteria. Physiological characterization and genome sequencing indicate a possible role for T49 as a scavenger that digests byproducts of cellulose degradation that diffuse through the soil. The EPS secreted may protect T49 from sudden environmental fluctuations as well as allowing substrate digestion.

1. Hugenholtz, P., et al., J Bacteriol, 1998. 180(2): p. 366-76.
2. Portillo, M.C., et al., Antonie Van Leeuwenhoek, 2009. 96(4): p. 559-67.
3. Stott, M.B., et al., Environ Microbiol, 2008. 10(8): p. 2030-41.

Abstract Category

11 Rare Microbes

PS.11.014 MICROBIAL DIVERSITY AMONG THE RARE MEMBERS OF A HYPERSALINE MICROBIAL MAT

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Microbial ecosystems consist of a few dominant microbial phylotypes and a larger population of less abundant (rare) phylotypes. The collective 'rare biosphere' consists of microbial phylotypes ('species') that individually comprise less than 1% of the microbial communities, yet it is thought to play important roles in the long-term stability of the communities. Here, we describe the structure of the rare biosphere of a Bahamian hypersaline microbial mat using pyrosequencing. These microbial mats are located in naturally formed shallow hypersaline lagoons and are often subjected to extreme changes in environmental conditions, such as temperature, UV radiation, salinity, and desiccation. Our examination of 194,835 sequence tags from the V3 region of archaeal and bacterial 16S rRNA genes revealed that the rare members of these communities (defined as phylotypes with <0.1% abundance) represented 51% and 48% of the total number of reads in the libraries, respectively. With few exceptions, rare phylotypes outnumbered abundant phylotypes in both archaeal and bacterial libraries. In addition, detailed phylogenetic analyses

indicated that the structure of the rare phylotypes was similar to that of the abundant phylotypes with 99.4% of the rare archaea community and 97.8% of the rare bacterial community having close relatives among the more abundant members of each community. Furthermore, when analyzed at both the domain and phylum level, the diversity of the rare biosphere is always greater than that of the abundant phylotypes. These findings suggest that the rare biosphere may be providing the metabolic plasticity needed for the community to adapt to extreme environmental change.

Abstract Category

11 Rare Microbes

PS.11.015 EXPLORING THE PROKARYOTIC DIVERSITY IN TROPICAL ESTUARIES BY PYROSEQUENCING

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The aim of this work was to determine the eubacteria and archaea diversities of two distinct tropical estuaries. Surface water samples were collected in traditional well studied sites as Guanabara Bay (Microbial Observatory of Rio de Janeiro - MORio project) and Amazonas river estuary (CARBAMA project). Another objective was to compare the microbial diversity from these two aquatic ecosystems and then use these diversities as models of tropical estuarine prokaryotic diversity. We used massively parallel pyrosequencing of hypervariable V6 region of rRNA to characterize the phylogeny of free-living prokaryotes. We obtained 17,631 and 7,220 sequences of eubacteria, respectively, in Guanabara Bay and Amazonas river estuary. *Proteobacteria* corresponded to 48% of total sequences. *Actinobacteria*, *Verrucomicrobia*, *Bacilli*, *Flavobacteria*, *Cyanobacteria*, *Mollicutes*, *Erysipelotrichi*, *Bacteroidetes*, *Alpha-*, *Beta-*, *Gamma-*, *Delta* and *Epsilon-Proteobacteria*, *Sphingobacteria*, *Clostridiae*, *Lentisphaerae* e *Planctomycetes* were found in both environments. *Gamma-Proteobacteria* predominated in Guanabara Bay while a more homogeneous eubacteria distribution occurred in Amazonas water. Sequences were evaluated by rarefaction analysis. At 80% cut-off, the most dominant eubacterial phyla were detected, however, the diversity was not fully observed at higher 97% cut-off. We retrieved 20,137 sequences of archaea in Guanabara Bay with dominance of Euryarchaeota. In Amazonas sample 15,037 sequences of archaea were found with the predominance of *Crenarchaeota*. This study provides, for the first time, a highly detailed evaluation of prokaryotic diversity in tropical estuaries, allowing a better understanding about microbial community composition, interactions and ecological function

Abstract Category

11 Rare Microbes

PS.11.016 EVALUATION OF ARTIFACTS GENERATED DURING 16S RRNA GENE AMPLICON SEQUENCING USING 454 TITANIUM CHEMISTRY

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Pyrosequencing studies have recently revealed the extent of the "rare biosphere", but concerns remain that sequencing errors could artificially inflate species richness estimates. Moreover, distinguishing the potentially important rare 16S rRNA gene sequences from sequencing errors or Taq polymerase errors is difficult. Clustering of unique sequences into operational taxonomic units (OTUs) is currently used as a way of minimizing these artifacts at the expense of greater resolution. Development of data analysis methods that minimize error while maintaining accurate diversity and richness estimates is therefore crucial to improving environmental genomic studies. We present an error rate analysis of GS FLX Titanium amplicon pyrosequencing data using the v5-v6 region of a known 16S rRNA sequence. A total of 111,006 reads without N's that passed the instrument's quality control (QC) filters were analyzed from four pyrosequencing runs. Of these reads, 73,527 did not pass post-instrument, custom-designed QC filters, including 62,531 correct parent sequences and 6,623 containing homopolymer errors. Longer homopolymers had higher error frequencies, with the most common error an extension of a 5-base homopolymer. Out of the 37,479 reads that passed the quality filters, 33,484 reads (or 89%) were correct, while 3,995 contained sequence errors. Remarkably, very few homopolymer insertion errors were seen. The single most common error was a homopolymer deletion (4Ts to 3Ts) but the remaining dominant errors were substitutions, predominantly A to G and C to T. We conclude that while a certain amount of sequencing error is to be expected with such large data sets, strict quality filters can effectively reduce many of the most common errors. When combined with additional post processing informatics, the stringent QC filters tested may avoid the need for combining sequences into OTUs for community analysis.

Abstract Category

11 Rare Microbes

PS.11.017 MICROBIAL ASSESSMENT OF FIVE GEOTHERMAL AREAS IN ICELAND USING CULTURE INDEPENDENT METHODS

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This project is a part of the governmental policy of comparative evaluation of geothermal areas in Iceland. Decisions of conservation or utilization of power from these areas will be partly based on microbiological assessment. The aim of the project was to estimate the biodiversity, composition and novelty of prokaryotic species in samples from five high temperature geothermal areas in Iceland and to estimate their uniqueness. Samples were collected from diverse sites within five geothermal areas. DNA was extracted and the 16S rRNA gene amplified, cloned and partially sequenced. BLAST searches were performed for the identification of species or their closest relatives. Novel or "rare species" were defined at $\leq 95\%$

GenBank similarity level. Shannons' Biodiversity Index (H) and collector's curves were calculated for each area to estimate the biodiversity. A total of 5075 prokaryotic 16S rRNA gene sequences were retrieved from 129 samples. Sequences representing rare species were found in samples from all areas and most likely represent new genera. Five novel genera were found within the Archaea, the similarity to their closest Genbank relatives ranged from 79-95%. The Biodiversity Index (H) for individual areas ranged from 2,7-3,6. Comparison of collectors curves calculated for each area confirmed the H ranking in most cases. The general pattern observed in the samples was typical of extreme ecosystems with a dominating species and a number of sporadic ones. In most cases rare species were confined to distinct geothermal areas or hot springs. The Biodiversity Indices (H) calculated for the 16S rRNA gene sequences from the individual geothermal areas were rather low. However, their range increased when calculated for individual samples, reflecting the diverse spectra of hot spring types examined.

Abstract Category
11 Rare Microbes

PS.11.018 PYROCOCCUS YAYANOSII SP. NOV., THE FIRST STRICT PIEZOPHILIC HYPERTHERMOPHILIC ARCHAEON ISOLATED FROM A DEEP-SEA HYDROTHERMAL VENT.

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A strict piezophilic anaerobic hyperthermophilic archaeon (strain CH1T) was isolated from a hydrothermal vent site named "Ashadze" located on the mid-Atlantic ridge at a depth of 4100 m. Enrichment and isolation were carried out at 95°C under an hydrostatic pressure of 42 MPa. CH1T cells are highly motile irregular cocci with a diameter of about 1-1.5 µm. Growth was recorded within a temperature range of 80 to 108°C and a pressure range of 20 to 120 MPa. Temperature and pressure optima for growth were 98°C and 52 MPa respectively. No growth was observed under atmospheric pressures for temperatures ranging from 60 to 110°C. Growth was observed from pH 6 to 9.5 (optimum 7.5-8) and salt concentrations from 2.5 to 5.5 % NaCl (optimum 3.5%). Strain CH1 is strictly anaerobic and grows by fermentation of various organic compounds. The G+C content of the genomic DNA is 49.0 + 0.5 mol%. Sequencing of the 16S rRNA gene revealed that strain CH1T belonged to the genus *Pyrococcus*. Comparisons of ribosomal proteins sequences with those from close relatives within the Thermococcales indicated that strain CH1 is a novel species for which the name of *Pyrococcus yayanosii* has been proposed. Genome sequencing is under progress and first available data will be presented. For our knowledge, *P. yayanosii* strain CH1 is the first obligate piezophilic hyperthermophile isolated so far.

Abstract Category
11 Rare Microbes

PS.11.019 LIVING IN THE DEAD ZONES: V6 TAG SEQUENCING OF MICROBIAL COMMUNITIES IN HOOD CANAL, WA

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Hood Canal is a narrow fjord in Puget Sound, Washington, that experiences seasonal hypoxia. Extreme hypoxic events at the southern end of Hood Canal have resulted in several fish kill events in the last five years. Here we use 454 16S rRNA V6 tag Sequencing to examine the relative influence of biotic and abiotic parameters (including dissolved oxygen, nutrients, chlorophyll a, salinity, temperature and phytoplankton community composition and abundance) on the ecology of marine Bacteria. Our sequencing strategy includes paired samples in surface and bottom (ranging from 10-140 m) waters at stations along a N-S gradient that experience varying levels of oxygen depletion throughout the year. We have sampled two stations in April, June, and October 2007 to observe seasonal variation in Bacterial composition. Two additional stations serve as more extreme end members and were examined in June 2007. In this study Oxygen concentrations ranged from 3-7 mg L⁻¹ in bottom waters and from 5-13 mg L⁻¹ in surface waters. Between 4000 and 16,000 reads were sequenced per sample. Our preliminary analysis indicates surface communities differ significantly from those at the bottom, even when plastid reads are removed. Although we do not observe a strong geographic signal in surface communities, bottom communities do differ along the N-S transect. We will also present comparison of organelle derived reads with phytoplankton species counts obtained at the same time via microscopy. The V6 tag deep sequencing approach has greatly expanded our ability to access the diverse estuarine bacterial community in Hood Canal beyond the dominant and most easily amplified community members.

Abstract Category
11 Rare Microbes

PS.11.020 THREATENED CORALS PROVIDE UNDEREXPLORED MICROBIAL HABITATS

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Communities in marine environments are commonly composed of a few dominant taxa and a high number of taxonomically diverse, low-abundance organisms. However, studying the roles and genomic information of these "rare" organisms remains challenging, because little is known about their ecological niches and the environmental conditions to which they respond. Given the current threat to coral reef ecosystems, we investigated the potential of corals to provide highly specialized habitats for bacterial taxa including those that are rarely detected or absent in surrounding reef waters. The analysis of more than 350,000 small subunit ribosomal RNA (16S rRNA) sequence tags and almost 2,000 nearly full-length 16S rRNA sequences revealed that rare reef water biosphere members are highly abundant or even dominant in diverse Caribbean corals. Closely related corals (in the same genus/family) harbored similar bacterial communities. At higher taxonomic levels, however, the similarities of these communities did not correlate with the phylogenetic relationships among corals, opening novel questions about the evolutionary stability of coral-microbial associations. Large proportions (28.7-49.1 %) of operational taxonomic units (OTUs), i.e. sequences clustered at the 97% similarity level, were unique to the coral species of origin. Analysis of the most dominant ribotypes suggests that many uncovered

bacterial taxa exist in coral habitats and await future exploration. To date, this study represents the most comprehensive census of coral-associated bacteria. We have shown that corals can provide specialized habitats of otherwise rare microbes in surrounding waters, and furthermore, that many bacterial taxa harbored by corals had previously been unidentified. Given that two of the coral species investigated are listed as threatened under the U.S. Endangered Species Act, our results add an important microbial diversity-based perspective to the significance of conserving coral reefs.

Abstract Category

11 Rare Microbes

**PS.11.021 *RHIZOMICROBIUM PALUSTRE* GEN. NOV., SP. NOV., A
FACULTATIVELY ANAEROBIC, STALKED BACTERIUM ISOLATED FROM RICE
PLANT ROOTS IN IRRIGATED SOIL**

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In Japan, rice is widely cultivated as the principal food using irrigated fields, which develop a highly reduced condition in soil during the flooding period. The anoxic rice-field soil harbors anaerobic microbial communities composed of diverse species of microbes including fermentative bacteria and methanogenic archaea. We isolated three strains of facultatively anaerobic, stalked bacterium from living rice roots, which were assigned to the class *Alphaproteobacteria* and phylogenetically distant from any recognized species. Strains were isolated and cultivated by the strictly anaerobic method using oxygen-free gas. PY medium was used as a basal medium for the cultivation. The strains were comprehensively characterized for cell morphology, physiology and chemotaxonomy as well as phylogeny. Cells of the strains were Gram-negative, non-spore-forming rods. Reproduction of cells was by binary fission as well as by budding. Some of cells, including dividing cells, were motile with a single polar flagellum. Cells developed a polar prostheca (stalk) with a holdfast-like structure and the cell with the stalk budded a daughter cell. The strains utilized various sugars as growth substrates. The strains fermentatively produced acetate and lactate as well as a small amount of H₂. Catalase was not detected. The strains reduced Fe(III) to Fe(II) in the presence of glucose. Ubiquinone Q-10 was the major respiratory quinone and the genomic DNA G + C contents were 54 mol%. Phylogenetic analysis based on 16S rRNA gene sequences placed the strains in the class *Alphaproteobacteria*. The closest relative was *Hyphomicrobium hollandicum* in the *Rhizobiales* with sequence similarity of 88.2%. Based on the 16S rRNA gene sequences and the phenotypic properties of the novel strains, we proposed that the strains should be assigned in the novel genus and species as *Rhizomicrobium palustre* gen. nov., sp. nov. Type strain: A48^T = JCM 14971^T = DSM 19867^T.

Abstract Category

11 Rare Microbes

PS.12.001 EXPERIMENTAL EVIDENCES OF PREDATOR-DRIVEN AGGREGATION AND FILAMENTATION OF FRESHWATER PLANKTONIC BACTERIA

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The success of microbial populations in aquatic environments partly depends on their ability to minimize the cell mortality inflicted by protistan grazing. Thus, the mechanisms defining microbial vulnerability or resistance to grazers could affect the energy transfer to higher trophic levels within the water column. We experimentally investigated the structural changes of freshwater bacterial communities exposed to high grazing pressure. Batch cultures were set up in triplicated microcosms containing 1:10 diluted and enriched lake water (Lake Zurich) and about 500 cells/ml of the filter-feeding nanoflagellate *Poterochromonas* (i.e., at a ratio of 1:500 of bacterial abundance). The number of free-living bacterial cells increased (max 5.6×10^6 cells/ml) until grazers started controlling bacterial growth, and predators reached the highest concentration (1.1×10^4 flagellates/ml) at 96 h. As visualized by epifluorescence microscopy and flow cytometry, filamentous cells bloomed at 48 h, while the number of bacterial aggregates increased significantly (up to about 800 aggregates/ml) at 120 h. Analysis by fluorescence *in situ* hybridization revealed that aggregates were comprised mostly of members of the *Alpha*, *Beta*, and *Gamma* subclasses of *Proteobacteria*. Surprisingly, the addition of the aggregate-forming bacterial strain *Sphingobium* sp. Z007, isolated from Lake Zurich, inhibited both aggregation and filamentation in the experimental assemblages. A better understanding and modelling of these mechanisms are important to define the functional role of microbial communities as the base of aquatic microbial food webs.

Abstract Category

12 Interkingdom Interactions

PS.12.002 SMALL MOLECULES AND ALTERNATIVE METABOLIC PATHWAY OF YEAST, DRIVE THE ESTABLISHMENT OF EXPERIMENTAL FUNGAL-BACTERIAL COMMUNITY.

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Fungi and bacteria co-inhabit a wide variety of environments, coexisting as communities usually within biofilms, where they compete for resources. Interactions between bacteria and fungi can affect survival, colonization, pathogenesis, and symbiosis. Little is known at the molecular level how these interactions affect their biological development. A dual-species cocultivation model was developed using plant symbiotic bacteria *Rhizobium etli* and eukaryote *Saccharomyces cerevisiae* as a tractable system to identify molecular mechanisms that underlie multispecies microbial associations. We discovered that *S. cerevisiae* and *R. etli* in coculture form a mixed biofilm with a 10 fold increase in biomass that is better structured and stable as compared to single species biofilms. We found that this interaction is not unique to laboratory strains, as it also establishes with wild yeasts isolated from rainforest. The increase in biomass in the mixed biofilm is the result of the establishment of a commensal interaction of the bacteria with the yeast. This interaction depends on the production of small molecules secreted by the fungus along with the function of genes encoded on the symbiotic plasmid of the bacterium. Furthermore, using *S. cerevisiae* single knockout strains, we found that respiratory metabolism is essential for the establishment, development, and maintenance of the fungus-bacteria interaction. These results provide an understanding of microbial interactions that resulted in molecular elements not specific to this system.

Abstract Category

12 Interkingdom Interactions

PS.12.003 SEASONAL DYNAMICS AND BACTERIAL DIVERSITY OF BIOFILMS ON THE KELP *LAMINARIA HYPERBOREA*

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The kelp *Laminaria hyperborea* forms extensive underwater forests along the Atlantic coasts of Europe. These kelp forests are hotspots for primary production and biodiversity, and many species of invertebrates, fish and epiphytic seaweeds depend on kelp for shelter, nutrition and as a growth substrate. Biofilms consisting mainly of bacteria coat the surfaces of the kelp. These bacterial communities are believed to be important in the kelp forest food chain by making kelp-produced carbon available to animal consumers yet very little is known about their biodiversity and ecology. Kelp surface biofilms form an excellent system to study the formation and succession of biofilms on living surfaces because of the seasonal growth cycle of the kelp. The aim of this study was to uncover some of the fundamental aspects of the biodiversity and seasonal dynamics of kelp surface biofilms. Denaturing gradient gel electrophoresis in combination with 454 pyrosequencing of amplicons of the 16S rRNA gene was used to study the seasonal variation of the bacterial diversity and community composition of the biofilms. Counting of biofilm bacteria by fluorescence microscopy was used to follow the development of the biofilm during the year. Fluorescence *in situ* hybridization was further used to quantify dominant bacterial groups. The results show an apparent seasonal "cycle" of bacterial community composition that appears to be closely linked to the growth cycle of the kelp. Bacterial diversity is also strongly influenced by seasonal aspects. Bacterial cell counts reveal sparse biofilms during the active growth period of the kelp and denser biofilms as the kelp surface ages. Planctomycetes are abundant in all seasons and dominate the biofilm communities in summer. In conclusion, kelp surface biofilms are intimately controlled by the growth cycle of the kelp and consist of a specialized community. The findings give important insights into the dynamics of marine biofilms on living surfaces.

Abstract Category
12 Interkingdom Interactions

PS.12.004 A SMALL MOLECULE REGULATES THE SYMBIOSIS-PATHOGENESIS SWITCH IN AN ALGAL-BACTERIAL INTERACTION

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Bacteria of the roseobacter clade and marine algae are commonly associated in the marine environment and engage in intermittent symbiotic relations. Algal derived compounds have been shown to be colonisation signals for roseobacters. However, the molecular details of colonization are unknown. Here we show that common breakdown products of lignin stimulate the model roseobacter *Phaeobacter gallaeciensis* BS107 to produce a potent antialgal compound with an unusual structure. This finding indicates that algal compounds produced in senescence convert *P. gallaeciensis* BS107 from a mutualist into an opportunistic pathogen of its algal host and serves as a regulator of small molecule production.

Abstract Category
12 Interkingdom Interactions

PS.12.005 PSEUDOMONAS AERUGINOSA VIRULENCE MODIFIED BY INOSITOL PHOSPHATE-CHELATED IRON

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Iron is an essential nutrient for all cellular organisms and the ability of microbes to sequester this ion in different environments becomes critical for survival. One microbe that excels at this task within the human host is *Pseudomonas aeruginosa*. The *P.aeruginosa* PAO1 genome codes for 35 TonB-dependent receptors. Many of these have been characterized to bind iron-chelating ligands for import of iron across the outer membrane. We investigated one of the putative TonB-dependent receptors that had not previously been investigated. Using gene deletion mutants and examining the expression of the outer membrane protein under different inducing conditions, we investigated possible host derived factors as ligands for the putative receptor. Inositol phosphates were

examined as potential ligands for this receptor. These molecules are known to participate in critical signaling pathways in eukaryotic organisms including those implicated in immune function and microbial killing mechanisms. *P.aeruginosa* exhibited an increased growth rate when inositol phosphates were supplied in defined media, and iron transport seemed to be facilitated by these molecules. Additionally, production of the *P.aeruginosa* virulence factor pyocyanin was altered when Fe³⁺-inositol phosphate chelate was supplied to the growth media. *P.aeruginosa* is a leading source of chronic infection in cystic fibrosis patients. Utilization of inositol phosphates by *P.aeruginosa* may contribute to the persistence of this bacterium in settings such as the cystic fibrosis lung where intracellular contents of host cells are expected to be released due to sustained inflammation. Possible perturbation of signaling pathways via the interaction of *P.aeruginosa* and various inositol phosphate isomers opens new avenues for research into the intricate relationship between host and pathogen.

Abstract Category
12 Interkingdom Interactions

PS.12.006 BACTERIAL COMMUNITIES ASSOCIATED WITH *MNEMIOPSIS LEIDYI*

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A recent reorganization within the animal tree of life places ctenophores as the extant form of earliest diverging multi-cellular animals. These gelatinous zooplankton reside in a phylum of their own, and are known to be parasitized by variety of organisms (amoebae, dinoflagellates, and sea anemones). To date, no studies have examined the bacterial communities associated with ctenophores. This study describes the bacterial communities associated with *Mnemiopsis leidyi*, a lobate ctenophore, widely distributed throughout the Gulf of Mexico, Caribbean, and along the eastern coasts of North and South America. *Mnemiopsis leidyi* were collected in April 2009 via plankton tows in Bayboro Harbor, Florida. Specimens were rinsed in sterile seawater and allowed to empty their guts before cryo-preservation and DNA extraction. Sequencing of 16S ribosomal DNA clone libraries from several ctenophores demonstrated that ctenophores contain a specific microbiota that is different from the surrounding water column. Both water column and ctenophore-associated bacterial communities were dominated by α -proteobacteria; however, the α -proteobacteria associated with the ctenophores were distinct from those in the surrounding water. Although individual ctenophores contained unique bacterial communities, a specific bacterial sequence with 99% identity to a marine *Roseobacter* was detected in all ctenophores. This sequence was absent from the surrounding water column, suggesting this may be a bacterial associate of *Mnemiopsis leidyi*. All ctenophores also contained sequences from ϵ -proteobacteria, which were absent from the water column. A number of sequences in the ctenophore libraries were closely related (96-99% identity) to bacteria previously described in corals. Future work will need to determine the mode of acquisition of ctenophore-associated bacteria, and the roles that bacterial associates play in ctenophore ecology.

Abstract Category
12 Interkingdom Interactions

PS.12.007 MICROBIAL MATS FROM HOT SPRINGS PRODUCE BIOACTIVE COMPOUNDS UNDER NATURAL CONDITIONS

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Microbial mats are highly structured and diverse biofilm communities living within sharply-defined geochemical gradients of oxygen, pH and sulphate that periodically fluctuate over time. In this study, we investigated the ability of mat microorganisms from hot springs of the Sultanate of Oman to exhibit anti-bacterial, anti-diatom and anti-quorum sensing bioactivities under in situ conditions. The studied springs differed in their physical-chemical conditions (temperature, water flow, concentration of anions, and pH) and each had its own distinct microbial communities as visualized

by Automated Ribosomal Intergenic Spacer Analysis (ARISA) technique. Microbial mats were harvested and extracted with polar (water) and non-polar (ethyl acetate) solvents and activity of these extracts was tested in laboratory experiments. Only extracts from two springs inhibited growth of heterotrophic bacteria, while all extracts inhibited growth of the benthic diatom *Amphora coffeaeformis*. Some extracts inhibited quorum sensing of the reporter strains *Chromobacterium violaceum* CV017 and *Agrobacterium tumefaciens* NTL4. The highest bioactivity was recorded for ethyl acetate extracts of a spring mat that had the lowest microbial diversity. We conclude that microbial mats have the ability to produce bioactive compounds under in situ conditions and differences in environmental conditions and microbial communities have led to differential bioactivity of community extracts. The produced compounds could be involved in the regulation of species to species relationships and of protecting mat communities from invasive species.

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Abstract Category
12 Interkingdom Interactions

PS.12.008 INTERACTIONS BETWEEN *EISENIA ANDREI* EARTHWORMS AND SOIL METHANOGENS

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Animal treading associated with a high input of organic matter was found to favour methanogenesis in soils used as overwintering pasture in outdoor cattle husbandry. Such soils typically show high abundance of methanogenic archaea and high methane emission, especially at the end of overwintering period in spring. The bioremediation ability of the earthworms *Eisenia andrei* and their effects on the methanogenic microbial community in cattle-impacted soil were studied in a six month microcosm experiment. Methanogens were identified using the phylogenetic microarray AnaeroChip and the most abundant genus *Methanosarcina* was quantified by real-time polymerase chain reaction. In addition, the rate of methane production was measured by gas chromatography and total biomass of anaerobic microbes was assessed using unsubstituted non-ester-linked phospholipid fatty acids analysis. The earthworms reduced significantly the diversity of methanogens and the quantity of *Methanosarcina* gene copies already two months since the start of soil incubation. The biomass of total anaerobic microorganisms significantly decreased after six months. The activity of *Eisenia andrei*, an earthworm inhabiting various nutrient-rich substrates like composts and manures, was found as an effective actor in the reduction of methanogens in cattle impacted soils. A reduction of methane production rate due to earthworm activity was, however, not found in our experiment.

Abstract Category
12 Interkingdom Interactions

PS.12.009 MICROBIAL COMMUNITIES IN THE DIGESTIVE TRACT OF THE AQUATIC CRUSTACEAN *DAPHNIA MAGNA*

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In aquatic systems, bacteria play a key role in the carbon cycle but their importance in zooplankton guts remains mostly unknown, although their presence was regularly documented. Recently, denitrification by ingested bacteria in anoxic guts of benthic aquatic invertebrates was demonstrated indicating their possible symbiotically participation in digestion. However, the guts of most important zooplankton, e.g. small *Daphnia spp.* which are a significant trophic link in freshwater systems, are probably only partly anoxic if at all. This leads to the question which microorganisms are present in the *Daphnia* gut and how they interact with their host, i.e., do they symbiotically

participate in digestion, do they prevent success of pathogens or do they compete for food. The aim of this study was to characterise the intestinal microbial community and to estimate if *Daphnia* have a specialised stable gut microbiota or if incubation conditions will alter or even completely restructure the community. Therefore, the intestinal microbial community of *D. magna* clones was analysed via 16S rDNA clone libraries. To investigate the stability of their microbiota, *Daphnia* were incubated under different conditions (food sources, exposure to defined bacteria) while changes in the intestinal community composition were followed by T-RFLP. The *D. magna* microbiota was dominated by clones affiliated to the β -proteobacteria *Limnohabitans* sp., which were described to respond rapidly to environmental changes. Overall, most clones were closely related to facultative anaerobic bacterial strains. *Limnohabitans* were also always prominent in the T-RFLP profiles despite of changing food sources and independent of applied bacteria, thus indicating that there are specialised stable community members. Other intestinal microorganisms were stimulated due to differing food sources but never dominated the community. Just when *Daphnia* spp. were starved to death their microbial community changed distinctly.

Abstract Category
12 Interkingdom Interactions

PS.12.010 BACTERIAL COMMUNITY STRUCTURE AND COMPOSITION IN VARIOUS VERMICOMPOSTS

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Vermicomposting is as a low-cost technique to transform biowastes into revalued agronomic products. There is scarce information about the bacterial communities involved in this process. It was our aim to elucidate the influence of initial substrates and process parameters on the bacterial community of mature vermicomposts produced by *Eisenia fetida*. Four vermicomposts were tested: two of them were produced at a laboratory-scale by traditional vermicomposting using winery or olive mill wastes, one was obtained from tomato-fruit wastes under continuous feeding conditions at a pilot scale, and one was a commercial vermicompost from cattle manure. The 16S rRNA gene was PCR amplified to study the structure of the bacterial communities by DGGE. The dominant bacterial taxa were identified using the CompoChip, an oligonucleotide microarray targeting microbes frequently found in composts. Cluster analysis of the DGGE profiles revealed that replicates were highly similar (>95%) and all four vermicomposts were medium to highly similar (61-93%). Winery and tomato vermicomposts clustered together, as did olive mill waste and manure vermicomposts. This was confirmed by principal component analysis of microarray data, which discriminated the vermicomposts mainly based on the abundance of the decomposers *Sphingobacterium*, *Streptomyces* and *Micromonospora*, and the potential pathogens *Pseudomonas aeruginosa*, *Salmonella* sp and *Agrobacterium tumefaciens*. The olive mill waste vermicompost had the highest levels of decomposers and the lowest of pathogens. All vermicomposts showed positive signals for *Xylella*, *Xanthomonas*, *Stenotrophomonas*, *Pseudomonas*, *Microbacterium*, *Sphingobacterium*, *Streptomyces*, *Acidovorax avenae* and *Stenotrophomonas maltophilia*. These results indicate that the bacterial community was both affected by the initial substrates and the type of vermicomposting, but all mature vermicomposts shared bacterial taxa possibly related to the activity of *E. fetida*

Abstract Category
12 Interkingdom Interactions

PS.12.011 LINK BETWEEN MICROBIAL DIVERSITY AND ANIMAL SIZE

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The link between microbial diversity and yield of the biological processes is often related. Considering studies related to the ecology of macro-organisms, those undertaken on insular population have already demonstrated the link between the size of the ecosystem and diversity. On the contrary at the microbial level, the link between process size and microbial diversity is rarely evaluated. This lack is due on one hand to the difficulty of designing innovative experimental set

ups and on the other hand, to the difficulty of measuring microbial diversity. To overcome experimental difficulties and to describe links between size and diversity, we chose to analyze animal digestive tracks. Digestive tracks are anaerobic digestion 'processes' which are, in many ways, similar to industrial anaerobic processes. Moreover, these models describe important size gradients (there is for example a factor 100 000 between an elephant and a passerine) independently of feeding habits. Diversity measurements were undertaken by molecular fingerprints (SSCP) performed on DNA extracted from animal feces. Microbial diversity measurements were provided from the Simpson index which was directly calculated from each fingerprint. Around 180 feces from 70 species with different feeding habits were analyzed. Assuming that the gut volume is correlated to animal weight and that microbial diversity is similar between feces and gut, the results indicate that : (i) the diversity value ranged from 1 to 7.5, (ii) animal weight and diversity seemed to be positively correlated, (iii) samples from the same species but from different individuals vary but remain in a similar range of diversity, (iv) neither the feeding habits nor the animal phylogeny affect the bacterial diversity.

Abstract Category
12 Interkingdom Interactions

PS.12.012 INTERKINGDOM-SIGNALLING: THE QUORUM-SENSING MOLECULE OF P. AERUGINOSA, N-3-OXODODECANOYL HOMOSERINE LACTONE, ACTIVATES HUMAN PHAGOCYtic CELLS

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To induce virulence factors or biofilm formation bacteria communicate with each other via "quorum-sensing molecules". We now report that a quorum-sensing molecule of *P. aeruginosa*, N-3-oxododecanoyl homoserine lactone (AHL-12), also activates phagocytic cells, the main protagonists of the innate host defence. AHL-12 was synthesized and its effect on isolated human phagocytic cells was tested. AHL-12 is chemotactic for phagocytic cells and enhances their phagocytic, and hence their bactericidal activity. AHL-12 executes its activity via the modulation of a central intracellular signalling pathway of phagocytic cells, the p38 mitogen activated protein kinases. Phosphorylation of p38 was seen within minutes after exposure of cells to AHL-12, followed by translocation into the nucleus of a crucial transcription factor NfκB. Only AHL-12 stimulated the phagocytes, but not its 3-deoxy-isomer, nor homoserine lactone lacking fatty acids. Uptake studies with radiolabelled AHL-12 (at 4° C) revealed a specific and saturable cellular binding. Taken together, the data argue for a surface receptor for AHL-12 on phagocytes, as does the fact that all chemokines known so far require surface receptors to induce polarisation as a prerequisite for directed cellular migration. Binding to a putative surface receptor does not exclude other interactions of AHL-12 with the cells, e.g. binding to the intracellular peroxisome proliferator-activated receptor (PPAR)γ, as reported by others. Novel, however, is the fact that human phagocytic cells recognise and respond to a molecule designed for bacterial communication. The benefit of this molecular eavesdropping for the host is obvious: phagocytic cells are attracted to the site of bacterial colonisation, which, in turn, warrants an early onset of the host defence.

Abstract Category
12 Interkingdom Interactions

PS.12.013 ENDOGENIC BACTERIA PROTECT CHIRONOMIDS FROM TOXICANTS

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Chironomids (non-biting midges) are the most widely distributed insects in freshwater. Females deposit egg masses at the water's edge, each egg mass containing hundreds of eggs embedded in a gelatinous matrix. They undergo complete metamorphosis of four life stages: eggs, larvae, pupae and adults. The larvae of *Chironomus* sp. live in tubes in the benthos of freshwater habitats. Chironomid as a group are considered pollution tolerant, however, little is known about their protective mechanisms in contaminated environments. Chironomid egg masses serve as a reservoir

for *Vibrio cholerae* (Broza & Halpern, 2001, Nature) as well as for *Aeromonas* pathogenic species (Senderovich et al. 2008, ISME J). In order to determine the diversity of bacterial communities associated with chironomid egg masses and larvae we used culture dependent and independent (PCR-DGGE and cloning) methods. A great similarity was found between the bacterial communities of different egg masses that were sampled during a five months period. This implies that those bacteria are stable residents in this niche. Using cloning methods it was found that ca. 40% of the species that inhabit the egg masses and the larvae had a potential ability to detoxify pollutants. Koch's postulates were applied to test whether bacteria play a role in protecting chironomids from toxicants. When chironomid larvae that were alcohol sterilized were incubated with 0.05mM lead, they did not survive. However, the control larvae without lead or with lead and without sterilization survived. *Chromobacterium* sp. was isolated from the unsterilized control larvae that survived in the presence of the lead. The addition of this bacterium to sterilized larvae with lead resulted in the larval survival. We conclude that the bacteria that inhabit chironomids have a role in protecting them from pollutants. These findings could open new horizons in our understanding of the relationships between bacteria and insects.

Abstract Category

12 Interkingdom Interactions

PS.12.014 TOXIC EFFECTS OF THE CRYSOPHYCEAN *OCHROMONAS* SP. ON FRESHWATER PLANKTON

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Chemical defenses or toxic metabolites of toxic algae and cyanobacteria can have widespread inter-kingdom effects in aquatic food webs. Less is known about the toxicity of mixotrophic flagellates, and we studied *Ochromonas* sp. to determine the effects of its toxins on a variety of planktonic freshwater organisms, and whether these effects depend on the trophic mode (autotrophy, osmotrophy, phagotrophy).

The effects of either *Ochromonas* cells or their culture medium, "filtrate" (minus the cells themselves) were tested, using growth rate and survival experiments, on organisms from four kingdoms: eubacteria, ciliates, green algae and zooplankton. *Ochromonas* was chemostat cultured in environments in which we manipulated light availability (no light or saturating light) and food source (either bacteria or peptone).

We found that *Ochromonas* cells were highly toxic to zooplankton (*Daphnia pulex* and *Brachionus calyciflorus*), their effect on other algae ranged from non-toxic to toxic depending upon the target species, and they were not alleopathic to bacteria. Interestingly, the effect of *Ochromonas* filtrate on ciliates depended on how the target organism acquired its nutrition. When *Paramecium aurelia* fed phagotrophically on detritus, we observed no effect, but for *Tetrahymena thermophila* feeding osmotrophically on peptone we found a strong negative effect. For zooplankton, we also found that light availability can have an effect on the toxicity of *Ochromonas* indicating that the feeding mode of the mixotroph can play a role in determining toxicity. Our study used *Ochromonas* densities (10^4 mL⁻¹) common in productive lakes, and one implication of our results is that the common assumption in studies of *Daphnia* resource use, that plankton < 30 µm are "edible", is flawed, at least when *Ochromonas* is present.

Abstract Category

12 Interkingdom Interactions

PS.12.015 TROPHIC RELATIONSHIPS IN THE RHIZOSPHERE: EFFECT OF FUNGAL, BACTERIAL AND NEMATODE INTERACTIONS ON MINERAL NUTRITION OF *PINUS PINASTER*.

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In terrestrial ecosystems, especially forest ecosystems, the availability of macro nutrients such as nitrogen and phosphorous is often limiting to the plant growth. This nutrient acquisition occurs in a high competitive environment, the rhizosphere, characterized by interactions between the roots, soil microorganisms and macrofauna. So far, this soil food web has been poorly studied in woody plants such as gymnosperms that always form symbiotic associations with mycorrhiza. These ectomycorrhiza live with specific bacterial populations called bacteria associated with mycorrhiza (BAM). In the rhizosphere, these bacterial populations are regulated by predation by microfauna like bacterial-feeding nematodes. We hypothesize that, in a depleted environment, these bacteria represents a pool of nutrients for the woody plants which is regulated by predation. To test this hypothesis, we used a simplified system including diverse combination of young *Pinus pinaster* plants, an ectomycorrhizal fungus (*Hebeloma cylindrosporum*), a BAM bacteria (*Bacillus subtilis*) previously labelled with ¹⁵N, and a population of bacterial-feeding nematodes (*Rhabditidae*). In this study, we demonstrated that nematodes exerted a positive effect for the mineralisation of ¹⁵N contained in bacteria with a value of 14 µg/plant. Our result suggests that this positive effect is not simply due to ¹⁵N uptake but also probably due to a better exploitation of nitrate from the medium. Mycorrhizae seem a better provider of nutrients for plants than predated bacteria as maximum accumulation of total nitrogen and phosphorous occurred in mycorrhizal pine seedlings (with or without bacteria). This study shows that the nutrients release by bacterial predation in the rhizosphere represents a suitable pool of nutrients for plants. This study underlines the importance of taking into account a certain level of biocomplexity like multitrophic interactions when functional processes like nutrient acquisition

Abstract Category

12 Interkingdom Interactions

PS.12.016 EVALUATION OF ANTIFUNGAL LACTIC ACID BACTERIA DIVERSITY IN RAW MILK SAMPLES

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Replacing chemical preservatives by antifungal lactic acid bacteria (ALAB) as bio-protective cultures on dairy products appears to be a promising way to fulfill consumers demand. Raw milk is likely to be a natural container of ALAB. The aim of this study was to evaluate the biodiversity of ALAB in raw milk from cow, goat and ewe. Raw milk samples (27 in total) were collected at the beginning, middle and end of lactation. For each sample, total bacteria were enumerated on non selective medium, whereas lactic acid bacteria were enumerated on 8 semi-selective media. After counting, 18,000 colonies were overlayed with *Mucor plumbeus*, *Kluyveromyces lactis* or *Pichia anomala* for antifungal activity screening. More than 600 active colonies were purified and their 16S rRNA gene sequenced for identification. Cow and goat milks harbored 51 % and 43% of active strains respectively, whereas ewe's milk contained some less (6%). Most isolates (86%) came from MRS containing media. Isolates were active against *Mucor plumbeus* (52%), *Kluyveromyces lactis* (28%) and *Pichia anomala* (21%) in decreasing order. Most of them belonged to the *Lactobacillus casei* (55%), *Lb. fermentum* (21%) and *Lb. plantarum* (14%) groups. The last 10% belonged to the *Lb. buchneri* and *Lb. acidophilus* groups, *Enterococcus*, *Lactococcus*, *Leuconostoc* and *Pediococcus* genera. The *Lb. casei* group contained strains active against all fungi, whereas most strains from the *Lb. buchneri* group targeted *P. anomala* and that from the *Lb. plantarum* group targeted *K. lactis*. Moreover, strains targeting *P. anomala* were mainly isolated during the middle of lactation, whereas that active against *K. lactis* were isolated at the beginning of lactation. In conclusion, raw milk from cow and goat are natural container of ALAB (ca 5 % of tested colonies). The great majority of active strains belonged to the *Lactobacillus* genus moreover targeted fungal species differed according to the *Lactobacillus* group tested.

Abstract Category

12 Interkingdom Interactions

PS.12.017 BACTERIA ASSOCIATED WITH THE PIG PARASITE ASCARIS SUUM

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Nematodes (or roundworms) are the most abundant metazoans on earth and commonly associate with bacteria as a source of food and/or in symbiotic relationships. Those nematodes that are parasites of the gastro-intestinal tract of vertebrates encounter environments that are exceptionally rich in microbial life. *Ascaris suum* is the most common parasite of pigs and resides in the small intestine of its host. Due to nutrient acquisition from the host, they cause growth retardation and weight loss resulting in higher feed and veterinary costs to the farmer and the pig farming industry. Treatment of *A. suum* infection relies on the use of anthelmintic drugs, but many nematode parasites are becoming resistant to the available chemotherapies. These developments have triggered interest in symbiotic bacteria such as *Wolbachia*, which play key roles in the survival and pathogenicity of a number of nematodes and which may represent novel therapeutic targets for parasite control.

The aim of this research is to investigate the relationship between *A. suum* and bacteria. The microbial diversity associated with *A. suum* pseudocoelomic fluid has been assessed using Denaturing Gradient Gel Electrophoresis (DGGE). This has allowed comparison of the bacteria present in both male and female nematodes from different pig herds showing the presence of a least ten bacterial species. Sequencing of individual bands has identified bacteria belonging to the genera *Escherichia*, *Pseudomonas* and *Haemophilus*, with limited similarities existing across the spectrum of worms and farms tested. DGGE results obtained from different samples, including eggs, will also be presented.

Abstract Category
12 Interkingdom Interactions

PS.12.018 CHEMICAL CUES PRODUCED BY TETRAHYMENA SP. INDUCE BIOFILM FORMATION AND ACTIVITY OF VIBRIO CHOLERAЕ

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Predation of bacteria in aquatic habitats by protozoa has resulted in the evolution of defence mechanisms e.g. toxin production, size variation and biofilm formation. Previous results showed that grazing results in an increase in *V. cholerae* biofilm biomass. Here we further investigate the effect of grazing on *V. cholerae* biofilms by the browsing feeding ciliate *Tetrahymena pyriformis*. *V. cholerae* (10^5 cells ml⁻¹) and *T. pyriformis* (10^3 cells ml⁻¹) were inoculated into 24-well microtitre plates at room temperature. The grazer abundance was calculated using an inverted microscope and biofilm biomass quantified by crystal violet staining. Transcriptomics of grazed biofilms was investigated by direct sequencing of cDNA generated from mRNA extracted from the grazed biofilm. While previous results showed that *Rhynchomonas nasuta*, a surface feeding protozoan, was killed by the *V. cholerae* antiprotozoal factor, *T. pyriformis* was not killed, but growth was inhibited. The grazed biofilms had higher substrate coverage of living bacteria and reduced numbers of dead cells than non-grazed biofilms as assessed by Live/Dead staining. The number of microcolonies increased while the size of the microcolonies did not. When *T. pyriformis* was physically separated from *V. cholerae* by a membrane which allowed chemical exchange, there was a large increase in the biofilm biomass. Planktonic cell numbers also increased when the predator and prey were separated. These results suggest that there is a chemical component that induces biofilm growth or metabolic activity. However, the biofilm biomass did not increase to the same extent as those that were physically grazed, suggesting stimulation of the biofilm is probably due to a combination of physical and chemical manipulation of the biofilm. Transcriptomic analysis identified genes expressed in response to grazing. The results suggest a complex relationship between grazing pressure, central metabolism, biofilm genes and stress responses.

Abstract Category
12 Interkingdom Interactions

PS.12.019 INCREASED SOIL pH IS A CONSEQUENT RESULT OF FUNGAL COLONIZATION THAT CREATES SUITABLE NICHE FOR ACID SENSITIVE BACTERIA

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Soil fungi and bacteria are key microbial groups with great environmental significance. There is a recent investigation about bacterial versus fungal abundances along a natural pH gradient in field soil showing pH 4.5 as a clear-cut determinative (threshold) point.

The objectives of the present study are (1) to evaluate the fungal effect on soil pH and (2) to assess whether *Lyophyllum karsteni* is able to increase soil pH to a level that raises the bacterial survival and/or growth, thus allowing them to benefit from locally-available nutrients. Soil pH is measured by conventional established method i.e. pH-H₂O, using a calibrated electrode while bacterial numbers are quantified by plating appropriate dilutions of sampled soil onto R2A (Difco) medium and counting CFUs/g soil after 3-days incubation at 28°C.

Bacterial strains *Variovorax paradoxus*, *Burkholderia terrae* and *Dyella japonica* rapidly dropped in CFU/g soil coming to the lowest detection limit in two days after inoculation in acidic soil (pH 4.5) without fungus but survived very well and even increased in CFU count in the presence of *Lyophyllum karsteni*. When the soil was limed to increase the pH along with 2% exogenous glycerol, *Variovorax paradoxus* survived well and got benefit of C-source only at increased pH values i.e. 6.5 and 5.5 compared to the original pH 4.5, indicating pH as important factor for bacterial survival and/or growth in soil. We also analyzed the actual increase in soil pH by the fungus *Lyophyllum karsteni* over time, and found that increase in pH is consequently more with more colonization by fungus over time. We, thus, conclude that soil fungus like *Lyophyllum karsteni* increases the environmental pH by its physiological activity upto the level where soil bacteria can happily live and utilize the available nutrients.

Abstract Category

12 Interkingdom Interactions

PS.12.020 WHY DO SOME MANY INVERTEBRATE SETTLE TO CORALLINE ALGAE?

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Many invertebrates have a biphasic life cycle that involves the transition of a planktonic swimming larva into a benthic adult mode of life. Coralline algae are important cues for this transition for a surprisingly diverse group of marine invertebrates. We conducted a quantitative literature review (meta-analysis) of sea urchin (Phylum Echinodermata) settlement which emphasised the importance of coralline algae as settlement cues. We then set out to determine whether different species of coralline algae induce settlement at similar rates, the nature of coralline algae as settlement cues and whether settlement responses are similar for different sea urchins. Settlement experiments using the sea urchins *Heliocidaris erythrogramma* and *Holopneustes purpurascens* showed greater settlement towards coralline algae with similar settlement among different coralline species. By reducing the abundance of live bacteria and disrupting the community that resides on the surface of the algae, we have shown that settlement is microbially driven for both urchin species. DNA fingerprinting (terminal restriction fragment length polymorphism) of the bacterial communities on coralline algae and co-occurring non-corallines revealed minor similarities in community composition refuting the idea that settlement is a community response and raising the hypothesis that only a small number of bacterial genera, present on all coralline species, may be responsible for settlement induction. Bacterial isolates from coralline algae were also tested and only one isolate was inductive for both urchin species. This isolate can be repeatedly isolated from all coralline algae tested. These results suggest a common cue among different coralline algae that larvae choose for settlement.

Abstract Category

12 Interkingdom Interactions

PS.12.021 ANAEROBIC HETEROTROPHIC NANOFLAGELLATES: THEIR ABUNDANCE AND BACTERIVORY IN A JAPANESE MEROMICTIC LAKE

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The abundance and bacterivory of anaerobic heterotrophic nanoflagellates (HNF) were investigated in an anoxic layer of Lake Suigetsu, a meromictic lake in Japan characterized by a permanent oxycline at a depth between 3 and 8 m separating the oxic freshwater layer from the anoxic, saline, sulfidogenic layer. Water samples were taken from anoxic layer (10 m) at the central basin of the lake between May 2008 and March 2010. Water samples were also taken from oxic layer (1 m) as a reference. Special care was made for determination of bacterivory in anoxic layer using an anoxically gassed bottle to prevent contact with air. HNF densities in anoxic layer varied from 2.2×10^2 to 7.1×10^2 cells ml^{-1} , whereas higher density of HNF (7.6 - 52×10^2 cells ml^{-1}) were detected in oxic layer. HNF densities in oxic layer decreased from summer to autumn and increased to spring, while that of anoxic layer did not show any distinct seasonal changing pattern. Bacteria densities in anoxic layer were lower than those of oxic layer. HNF bacterivory determined by the fluorescently labeled beads method showed clearance rates ($\text{nl HNF}^{-1} \text{h}^{-1}$) as 0.20 ± 0.28 in anoxic layer and 0.24 ± 0.35 in oxic layer. Significant difference in the clearance rate between the two layers was not found, suggesting that HNF grazing is also active in the anoxic water. The mean community grazing rate in anoxic layer was lower ($5.0 \pm 4.0 \times 10^2$ bacteria $\text{ml}^{-1} \text{h}^{-1}$) than that of oxic layer ($1.4 \pm 1.3 \times 10^4$ bacteria $\text{ml}^{-1} \text{h}^{-1}$). Bacterial production which drives microbial loop depends on the supply of organic matter derived from photosynthetic primary production. Low community grazing rates by HNF in anoxic layer were attributable to low bacterial density which might be due to low organic carbon supply from the euphotic layer. To our knowledge, this is the first study on the bacterivory by HNF in sulfidogenic, anoxic environments.

Abstract Category
12 Interkingdom Interactions

PS.12.022 ALGAL POPULATIONS INFLUENCE LAKE BACTERIAL COMMUNITY STRUCTURE

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Previous research has suggested that phytoplankton may be centrally important in determining the structure of aquatic bacterial communities. While correlations between algal and bacterial communities have been observed in natural as well as experimental systems, observational and experimental approaches are rarely combined to demonstrate both causality and ecological relevance. This study aimed to determine if correlative community patterns in humic lakes could be attributed to the influence of phytoplankton on bacteria by coupling algal manipulation experiments and observations of algal and bacterial communities in situ. Bacteria ($<1 \mu\text{m}$ size fraction) from three humic lakes were incubated with phytoplankton (20-100 μm size fraction) from the same three lakes and a no-phytoplankton control in a full factorial mesocosm experiment ($n=3$). Mesocosms were incubated in situ for 7 days. Initially distinct bacterial communities became more similar when incubated with the same phytoplankton assemblages. Correspondence analysis determined that algal treatment accounted for 32% of variation in bacterial community composition following incubation, indicating that algal assemblages structure bacterial communities. Each phytoplankton treatment consistently enriched for 3-4 bacterial populations in all bacterial treatments. Correlations among pelagic algal and bacterial populations were determined from community composition data collected weekly May-August 2003 and 2008 from each lake. Correlation of individual bacterial populations with specific algal species during seasonal algal succession agreed with observations from algal incubation experiments. Experimental manipulations provided evidence for a causal relationship driving correlated algal and bacterial community dynamics in humic lakes. Each algal population may represent a specific set of resources that enriches for different bacterial populations during seasonal succession.

Abstract Category
12 Interkingdom Interactions

PS.12.023 GENERAL SUPPRESSION OF FREE-LIVING NEMATODES BY BRASSICACEAE SEED MEAL

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Biologically-based management systems such as soil-organic residue amendments or "biofumigation" are promoted as alternatives for control of soil-borne pathogens in agro-ecosystems. *Brassica* plant residues contain glucosinolates which upon hydrolysis by endogenous myrosinase yields broadly-active biocidal compounds such as isothiocyanates (ITC). Incorporation of *brassicaceae* seed meal (BSM), a waste product of oil extraction processes (including biofuel), has been shown to suppress plant pathogenic fungi, oomycetes and nematodes. Although effective control of lesion (*Pratylenchus*) and root knot (*Meloidogyne*) nematodes is advantageous and well documented, the impact on plant beneficial or free-living populations is not well defined. Suppression of free-living bacterivorous nematodes may be detrimental to overall soil health since these populations contribute significantly to the process of nitrogen mineralization and plant bioavailability. Terminal restriction fragment length polymorphism and quantitative polymerase chain reaction analyses were utilized to determine the effect of BSM amendment on nematode community structure and abundance in orchard soils. The study employed BSMs with high (*B. juncea*) and low (*B. napus*) glucosinolate content, as well as the corresponding autoclaved BSMs possessing inactivated myrosinase function and subsequently diminished levels of ITC. As evidenced by decreased nematode abundance and diversity in lab-scale studies, *B. juncea* seed meal (SM) was an effective nematicide against free-living populations and performed equivalent to a commercially available product. The biocidal activity was dependent on generation of allyl ITC since both *B. napus* SM and heat-inactivated *B. juncea* SM enhanced overall nematode community diversity. This study indicates that the biocidal activity of *B. juncea* SM is non-specific to plant parasitic nematodes and further studies are required to assess the impact on N-mineralization and overall soil health.

Abstract Category
12 Interkingdom Interactions

PS.12.024 EARLY BLOOM GENES IN THE DINOFLAGELLATE GYMNODINIUM CATENATUM - A STEP TOWARDS BLOOM PREDICTION

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Gymnodinium catenatum is a paralytic shellfish toxin-producing dinoflagellate that was introduced into Australian waters via ballast water. Blooms cause economic loss but their dynamics are poorly understood. Cultures of *G. catenatum* contain mixes of auxiliary marine bacteria. We have shown that *G. catenatum* cysts surface sterilised and then forced to germinate will not propagate without bacteria. As a screening system this has allowed the identification of various bacterial strains, including *Marinobacter 879*, that are solely capable of supporting *G. catenatum* growth. Reasoning that communication between *Marinobacter 879* and *G. catenatum* must be a crucial factor in bloom dynamics, we investigated the nature of the signalling system. A pure culture of *G. catenatum* and *Marinobacter 879* was established by surface sterilising cysts and then inoculating them with *Marinobacter 879* prior to germination. Cultures were grown and then treated with a cocktail of antibiotics, predetermined to be toxic to *Marinobacter 879*. The addition of antibiotics resulted in a sudden and catastrophic collapse in the *Marinobacter* population within the culture. This was accompanied by a reduction in growth rate of *G. catenatum*. Cells were then resuspended in antibiotic-free medium and re-inoculated with *Marinobacter 879*. *G. catenatum* growth was revived. Reasoning that this system simulates the early phases of bloom development, we isolated mRNA at various timepoints, performed differential display and sequenced the *G. catenatum* transcriptome to identify genes differentially expressed during early bloom development.

Abstract Category
12 Interkingdom Interactions

PS.12.025 CHARACTERIZATION OF THE MICROBIAL DIVERSITY ASSOCIATED WITH ALGAL BLOOMS IN A EUTROPHIC FRESHWATER LAKE

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Freshwater quality and biodiversity are dependent on adequate nutrient levels. However, increasing rates of nutrient supply fuel accelerating primary production or eutrophication. A symptom of eutrophication is the development of large algal populations, called blooms. Blooms are a significant cause of water quality deterioration. However, freshwater algae are also a potentially rich source for

biodiesel and hydrogen production. Thus a better understanding of the organisms and processes involved in development and decline of freshwater algal blooms could be applied to improve management of both beneficial and detrimental aspects of algal growth. We characterized the planctomycete populations associated with a 2008 freshwater algal bloom in LaBonte Lake, WY. Using pyrosequencing, 9,488 planctomycete 16S rRNA gene sequences were recovered during the course of the bloom. Distinct lineages related to the genus *Rhodopirellula* were enriched in the algal mat, suggesting that these organisms may play a role in bloom dynamics. In 2009 we extended the analysis to characterize the algal, bacterial, and archaeal communities associated with a bloom event in the same lake. Bacterial and archaeal populations were characterized by 16S rRNA sequencing, while algal populations were described by plastid 23S rRNA sequencing. Samples were collected prior to the development of the bloom and progressing into the bloom decline. Over the season we obtained 81,511 archaeal, 158,634 bacterial and 150,058 algal sequence tags. Preliminary analyses suggest that the microbial populations display biogeography in the lake, with specific organisms enriched in the algal mat. For example, bacteria assigned to the *Alphaproteobacteria* were the dominant non-algal bacteria in the mat, but were a minority component of other samples. Currently, metagenomic and metatranscriptomic analyses are being conducted to define the metabolic processes occurring during different developmental stages of the bloom.

Abstract Category
12 Interkingdom Interactions

PS.12.026 CONTROL OF CELL-BOUND NITRATE IN COASTAL MARINE SEDIMENT BY THE POLYCHAETE WORM *NEREIS DIVERSICOLOR*

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We propose a mechanism of nitrogen cycling in coastal marine sediments that increases the concentration of nitrate stored in the cells of sedimentary organisms. This mechanism involves the interaction of different trophic levels (i.e., worms, bacteria, and diatoms) and converts particulate organic nitrogen (PON) into reactive nitrogen that fuels microbial processes. Sediment microcosms were set up with or without *N. diversicolor*, a polychaete worm abundant in intertidal sediment. Additional treatments were amended with ammonium to mimic animal excretion or with allylthiourea to inhibit nitrification by sediment bacteria. Cell-bound nitrate concentrations, as measured by a freeze-thaw technique, generally exceeded porewater nitrate concentrations as measured with microsensors. Notably, cell-bound nitrate was present at depths at which porewater nitrate was depleted. Pigment analysis in different sediment layers revealed that cell-bound nitrate was correlated with the abundance of diatoms. Addition of *N. diversicolor* or ammonium to microcosms increased cell-bound nitrate concentrations, whereas addition of *N. diversicolor* and nitrification inhibitor decreased cell-bound nitrate concentrations. Microsensor profiles of porewater nitrate further substantiated that cell-bound nitrate was correlated with bacterial nitrification, which was particularly active and reached deeper into the sediment in microcosms with *N. diversicolor*. Apparently, the worms stimulate nitrification by feeding on PON and excreting ammonium and by oxygenating the sediment through pumping behavior. Ammonium is then nitrified to nitrate in the oxic sediment layer and stored intracellularly by diatoms, and possibly by foraminifers and sulfur bacteria. This mechanism may accelerate the conversion of PON into reactive nitrogen that fuels benthic primary production, but may, after cell lysis, also serve as an electron acceptor in anoxic sediment layers.

Abstract Category
12 Interkingdom Interactions

PS.12.027 ENVIRONMENTAL AND EVOLUTIONARY FACTORS THAT SHAPE FISH GUT BACTERIAL COMMUNITIES

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Gut bacteria play important physiological roles in their animal hosts, influencing metabolic processes either by altering host gene expression or by directly breaking down digested food items. Because of their contribution toward energy extraction, it is important to assess the factors that influence bacterial gut communities within digestive tracts. Previous literature has shown that host taxonomy shapes gut microbes in the mammalian gut indicating co-evolution between microbes and their hosts. Mammalian gut communities are also influenced by trophic level. To date, it is unclear whether such relationships hold in other organisms, such as fish. In this study, we compiled 16S rRNA bacterial sequences from fish gut associates that had been deposited in GenBank as well as those that have been isolated in our lab. These data allowed us to investigate the phylogenetic relationships of fish gut bacteria. We then explored factors shaping fish gut communities, focusing on the fish hosts' taxonomy, environment (i.e. freshwater, marine, farm-reared or wild caught) and diet. Our analyses reveal that various factors influence fish gut bacterial communities. Using UniFrac analyses, we found significant differences among bacterial communities from predatory versus omnivorous fish. Also, wild caught fish appeared to have different communities than lab- or farm-reared fish. Similarly, bacteria from saltwater fish were distinct from those of freshwater fish. Although we found a suggestive effect of host taxonomy on the composition of gut communities, it was not significant. In summary, a variety of environmental factors appear to shape the gut flora of fish, but a more thorough examination of closer related fish may elucidate a stronger relationship between gut bacteria and fish phylogeny. Through future analyses, we aim to disentangle the effects of these variables, thus attempting to determine if gut bacteria have facilitated the evolution of various fish diets.

Abstract Category

12 Interkingdom Interactions

PS.12.028 INDIRECT POSITIVE EFFECT OF ALGA ON VIABLE BUT NON-CULTURABLE STATE OF A BACTERIAL STRAIN CAUSED BY AN ACTINOMYCETE AND VISIBLE LIGHT ILLUMINATION

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Combined effects of other microorganisms and visible light illumination on a viable but non-culturable (VBNC) state of a bacterial strain of concern was studied. *Burkholderia* sp. strain DP-4 decreased 3.6×10^5 mL⁻¹ of colony forming unit (CFU) 1/100-fold in a mixed culture with ca. 10^5 mL⁻¹ of actinomycete, *Rhodococcus* sp. strain LM-1, under the illuminated condition of visible light (6,500 lux, 12 hr d⁻¹). On the other hand, the CFU of DP-4 was constant at ca. 10^5 in a mixed culture with 10^5 of LM-1 under the non-illuminated condition and in a pure culture irrespective of the illuminated condition. In a filter-sterilized medium in which 10^5 of LM-1 was cultured prior to filtration, DP-4 decreased the CFU 1/100-fold under the illuminated condition, while it decreased the number of viable cells estimated by cell membrane integrity only 2/3-fold. Thus, DP-4 may enter into a VBNC state by combined effect of exudate of LM-1 and visible light illumination. However, in a mixed culture with LM-1 and an alga, *Chlorella ellipsoidea*, the CFU of DP-4 was constant at 1.1×10^6 mL⁻¹ even under illuminated condition, while the CFU of LM-1 was at 10^3 . The CFU of LM-1 in a mixed culture with *C. ellipsoidea* was 1/100-fold lower than in pure culture. In a filter-sterilized medium in which *C. ellipsoidea* was cultured prior to filtration, the CFU of LM-1 was constant at ca. 10^3 , but that was at 10^5 when phosphate was supplemented. On the other hand, the CFU of LM-1 was constant at 10^5 in a fresh medium with 1/100-fold concentration of phosphate. Thus, the lower level of CFU of LM-1 cultured with *C. ellipsoidea* may be attributable to the deficiency of phosphate available to LM-1 resulting from consumption by the alga, or to the suppressive effect of algal exudate on LM-1. In conclusion, DP-4 may not enter into a VBNC state due to indirect effect of *C. ellipsoidea*, i.e., the alga suppresses the cell number of LM-1.

Abstract Category

12 Interkingdom Interactions

PS.12.029 PROTEOME ANALYSIS OF MIXED BIOFILM CANDIDA ALBICANS - PSEUDOMONAS AERUGINOSA.

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The interactions among microbes inside the human body are of great importance for the human health and the diseases development. The study of microbe-microbe interactions is essential for understanding *in vivo* activities of commensal and pathogenic microorganisms. *Candida albicans*, a polymorphic fungus, and *Pseudomonas aeruginosa*, a Gram-negative bacterium, are two opportunistic pathogens that can cause infections in different sites within the human host. In order to determine how *P. aeruginosa* affects the physiology of *C. albicans* and vice versa, we compared the proteome of mixed biofilms versus single species biofilms. We found that in mixed biofilms *P. aeruginosa* overexpressed proteins involved in the synthesis of the siderophore pyoverdine, receptors for hydroxamates, outer membrane proteins involved in drug resistance, proteases, and other elements of lipid metabolism. We detected double the concentration of pyoverdine in the mixed biofilm. Furthermore, we demonstrated that *C. albicans* secretes small molecules that strongly induce synthesis of pyoverdine. Interestingly, one of these molecules has the ability to interact with iron ions suggesting it is siderophore produced by *C. albicans*. Overall results suggest that *P. aeruginosa-C. albicans* interaction is that of competition for iron capture and that virulence factors from both organisms are involved. It is important to note that competition for the metal involved a probable signaling mechanism by which the bacterium senses molecules secreted by its competitor.

Abstract Category

12 Interkingdom Interactions

PS.13.001 LOW CELL NUMBERS AND DOMINANCE OF ARCHAEA IN OLIGOTROPHIC MARINE SEDIMENTS OF NORTH POND; MIDDLE ATLANTIC RIDGE

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Abundance and distribution of microorganisms are of interest to study life in oligotrophic marine sediments which comprise a major part of the deep biosphere. Therefore, the microbiology of the oligotrophic, carbonate rich sediments of the basin North Pond on the western flank of the middle Atlantic ridge is subject of this study. Sediments were sampled during the IODP site survey cruise in 2009 up to a depth of more than 8 m below seafloor. The application of published protocols for total cell counts and real-time PCR quantification (qPCR) of *Bacteria* and *Archaea* resulted in a high variability and a low reliability of the data. For improvement, published protocols were modified. To validate these protocols, sterilized sediments were spiked with cells of pure cultures. A comparison of cell recovery for the different protocols was carried out. Concerning qPCR, a high cell recovery was achieved using the protocol by Webster et al. (2003) with a previous washing step with 0.1 mM HCl or HI for extraction of prokaryotic DNA. Concerning total cell counts, a good cell recovery was obtained with modifications of the protocols described by Weinbauer et al. (1998) and Kallmeyer et al. (2008) especially for samples with low cell numbers. Depth profiles of cell numbers for four sites with the optimized protocols show low cell numbers (104 to 107 cells per mL sediment) with dominance of *Archaea* over *Bacteria* with an average difference in cell numbers of two orders of magnitude. *Archaea* have been described to be better adapted to low energy fluxes in oligotrophic extreme environments.

Kallmeyer et al. 2008. *Limnology and Oceanography Methods* 6: 236-245. Webster et al. 2003. *Journal of Microbiological Methods* 55: 155-164. Weinbauer et al. 1998. *Applied and Environmental Microbiology*. 64: 5000-5003.

Abstract Category

13 Life at the Energetic Edge

PS.13.002 PHOTOSYNTHESIS ON THE EDGE OF LIGHT: THE CHROMATIC EFFICIENCY OF A LOW-LIGHT SPECIALIST

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Two decades ago, a strain of obligately phototrophic green sulfur bacteria *Chlorobium phaeobacteroides* was found living in the deep chemocline of the Black Sea and was later shown to fix CO₂ at light levels as low as 0.015 μE/m²/s (~0.005% of the daylight level of radiation between 400-700nm). Strain BS1 has held the record for low-light photosynthesis ever since.

This tenacious organism uses an impressive arsenal of tricks to efficiently harvest and use the low levels of energy from photons that it receives in its dark home including unusually large chlorosomes and low maintenance energies. What is still unknown, however, is how efficiently BS1 uses the different wavelengths of light available to it. How efficiently organisms can funnel the energy received by antenna pigments (which, together, absorb a wide range of wavelengths) to the far more selective reaction center pigments determines the relationship between incident light and the energy that is made chemically available for the organism.

This poster presents the preliminary results of experiments investigating the response of BS1 to different wavelengths of light at sub-saturating levels ($<6E17$ quanta/m²/s). Batch cultures of BS1 were exposed to controlled, sub-saturating levels of light from 30 different light-emitting diodes with peak wavelengths of light covering the spectrum from 370-960nm (spectral full-width half-max of the light sources ranged from 15-35nm). The rate of sulfide oxidation - a key reaction in the photosynthetic pathway of green sulfur bacteria - was measured and used to determine the rate of photosynthetic electron flow. By comparing the resulting action spectra with the in vivo absorbance spectrum of BS1, the organism's light-harvesting efficiency at different wavelengths was determined.

Abstract Category

13 Life at the Energetic Edge

PS.13.003 A CLASSIFICATION METHOD USING MALDI-TOF MASS SPECTROMETRY AND SUBSEQUENT MULTIVARIATE DATA ANALYSIS FOR ENTEROCOCCUS FAECALIS AFTER STRESS

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As previous experiments proved, MALDI/TOF-MS is capable to distinguish between different cell states of a single species population. We use this technique to classify the viable but not culturable (VBNC) stage as a survival state of bacteria caused by starvation and cold as well as their resuscitation in comparison to cells during exponential growth phase.

In this study *Enterococcus faecalis* was selected as model organism. The generated "molecular fingerprint" spectra were subjected to multivariate data analysis without targeting single bacterial molecules or molecule structures and were compared to the corresponding growth curve afterwards. *E. faecalis* arrested in VBNC state for 42 days was recultivated by incubation in BHI media at 37 °C under shaking. After 3 h and 6 h, respectively, an aliquot of bacterial suspension was analysed by MALDI/Tof-MS. As a control, bacteria in the exponential growth phase were analysed. For an effective data analysis a multivariate approach using hierarchical cluster analysis and principle component analysis was applied in order to classify each state in comparison to each other.

The present method enables a rapid classification of bacteria which were kept in VBNC state and within different stages of recultivation.

Abstract Category
13 Life at the Energetic Edge

PS.13.004 "THE STARVING MAJORITY" PUT TO THE TEST

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The deep biosphere is potentially the largest prokaryotic habitat on Earth [1] and active microorganisms have been found in very old and deep sediments [2]. With extremely limited energy sources these prokaryotes face starvation and ultimately death. So how do they survive? Starvation-survival experiments have mostly used aerobic pelagic microbes and subsurface prokaryotes may differ considerably. Therefore, we compared a range of typical deep subsurface isolates and their near surface counterparts to substrate deprivation under anaerobic conditions. Some 30 prokaryotes were investigated covering several physiological groups (e.g. sulphate reducers, methanogens, heterotrophs). These included both type strains and isolates from Aarhus Bay, Black Sea, Mediterranean Sea, Wadden Sea, and an ultra-oligotrophic cold-water carbonate mound (IODP Leg 307, [3]). Our results show considerable resistance towards starvation not only for deep biosphere isolates but also for near-surface strains. Often no significant decline in total cell numbers occurred up to a maximum of two years of starvation. There was a high proportion of "live" (20-100%) and FISH-detectable cells (0-70%). Culturability did decrease over time, but interestingly, was higher under oxic than anoxic conditions for facultative anaerobes. *Photobacterium* strains could remove volatile fatty acids from the medium during starvation despite apparently not being able to utilise these compounds under normal anaerobic cultivation. Experiments are underway assessing intracellular enzyme activities (Celltracker™) and substrate uptake and turnover (radiotracer experiments). Thus far deep biosphere isolates do not seem to be better adapted for starvation survival than their near-surface relatives. All prokaryotes exhibited a remarkable resilience to long-term starvation under anaerobic conditions. [1]Whitman et al.1998 PNAS 95: 6578ff [2]Roussel et al.2008 Science 320: 1046 [3]Webster et al.2009 Env. Microbiol. 11: 239ff

Abstract Category
13 Life at the Energetic Edge

PS.13.005 GENE EXPRESSION OF LONG TERM STARVED *VIBRIO VULNIFICUS*

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Ingestion of oysters harboring *V. vulnificus*, a bacterium existing naturally in estuarine environments, causes 95% of all seafood-related fatalities in the USA. This pathogen has two genotypes; C types associated with clinical isolation and E types with environmental sources. In the environment, planktonic *V. vulnificus* cells must be able to survive long periods in low nutrient seawater. The purpose of this study was to observe the effects of long term (to 19½ years) starvation of C and E genotype strains of *V. vulnificus* cells. At

intervals, RNA was extracted and used for RT-PCR analysis to examine expression of 23 genes. These included *vvhA* (hemolysin), *wzb* and *wza* (capsule), *rpoD*, *rpoE*, and *rpoS* (sigma factors), *pilA* and *flgE* (pilin and flagella, respectively), *mutL* and *mutS* (mismatch repair), *luxS* and *smcR* (quorum sensing), *rtx* (toxin), *ompU* (outer membrane protein), *recA* (SOS response), *relA* (stringent response), *oxyR* (oxidative stress), *groEl* and *hfq* (stress related chaperones), *cspA* (cold shock protein), *katG* (catalase), *trkH* (potassium transport), and *venB* (siderophore). Some of these (e.g. *mutL*, *mutS*, *groEl*, *wza*, *recA*, *flgE*, *relA*, *venB*) were not expressed in starved cells, whereas others (e.g. *vvhA*, *rpoS*, *rpoD*, *rpoE*, *oxyR*, *luxS*, *rtx*, *katG*, *ompU*, *trkH*) were expressed for at least 2 months and several (*wzb*, *pilA*) for up to 31 months. One gene involved in quorum sensing, *smcR*, was not expressed during early starvation, but was expressed later and was still observed at a low level for up to 19 years. These 19 year old samples also expressed *hfq*, while *cspA* was expressed through 6 years of starvation. The significance of each of these findings will be discussed relative to starvation survival and their role in virulence. Based on this study, however, it is clear that *V. vulnificus* is able to maintain metabolic activity, including the expression of virulence-related genes, in low nutrient seawater conditions for extended periods of time.

Abstract Category

13 Life at the Energetic Edge

PS.13.006 AN INCUBATION SYSTEM TO DETERMINE THE TEMPERATURE AND PRESSURE AS MICROBIAL PHYSIOLOGICAL VARIABLES IN LOW-ENERGY CRUSTAL FLUIDS

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Advanced Integrated Ocean Drilling Program (IODP) circulation obviolation retrofit kit (CORK) observatories recently installed on the flanks of Juan de Fuca Ridge provide a unique opportunity to sample the otherwise inaccessible pore fluids (altered seawater) from oceanic basaltic crust. Aging porewaters remain isolated within sediment-buried upper oceanic basement, subjected to increasing temperatures and pressures as plates move away from spreading ridges. Strong physical gradients acting at different spatial and temporal scales may influence the metabolic processes potentially active in these habitats.

High pressure thermal gradient systems are commonly used to study the pressure and temperature response of microbial metabolism. Current designs, however, have several practical limitations related to the maximum operational pressures and, most important, to the monitoring of multiple samples under constant pressure during long-term incubation experiments. Here we introduce an advanced instrument for the incubation of liquid samples at ultra high pressures (1000 Bar) and temperature gradients (up to 150 °C) that allows continuous sampling during time-course experiments. The latter is a

desirable feature for exploring the potential for microbial metabolisms in energy-limiting habitats where prolonged incubation periods are often required.

During an upcoming cruise to the Juan de Fuca Ridge flanks (June, 2010), we will explore the correlation between active microbial metabolic processes in fluids from ocean crust and the physical parameters that characterize these habitats. We will present the results from measurements of potential rates of microbial energy metabolism over a wide range of pressures and temperatures.

Abstract Category

13 Life at the Energetic Edge

PS.13.007 COMMON EUTROPHIC BACTERIA CAPABLE TO GROW UNDER EXTREMELY OLIGOTROPHIC CONDITIONS

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The isolation and cultivation of marine, planktonic bacteria is always a challenge, because the natural habitat - the open ocean - is strongly limited in available energy sources. Whenever a complex, organic-rich medium is used for isolation and cultivation of heterotrophic bacteria, the available substrate concentrations are much higher than under natural conditions and might even inhibit the growth of some oligotrophic bacteria. However, if an extremely oligotrophic medium is used, which resembles the conditions of the original habitat, the substrate concentrations and cell numbers are close to detection limit and require the development of new techniques and routines to study bacterial growth. We isolated several bacteria from marine environments on an extremely oligotrophic medium. Surprisingly, the obtained oligotrophic isolates, which grow in pure artificial seawater with an organic carbon content of about 0.1 mg C l⁻¹, are also able to grow under eutrophic conditions on complex media. All isolates are able to switch within days from eutrophic to oligotrophic growth and vice versa. This special adaptation to changing substrate concentrations was employed as an isolation strategy. To avoid contamination of the oligotrophic medium by organic substances, all glassware used for medium preparation was acid-cleaned, and glassware and sodium chloride was combusted prior to use. Additionally, the gas phases in media bottles were exchanged with synthetic air to prevent contamination from the laboratory air. With this technique we were able to isolate 15 strains from different phylogenetic groups, as demonstrated by 16S rRNA analysis. This leads us to suspect, that the ability to grow under extreme substrate limitation is more widespread among heterotrophic bacteria than previously recognized. We have now initiated a proteomic study to find out more about the physiology of the oligotrophic growth and involved enzymes.

Abstract Category

13 Life at the Energetic Edge

PS.13.008 GROWTH AND METHANE PRODUCTION CHARACTERISTICS OF A NOVEL METHANOCALDOCOCCLUS SPECIES ISOLATED FROM AXIAL VOLCANO

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A hyperthermophilic methanogen, strain JH146, was isolated from low-temperature hydrothermal fluid collected from Marker113/62 vent at Axial Volcano. As determined by 16S rRNA gene sequence analysis, this organism is closely related to Methanocaldococcus species. The new isolate is a strictly anaerobic, methanogenic autotroph capable of using hydrogen and carbon dioxide as its sole energy and carbon sources. In the presence of ammonia, growth and methane production were observed between 58 and 90°C (optimum of 82°C, 24 min doubling time), between pH 4.5 and 9.0 (optimum pH 6.0-6.5), and between 4.5 to 54 g/L NaCl (optimum 18 g/L NaCl). Under non-stressful conditions, methane was produced at a uniform rate (0.16 ± 0.08 pmol CH₄/cell/doubling). At the limits of growth, where doubling times increased, the methane production rate increased up to 0.98 pmol CH₄/cell/doubling indicating that the organism was stressed and compensated for this by increasing its rate of metabolism. The organism's growth was also evaluated under different hydrogen concentrations (10-225 μM) in a 2-L gas-flow controlled bioreactor. With decreasing hydrogen availability, maximum cell concentrations decreased and doubling times increased. There was no growth at 10 μM hydrogen. This study emphasizes the importance of determining the environmental constraints on growth with concomitant methane production rates. This is valuable for quantitative modeling of growth in hydrothermal environments and refining our understanding of microbial distribution, thermodynamic models, environmental geochemistry data sets, and microbial interactions with other microbes and their environment.

Abstract Category

13 Life at the Energetic Edge

PS.14.001 CHARACTERISING THE MICROFLORA OF AEOLIAN DUST AND ITS SOURCES IN AUSTRALIA

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Various microorganisms have been isolated from dust and aerosols globally. Australia is the main source of dust in the Southern hemisphere with the primary source being inland salt lakes and deserts of the Lake Eyre basin (LEB) and central and western South Australia (CWSA), however, little is known about the microflora of Australia's dust and its sources. The aim of this study was twofold: 1) to determine if Automated Ribosomal Intergenic Spacer Analysis (ARISA) could be used to "fingerprint" the source of dust; and 2) to investigate the microflora of various different dust source samples. ARISA profiling was performed on LEB and CWSA source samples, a dust sample collected in Canberra, and dust and source samples collected from a small salt lake in Victoria, which regularly deflates and deposits material locally. For the source samples, the corresponding pH, nutrient and pigment analyses were also added to the database. The ARISA profiles of the source and dust samples were compared using a Nonmetric Multidimensional Scaling (NMDS) plot. The Canberra dust sample clustered separately to the source samples suggesting that the microflora represented a mixture of source organisms and/or a conglomerate sample comprised of LEB/CWSA sources and other organisms deflated at the front of the dust storm. In contrast, the ARISA profiles of dust and source samples collected from the Victorian salt lake clustered together suggesting that the microflora of the dust sample was a subset of its source. Multivariate statistical analysis was used to compare the ARISA profiles, pH, and nutrient content. Preliminary results suggest that microbial communities differed among different provinces in Australia. Collectively, the results indicate that dust and dust sources in Australia have a diverse microflora. While ARISA is useful in investigating source microflora, analysis of dust at the site of deposition requires a multidisciplinary approach to accurately determine its source.

Abstract Category

14 Microbe-Mineral Interactions

PS.14.002 A PEPTIDE-BASED PROBE FOR THE CAPTURE OF MANGANESE OXIDES AND ASSOCIATED MICROORGANISMS.

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Microorganisms mediate important biogeochemical transformations. Mn-oxidizing microbes, including members of the genera *Bacillus*, *Aurantimonas*, *Pseudomonas*, *Roseobacter* and *Erythrobacter*, catalyze the conversion of Mn (II) to insoluble Mn (IV) oxides. Studies using these and other cultured taxa are providing valuable insights into oxidation mechanisms. Phylogenetic analyses alone are insufficient to identify which microbes may be involved in Mn oxidation *in situ*. This study aimed to identify the microorganisms associated with naturally occurring Mn oxides in environmental samples using a peptide probe specific for biogenic Mn oxides. An Mn oxide specific peptide probe was selected from a phage display library expressing random dodecapeptides by multiple rounds of panning. The selected peptide probe was coupled to magnetic beads and its oxide-capture capability optimized using Mn-oxidizing bacteria. The probe was then used to select for Mn oxide particles and associated bacteria in environmental samples, including microbial mats from Loihi Seamount (HI), Carter Salt Peter Cave (TN) and the Columbia River (OR/WA). Captured microbes were identified with 16S rRNA-based techniques and by culturing. Results demonstrated that significantly more material hybridized to the probe-coated magnetic beads, compared to controls with no beads, with beads only and no probe, or with beads and a random probe. Phylogenetic analysis of the bacterial communities found in the Mn-probe captured material from the study sites revealed an abundance of *Alphaproteobacteria*, and a modest enrichment of *Pseudomonas* spp., relative to material captured in a control experiment with no probe. These analyses have identified putative new Mn oxidizers among the *Rhodobacter/Roseobacter* clade. Results are supported by the isolation of a new Mn oxidizer in this clade, whose 16S rRNA gene sequence has 94% identity to a sequence in the Mn probe captured library.

Abstract Category
14 Microbe-Mineral Interactions

PS.14.003 SIDEROPHORE BIOSYNTHESIS IS INVOLVED IN MANGANESE OXIDE REDUCTION IN *SHEWANELLA ONEIDENSIS* MR-1

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Dissimilatory metal-reducing bacteria, such as *Shewanella oneidensis* MR-1, are of great interest for their importance in the biogeochemical cycling of metals and their utility in biotechnological processes, such as, bioremediation, and microbial fuel cells (MFCs). Extensive studies have been performed to identify cellular components in MR-1 that are involved in extracellular electron transfer and metal respiration, it is still far from complete understanding of molecular mechanisms underlying these complex physiological processes. In this study, we screened a random mutant library of MR-1 for identifying as-yet-unknown genes involved in metal reduction. A transposon mutant library of MR-1 was grown on lactate minimal medium plates containing MnO₂ under aerobic and anaerobic conditions for isolating mutants deficient in the MnO₂-reduction ability. We isolated a mutant deficient in MnO₂ reduction from a plate incubated under the aerobic condition. This mutant, named N22-7, also showed the decreased MnO₂-reduction rate in anaerobic liquid cultures. Determination of the transposon-insertion site in N22-7 followed by deletion and complementation experiments revealed that the disruption of SO3030, a putative siderophore biosynthesis gene, was responsible for the decreased MnO₂-reduction rate. The LC-MS analysis of the supernatant extracts from the wild-type and ΔSO3030 cultures revealed that SO3030 was involved in the biosynthesis of putrebactin, a siderophore that is also produced by other *Shewanella* species. In ΔSO3030 cells, iron and cytochrome contents were decreased to 50% of those in the wild-type cells when incubated under MnO₂-reduction condition. In addition, the expression of *c*-type cytochrome genes necessary for metal reduction, e.g., *omcA* and *mtrC*, was repressed in ΔSO3030 under MnO₂-reduction conditions. These results suggest that siderophore is important for iron acquisition and cytochrome biosynthesis under anaerobic metal-reducing conditions.

Abstract Category
14 Microbe-Mineral Interactions

PS.14.004 CHARACTERIZATION OF THE C-TYPE CYTOCHROME UNDA-HRCR-6 OF A *SHEWANELLA* STRAIN ISOLATED FROM THE HANFORD REACH OF THE COLUMBIA

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The outer membrane *c*-type cytochromes (*c*-Cyt), such as MtrC and OmcA of *Shewanella oneidensis* MR-1, play critical roles in extracellular reduction of Fe(III) oxides and U(VI). In an effort to characterize the outer membrane *c*-Cyts found in the *Shewanella* strains isolated from the Hanford Reach of the Columbia River (HRCR), we tested 7 HRCR isolates for the presence of *mtrC/omcA/undA/undA1*-like genes. PCR and sequencing results showed that all 7 screened strains possessed *mtrC*-like genes, while 3 strains had *omcA*-like genes and the remaining 4 strains contained *undA/undA1*-like genes. The entire coding region of an *undA/undA1*-like gene from HRCR isolate 6 (HRCR-6) was sequenced, because this strain was isolated from a uranium seep near the Hanford 300A and reduced both U(VI) and Tc(VII). These radionuclide contaminants are of particular concern at DOE's Hanford Site. The results showed that this *c*-Cyt (i) contained 843 amino acid residues, (ii) had 11 putative heme-binding sites, and (iii) was 73% and 93% identical to the UndA1 of *S. putrefaciens* CN-32 and the UndA of *S. baltica* OS223, respectively. Based on these results, we named this protein UndA-HRCR-6. UndA-HRCR-6 was overexpressed in *S. oneidensis* MR-1 cells and purified as a functional *c*-Cyt from the membrane fraction, indicating that it is most likely an outer membrane *c*-Cyt. In vivo, UndA-HRCR-6 complemented, at least in part, *S. oneidensis* MR-1 mutant lacking MtrC and OmcA in Fe(III) oxide reduction as well as extracellular U(VI) reduction. The recombinant UndA-HRCR-6 was translocated across the bacterial outer membrane by the MR-1 type II secretion system. Collectively, these results indicate that UndA-HRCR-6 is a functional extracellular metal reductase.

Abstract Category
14 Microbe-Mineral Interactions

**PS.14.005 RESPONSE OF THE MICROBIAL COMMUNITIES OF THE SEINE ESTUARY
SEDIMENTS TO METAL STRESS**

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The "RHAPSODIS" multidisciplinary project of the "Seine Aval" scientific program aims to study the transfer and deposit processes of fine sediments and its associated chemical and bacterial contaminants in the Seine estuary (France). The aim of this work is to investigate the occurrence of bacterial heavy metal resistance genes in relation with the chemical contamination of sediments. The prevalence of these genes was assessed in sediments collected along a 442-cm-long core sampled in the fluvial Seine estuary.

The heavy metals Cd²⁺, Ag²⁺ and Hg²⁺ concentrations were measured and are more important in depth and tend to decrease towards the surface of the core. Crustal enrichment factors are very high in particular for Cd²⁺ and Ag⁺ (300 and 400 respectively).

The *czcA* gene encoding a heavy metal efflux pump (HME-RND) which confers bacterial resistance to Cd²⁺, Zn²⁺ and Co²⁺ was detected all along the core. The *merA* gene, encoding the mercuric reductase involved in bacterial mercury detoxification from β/γ *Proteobacteria* was only detected in recent sediment deposits. In contrast, the *Actinobacteria merA* genes were amplified in depth as well as in surface sediments. The bacterial resistance genes to silver (*silA*, HME-RND) were also investigated and were detected in all analyzed sediments.

Results suggest that the presence of *Actinobacteria merA*, *czcA*, and *silA* genes in the mudflat sediments of the Seine estuary, could result from the response of the microbial communities to high concentrations in Cd²⁺ and Ag⁺ and Hg⁺. A quantification of the *czcA* and *silA* genes is planned in order to compare the abundance of these genes and the concentrations in heavy metals along the core. Furthermore, the impact of the heavy metals contamination on the *merA* and *czcA* genes diversities will be investigated.

Abstract Category
14 Microbe-Mineral Interactions

**PS.14.006 HEAVY METAL BIOREMEDIATION BY GENETICALLY MODIFIED
CUPRIAVIDUS METALLIDURANS CH34**

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Heavy metals are widespread pollutants, that are non-degradable and thus persistent in the environment, with severe consequences for human and animal health, besides causing negative impacts for natural ecosystems. Conventional chemical or physical remediation procedures are inherently problematic in their application because they are often inadequate to reduce heavy metal concentrations to acceptable regulatory standards and are in general cost-expensive and potentially risky. Alternative environmentally friendly methods for treatment, known as bioremediation, have been developed using microorganisms as biosorption agents because of the lower costs, good performance and safety. The present work describes the targeting of a synthetic metal-chelating protein to the cell surface of the heavy metal-resistant *Cupriavidus metallidurans* CH34 bacterial strain, viewing to metal bioremediation. For this purpose, an analog gene of the natural phytochelatin (Glu-Cys)_nGly with n=20, EC20sp, was synthesized in vitro and fused to the autotransporter β -domain coding sequence of the *Neisseria gonorrhoeae* IgA protease, which successfully targeted the hybrid protein towards the bacterial outer membrane. The translocation, surface display, and functionality of the EC20sp- β -domain protein were initially demonstrated in *Escherichia coli* upon insertion of the fused protein under control of the *plac* promoter. As for the *Cupriavidus metallidurans* CH34 strain, the EC20sp- β -domain gene fusion was placed under the

control of a strong constitutive heterologous promoter which allowed high levels of basal expression, that were even higher in the presence of metal ions. In comparison to the wild type, the recombinant strain, *C. metallidurans*/pCM2, proved to have significantly enhanced ability for immobilizing, Co (II), Cd(II), Mn(II), Zn(II), Cu(II), Ni(II), Pb(II), and Fe(II) ions from the external media. Support: VALE Mining Company.

Abstract Category
14 Microbe-Mineral Interactions

PS.14.007 A UNIQUE NICHE FOR THE METAL REDUCING BACTERIUM *GEOTHRIX FERMENTANS* BASED ON ELECTROCHEMICAL EVIDENCE

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Bacteria able to transfer electrons beyond their outer surface can utilize insoluble metals in anaerobic respiration, affecting redox geochemistry. Many metal-reducing bacteria also use electrodes as electron acceptors, providing opportunities to produce electrical current, and use electrochemistry to understand the effect of thermodynamic driving force on the kinetics of microbial electron transfer. We developed techniques for cultivating biofilms of the one known representative of anaerobic *Acidobacteria*, *Geothrix fermentans*, using poised electrodes. Electrochemical analysis revealed a more complex response to redox potential than *Geobacter* or *Shewanella* species, and a preference for much higher redox potentials than what has been reported for any other metal-reducing organism. While the *Geobacteraceae* consistently demonstrate a simple Nernstian response to potential, reaching a maximum electron transfer rate near potentials of 0 V (vs. SHE), *G. fermentans* required potentials of nearly +0.5 V to achieve maximum rates of electron transfer. Electron transfer by *Geothrix* also responded in a biphasic manner, suggesting at least two potential-dependent pathways. When medium surrounding biofilms was replaced, electron transfer dropped over 50%, consistent with a soluble compound mediating part of the reaction. Medium replacement predominantly affected electron transfer in only one of the two potential windows. These results provide insight into a possible environmental niche, suggesting *Geothrix* should be competitive where electron acceptors are at a redox potential greater than +0.2 V, such as those containing Mn(IV) or chelated Fe(III) metals, or close to the oxic-anoxic interface. These electrochemical results show how two metal-reducing organisms capable of oxidizing electron donors with identical midpoint potentials (acetate), can still be adapted to unique electron acceptor potentials

Abstract Category
14 Microbe-Mineral Interactions

PS.14.008 EFFECT OF ZINC AND CADMIUM AT LOW CONCENTRATIONS ON BACTERIAL PROTEASES IN SEAWATER

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Metals contamination in the sea affects bacterial activity and also the material cycling in microbial loop. Zinc (Zn) is an essential and Cadmium (Cd) is a toxic metal for biota including bacteria. These metals accelerate or inhibit enzyme activities. However, very little is known about the effects of these metals at low concentrations than the available knowledge on toxic doses mainly focusing on bacterial extracellular enzymes. Aim of this study is to clarify the effects of Zn and Cd on bacterial protease, which has important role in protein processing in the marine environment. The dose dependent effect of Zn and Cd on bacterial abundance and proteolytic activity were studied for up to 72 hr by passing 0.6- μ m fraction of seawater which is grazers free and contains only dissolved matter and bacteria. Protease activities (aminopeptidase-, trypsin- and chymotrypsin-type activities) were measured by methylcoumarylamide (MCA)-substrate method. Bacterial number was enumerated using epifluorescence microscopy. As a result it was found that Zn and Cd did not cause any significant change on the bacterial abundance, all through the 72 hr incubation after the addition of Zn and Cd. However, Zn and Cd clearly suppressed the increase in aminopeptidase activity at low concentrations (1.36 μ g/L and 5 μ g/L of Zn and Cd, respectively). Trypsin activity was high at the initial stage of the experiment and decreased over incubation time with a decrease in enzyme activity at high concentration of Zn (1360 μ g/L). No change was observed all through the

72 hr experiment on the Chymotrypsin activity by both Zn and Cd. This suggests that Zn and Cd have direct impact on aminopeptidase production by bacteria at 1.36µg/L and 5µg/L, respectively. Thus, it can be stated that Zn and Cd contamination would have effect on the microbial enzymes and biogeochemical cycle of organic matter in the marine ecosystem.

Abstract Category
14 Microbe-Mineral Interactions

PS.14.009 INVESTIGATION OF OSMOTIC STRESS RESPONSE IN THE ANAEROBIC METAL-REDUCING MICROBE *GEOBACTER METALLIREDUCTENS*, STRAIN GS -15

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As part of our ongoing project on determination of stress response pathways in dominant metal reducing bacteria, we compare and contrast effects of common environmental stressors on microbes co-existing in DOE-contaminated sites. In this study we present results of osmotic stress response by *Geobacter metallireducens* strain GS15 (GM). A comparison of these results with those from *Desulfovibrio vulgaris* and *Shewanella oneidensis* will provide a better understanding of bioremediation by these microbes in the field. For this study, GM was grown in defined medium containing 10mM acetate and 10mM Fe-NTA, and the minimum inhibitory concentration of NaCl was determined to be 100mM. To examine salt stress response of GS15 in detail, 100mM NaCl was added to a midlog-phase culture and cells harvested after 1, 2, 4 hours for analyses of Fe(III) reduction, transcriptomics and phospholipid fatty acid analysis (PLFA). After 4 hours, stressed cultures showed a marked decrease in cell number, total cell protein and Fe(III) reduction compared to non-stressed controls. Transcriptomic analysis revealed that the most up-regulated genes were those encoding protein folding, trafficking and degradation. Also up-regulated were genes encoding amino acid biosynthesis. The most down-regulated genes included those encoding inorganic ion transport, energy production, cell motility, chemotaxis and several signal transduction proteins. PLFA analysis of stressed cells compared to non-stressed cells showed small shifts in relative levels of saturated and unsaturated fatty acids in response to salt stress. Additions of common osmoprotectants like glycine betaine, ectoine, yeast extract and Potassium to cells did not alleviate the stress in contrast to observations with *Desulfovibrio vulgaris*.

Abstract Category
14 Microbe-Mineral Interactions

PS.14.010 BACTERIAL COMMUNITY ANALYSIS OF CONTAMINATED SOILS FROM CHERNOBYL

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Background and aims: Shortly after the Chernobyl accident in 1986, contaminated soil and other radioactivity-contaminated debris were buried in situ in several trenches and then covered with a screen of fine sand. In this area, the trench n°22 constitutes a pilot site studied for the migration behaviour of radionuclide since 1999. The aims of this work are (i) to analyse the structure of bacterial communities evolving in this environment since 20 years, and (ii) to evaluate the potential role of microorganisms in radionuclides migration in soils.

Methods: Soil samples exhibiting contrasted radioactivity levels were collected in and around the trench n°22 in October 2008, April and October 2009. Total DNA purification, cultivation of bacteria, pH, TOC and 137Cs activity measurements were carried out in Chernobyl immediately after collection of the samples. The bacterial diversity was analysed using a combination of a genetic fingerprinting method (DGGE) and cultivation methods.

Results - Conclusion: Bacterial communities were examined using DGGE with universal and group-

specific PCR primers. Our results indicate that Chernobyl soil samples host a wide diversity of Bacteria, even in highly contaminated samples. For each soil sample, the total bacterial community structure appeared to be very similar at different time of sampling, with only few different bands between October and April (<5). Similar DGGE patterns were observed along the trench for *Firmicutes*, *Actinobacteria* and more variable for *Proteobacteria*. A phylogenetic analysis of 250 heterotrophic aerobic isolates revealed that 5 phyla are represented: *Beta*-, *Gammaproteobacteria*, *Actinobacteria*, *Bacteroidetes* and spore-formers *Firmicutes*, representing the dominant group of the culturable population. This collection will be screened for the presence of radionuclide-accumulating species in order to estimate the potential influence of microorganisms in radionuclides migration in soils.

Abstract Category
14 Microbe-Mineral Interactions

PS.14.011 MICROBIALLY ENHANCED CALCITE PRECIPITATION IN POROUS MEDIA

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A strategy for remediating strontium-90 (Sr-90) from contaminated aquifers is to co-precipitate the radioactive element into calcite, sequestering it in mineral form. Calcite precipitation in aquifers can be enhanced by encouraging native microorganisms to hydrolyze urea thereby increasing the aquifer pH and shifting the bicarbonate equilibrium. However, little is known about how to manage this process to permit the precipitation to occur optimally throughout an aquifer. Our aim is to develop a model system that will allow examination of mixing strategies and amendments needed to optimize microbial calcite precipitation in porous media on a centimeter scale that will translate to an effective remediation process at larger scales. In flask studies with microbes known to possess the genes for urease we have tested conditions such as temperature, urea concentrations, and initial ammonia levels to determine how to promote urease expression. Cultures include *Escherichia coli*:pURE14.8 with a urease constitutive plasmid, *E. coli* without the plasmid, *Sporosarcina pasteurii* that is constitutive for urease production and *Pseudomonas aeruginosa*. Urea added at a minimum of 10 mM to incubations at 20°C in artificial ground water resulted in urease activity and calcite precipitation using *E. coli*:pURE14.8. *P. aeruginosa* has not expressed urease to the extent that calcite would precipitate under any of the conditions tested thus far. Finding the conditions that will derepress the urease enzyme in an environmental organism such as *P. aeruginosa* will help in eventually determining the response of aquifer organisms to potential urease stimulating amendments. We are currently developing direct urea and high throughput (96-well plate) ammonia assays to examine the optimal conditions required for urease expression and the kinetics of urea hydrolysis in model systems and native aquifer communities during Sr-90 remediation.

Abstract Category
14 Microbe-Mineral Interactions

PS.14.012 MOLECULAR DIVERSITY OF CORRODED CONCRETE BIOFILMS AND THE ROLE OF ACID-PRODUCERS IN CONCRETE CORROSION

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Concrete corrosion has an enormous economic impact worldwide, especially when the repair of municipal sewer, bridges, and other concrete systems is required. Sulfate reducing bacteria that exist in the slime layer in the submerged part of the concrete convert naturally occurring sulfate to H₂S and thus formed H₂S volatilizes and reacts with oxygen above the water line at the concrete surface to form elemental sulfur and thiosulfate. Sulfur-oxidizing bacteria oxidize the dissolved H₂S, thiosulfate and elemental sulfur to sulfuric acid which reacts with calcium hydroxide (Ca(OH)₂) in the concrete to form gypsum (CaSO₄ · 2H₂O), ettringite or thaumasite. Formation of these compounds results in cracks and corrosion of concretes. The primary goal of this research was to

determine the microbial diversity of deteriorated concrete and enrich for microorganisms responsible for corrosion. Highly deteriorated concrete samples were collected from six different bridges in Texas. Non-deteriorated samples were also collected in the vicinity of deteriorated concrete as controls. Clone libraries were constructed from deteriorated and non-deteriorated concrete by PCR using universal primers (515F-1391R). Analyses showed the presence of highly diverse microbial communities with sulfur-oxidizing, ammonia oxidizing, cyanobacteria, and chloroflexi as the dominant members of the concrete biofilm assemblages. To study the corrosion process, bottles were prepared with mineral salts medium containing concrete and thiosulfate. Bottles were monitored for pH changes and accumulation of sulfate and calcium. Results showed drop in pH to below < 2.5 within 6 weeks. Also elevated levels of sulfate and calcium were measured in inoculated bottles. A better understanding of naturally occurring microbial assemblages of deteriorated concretes and their role in acid-production is essential for the efficient control of microbial corrosion.

Abstract Category
14 Microbe-Mineral Interactions

PS.14.013 MICROBIAL ECOLOGY AND METAL TRANSFORMATIONS AT A SIMULATED LOW-LEVEL WASTE SITE

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Low-level radioactive waste sites frequently contain cellulosic waste in the form of paper towels, cardboard boxes, or wood contaminated with heavy metals and radionuclides. The goal of these studies was to gain a better understanding of how the soil microbial community is influenced by the presence of cellulosic waste products, as well as determine the potential role played by these microorganisms in hexavalent chromium and uranium reduction. Soil samples were obtained from a non-radioactive model low-level waste test pit at the Idaho National Laboratory and were analyzed using 16S rRNA gene clone libraries, a 16S rDNA microarray (PhyloChip) and quantitative PCR. Isolates were obtained from enrichments and characterized based on their abilities to reduce chromium and uranium in the presence of various potential cellulose degradation products. Both the clone library and PhyloChip results revealed significant changes in the bacterial community structure with depth. The PhyloChip detected greater relative diversity than the clone libraries. Calculated diversity indices, principal coordinates analysis and lineage specific analyses identified changes in microbial community structure with depth. The *Bacteroidetes* and *Actinobacteria* phyla accounted for most of the significant differences observed. Batch studies with the isolates demonstrated that the type of electron donor (carbon source) influences the extent of uranium and chromium toxicity as well as the organisms' ability to reduce these contaminants. Overall, these results suggest that the presence of the cellulosic material significantly influences the bacterial community structure in a stratified soil system. Additionally, the batch isolate studies suggest that the interactions between cellulose degraders and metal reducers may be important at these sites and will be evaluated further as the degradation products may influence the mobility of these contaminants.

Abstract Category
14 Microbe-Mineral Interactions

PS.14.014 UNTANGLING THE POPULATION DYNAMICS OF *LEPTOTHRIX OCHRACEA* AND *GALLIONELLA FERRUGINEA* IN A WETLAND IRON SEEP

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Thick iron mats (>10 cm depth) are a common feature of freshwater environments where iron-rich hypoxic water emerges from the subsurface to mingle with oxygenated surface water. The mats are composed primarily of biogenically produced Fe-oxides, most often the tubular sheaths of *Leptothrix ochracea* or the twisted stalks of *Gallionella ferruginea*. Analysis of a number of different wetland sites indicates that *L. ochracea*-type sheaths are predominant; however the host organism, *L.*

ochracea is conspicuous by its absence (> 90% of sheaths are empty), and is very recalcitrant to laboratory culture; therefore little is known about it. More is known about the identity and physiology of *G. ferruginea*; however its ecological interactions with *L. ochracea* are not understood. Using a combination of SSU rRNA gene tagged pyrosequencing, conventional SSU rRNA clone libraries, and single cell genomics, we have been able to establish the phylogeny of uncultivated *L. ochracea*, and confirm its identity in these systems with a custom designed FISH probe. We studied a local iron seep in Maine where *Gallionella*-like stalks dominated the mat from mid-April when they comprised literally 100% of the sheath/stalk biogenic structures. By early June, the relative abundance of sheaths was 75% of the total, and sheaths predominated the remainder of the summer. Through the spring transition waters were 10 C and averaged pH 6.24. High concentrations of ferrous iron were consistently present in the mat (0.27 mM, on average). FISH analysis indicated in the *L. ochracea* dominated mats *L. ochracea*

Abstract Category

14 Microbe-Mineral Interactions

PS.14.015 COMPARISON OF THE METABOLIC POTENTIAL IN LITHIFYING AND NON-LITHIFYING STROMATOLITIC MICROBIAL MATS OF HIGHBORNE CAY, BAHAMAS

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The stromatolites of Highborne Cay, Bahamas are characterized by the presence of three distinct surface microbial mat communities, Type 1, 2, and 3. These mat types are distinguished by the diversity of the mat community and the extent of carbonate mineralization. In this study, we examined the metabolic and molecular potential in these stromatolitic mats by comparing the substrate (C, N, S, P) utilization patterns in Type 1 (non-lithifying) and Type 3 (lithifying) mats using Phenotypic MicroArrays. These metabolic utilization profiles were confirmed by functional metagenome analysis in Type 1 and 3 stromatolitic mats. Incubation of Type 1 and Type 3 mats for 72 h with a single carbon source revealed that 21% of the 190 C substrates tested were metabolized by both mat types. Of these 21% the fastest respiration rates occurred when mats were grown on carboxylic acids. Type 3 mats, however, used an additional 11% C substrates, whereas Type 1 mats only utilized an additional six substrates. Nitrogen utilization was also compared between mat types and most of the N substrates were metabolized by both mat communities (64%) by 48 h. Type 3 mats utilized 14 additional N substrates that were not metabolized by Type 1 mats. This trend of higher metabolic diversity in lithifying Type 3 mats continued in the utilization of S substrates. Of the 35 S substrates, 57% were used by both mats after 48 h. Type 3 mats utilized an additional 9 (26%) substrates, whereas only one S substrate was specific to Type 1 mats. There was no statistical difference between the two mat types with regard to the utilization of P substrates. While both mats types were able to use a wide range of metabolic substrates, the results indicated metabolic diversity was greater in the Type 3 mats. Isolating the metabolic and molecular pathways unique to Type 3 lithifying mats may delineate many of the molecular and metabolic pathways underlying organomineralization in these modern stromatolitic mats.

Abstract Category

14 Microbe-Mineral Interactions

PS.14.016 EVOLUTION OF INTERFACIAL FEATURES OF PYRITE COLONIZED BY ACIDITHIOBACILLUS THIOOXIDANS BIOFILMS

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Interfacial studies of biooxidized minerals are important for mining industry due to potential enhancement of metal bioleaching rates. In the bioleaching of base and precious metals from

metallic sulfides, the main role of Fe(II) oxidizers (*Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans*) is the regeneration of the oxidizing agent, the Fe(III) ions, while the sulfuroxidizing (as *A. thiooxidans* and *A. caldus*) contribute to the overall process by the oxidation of the intermediary (reduced) sulfur compounds to sulfuric acid. This work develops a complete analysis of interfacial features associated with the colonization of pyrite by the sulfuroxidizing *A. thiooxidans* under acidic conditions, at different stages of mineral biooxidation. This goal was achieved by combination of atomic force microscopy, Raman spectroscopy and confocal laser scanning microscopy, as well as biochemical (carbohydrates and proteins) characterization of the extracellular polymeric substances secreted by the biofilms. *A. thiooxidans* was exposed during 1, 15, 24, 72 and 120 hours to a previously electrooxidized massive pyrite electrodes. Our results confirmed the role of extracellular polymeric substances during the cells attachment and consequently, the biofilms formation. Hence, biofilms development involves a strong adhesion force of 467 pN at 120 hours, an intimate contact between bacterium and mineral surface, as well as an over expression of the extracellular polymeric substances during cells attachment, in comparison with planktonic cells (84.5% and 100% overproduction at 24 and 120 hours, respectively). Meanwhile, the Raman analysis indicated the complete consumption of sulfur reduced compounds after 15 hours of the inoculation; in contrast, the non-inoculated (control) surfaces showed the presence of sulfur compounds.

Abstract Category

14 Microbe-Mineral Interactions

PS.14.017 INTERFACIAL STUDY OF ELECTROOXIDIZED CHALCOPYRITE COLONIZED BY ACIDITHIOBACILLUS THIOOXIDANS BIOFILMS

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Interfacial processes associated with bacteria-mineral contact is useful for determinate the pathways of biological sulfide oxidation. The bioleaching of copper sulfides can be enhanced in bioleaching tanks. The most utilized leaching bacterium is *Acidithiobacillus ferrooxidans*, an iron- and sulfuroxidizing. Sulfuroxidizing bacteria as *A. thiooxidans* have received much lesser attention, nevertheless their importance in the global process. We studied interfacial insights associated with attachment of *A. thiooxidans* on electrooxidized chalcopryrite combining atomic force microscopy, confocal laser scanning microscopy and Raman spectroscopy, after different stages of colonization. Biotic and abiotic assays were performed for 1, 12, 24, 48 and 120 hours using electrooxidized massive chalcopryrite electrodes. The atomic force microscopy was also used to quantify the adhesion forces of attached cells. The results indicated an over expression of lipids and other extracellular polymeric substances during cells attachment, e.g. 343 % at 48 hours, in comparison with planktonic cells; the adhesion forces of *A. thiooxidans* forming biofilms is directly associated with this over production. Raman analysis indicated the presence of sulfur (reduced) species: elemental S, polysulfides, on both biotic and abiotic assays; however, in surfaces with biofilm it was found further transformations of polysulfide defined by *A. thiooxidans*-chalcopryrite contact, suggesting a dynamic process during biofilms development. Hence, the combination of the microscopic and spectroscopic techniques allowed us to identify the active role of EPS during the formation of *A. thiooxidans* biofilms that is important due to bioleaching rates depends mostly of leaching bacteria-chalcopryrite interactions.

Abstract Category

14 Microbe-Mineral Interactions

PS.14.018 CIRCUMNEUTRAL SULFUR OXIDAZING MICROORGANISMS FROM MINE TAILINGS AND THEIR ACTIVITY ON IRON CONCENTRATE

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The iron concentrates are iron-oxides that commonly contain sulfur impurities, which notably affect the physical quality of iron *pellets* and provoke the emission of sulfur dioxides during steel production and, consequently, the generation of sulfuric acid; this acid affects the high-life of blast furnaces due to their corrosion. Besides, the emission of sulfur dioxides to the atmosphere is of environmental and public concerns. We evaluated the potential of sulfuroxidizer microorganisms to remove sulfur impurities from an iron concentrate and under circumneutral conditions. Sulfuroxidizer were previously isolated from mine tailings. In presence of sulfuroxidizer, the insoluble sulfur compounds of the iron concentrates decreased just 2% after 7 days, while an over production of gypsum and iron-oxyhydroxides were recorded. Because the percent of pyrrhotite in the concentrate was lowest in presence of the microorganisms than in abiotic trials, we suggested that pyrrhotite was the main mineral sulfur biooxidized. The sulfuroxidizer were identified by PCR using universal primers designed on conserved regions of bacterial 16S rDNA. From a total of 56 clones we identified several sequences that belong to thiosulfate-oxidizing bacteria. We also found alpha proteobacterium from the genera *Bocea* spp. that has been calcsified as obligate chemolithoautotrophs and were closely affiliated with *Thiobacillus thiooparus*, a sulfuroxidizer microorganism. From the alpha-protobacteria group we also found several clones related to the known metal-oxidizing genera *Hyphomicrobium*. This is the first report of sulfuroxidizer isolated and identified from mine tailings, which oxidize mineral sulfur under circumneutral conditions.

Abstract Category

14 Microbe-Mineral Interactions

PS.14.019 DYNAMICS OF MICROBIAL IRON CYCLING IN MARINE SEDIMENT

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Iron oxides are an important electron acceptor in marine sediments. Rapid cycling of iron can occur between oxidized and reduced iron species with changes in the position of the chemocline, for example as a result of tidal cycles. Ferrous iron is rapidly oxidized by oxygen through both microbial and abiotic oxidation reactions. In the absence of oxygen, Fe (hydr)oxides are used as electron acceptor by heterotrophic bacteria. Moreover, microbial ferrous iron oxidation may be coupled to the reduction of nitrate, resulting in the possibility of cycling of iron in anoxic conditions. However, the microbial community that is responsible for these conversions in marine ecosystems is not well known.

In this study, we assess the rates of conversion and the microbial community involved in the utilization of iron in sediment from a North Sea flat. Biomass extracted from non-sulfidic sediment is incubated in a bioreactor that is continuously supplied with nutrients, organic C and with additional inoculum, to allow re-selection of the microbial community during the experiment. Initially, amorphous iron hydroxide is supplied as the sole electron acceptor, and subsequently, the system will be perturbed by the addition of nitrate. In addition to measuring Fe and nitrate conversion rates, the oxidation of organic C is monitored by on-line mass spectrometry of the off-gas, and the efficiency of respiration is determined by high-resolution calorimetry. The composition of the microbial community is assessed using ARISA fingerprinting and metagenomic sequencing. The results give insight in the importance and rates of the utilization of iron as electron acceptor and the scope for anoxic iron cycling in marine sediments. Furthermore, the information on the composition of the microbial community will be compared with what is known for Fe-reducing and Fe-oxidizing microorganisms in other environments.

Abstract Category

14 Microbe-Mineral Interactions

PS.14.020 MICROBIAL COMMUNITY SIGNATURES ASSOCIATED WITH DISCRETE PHASES OF CARBONATE MINERALIZATION IN METHANE SEEP ENVIRONMENTS

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The precipitation of authigenic carbonates in marine methane seep environments has been linked to microbial activity - primarily the anaerobic oxidation of methane (AOM). We present environmental clone library and Terminal Restriction Fragment Length Polymorphism (TRFLP) 16S rRNA gene data for sediments, sediment-hosted carbonate nodules and carbonate crusts from methane seep localities of the Eastern Pacific margin. Variations in Archaea and Bacteria between these three samples types represent distinct stages of carbonate crust formation in methane seep environments.

Archaeal diversity was not found to vary systematically between sample types, suggesting that authigenic carbonates largely retained the community signature of original sediment prior to carbonate mineralization. However, bacterial diversity frequently varied between sediments, nodules and crusts. Sediments showed increased abundance of TRFLP fragments associated with δ -proteobacteria and Candidate Division JS1. Bacterial communities of weathered carbonate crusts outside areas of active seepage were enriched in α -proteobacterial and γ -proteobacterial phylotypes related to taxa found in seafloor basalts. Therefore, carbonate crusts may present a record of temporal changes in marine bacterial communities that may provide some insight into the ecology of these taxa in other rock-hosted marine environments.

Bacterial and Archaeal TRFLP signatures varied between discrete cementation phases of carbonate crusts. Most changes occurred between δ -proteobacterial phylotypes and ANME archaeal groups while other taxa remained relatively constant, suggesting that changes in AOM activity may be a driving factor in determining the character of carbonate precipitation. Further work may yield insight into the role of the microbial community in various stages of carbonate mineralization in methane seep communities.

Abstract Category
14 Microbe-Mineral Interactions

PS.14.021 INTERSPECIES ELECTRON TRANSFER MEDIATED BY ELECTRIC CURRENTS THROUGH CONDUCTIVE PARTICLES

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Background and aims: Microbial interspecies electron transfer (IET) is an important catabolic reaction in anaerobic biota, where multiple microorganisms have to share limited energy for their lives. IET mechanisms described so far are based on redox reactions of chemical compounds, such as redox pairs of H_2/H^+ , Fe^{2+}/Fe^{3+} , S^{2-}/SO_4^{2-} . In this study, we aimed to verify a conceptually novel IET mechanism which is mediated by electric currents through conductive solid materials, such as magnetite and graphite. Methods: An iron-reducing bacterium *Geobacter sulfurreducens* and an iron-oxidizing bacterium *Thiobacillus denitrificans* were grown together in a medium supplemented with acetate as the sole electron donor and nitrate as the sole electron acceptor. In this system, acetate oxidation could be coupled with nitrate reduction only when IET occurred between the two microbes, because *G. sulfurreducens* can oxidize acetate but cannot reduce nitrate, vice versa for *T. denitrificans*. Results: Acetate oxidation and nitrate reduction did not occur under a control condition (no possible IET mediators were added), indicating IET did not occur between them. Addition of magnetite or graphite nanoparticles facilitated acetate oxidation and nitrate reduction by the coculture, suggesting that IET can be mediated by electric current via the solid conductive materials. Conclusion: The results suggest that electric currents through conductive particles can mediate microbial IET. The IET based on electric currents would have advantages over diffusion of redox chemical mediators, in terms of the reaction rate and flexibility of electric potential. Considering that conductive materials (such as magnetite) and also mineral-respiring bacteria are commonly found in natural environment, it is likely that IETs via electric currents largely contribute to the establishment and functioning of natural microbial communities.

Abstract Category
14 Microbe-Mineral Interactions

PS.14.022 PHYSICAL-CHEMICAL BASED MODELING OF BIOFILM INDUCED MINERALIZATION

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Biofilms, via their inherent spatially heterogeneous chemical environment, can produce pH microenvironments where mineral formation or loss may occur. Such deposits can be important in environmental, medical, and engineering contexts. Modeling, however, of mineralization processes is difficult due to the inherent juxtaposition of physical, chemical, and biological influences (including electric fields induced by ion transport), often all embedded in a moving fluid environment. This poster presents a full, conservation-law based modeling system to study biofilm induced mineralization.

Here we combine conservation law-based models of biofilm growth and mechanics, chemistry including pH, with a free energy mineralization model. Equations are solved numerically in a pore-scale geometry.

We are able to simulate mineralization in a system including fluid transport and shear stress. We can predict effects of variation of, for example, flow rate and nutrient level on pH microenvironment and mineral deposition.

We are able to demonstrate a fully integrated physical-chemical-hydrodynamical pore-scale biofilm model that provides a useful in silico system to test dynamics of biofilm induced mineralization processes. We can use this model to test hypotheses regarding, for example, significance of electrochemistry in controlling mineralization processes.

Abstract Category

14 Microbe-Mineral Interactions

PS.14.023 DETECTION AND QUANTIFICATION OF MICROORGANISMS IN HIGH SALINE MINE TAILINGS AND TWO DIFFERENT OPEN PITS IN THE DESERT ATACAMA, CHILE

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A marine shore copper mine waste tailings deposit in arid climate at Chañaral as well as the two open pits Chuquicamata and Exotica, Chile were studied to understand the influence of high salinity on the microbial community composition and biogeochemical processes in these extreme environments. Samples were taken from the oxidized zones at several sites which had a paste pH in the range of 2-8. The microbial community was quantitatively analyzed using different methods: 1) total cell numbers by SYBR Green II direct counting, 2) quantitative real-time PCR (Q-PCR), 3) most probable number cultivation of acidophilic Fe(II)-oxidizers. The results showed that the composition of microbial communities and the cell numbers of different microbial groups are highly variable at different sampling sites. Depth profiles of cell numbers of the mine tailings deposit showed total cell numbers in the range of $10^4 - 10^8$ cells g⁻¹ tailings. Cultivable acidophilic Fe(II)-oxidizing microorganisms were present in numbers of up to 10^4 cells g⁻¹ tailings. *Bacteria* dominated over *Achaea* in the mine tailings. The acidophilic Fe(II)- and/or sulfur-oxidizing *Acidithiobacillus spp.* dominated over the acidophilic Fe(II)- oxidizing *Leptospirillum spp.* among the Gram-negative *Bacteria*. The acidophilic Fe(II)- and sulfur-oxidizing Gram-positive *Sulfobacillus acidophilus* was generally less abundant. Microcalorimetric pyrite oxidation rate measurements revealed microbial activity in distinct layers of the tailings. In the open pits, cells were not detected by Q-PCR or cultivation, while total cell numbers were up to 10^7 cells g⁻¹ in a few samples. In parallel to the microbial community analyses, novel, acidophilic halotolerant Fe(II)- oxidizing microorganisms were enriched at salt concentrations of up to 1 M probable suitable for metal bioleaching using seawater.

Abstract Category

14 Microbe-Mineral Interactions

PS.14.024 PROTEOMICS OF EXTRACELLULAR ELECTRON TRANSPORT STRUCTURES IN AN ACIDOPHILIC BACTERIUM

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Elucidating the structure and assembly of abundant extracellular electron transport complexes in iron-respiring microbes is important to understanding global biochemical and ecological features of these unique systems. While progress has been made in neutrophilic systems, extremophilic iron-respiring bacteria have not been as thoroughly explored. The acidophilic iron respiring Alphaproteobacterium *Acidiphilium cryptum* was grown in biofilm reactors with Fe(III) as electron acceptor, and biofilms were used for fractionation and proteomic analysis. Biofilm material was selectively extracted in order to remove membrane vesicles from intact cells. Vesicles were analyzed for cytochrome c content using electrophoretic detection, and proteomic profiling was done using electrospray mass spectrometry. Peptides were examined using two bioinformatics packages to confirm identity. Results show the presence of several c-type cytochromes in the vesicle fractions, as well as a variety of other enzymes potentially involved in metal transformation. Vesicles were not a random packaging of proteins resulting from cell lysis; rather, they appear to have a distinct and orchestrated protein composition. Our findings suggest that membrane vesicles provide a packaging and transport mechanism for extracellular redox proteins, and that this theme may be shared among physiologically distinct groups of metal reducing bacteria. Moreover, the vesicles also contain many other functions that serve in cell-mineral interactions and transformations. It is clear that a membrane vesicle mechanism of placing key electron transfer proteins near their natural mineral substrates is used by *A. cryptum*, and that this mechanism should be considered for other metal respiring isolates and complex microbial communities where metal transformation represents the dominant physiologic process.

Abstract Category

14 Microbe-Mineral Interactions

PS.14.025 ISOLATION AND CHARACTERIZATION OF METAL- AND RADIONUCLIDE-REDUCING MICROORGANISMS FROM 300 AREA SEDIMENTS WITHIN THE HANFORD SITE

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Historically, significant quantities of uranium (U) and technetium (Tc) were released into the environment at the U. S. Department of Energy's Hanford Site. The anoxic bioreduction of U(VI) or Tc(VII) to insoluble oxides by dissimilatory metal-reducing bacteria (DMRB) significantly alters the groundwater mobility of these radionuclides. DMRB also indirectly influence radionuclide mobility via the reduction of Fe(III)-containing sediments. This project focuses on the isolation and characterization of subsurface microorganisms involved in metal and radionuclide biotransformation from the 300 Area of the Hanford Site. Subsurface sediment samples from the aerobic Hanford formation, the oxic/anoxic transition of the Ringold formation, and the deep Ringold formation were added to a series of microcosms to isolate relevant microorganisms which are able to reduce Fe(III)-oxides either directly or indirectly. Microcosms were transferred into a defined minimal media containing Hanford 300 Area synthetic groundwater and amended with a synthetic ferrihydrite (FH) electron acceptor. Microcosms were provided with one of fourteen different electron donors to stimulate growth and activity of subsurface organisms capable of FH reduction. We have determined that three organic acids and four alcohols were electron donors supporting the reduction of FH and that responsiveness to the suite of electron donors varied between the microbial communities in the three sediment strata. FH reduction was also influenced by biotic interactions with sulfate, a component of Hanford groundwater. Currently, we are analyzing the consortia in positive enrichments to isolate pure FH reducing organisms. The near-term objective is to analyze physiological differences in biotransformation rates for both metal and radionuclides and subsequently identify the genetic basis for those differences, in order to better understand the biological processes influencing contaminant mobility at the Hanford Site.

Abstract Category
14 Microbe-Mineral Interactions

PS.14.026 ISOLATION AND IDENTIFICATION OF HEAVY METALS RESISTANT BACTERIUM FROM WASTEWATER AND MEASUREMENT OF RESISTANCE CONCENTRATION

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Resistance to heavy metals in environmental bacteria lead to survival of resistance and transferable power of these genes to pathogens could be hazardous for community health. on the other hand, some bacteria can be applied for bioremediation and removal poisonous heavy metals from the environments contaminated various pollutants. the aim of this study was isolation and characterization of heavy metals resistant bacterium from wastewater and measurement of resistance to different concentrations.

In this study, Sampling was performed from soils contaminated with wastewater and added to a flask containing 100 ml basal salt medium with 200ppm of CdCl₂, MnCl₂, Pb(NO₃)₂, HgCl₂, Ag₂SO₄ and CoCl₂. Then, for purification, Samples were incubated at 35°C with 150rpm shaken for 48h.

Experiments showed this bacterium has similarity to pseudomonas genus but, against of pseudomonas genus, this strain is nonmotile. Minimal inhibitory concentration (MIC) indicated that among 14 experimented heavy metal, maximum resistance was shown to VO₃⁻, WO₄²⁻, Co²⁺, Fe²⁺, Cu²⁺, Cd²⁺, and pb²⁺ up to 500ppm and resistance to Cro₄²⁻, MoO₄²⁻, Zn²⁺, and Hg²⁺ between 200-300ppm was obtained. But, growth at presence of Mn²⁺ and Ag⁺ was not observed.

Although low concentrations of heavy metals is essential for growth of bacteria, but, high heavy metals level lead to inhibit growth. The presence of these bacteria, due to property has closely relationship with antibiotic resistance can be hazardous for community health.

Abstract Category
14 Microbe-Mineral Interactions

PS.14.027 IN VIVO ELECTRICAL CONDUCTION ACROSS BACTERIAL NETWORKS WITH NANOSIZED IRON OXIDE AND SULFIDE MINERALS

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Owing to its influence on microbial ecosystems in soils and sediments, bacterial extracellular electron transfer to Fe(III) oxide minerals has gained considerable attention in the past few years. Several recent studies have provided genetic evidences supporting the view that dissimilatory metal-reducing bacteria (DMRB), Shewanella and Geobacter, exploit the cytochrome rich outer-membrane for specific attachment to Fe(III) oxide surfaces, followed by electron injection as a terminal step for metabolism. Although a great deal of research has been focused on the extracellular ET reaction of DMRB, few studies have been performed by directly monitoring the ET process of intact cells, and therefore the mechanism of this process has largely remained unsolved. Here, we report the first experimental verification for the ability of *S. loihica* PV-4 and *S. oneidensis* MR-1 to self-assemble into an electrically conductive network in the presence of Fe(III) oxides, and demonstrate the role of semiconductive iron-oxide nanocolloids, hematite, in promoting a long-distance ET process using direct photochemical monitoring of the extracellular ET in the bacterial network [1, 2]. Beside the semiconductive iron-oxide, we also demonstrate that *Shewanella* can utilize the biogenically synthesized metallic iron-monosulfide, mackinawite, to promote a long-distance extracellular ET longer than 20 mm in cellular networks [3]. Semiconductive and conductive nanocolloids are abundant in natural minerals, but their in vivo functions have been never investigated. Thus these findings shed light on impact of electron conduction of Fe oxide and sulfide nanominerals on microbial ecosystems and chemical processes in the soil and sediment environments.

References: [1] R. Nakamura, et al. Angew. Chem. Int. Ed. 2009, 121, 516. [2] R. Nakamura, et al. Angew. Chem. Int. Ed. 2009, 121, 1634. [3] R. Nakamura, et al. ChemBioChem, 2010, 11, 643.

Abstract Category
14 Microbe-Mineral Interactions

PS.14.028 DIRECT ELECTROCHEMICAL CHARACTERIZATION OF A SINGLE SHEWANELLA USING OPTICAL TWEEZERS

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Genus *Shewanella* is a gram-negative microorganism which has a unique property of utilizing solid state metal-oxide as a terminal electron acceptor. The extracellular electron transfer occurs via cytochromes located in the outer membrane (outer-membrane-cytochrome, OMC). Due to the unique property, *Shewanella* has attracted a lot of attentions from the viewpoint of biogeochemical cycle and microbial fuel cell. Electrochemical techniques have been used to investigate the electron transfer between the microbes and the (solid) electrode. So far, two different pathways have been proposed: mediated electron transfer by self-secreted flavin (mediator pathway) and direct electron transfer from the OMC to solid surfaces (direct pathway). For the mediator pathway, many experimental supports have been carried out. However, the direct experimental evidence for the electrical connection between genus *Shewanella* and external acceptor is still lacking, mostly due to the extreme complexity and unpredictable dynamic property of the living system. Here, by using optical tweezers, we successfully monitored the ET process between a single cell of *Shewanella loihica* PV-4 and a poised electrode, and firstly gave a direct experimental evidence to show that the *S. loihica* PV-4 can use direct extracellular ET path to respire. When the electrode potential (E) was kept constant at +0.2 V, which is more positive than the redox potential of the OMC (~ -0.17 V vs. Ag|AgCl), the microbe gave ca. 200 fA current jump. We also performed the experiments at various potentials, and found that the current jump is observed only in the potentials more positive than the mid-point potential of the OMC. This result indicates that the current jump observed at the moment when the microbe is attached on the electrode is due to the direct contact between the OMC and the electrode.

Abstract Category
14 Microbe-Mineral Interactions

PS.14.029 ARSENIC BIOREMEDIATION USING IN-SITU BIOPRECIPITATION WITH FE-(OXYHYDR)OXIDES AND SULFIDES

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Millions of people globally are exposed to groundwaters that exceed the World Health Organization of limit of 10 ppb for arsenic. Here we report on a study aimed at evaluating the use of biogenic Fe-(oxyhydr)oxides (HFOs) and sulfides for the remediation of As-contaminated groundwaters. We used microcosms containing sediment from an aquifer in Cambodia with naturally elevated levels of arsenic in the groundwater. Fe(II) oxidation and sulfate reduction were stimulated by the addition of either nitrate or sulfate and lactate respectively. Prior to treatment, soluble As and Fe concentrations were 150-170 ppb, and 50-80 ppm, respectively. The microbial community was dominated by known Fe(II)-, and As(V)-reducing bacterial species, such as *Geobacter* and *Sulfurospirillum*. Within two weeks of nitrate addition, HFO formation had started; resulting in a 100% decrease in soluble As, presumably associated with the newly formed biogenic HFOs. This was followed by a 100% removal of soluble Fe a month later. 16S rRNA gene analysis revealed a shift towards organisms related to the nitrate-dependent Fe(II)-oxidizing bacterium *Sideroxydans ghiorisii*. Sulfide production started within two weeks, when stimulated with lactate and sulfate. This resulted in a 70-80% decrease in soluble As and Fe, as orpiment and presumably arsenic sorbed to insoluble iron sulfide phases. After 120 days, soluble As and Fe concentrations were 25-90 ppb, and 3 ppm, respectively. The onset of sulfate reduction occurred with the appearance of sulfate-reducing bacteria related to *Desulfobulbus* and *Desulfovibrio* species. Soluble As and Fe concentrations in

untreated microcosms were 270-300 ppb and 40-60 ppm, respectively. Our results indicated that microbial communities under both conditions reacted quickly to biostimulation, and effectively controlled soluble arsenic levels. However, only treatment through HFO production maintained As levels below the WHO limit of 10 ppb.

Abstract Category
14 Microbe-Mineral Interactions

PS.14.030 GEOCHEMICAL TRANSFORMATION OF LIMESTONE BY BIOFILM-ASSOCIATED FUNGI FROM MAYA SITES

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Extensive coverage of microbial biofilm is often seen on walls, ceilings, stelae and carved statues on most buildings at Mayan sites. This microbial growth may have a range of consequences, ranging from deterioration to protection of this cultural heritage. We carried out a characterization of the biogeochemical impact on limestone by selected fungi isolated from biofilms retrieved from Chichen Itza monuments. Our survey yielded 63 fungal isolates derived mainly from epilithic black biofilms, from which ribosomal intergenic spacer analysis (RISA) showed that *Penicillium oxalicum* TM1H52, *Annulohyphoxylon stygium* TM1H19 and *Rosellinia* sp TM1H05 were very active in calcium carbonate-dissolving activity with subsequent formation of mycogenic fabrics. Further incubation of limestone and electron scanning microscopy (SEM) and X-ray diffraction (XRD) of crystalline precipitates indicated the presence of a mixture of calcite and calcium oxalates. In addition to calcium oxalates, Fourier transform infrared (ATR-FTIR) spectroscopy showed spectral features that are consistent with the presence of citric acid on the limestone surface incubated in the presence of *Penicillium oxalicum* TM1H52, as evidenced by evolution of strong bands developing at 1590 cm⁻¹ and 772 cm⁻¹. *Annulohyphoxylon stygium* TM1H19 showed similar bands to the *Penicillium oxalicum* TM1H52, however its bands are much weaker in intensity, which suggests that citric acid may develop at a lower concentration. These results provide experimental evidence for the formation of secondary mycogenic minerals by naturally occurring fungi derived from microbial biofilms associated with Chichen Itza buildings, suggesting a potential role in limestone transformation.

Abstract Category
14 Microbe-Mineral Interactions

PS.14.031 THE RELATIONSHIP BETWEEN PARTICLE SIZE DISTRIBUTION IN SUBSURFACE SEDIMENTS AND THE COMPOSITION OF INDIGENOUS MICROBIOTA

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To investigate the relative influence of local site geochemistry versus the indigenous microbial community on maintaining stable uranium bioremediation, we are conducting a reciprocal transplant experiment using subsurface sediment from three sites (Oak Ridge, TN, Rifle, CO, Hanford, WA). To determine the relationship between particle size distribution and the composition of microbial communities in the three sediments, we fractionated the sediments using a minimally-disruptive procedure to maintain the microbial community associated with each of the three fractions (0.2-2 mm, 20-200 µm, <20 µm). The microbial community composition was analyzed by microscopic cell counts, qPCR and PhyloChip microarray analysis of 16S rRNA genes, while sediment chemistry was determined by X-ray fluorescence. The three sediments differ markedly in their geochemistry and indigenous microbial communities. Oak Ridge sediment has significantly more microbial cells, while Hanford sediment has a significantly lower proportion of Archaea and Geobacteraceae. They also differ in their particle size distributions with Rifle being mostly sand and Hanford being mostly silt. The number of microbial cells in the sediments was strongly correlated with the amount of iron

present, as well as the proportion of high-surface area clay. However, the microbial densities on the particle size fractions did not correlate with iron and the microbial densities on the Hanford fractions did not correlate with surface area. There was a strong positive correlation across all fractions with certain micronutrients, such as copper, which is a necessary cofactor for a number of important microbial enzymes. The localization of micronutrients in the subsurface may determine the extent of microbial proliferation. Understanding how the distribution of sediment particles impacts the composition of the subsurface communities may help elucidate the constraints on microbial abundance and their distribution in the subsurface.

Abstract Category

14 Microbe-Mineral Interactions

PS.14.032 TRANSIENT AND MINOR EFFECTS OF COPPER ON BACTERIA BELONGING TO THE GENUS *PSEUDOMONAS* IN AGRICULTURAL SOILS

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This work is part of the multidisciplinary *Bioindicateurs* program, which aims to establish an index of the soil biological status in order to evaluate the impact of pollutants on soil quality. The chosen model pollutant is copper, a metal trace element widely found in agricultural soils. So, the objective of this work is to evaluate the impact of a single copper contamination on soil bacterial communities, and particularly on bacteria belonging to the genus *Pseudomonas*, in a soil undergoing two different agricultural management practices. This study was held both in non perturbed soil microcosms, to mimic an single in situ contamination, and in mechanically perturbed soil microcosms, to check whether the way microcosms were set up had an influence on the interaction between copper and soil bacterial communities. These communities were examined through their abundance (cultivable bacteria enumeration and bacterial DNA quantification by qPCR) and diversity (potential functional diversity, using the Biolog system, and genetic diversity by tRFLP). The *Pseudomonas* genus was studied following the same approaches. In undisturbed soils, results showed a strong impact of agricultural practices on soil bacterial communities' structure, a medium impact of the incubation time, and no impact of the copper contamination. In the physically disturbed soil, the same gradient was observed, with a weak and transient impact of copper on soil bacteria and *Pseudomonas*. This study shows the transient and minor impact of a single copper contamination on soil bacterial communities, and particularly the *Pseudomonas*. It was thus possible to establish a hierarchy of anthropic impacts on soil bacteria and *Pseudomonas*. This study also highlights the need of a reference system and the importance of experimental procedures when assessing the impacts of a metal on soil bacterial communities.

Abstract Category

14 Microbe-Mineral Interactions

PS.14.033 BIOGEOCHEMICAL TRANSFORMATIONS OF TECHNETIUM-99 IN HANFORD 300 AREA SUBSURFACE SEDIMENTS

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Technetium-99 (⁹⁹Tc) is an environmental contaminant from civilian and defense nuclear fuel processing. At the U.S. Department of Energy's Hanford Site, ⁹⁹Tc is a major risk driver, due to its quantity, long half-life, mobility as the oxic anion pertechnetate (TcO₄⁻), and potential to enter the food chain as a sulfate (SO₄²⁻) analogue. However, in anoxic subsurface sediment and groundwater, reductive precipitation of Tc(VII)O₄⁻ as sparingly soluble Tc(IV)O₂ can retard the migration of Tc. In anaerobic laboratory microcosms, we examined the fate of TcO₄⁻ in oxic sediments from the Hanford 300 Area. To stimulate anaerobic biological activity, sediment microcosms in synthetic groundwater were amended with various combinations of organic carbon, hydrogen (H₂), and SO₄²⁻, along with TcO₄⁻ (20 μM). Soluble Tc was monitored via 0.2-μm filtration, and Tc reductive products were examined via X-ray Absorption Spectroscopy. In H₂ or OC amended Hanford formation sediments,

solution Tc decreased to $\sim 5 \mu\text{M}$ in both live and autoclaved sediments, but in oxidized Ringold formation material amended with H_2 or OC, $\text{Tc}_{(\text{aq})}$ did not change significantly. In live Hanford formation microcosms amended with OC, with or without SO_4^{2-} , $\text{Tc}_{(\text{aq})}$ decreased to $\sim 5 \mu\text{M}$, while in the absence of OC, $\text{Tc}_{(\text{aq})}$ decreased to $\sim 15 \mu\text{M}$; in similar heat treated (100C) samples, $\text{Tc}_{(\text{aq})}$ remained relatively unchanged in the presence of OC and SO_4^{2-} , but $\text{Tc}_{(\text{aq})}$ decreased to $\sim 15 \mu\text{M}$ in the absence of SO_4^{2-} . When TcO_4^- (20 μM) was added to heat treated Hanford ultra-fine bioreduced material at various sediment densities with and without SO_4^{2-} , $\text{Tc}_{(\text{aq})}$ decreased to $\sim 0.005\text{-}2 \mu\text{M}$, and XAS analysis indicated both Fe-Tc and S-Tc bonds, suggesting that sulfide or iron sulfide minerals contribute to Tc reduction in the sediments bioreduced in the presence of SO_4^{2-} . Our results suggest that both iron and sulfate reduction may retard Tc migration in Hanford formation sediment.

Abstract Category
14 Microbe-Mineral Interactions

PS.14.034 MECHANISM OF PALLADIUM (II) REDUCTION BY MICROORGANISMS

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Palladium paucity in nature and high demand from different industries increased the interest in palladium recovery. Most methods of Pd recovery require high temperatures and hazardous chemicals. Recently, bio-recovery was considered an environmental friendly alternative. Presently, the mechanism of Pd (II) reduction at cell surfaces is poorly understood. It was suggested that [Ni-Fe]-hydrogenases nucleate the reduction of Pd (II) to Pd (0), followed by autocatalytic growth. Hence, cells pre-treated with an unselective inhibitor for hydrogenases, Cu (II), did not sustain Pd (II) reduction. Secondly, the location of Pd (0) nanoparticles (NPs) in hydrogenase mutants (inner membrane) differed tremendously from untreated cells (periplasmic). The aim of this study was to identify the factors which influence the initial nucleation of Pd (0) NPs. Pd (II) reductive recovery was studied under anaerobic conditions and acidic pH. Pd (0) nanoparticles were identified on biological and non-biological surfaces via transmission electron microscopy. The NPs composition was determined by energy dispersive spectroscopy. In this study we investigated if certain chemical groups could nucleate Pd (II) reduction in an abiotic system. We observed that both carboxyl- and amino-functionalised polystyrene microbeads could be used as sorbents for reduction alike bacterial cells. Pd (II) reduction was faster in the presence of bacterial cells and carboxyl- or amino-functionalized microbeads compared to cell-free controls. Furthermore, Pd (II) was reduced in the presence of dead and living cells. Our results suggest that the mechanism of nucleating Pd (II) reduction involves physico-chemical interactions and does not require active enzymes as previously thought.

Abstract Category
14 Microbe-Mineral Interactions

PS.14.035 GEOMICROBIOLOGY OF SULFIDIC MINE WASTE AND BIOLEACHING DUMPS AND HEAPS

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More than 70 microbiological studies of sulfidic mine dumps and heaps have been published. The microbiology and the relevant biogeochemical processes in the dumps and heaps have to be understood for improving copper heap leaching operations and to develop and control countermeasures for the formation of acid mine drainage (AMD). Pyrite or pyrrhotite oxidation is the most relevant biogeochemical process in sulfidic mine waste dumps and heaps and different techniques have been applied to measure reaction rates: column experiments, humidity cells, heat flow measurements, or oxygen consumption measurements. Acidophilic Fe(II)- and sulfur compounds-oxidizing microorganisms are most relevant for metal sulfide oxidation. Anaerobic biogeochemical processes in sulfidic mine dumps and heaps are Fe(III)- and sulfate-reduction, but little is known about the reaction rates. Fe(III)-reducing microorganisms dissolve Fe(III)(hydr)oxides and may thereby release adsorbed or precipitated metals. Sulfate-reducing microorganisms precipitate and immobilize metals. Mainly culturing approaches were used to study the microbial communities in sulfidic mine dumps and heaps. More recently, molecular biological techniques have been applied to investigate the microbial diversity and to quantify and monitor

particular microorganisms. A state-of-the-art overview of the microbial ecology of sulfidic mine dumps and heaps will be given.

Abstract Category
14 Microbe-Mineral Interactions

PS.14.036 DELINEATING THE SERPENTINITE-HOSTED MICROBIAL BIOSPHERE

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The aqueous alteration of ultramafic rocks (serpentinization) produces copious quantities of hydrogen and small organic molecules, and leads to highly reducing, highly alkaline conditions (up to pH 12). Several environments provide insight into the relationships between serpentinization and microbial life including slow-spreading mid ocean ridges, subduction zones, and ophiolite materials emplaced in continental settings. The Tablelands serpentinite, an ophiolite in Newfoundland, Canada provides an opportunity to carefully document and map the relationships between geochemical energy, microbial growth, and physiological adaptations. Alkaline fluids at the Tablelands originate from 500-million year old oceanic crust and accumulate in shallow pools or in seeps beneath serpentinized rubble. Fluids, rocks, and gases were collected from the Tablelands during a series of field excursions and geochemical, microscopic, molecular, and cultivation-based approaches were used to study the serpentinite ecosystem. Preliminary data indicate that a low but detectable stock of microorganisms inhabit the high pH pools. Enrichment cultures yielded brightly pigmented colonies related to *Alphaproteobacteria*, presumably carrying out anoxygenic photosynthesis. Culture independent analyses of SSU rRNA indicated low diversity communities of *Firmicutes* and Archaea in standing alkaline pools, communities of *Beta*- and *Gammaproteobacteria* at high pH seeps, and assemblages consisting of diverse taxa at control sites. Terrestrial serpentinite-hosted microbial ecosystems with their accessibility, their low phylogenetic diversity, and limited range of energetic resources provide an excellent opportunity to explore the interplay between geochemical energy and life. Furthermore, because ultramafic rocks originate from the deep Earth, serpentinite hosted microbial communities may play critical roles in controlling the flux of carbon and energy between the subsurface and the surface biosphere.

Abstract Category
14 Microbe-Mineral Interactions

PS.14.037 BASALT MINERAL OXIDATION BY OCEAN CRUST BACTERIA

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Ocean crust microbial biomass is more substantial than that of microbial life in the deep sea. The most likely source of energy for sustaining sub-seafloor microbial populations comes from redox-active iron that is slowly released from basalt glasses and minerals, including olivine and fayalite. Fluid flow creates a transport mechanism for microbes to colonize new crust as it forms, and it has been shown that older crusts have the highest biomass and greatest alteration. The main purpose of this study was to determine which minerals in basalt are preferentially colonized by bacteria, and why. A variety of igneous minerals and glasses were incubated in microbial flow cells for four years in IODP borehole site 1301A on the eastern flank of the Juan de Fuca ridge. The total number of cells on each mineral were stained with DAPI and counted using epifluorescent microscopy. Oligotrophic mesophiles and thermophiles were isolated on marine R2A plates and reported as colony forming units (CFUs) per gram of mineral. Isolate 3d.6 from fayalite was incubated in olivine cultures to determine iron-oxidation and iron-reduction capabilities. Total cell counts reflect a preference among bacteria for iron-rich minerals from basalt. Thermophilic bacteria were isolated from olivine and fayalite only, and mesophilic bacteria were present in larger numbers on the iron-rich minerals. Our results also indicate iron oxidation of olivine minerals by 3d.6, a deep sea borehole bacteria isolated from the Juan de Fuca Ridge. 3d.6 is was not found to be capable of iron

reduction in olivine cultures with either lactate, acetate, succinate, or pyruvate. Our results suggest iron is an important source of redox energy in the ocean crust which helps support a greater biomass. Our 3d.6 results also indicate that olivines in basalt are capable of supporting iron-oxidizing populations of bacteria.

Abstract Category
14 Microbe-Mineral Interactions

PS.14.038 MANGANESE OXIDES ASSOCIATED WITH MANGANESE OXIDIZING BACTERIA

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The objective of this research is the characterization of Mn(IV) oxide formation relating to cell surface biopolymers as templates for Mn(IV) oxide formation, and processes governing both mineral precipitation and biofabric formation. Mn is an important environmental driver of redox reactions. Microorganisms catalyze the oxidation of Mn(II) to Mn(IV). Mn(IV) oxides collected from both Mn-oxidizing bacteria in laboratory cultures and environmental samples were analyzed using optical (OLM) and electron microscopy (EM) to characterize microbe-mineral associations and BET analysis measuring surface area. Microbial communities catalyze the precipitation of Mn(IV) oxides influencing the biofabric of geologic formations. Recent studies have shown biogenic Mn(IV) oxides possess novel nanosheet architectures exhibiting layer thicknesses extending from a few to hundreds of nm laterally, with exceedingly high surface areas. Environmental samples from two Mn-precipitating environments, an 8°C terrestrial cold-seep and a 4°C submarine volcano were analyzed and compared to model laboratory cultures, allowing for visualization of microbe-mineral associations in a controlled environment. Environmental samples prepared as petrographic thin sections and characterized using OLM showed the distribution of Mn(IV) oxides and their association with microbial communities. Mn(IV) oxides from the cold-seep formed porous structures closely associated with microbial communities and secreted polysaccharides. Mn(IV) oxides from the submarine volcano formed thin granular layers of oxides possibly settled from the water column. Laboratory cultures cultivated with and without dissolved Mn were analyzed by EM characterizing microbe-mineral associations, Mn(IV) structures and cell surface characteristics important for mineral precipitation. Microorganisms and their polysaccharides affect the precipitation of Mn(IV) oxides influencing the nanostructure, reactivity and surface area of precipitates.

Abstract Category
14 Microbe-Mineral Interactions

PS.14.039 CHARACTERIZATION OF OUTER MEMBRANE PROTEINS INVOLVED IN IRON REDUCTION AND BIOFILM FORMATION IN *GEOBACTER SULFURREDUCTENS*

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Geobacter sulfurreducens is an iron reducing bacterium commonly found in subsurface environments that is capable of utilizing iron and other metals as electron acceptors for respiration. It has been demonstrated that *Geobacter* reduces iron oxides via direct contact and that biofilm formation facilitates interaction with solid electron acceptors. There are several outer membrane c-type cytochromes (Omc) and a pilin protein that been linked to iron oxide reduction in *G. sulfurreducens*. These proteins include OmcB, OmcE, OmcS and PilA. The loss of OmcE, OmcS and PilA does not affect the reduction of iron citrate, a soluble electron acceptor, while loss of OmcB affects both soluble and insoluble iron reduction. Although it is known that these proteins are important for the reduction of solid electron acceptors, how their expression changes throughout a multi-layered biofilm during growth remains to be discovered. As a result, our research is focused on differential protein expression in multi-layer *Geobacter* biofilms. The genes encoding OmcB, OmcE, OmcS and PilT were amplified from the genomic DNA, subcloned into an expression vector

and expressed as a His-tag fusion protein in *E. coli* cells. Once expressed, proteins were purified on a Ni-NTA column and antibodies were generated against them. Antisera were affinity purified and the antibodies were tested for their specificity against their respective protein in *Geobacter* via Western blotting, which demonstrated specificity for detection. The antibodies were used to immunolocalize these proteins in a *Geobacter* biofilm grown to a thickness of 20 µm over 72 h in a microbial electrolytic cell (MEC) on a polished graphite electrode. The pre-immune serum was used for the control biofilm. Immunogold labeling and transmission electron microscopy were used to visualize localization in the biofilm. Initial results show that the concentration of OmcB may vary significantly as a function of depth in the biofilm.

Abstract Category

14 Microbe-Mineral Interactions

PS.14.040 BIOLEACHING OF SUBMARINE HYDROTHERMAL DEPOSIT USING IRON- AND SULFUR-OXIDIZER

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Hydrothermal deposit in submarine is known to contain high concentration of Cu, Zn, Pb, and several precious and rare metals. In order to use the deposit as resources of metals, development of suitable metallurgical technology is needed. In this study, bioleaching using several kinds of iron- and sulfur-oxidizers were applied for a hydrothermal deposit. Solubility of Cu and Zn from the deposit and fate of some metals were investigated. Hydrothermal deposit was obtained from a chimney of Myojin knoll in Izu-Bonin back-arc, Japan, in 2003. This deposit contained 24% Zn, 5.4% Pb, 5.2% Cu and 10.8% Fe mainly, and Au, Ag, Sb, Mo, Ga, Ge, and others were also contained in the range of ten to hundred mg/kg. According to XRD analysis, sphalerite, chalcopyrite, pyrite and galena were detected. As iron- and sulfur- oxidisers, *Acidithiobacillus ferrooxidans* (DSM14882), *Sulfobacillus thermosulfidooxidans* (DSM9293) and *Acidinus brierleyi* (DSM1651) were used. Bioleaching experiments were carried out batch system using 300 ml of Erlenmeyer flasks containing 100 ml of medium and 1 g of deposit. *A. ferrooxidans* shown the highest leaching ability, extraction of Zn and Cu reached almost 100 % in 30 days cultivation. Cu existed as chalcopyrite in the deposit. Usually, Cu leaching from chalcopyrite was very difficult using *A. ferrooxidans*. The difference was seemed a catalyst of Ag dissolved into leaching solution. Although *A. brierleyi* has shown high ability of chalcopyrite leaching, neither of Zn and Cu did not dissolve in this study. These results showed that the hydrothermal deposit is different from Kuroko in despite of similar structure. In the residues, Au and Pb were detected. Au did not dissolve into the leaching solution, but Pb was precipitated with sulfate once leached. Some rare metals, Mo, Sb and so on, were mainly dissolved into the solution. However their mass balances were not estimated because some missed in chemical analysis. Therefore more consideration was needed.

Abstract Category

14 Microbe-Mineral Interactions

PS.14.041 TRANSIENT STORAGE OF ELECTRONS IN CULTURES OF *Shewanella oneidensis* MR1, TEMPORARILY DEVOID OF AN EXTERNAL ELECTRON ACCEPTOR

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The genus *Shewanella* contains aquatic microorganisms with worldwide distribution due to their unparalleled capacity to respire compounds found in the environment and their capacity to thrive at low temperatures. In addition to oxygen, *Shewanella* can respire various organic and inorganic substrates, as well as various soluble and solid metal electron acceptors.

Since solid electron acceptors do not have a continuous distribution in the environment, but appear rather as discrete packages at random locations, we postulate that *Shewanella* would profit from the ability to transiently store electrons during its search for solid electron acceptor.

The objective of this work consists in the study of the electron storage capacity of *Shewanella oneidensis* MR1 when no electron acceptors are present in the medium. This was analyzed using Microbial Fuel Cell (MFC) reactors.

Three MFC were employed with the same anode and cathode composition. The anode chamber contained a pure culture of *Shewanella oneidensis* MR1, with lactate as carbon source while the catholyte was formed by ferricyanide.

When electric circuit of a MFC is opened, electrons cannot flow from the anode to the cathode and, as a result bacteria are unable to use the anode as an external electron acceptor. The circuits of several MFCs were opened for variable intervals, and closed again for a period of one hour.

After closing the circuit, the current circulating through the cell increased sharply to a maximum value followed by a gradual decrease back to a basal level. The magnitude of the overcurrent, defined as the difference between the initial peak and the basal level, appears to correlate well with the time the fuel cell was kept in open circuit state.

Our results indicate that cultures of *Shewanella oneidensis* MR1 can store electrons when no electron acceptors are present. The amount of charge stored appears to be proportional to the time that he spends in the absence from these acceptors.

Abstract Category
14 Microbe-Mineral Interactions

PS.14.042 GEOCHIP-BASED ANALYSIS OF GROUNDWATER MICROBIAL COMMUNITIES ACROSS A GRADIENT OF PH, HEAVY METAL, AND NITRATE CONTAMINATION

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The Integrated Field Research Center (FRC) site at the U.S. DOE's Environmental Remediation Science Program at Oak Ridge, TN, is contaminated with radionuclides, heavy metals, and other contaminants due to historical U enrichment activities. To examine the impact of contaminant level on microbial communities, six contaminated and one uncontaminated well, providing a range of contaminant levels, were sampled. DNA from these samples was analyzed with a comprehensive functional gene microarray (GeoChip 2.0) containing probes for >10,000 genes involved in carbon, sulfur, nitrogen and phosphorus cycling, contaminant degradation and metal resistance and reduction. Results of principle component analysis of all functional genes detected showed that moderately contaminated wells clustered together while wells with high and relatively low contamination levels grouped together. These results suggest that individual contaminants may have a stronger influence on community structure than the overall level of contamination. Wells with higher levels of contamination were enriched in genes for sulfate reduction (*dsr*), contaminant degradation, nitrate/nitrite reduction, and metal resistance compared to wells with little to no contamination. Redundancy analysis indicated that dissolved oxygen, distance from the contamination source, Sr, and Cs were the most important environmental variables in determining community structure ($p=0.03$). Subsequent variance partitioning analysis indicated a significant correlation between the community structure and both Sr and Cs ($p=0.05$). These metals were able to independently explain almost 60% of the variation observed. This study provides an overall view of the functional genes present in a highly contaminated environment, and shows that the contaminant level (individually and as a whole) has significant effects on bacterial community structure.

Abstract Category
14 Microbe-Mineral Interactions

PS.14.043 IDENTIFICATION OF THE ACETATE-OXIDIZING MANGANESE-REDUCING BACTERIA IN MANGANESE OXIDE RICH MARINE SEDIMENT BY STABLE ISOTOPE PROBING

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Energetically, manganese oxide is a highly favorable electron acceptor and recent studies showed that with available manganese oxide microbial manganese reduction can dominate carbon oxidation over iron and sulfate reduction in marine sediments. However, manganese-reducing bacteria have rarely been studied and, since most iron reducers are able to reduce manganese oxides, most of their properties have been transferred to manganese reducers. We aimed to identify the acetate-oxidizing manganese-reducing bacteria in a manganese oxide-rich sediment by stable isotope probing (SIP). The upper 5 cm of sediment from Gullmarsfjorden (Sweden) were rich in manganese and iron oxides (both ~50 $\mu\text{mol cm}^{-3}$). In anoxic sediment incubations with and without addition of acetate sulfate reduction was insignificant for carbon oxidation and microbial manganese reduction was considered the single terminal electron accepting pathway. The addition of ¹²C- or ¹³C-labeled acetate stimulated carbon oxidation 3-fold compared to the control and the total dissolved inorganic carbon (DIC) production was calculated to result solely from acetate oxidation. RNA-SIP coupled to terminal restriction fragment length polymorphism (TRFLP) analysis identified 3 major TRFs in the ¹³C-labeled (heavy) RNA fraction of the ¹³C-acetate incubation after 2, 6 and 17 days of incubation. The 3 TRFs were identified as sequences related to the genera *Colwellia* and *Neptunomonas* within the *Gammaproteobacteria* and *Arcobacter* within the *Epsilonproteobacteria* by cloning. For none of these genera the ability to reduce iron or manganese oxides has been shown in pure cultures. The low number of sequences from known iron- and manganese-reducing bacteria (<5%) suggests that members of *Colwellia*, *Neptunomonas* and *Arcobacter* are important manganese reducers in the studied sediment.

Abstract Category

14 Microbe-Mineral Interactions

PS.14.044 MAPPING IRON BINDING SITES ON THE SMALL TETRAHEME CYTOCHROME (STC) OF SHEWANELLA ONEIDENSIS MR-1

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Shewanella oneidensis MR-1 has the metabolic capacity to grow anaerobically under Fe(III)-reducing condition in which it produces a variety of periplasmic c-type cytochromes that are thought to be involved in the Fe(III) respiratory process. Among those is the small tetraheme cytochrome (SoSTC) with unknown physiological role although its homologous form from *Shewanella frigidimarina* has been proved to participate in iron respiration. In this work, We use Isothermal Titration Calorimetry (ITC) to measure binding affinities between purified recombinant SoSTC and various Fe (III)-chelates including Fe (III)-EDTA, Fe (III)-NTA, and Fe (III)-Citrate. The ITC measurements demonstrate that SoSTC has a preferential binding with Fe (III)-EDTA and Fe(III)-NTA. In contrast with ferric species, ferrous chelates have shown about 10-100 times less in terms of binding affinity with SoSTC. With understanding of SoSTC binding with charged iron complex, we propose that the iron binding site should consist of charged amino acids. Through chemical modification of amine groups and carboxyl groups, we have found that SoStc with carboxyl residues modified shows no binding with Fe(III)-EDTA by ITC measurements. Moreover, the electron transfer rate constant of carboxyl-group modified SoSTC when reacting with Fe (III)-EDTA were retarded by 50 times. Carboxyl-group scanning studies were used to probe the putative iron-binding site by site-directed mutagenesis of glutamate and aspartate on SoSTC. SoSTC (D79N) shows no binding with Fe(III)-EDTA measured from calorimetric measurements. The imparity of the binding of SoSTC (D79N) with Fe (III)-EDTA was further confirmed by transient state kinetics of reduced SoSTC (D79N) reacting with Fe (III)-EDTA which demonstrates around 10 times decrease of second order rate constant. Thus, we suggest that Asp79 is involved in electron tranfer from SoSTC to Fe(III)-EDTA

Abstract Category

14 Microbe-Mineral Interactions

PS.14.045 MICROARRAY-BASED CHARACTERIZATION OF MICROBIAL COMMUNITY FUNCTIONAL STRUCTURE DURING IN SITU BIOSTIMULATION AT A URANIUM-CONTAMINATED AQUIFER

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The heavy contamination of U(VI) (5-10 μ M) in groundwater at the Oak Ridge National Laboratory in TN presents a great ecological and human health concern. To investigate bioremediation potential, a slow-released substrate (SRS; 60% vegetable oil, 4% lactate) was injected within 2 hrs into the subsurface and microbial communities in one upgradient and seven downgradient wells were monitored for 140 days using GeoChip, an array capable of detecting \sim 28,000 functional genes responsible for C, N, S, and P cycling, metal resistance, and organic contaminant degradation. The SRS amendment substantially decreased the U(VI) concentration in all seven downgradient wells and changed the overall microbial functional structure. Detrended correspondence analysis incorporating all functional genes detected separated the communities in the seven wells after injection from those in same wells but before injection, whereas the functional structure in the upgradient well remained similar throughout the experimental period and clustered closely with those in the seven wells before injection. Further analysis of the detected gene groups revealed long-term enrichment of sulfate-reducing, nitrate-reducing, and cytochrome genes and of cytochrome genes derived from species in *Desulfovibrio*, *Anaeromyxobacter*, and *Geobacter*. This was indicated by significantly increased relative abundance ($P < 0.05$) and diversity of these groups of genes in the communities. The functional gene results were highly coincident with the supplement of electron donor (acetate) from SRS biodegradation and subsequent reduction of multiple electron acceptors (U(VI), sulfate, Fe(III), and nitrate) in the aquifer, as evidenced by canonical correspondence analysis of the geochemical and functional gene data. GeoChip is a high throughput technology that provides the advantage of directly targeting multiple groups of functional genes responsible for complex biotransformations in the environment.

Abstract Category
14 Microbe-Mineral Interactions

PS.15.001 MODELING TEMPORAL DYNAMICS OF VAGINAL BACTERIAL COMMUNITIES IN HEALTHY WOMEN

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The beneficial effects of the indigenous microbiota on women's health are numerous and poorly characterized. Significant alterations or disruptions of the vaginal microbiome, such as in bacterial vaginosis, may increase a woman's risk to preterm labor and acquisition of infections. We sought to develop a mathematical model of temporal variations in the composition and abundances of indigenous bacterial populations in the human vagina and determine associations with environmental, behavioral, and life history characteristics. The Bayesian approach presented here has a hierarchical framework, which can be divided into two main parts. First we model the lower level of the hierarchy, which includes changes in the presence/ absence of bacterial phylotypes and their relative abundance. We suggest a mixture model, combining a Bernoulli distribution describing presence and absence of each phylotype and a Poisson distribution describing the corresponding abundances. In the second part we implement the meta-data into the model, which includes environmental, behavioral, and life history aspects using a state space model in conjunction with a generalized linear model. This approach allows us to detect dependencies between bacterial community structures and the metadata. The models will be applied to a longitudinal data set obtained by 454 pyrosequencing of 16S rRNA genes based on vaginal swab samples and questionnaires addressing environmental, behavioral, and life history aspects, both provided every 3rd day from 39 woman over a period of 15 weeks. Preliminary results suggest that the temporal dynamics of the bacterial communities are personalized, that is there are no obvious patterns in changes in the vaginal bacterial communities over time among woman but rather the dynamics are individual to each woman.

Abstract Category

15 Microbial Ecology and Ecological Theory

PS.15.002 NUTRIENT CYCLING INCREASES THE PERSISTENCE OF A MODEL MICROBIAL ECOSYSTEM

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Nutrient cycling is key feature of microbial ecosystems but from a theoretical point of view, the consequences of nutrient cycling for ecosystem dynamics remain poorly understood. Our aim is to use mathematical modeling to study the effects of nutrient cycling on the dynamics and persistence of simple model microbial ecosystems.

We have used computer simulations to study a simple mathematical model for a two-species, nutrient-cycling, microbial ecosystem. We compare this nutrient cycling model to the equivalent

one-species model system, without nutrient cycling. Our simulations include randomness due to the births and deaths of individual microbial cells, so are relevant to environmental communities inhabiting very small niches.

Unexpectedly, we find that the birth-death random noise in our simulations can cause population oscillations, in both the one-species and two-species models. Small communities are highly susceptible to extinction. We compare the average time to extinction (persistence time) for the one-species and two-species models. We find that nutrient cycling leads to increased ecosystem persistence, and that in environments where the resource supply is very intermittent, it may even be beneficial for a population to divert nutrients from its own growth to sustain the other partner in the cycle.

Simple mathematical models have great potential for shedding light on the fundamental behaviour of microbial ecosystems. We discuss the implications of our results for real environmental microbial ecosystems, from both an ecological and an evolutionary perspective.

Abstract Category

15 Microbial Ecology and Ecological Theory

PS.15.003 RESISTANCE AND RESILIENCE OF BACTERIAL COMMUNITIES DEPEND ON DISTURBANCE STRENGTH AND FREQUENCY

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Disturbances are important factors controlling biodiversity and ecosystem functioning. Even though bacteria are key components of ecosystems, systematic knowledge about how they respond to different types of perturbations is unknown. More specifically, little is known about their resistance and resilience in response to variations in disturbance intensity and frequency. We therefore implemented a transplant experiment with dialysis bags to investigate how the strength and frequency of a perturbation (an increase in salinity), affects the composition and functioning of aquatic bacterial communities. To investigate the effect of disturbance strength, microbial communities were exposed to six different salinity treatments (0, 1, 3, 5, 10, 20 psu). Dialysis bags filled with freshwater were moved from the control tank (0 psu) to the different disturbance treatments and incubated for 24 h. Afterwards the bags were moved back to the control tank and incubated for 36 h. To investigate the effect of disturbance frequency, another experiment was performed using the same set-up. Here, the dialysis bags were swapped between the control and the 20 psu treatment at different frequencies (0, 1, 2, 3 or 4 times over a 64 h period). In both experiments, water samples from the bags were taken before and after disturbance exposure to obtain a measure of resistance of the communities, and after the second incubation in the control tank to obtain a measure of resilience. For each sample, bacterial abundance, activity and community substrate utilization profile were measured. Moreover, bacterial community composition was determined by t-RFLP of the 16S rRNA gene. With these data we will test 1) how resistant and resilient bacterial communities are in terms of the measured functional properties as well as community composition, 2) whether resistance decreases with increasing disturbance rates and 3) whether resilience is higher in less disturbed communities.

Abstract Category
15 Microbial Ecology and Ecological Theory

PS.15.004 ARE SPECIES COHESIVE? WHAT MICROBIOLOGISTS AND ZOOLOGISTS CAN LEARN FROM ONE ANOTHER

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Evolutionary biologists have viewed species as cohesive groups of organisms, but it is not clear that either the macroorganisms or the bacteria form cohesive species. Here I address, through theoretical and bioinformatics approaches, whether species-like ecotypes (ecologically homogeneous populations) of bacteria are subject to these three modes of cohesion: speciation-quashing cohesion (preventing divergence), niche-transcending cohesion (sharing of adaptations), and intra-population cohesion (elimination of within-species diversity). Bacteria are not subject to speciation-quashing cohesion by recombination; theory of recombination-selection balance shows that recombination is too rare to prevent divergence in niche-specifying adaptations. Likewise, animals show little speciation-quashing cohesion, as interbreeding populations can usually diverge without bound in their niche-specifying adaptations. Genome comparisons show that bacteria share niche-transcending genes among ecologically distinct populations while maintaining their niche specificity; the difference between bacteria and animals is that sharing of niche-transcending genes is limited to close relatives in animals but not in bacteria. While most models of bacterial species assume intra-population cohesion, this is not yet proved; recent genome comparisons indicate that some bacterial species are not subject to intra-population cohesion. Species studies of animals and plants focus on the evolution of barriers to recombination, which emphasizes the end of niche-transcending cohesion and de-emphasizes the origins of ecological divergence. Species studies in bacteria instead focus on the origins of irreversible divergence, since there are no phylogenetic limits on the ability of bacteria to acquire niche-transcending genes. If zoologists were to follow the lead of bacteriology in de-emphasizing niche-transcending cohesion, this would allow them to focus on the origins of diversity.

Abstract Category
15 Microbial Ecology and Ecological Theory

PS.15.005 CO-AGGREGATION MAY FAVOUR THE SURVIVAL OF GRAZING-VULNERABLE BACTERIA IN FRESHWATER HABITATS

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Aggregation is a successful anti-predator strategy for freshwater bacteria against the smallest and most effective protistan predators (nanoflagellates). We enriched for grazing-resistant bacterial phenotypes by exposing natural microbial communities from a lake to predation by the voracious interception-feeding nanoflagellate *Poteroiochromonas*. Aggregates larger than the predators were sorted out by flow cytometry and plated on low-nutrient agar to obtain bacterial strains that were able to aggregate or to co-aggregate with others. A number of strains from different

bacterial taxa were isolated, identified, and tested in batch co-cultures with *Poteroiochromonas*. A wide spectrum of different responses was observed: besides aggregating strains, there were also edible, non-aggregating ones, albeit with different nutritional quality for the predator. Since all strains were derived from sorted aggregates, we hypothesized that some of the non-aggregating strains were able to co-exist with aggregate forming ones, thereby obtaining refuge against the predators. This was tested in batch culture experiments with predators and defined mixes of grazing-vulnerable and aggregate forming strains and subsequent analyses by fluorescence in situ hybridization combined with microscopy and flow cytometry. The survival of an edible strain was significantly favoured in co-culture with an aggregating strain (as compared to pure culture), providing evidence for the advantage of co-aggregation as a means to escape nanoflagellate predation. Interestingly, predators also seemed to profit from the simultaneous presence of aggregating and non-aggregating strains, possibly because the refuge within the aggregates allowed for a continued production of free-living, edible cells by the non-aggregating strain. The here described mechanisms might be important both for the maintenance of grazing-vulnerable bacterial populations in freshwaters and for their importance as food for bacterivores.

Abstract Category

15 Microbial Ecology and Ecological Theory

PS.15.006 CONSERVATION OF GENE REGULATION IN *BURKHOLDERIA*

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Understanding the common functions and regulatory features of larger groups (e.g., genera and families) enables extension of data from specific lab strains to more diverse natural assemblages. The rapid accumulation of genome sequences compels this expanded focus. We have begun deciphering the common properties of *Burkholderia*- a versatile group that thrives in diverse ecological niches including soils, water, protozoa, insects, plants, and animals. Using *Burkholderia thailandensis* as a model system, we applied comparative genomics, high-throughput experiments, and informatics to build a functional map of the genome and extend information across the genus. With this combined approach, we a) revised *Burkholderia* species gene maps to improve consistency across the genus; b) used these maps to compare the conservation of orthologous genes and corresponding intergenic regions, c) obtained ~170 global gene expression profiles for *B. thailandensis* under a wide range of growth conditions; d) used these profiles to validate operon structure and predict transcriptional regulatory interactions; e) experimentally identified DNA binding sequences for transcriptional regulatory proteins; f) and transitively mapped the information gained from *B. thailandensis* to closely related species. Our collection of *B. thaliandensis* Affymetrix gene expression data is comparable in size to the landmark collections for *Shewanella* and *E. coli*. Our aggregate data has substantially increased our understanding of gene regulation in *B. thaliandensis*, and has generated experimentally testable hypotheses about gene regulation in this and closely related species. We are now poised

to validate regulatory features conserved across the *Burkholderia* genus, and to identify regulatory divergence associated with speciation and niche specialization.

Abstract Category

15 Microbial Ecology and Ecological Theory

PS.15.007 DYNAMICS OF BACTERIAL COMMUNITY SUCCESSION AND INTERACTION IN A CHEMOSTAT MODEL

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Chemostat models on phenol were constructed to investigate the basis of bacterial ecosystem. Analyses of bacterial community structure using both culture-dependent and -independent methods revealed that the succession of dominant bacteria occurred in a chemostat enrichment added soil as a inoculum; first dominant bacterium was genus of *Pseudomonas*, next were *Ralstonia* and *Acinetobacter*, and final was *Variovorax*. Interestingly, kinetic parameters for phenol of the enrichment changed from low KS and high KI values to high KS and low KI values with oscillation, which corresponded to these parameters of strains isolated from the chemostat; *Pseudomonas* strains exhibited low KS and high KI values and *Variovorax* strains exhibited high KS and low KI values. How does the *Variovorax* become dominance in the culture? We thought that there is a clue for understanding microbial ecosystem. A mixed chemostat model culture was established with *Pseudomonas* sp. C8, *Ralstonia* sp. P-10, and *Variovorax* sp. chemo24. These λ values of strains C8, P-10, and chemo24 were 0.0155, 0.0556, and 0.147, respectively. The λ value was calculated according to dominating theory (Hansen & Hubbel 1980 Science 207:1491), which demonstrated that the bacterium exhibiting lower λ value become dominance. These population densities were monitored with real-time PCR targeting the gene coding α subunit of phenol hydroxylase. Bacterial succession was observed and chemo24 became dominance in the culture. Their interaction was evaluated by measuring the growth curve under the addition of supernatant or not. The supernatant of C8 strongly suppressed the growth of P-10 and chemo24, while each supernatant of P-10 and chemo24 hardly affected the growth of all three strains. Interestingly, the mixed supernatants of C8 and P-10 enhanced the growth of chemo24. This research demonstrated that bacterial interaction is an important key factor for microbial ecosystem.

Abstract Category

15 Microbial Ecology and Ecological Theory

PS.15.008 CROSSING ECOSYSTEM BOARDERS: A MICROBIAL COMMUNITY TRANSECT STUDY FROM THE TERRESTRIAL SHORELINE TO THE FRESHWATER COLUMN OF A LAKE.

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Terrestrial and aquatic ecosystems are two connected neighboring biomes linked via nutrient flows, water run-off, ground water, and flooding events. Environmental studies on microbial turnover and total carbon and nitrogen yields indicate sensitivities to environmental and climate changes. Hence it is important to understand the diversity and ecology of the microorganisms involved. Earlier studies from globally dispersed sites suggest that each of these biomes harbor a characteristic microflora mediating carbon and nitrogen turnover. In the present study we assess microbial dispersal along a horizontal transect from the terrestrial shoreline across the littoral of a lake towards the pelagic zone in a temperate Swedish freshwater lake, lake Erken, east of Uppsala. We have sampled soil cores on the shoreline and collected sediment and water samples in the littoral and open water. For each sampling site, pH, carbon and nitrogen contents were determined along with temperature and oxygen levels in the open water. These variables allowed determine the environmental context of the microbial community in this study. We here applied the modern TITANIUM pyro-sequencing technology to identify the bacterial and archaeal community, using the 16S rRNA gene sequence and the ITS region for the fungal community respectively. The data allow drawing a picture on the phylogenetic connectivity of microbial communities in geographically close terrestrial and aquatic environments. This study is incorporated in a project of the Uppsala Microbiomic Center (UMC) investigating microbial communities at terrestrial and aquatic sites, and their importance in chitin degradation.

Abstract Category

15 Microbial Ecology and Ecological Theory

PS.15.009 COMMENSALISM AND NICHE ADAPTATION IN *Escherichia coli*

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Commensalism and pathogenesis are important evolutionary and ecological adaptations that enable organisms to exploit new and different habitats. *Escherichia coli* is capable of existing as a free-living organism and as a commensal or pathogen in the colons of mammals and birds. The genome of the pathogenic strains of this species contains several genetic elements such as pathogenic islands and plasmids that encode an array of virulence factors. Surprisingly, the commensal strains also have some of these pathogenic elements in their genomes. The aim of the present research is to explore the evolutionary mechanisms that allow *Escherichia coli* to adapt to different niches. Using Pulsed Field Gel Electrophoresis, Southern blots, Multilocus Sequence Typing and dot-blot of several virulence factors, we surveyed a collection of 153 isolates. These isolates include wild mammalian and avian hosts as well as environmental isolates from mud, water and from healthy and diseased humans. Great variability in chromosome size and number of plasmids was found throughout the sample whereas no remarkable differences were found among commensal and pathogenic strains. We also obtained high levels of genetic diversity together with intermediate levels of recombination and genomic rearrangements. Defined genetic structures and a wide distribution of virulence factors were also detected in the isolates. All above suggests that *E. coli* presents considerable genome plasticity and genetic diversity which allows this species to adapt to different ecological niches. Furthermore, due to the astonishing

genetic information harbored, commensal strains carry out an important role in the evolution of the pathogenesis acting as a reservoir of potentially adaptive factors.

Abstract Category

15 Microbial Ecology and Ecological Theory

PS.15.010 A WHOLE LANDSCAPE IN A SINGLE MICROTITRE PLATE - THE DYNAMICS OF MICROBIAL DIVERSITY OF ARTIFICIALLY COUPLED ECOSYSTEMS WITH INCREASING DISPERSAL

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The emergence or the persistence of a high microbial diversity in natural ecosystems results in the balance between the probability of having the best adapted species of the local niche, and the colonization of new species through immigration from neighboring communities. Designing microbial microcosms seemed well adapted to delineate the respective role of the competitive exclusion at the local scale and the immigration events at the regional scale according to the theory of island biogeography. We started from an activated sludge of a domestic wastewater treatment plant as inoculum. It was repeatedly grown in either commercially available 96 wells microplates with 31 substrates (EcoPlate Biolog) or custom designed microplates. The productivity of each community was monitored on line with a microplate absorbance reader. The community structure and genetic diversity were assessed after each growth by CE-SSCP fingerprinting profiles. Successive inoculations allowed us to follow the dynamics of about 100 generations. In order to gradually increase the immigration pressure, we added increasing amounts of cells from neighboring wells (with bacteria having different history) for the successive inoculations. The inoculum diversity first decreased then stayed constant at equilibrium, but differed according to the substrate used (as expected by the niche theory). The immigration from the regional pool induced a larger diversity as compared with the undisturbed communities, but a slightly lower performance. These results were in accordance with the island biogeography theory; the dispersal of non adapted species increased the apparent local diversity but encountered unfavorable conditions. We were able to follow the dynamics of many communities in parallel with a perfect control of environmental conditions. This smart experimental set up allowed us manipulating artificial ecosystems to test whether some fundamental ecological theories applies to microbes.

Abstract Category

15 Microbial Ecology and Ecological Theory

PS.15.011 RESISTANCE AND RESILIENCE IN MICROBIAL ECOLOGY, THEORETICAL CONSIDERATIONS APPLIED TO IMPACTS OF MERCURY ON SOIL MICROBIAL COMMUNITIES

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Resilience of soil microbial communities is a major concept for managing ecosystems' durability. While it appears somehow difficult to give an estimation of the ecological resilience of an ecosystem, resilience can be easily defined as the time required for an ecosystem to recover from a stress while its resistance, the amount of perturbation tolerated. This is the bases of the engineering resilience concept. However, when applied to microbial communities, this concept must integrate the return to a steady state of multiple descriptors simultaneously. These descriptors may be as diverse as relative species composition and/or multiple functional activities. In addition, these descriptors may be more or less correlated, which increases the complexity of the task.

We thus developed a multivariate based estimation of microbial communities' resilience and resistance, which was validated on ECOLOG-based multiple substrate degradation analysis and biodiversity measurement using DGGE in tropical soils: Tropical soil samples were submitted to different levels of mercury and their functional diversity and genetic structure were followed for one month using ECOLOG plates and DGGE patterns, respectively. Dynamics of soil microbial community's global recovery were obtained by (i) performing Principal component (PCA) and factor analysis (FA) on the quantitative (ECOLOG) and qualitative (DGGE patterns) descriptive variables, respectively, and (ii) calculating an Euclidian distance between the individual's (the soil samples) projections using their factorial (or principal component) coordinates and (iii) representing the evolution of this distance during time.

While a sigmoid shape of this distance curve indicates an irreversible shift of ecological parameters, a dome shape allows the estimation of the resistance and the engineering resilience of a complex microbial community as the height and the width of the dome.

Abstract Category

15 Microbial Ecology and Ecological Theory

PS.15.012 RECOVERY OF METHANOTROPHS FROM DISTURBANCE: POPULATION DYNAMICS; EVENNESS; AND FUNCTIONING

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Earth's biodiversity is declining forcing humankind to consider how ecosystems' stability and services will change in future. Prokaryotic microbes can catalyze all biogeochemical cycles and have been assumed to be functionally redundant and virtually inextinguishable. However, recent work indicates that microbes may well be sensitive to environmental disturbance. Functional guilds like methane oxidizing bacteria with a well-defined substrate usage are prime candidates to study potentially endangered microbial functions. We simulated disturbance-induced mortality by mixing native with sterilized paddy soil in two ratios, 1:4 and 1:40, representing moderate and severe die-offs. Disturbed microcosms were compared to an untreated control. Recovery of activity and populations was followed over four months by process measurements, pmoA-qPCR, pmoA-based T-RFLP, and a pmoA-based diagnostic microarray (pmoA: gene encoding a subunit of the methane monooxygenase). Methanotrophs showed a remarkable resilience even over-

compensating for die-offs, yet resulting in a decreased evenness. Evenness of a microbial community has been found to be important for their functioning under environmental stress. Here, we show that upon disturbance different growth rates may lead to an uneven community that nevertheless is functioning better than the control. Linking microbial diversity or evenness to functional resilience should consider not only population structure but also population dynamics and controls of activity. While paddy soil methanotrophs supported by high methane concentrations have been found to be resilient, methanotrophs in upland soils living at extremely low methane concentrations was found to be vulnerable. We suggest that the energy flow through a microbial population and its effect on quantitative population dynamics plays a key role deserving increased attention in biodiversity-ecosystem-functioning studies.

Abstract Category

15 Microbial Ecology and Ecological Theory

PS.15.013 AGING WELL: METHANOTROPHIC POTENTIAL AND COMMUNITY STRUCTURE ALONG A PADDY SOIL CHRONOSEQUENCE OF 2000 YEARS.

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Given that rice paddies are anthropogenic methane sources and the inevitable need to increase rice production to sustain human population growth, it is pertinent to identify the effects of long term agriculture on the selection of methanotrophs. Methanotrophs play a crucial role in mitigating methane emission from rice paddies. Here, we analyzed the methanotrophic community along a chronosequence of paddy soils from China covering recently reclaimed sites to paddies under permanent culture since 2000 years (Cheng et al. 2009, *Geoderma* 151, 31-41). Fresh soil was incubated under 10 vol.% CH₄ atmosphere. Temporal changes in activity and population dynamics were followed by methane uptake measurement, pmoA-based T-RFLP and group-specific quantitative PCR (pmoA: gene encoding a subunit of the methane monooxygenase). Maximum potential methane oxidation rate was positively correlated to soil age. Long-term agriculture also imposes a selection pressure on different groups of methanotrophs. More evident in the ancient soil, type Ib methanotrophs were observed to multiply in correspondence with increasing methane uptake, while other groups showed a relatively constant community composition. Long term agriculture shapes the microbial community, selecting for sub-populations of methanotrophs and allows for an elevated level of potential methane oxidation.

Abstract Category

15 Microbial Ecology and Ecological Theory

PS.15.014 MICROBES AND MICROBIAL PATTERNS AS SENTINELS FOR GROUNDWATER ECOSYSTEM ASSESSMENTS

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Healthy aquifers deliver important ecosystem services, e.g. the purification of infiltrating water and its storage in high quality. Nowadays, environmental policy started to consider groundwater not only a resource but a living entity. For a comprehensive protection and management of groundwater ecosystems, the assessment of its ecological status is necessary, but ecological groundwater quality criteria have not defined so far. Aquifers are ecosystems harbouring a vast diversity of microorganisms which are involved in almost all geochemical processes occurring in the subsurface. Besides, microbes are characterised by short generation times and high metabolic rates when compared to classical indicator organisms such as invertebrates, providing a fast reaction to environmental changes. Subsurface microorganisms and microbial patterns are thus believed to represent ideal sentinels of groundwater ecosystem status. Within the framework of a current research project several aquifer systems located in geographically distinct areas of Germany are under investigation. The focus is on the development of an ecologically sound concept for the assessment of groundwater quality and ecosystem health. Our central working hypothesis is that in low dynamic habitats microbial communities reflect the in situ environmental conditions and their biomass, activity and diversity may serve as sensitive criteria for short-term dynamics in aquifers. Linking biodiversity patterns with environmental data is still a challenge for ecologists. Our first results indicate that groundwater microbial community patterns successfully mirror impacts from land use (e.g. agriculture) resulting in an elevated bacterial biodiversity atypical for pristine systems. The application of multivariate statistical approaches, such as multivariate regression trees and aggregated boosted trees, useful for the identification of indicator species/groups is introduced.

Abstract Category

15 Microbial Ecology and Ecological Theory

PS.15.015 IN SITU ABUNDANCE AND DISTRIBUTION OF HYDROCARBON-DEGRADING SRB AT MARINE GAS AND OIL SEEPS

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Hot spots of sulfate-reduction (SR) occur in anoxic marine sediments, where methane, short-chain alkanes or oil components dominate. In methane seeps, SR is tightly coupled to anaerobic oxidation of methane (AOM). In contrast, in sediments with natural oil seepage methane-dependent SR drops to less than 10% of total SR rates as an indicator for non-methane hydrocarbon degradation by sulfate-reducing bacteria (SRB). Although several SRB have been described to be capable of hydrocarbon degradation it is still unknown which SRB are relevant in the environment.

Here, we describe the distribution and abundance of SRB at marine oil and gas seeps as detected by CARD-FISH.

Non-aggregated Deltaproteobacteria made up between 4% and 23% of total single cells while SRB of the Firmicutes were close to the detection limit. SRB of the *Desulfosarcina/Desulfococcus* (DSS) branch strongly dominated gas as well as oil seeps with up to 17% of total single cells, indicating a major role in hydrocarbon degradation. Of these, members of SEEP-SRB1, including the sulfate-reducing partners of methanotrophic archaea but also members of unknown metabolic functions were abundant with up to 35% but only at gas seeps. Other DSS cells seemed to be highly diverse indicating the presence of numerous diverse

and specialized but low abundant SRB (e.g. relatives of str. BuS5) rather than the presence of a dominant subgroup or single species. SEEP-SRB-2, -3, and -4 as well as relatives of aromatics-degrading *Desulfobacterium anilini* were detected in remarkable numbers of up to 5% of total cells. cDNA-based clone libraries were constructed for ribosomal (16S) and functional genes (*aprA*, *dsrA*). Clone sequences confirmed our previous results, showing most sequences affiliated to the DSS branch, SEEP-SRB groups 1 and 2, *Desulfobacterium anilini* and *Syntrophobacterales*. The active sulfate-reducing community is currently further analyzed by stable-isotope probing and NanoSIMS.

Abstract Category

15 Microbial Ecology and Ecological Theory

PS.15.016 ENERGY FLOW AND DISTURBANCE SHAPE METHANOTROPHIC COMMUNITIES

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Methane is next to water vapor and carbon dioxide the third most important greenhouse gas. The largest single sources are natural wetlands and wetland rice fields. In wetland rice fields, aerobic methanotrophs play a key role oxidizing methane before it is released to the atmosphere. However, it has been shown that methanotrophs are affected by nitrogen fertilization, a common agricultural practice in rice fields. We tested two hypotheses: (1), nitrogen fertilization has an inhibitory effect, and (2), methanotrophs are more resilient at high energy flows. We used microcosms supplementing a thin layer of water-saturated paddy soil from below with methane and from above with air. We used two different source strengths of methane resulting in a different flux into the oxic-anoxic boundary, and, hence, different energy flows through the community. Ammonium-based fertilization was applied at high and low energy flows. We analyzed the different communities with a *pmoA* specific microarrays (*pmoA*: gene encoding a subunit of the methane monooxygenase), and used ordination and indicator species to unravel the effects of energy flow and fertilization. Different energy flows had a significant influence on the methanotrophic community, while fertilization had not. Fertilization did not affect methane oxidation activity. Indicator species analysis revealed the genus *Methylobacter* and an environmental cluster strictly affiliated with paddy soils as indicative for high energy flows. Based on these results we rejected the first (effect of fertilization), but could support the second hypothesis (energy flow). We suggest that methanotrophs are adapted to particular environments, and that changes in the energy flow have major effects for the community structure.

Abstract Category

15 Microbial Ecology and Ecological Theory

PS.15.017 THE ROLE OF MICROBIAL COMMUNITIES IN SOIL FOOD WEBS

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A growing body of food web theory studies the paradoxical idea that herbivory can have positive feedbacks on nutrient flow to plants. In more detail, the relationships between root grazing herbivores, microbial pathogens and microbial decomposers may heavily influence plant primary production. By developing a theoretical model combined with glasshouse experiments using dune grasses, ectoparasitic nematodes and dune microbial inoculum, we tested the idea that plants, microorganisms and plant herbivores are in a delicate balance between maximizing nutrient mineralization, inhibiting microbial pathogens and maintaining plant biomass. Microbial enemies of nematodes can control population size of root grazing nematodes, thus protecting the plant from overgrazing. Individual plant species have their own local microbial flora, and this flora interacts with grazing nematodes to affect their population size as well as plant growth. However microbial nematode control is dependent on plant and nematode species, and the mechanisms responsible for this variation have not been established. In fact, nematode grazing activity may stimulate microbial growth through mineralization of nutrients and increased availability of root carbon. In our research, we are resolving the mechanisms of interaction between root herbivores, microorganisms (the microbial loop including protozoa and bacteria) and the plant host. Specifically, we focus on the microbial loop for its critical role in nutrient mineralization. In the past, both positive and negative feedbacks between microorganisms and herbivorous nematodes have been established. Our research describes an unexplored interaction network in soil food webs where herbivorous nematodes and the microbial loop positively affect nutrient availability and plant productivity.

Abstract Category

15 Microbial Ecology and Ecological Theory

PS.15.018 USING ECOLOGY FOR THE UNDERSTANDING AND RATIONAL DESIGN OF BACTERIAL CONSORTIA FOR THE BIODEGRADATION OF WASTE METAL WORKING FLUIDS.

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Bacteria can be found in short term associations, established communities or may be deliberately assembled for a specific purpose. In these circumstances there will be a relationship between the diversity of bacteria present and their net effects such as biodegradation, and nutrient cycling. Many of these effects are ecosystem functions providing services such as bioremediation of wastes or promoting plant growth. In various situations these effects are important for an extensive list of services including human health, rumen function, or the local sewage plant. In all of these we are interested in the relative significance of the different ways in which bacterial diversity influences the activity of systems. Our aim is to study the impact of bacterial diversity by identifying the variance in effects due to different species identities, species richness and interactions among species. We will present results using the random partitions design (1, 2) that show the relative contribution of bacterial identities, diversity and interactions to the bacterial anaerobic reduction of metal working fluid. Our results show the potential for the rational design of biological systems using microbial ecology and ecological theory to establish communities effective in the biodegradation of metal working fluids and the generation of biogas (3). Metal

working fluids are lubricating coolants, extensively employed for manufacture of metal products. They are chemically complex (oils, amine propoxylate, biocides), toxic in nature, with high chemical oxygen loading (COD 20,000-50,000 mg/l), which makes their safe disposal problematic. 1) Bell, T., Newman J. A., Silverman B. S., Turner S. L., and Lilley A. K. 2005. *Nature* 436. 2) Bell T., Lilley A.K., Hector A., Schmid B., King L, and Newman J.A. 2009. *Am. Nat.* vol 174. 3) Curtis, T.P., Head I.M., Graham, D.W. 2003. *Environmental Science and Technology*.vol 65

Abstract Category

15 Microbial Ecology and Ecological Theory

PS.15.019 CURVE-BASED COMMUNITY FINGERPRINT DATA ANALYSIS IN THE FOLLOW-UP OF ECOLOGICAL SUCCESSION AND EVALUATION OF TREATMENT EFFECTS

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Community fingerprinting techniques produce data from large environmental sample numbers at a reasonable cost, enabling statistically valid study design. The technical details of fingerprinting methods are discussed actively; less attention has received the subsequent data processing step necessary to refine the fingerprint images into reproducible, representative and ecologically significant information on the phenomena.

Here we describe the possibilities of exploratory and hypothesis-driven analysis of fingerprints as curves (electropherograms) instead of binary or semi-quantitative peak data. To exemplify community succession and the effects of different treatments we utilise length heterogeneity PCR fingerprint and background data from a 21-week contaminated soil bioremediation experiment (10 sampling times and three treatments: oil+plant, unvegetated ctrl, uncontaminated ctrl). Pearson correlation was used for similarity, cluster and canonical analyses. Averaged fingerprints enabled recognition of community changes. The taxa responsible for the community shifts were identified by sequencing.

The 180 bacterial community fingerprints grouped into three clusters. Contaminated soils separated from the Unaltered Cluster into the Oil-Degrader Cluster mostly due to the rapid growth of *Aquabacterium*, an r-strategist whose relative abundance correlated with oil degradation rate. Short fragments were characteristic of the Developed Cluster and comprised likely K-strategic *Alfaproteobacteria*. On week 21 these were indifferent in all three treatments, demonstrating community recovery from the contamination.

Both similarity and diversity approaches showed that oil addition induced a rapid but reversible shift in the bacterial community. The gradual effect of the growing plant was less pronounced but also significant. Curve-based fingerprint analysis avoided the caveats and subjectivity of peak binning and quantitation, but enabled sensitive and equally extensive data analysis.

Abstract Category

15 Microbial Ecology and Ecological Theory

PS.15.020 INSIGHTS INTO THE IRON-OXIDIZING ZETAPROTEOBACTERIA: IS EVERYTHING EVERYWHERE?

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The *Zetaproteobacteria* represent a novel class of *Proteobacteria* found predominantly at sites of microbially mediated iron oxidation in marine environments. The chemoautotrophic, neutrophilic Fe-oxidizing representative isolate for this novel class, *Mariprofundus ferrooxydans*, was isolated from Fe-oxide encrusted microbial mats at Loihi Seamount, Hawaii. To date, environmental clones from the *Zetaproteobacteria* have been shown to be dominant at numerous sites with iron-rich microbial mats, including Loihi Seamount, Juan de Fuca Ridge, Tonga Arc and the Southern Mariana Backarc Spreading Center. However, no attempts at describing the phylogenetic relationships of these geographically distinct clones have yet been made. For this study, 66 full-length (>1300bp) small subunit rRNA gene sequences representing 228 *Zetaproteobacteria* clones were identified using the Ribosomal Database Project seqmatch algorithm. Aligned sequences were grouped into operational taxonomic units (OTUs) based on a minimum similarity of 97% (DOTUR). Sequences were also analyzed via principle component analysis (PCA) and analysis of molecular variance (AMOVA) to see if any regional/environmental trends could be correlated to *Zetaproteobacteria* OTU distribution. Seven OTUs contained clones from more than one geographic region. Three of these were widely distributed throughout the Pacific Ocean. The published representative *Zetaproteobacteria* isolates (*M. ferrooxydans* JV-1 and PV-1) grouped as the tenth most abundant OTU, and were found to be endemic to Loihi Seamount. Several other OTUs were also found to be endemic. PCA and AMOVA provided support for *Zetaproteobacteria* biogeography. Ultimately, in order to gain a more detailed understanding of *Zetaproteobacteria* biogeography we need—in addition to further sampling from more diverse geographic regions—more isolates from the dominant OTUs identified by this study.

Abstract Category

15 Microbial Ecology and Ecological Theory

PS.15.021 BIOGEOGRAPHIC PATTERNS IN *THERMOCOCCUS* ISOLATES FROM HYDROTHERMAL VENT SYSTEMS OF THE PACIFIC

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In terrestrial environments islands can be geographically isolated from one another leading to the divergence of populations. In marine environments hydrothermal vent systems and the organisms associated with them can exist as island-like ecosystems dispersed along plate boundaries and geographically isolated from one another over vast stretches of the seafloor. While geographic isolation is commonly accepted as an evolutionary force in the divergence of macroorganisms

the significance of geography in the structuring of microbial diversity remains unclear. *Thermococcus* are an Archaeal genus of hyperthermophilic microorganisms, found to have ubiquitous distribution in hydrothermal habitats. Although *Thermococcus* are anaerobic they can withstand exposure to oxygen and cold temperatures, allowing them to survive outside of hydrothermal habitats and resulting in high dispersal potential. DNA analysis of *Thermococcus* isolates from the Juan de Fuca Ridge, Gorda Ridge, East Pacific Rise, Mid-Atlantic Ridge, Loihi Seamount, and Mariana Arc, were used to determine the relationship between geographic distribution and relatedness. Amplified fragment length polymorphism (AFLP) analysis was used to resolve genomic differences at the species and strain level in 85 *Thermococcus* isolates, allowing for the detection of biogeographic patterns. Population genetic analyses were used to test for geographic genetic structure in isolates at varying geographic scales. AFLP fingerprints and population genetics analyses differentiated isolates from different regions thus supporting the pattern of endemic populations resulting from geographic isolation, while raising new questions about distinct lineages observed and the identification of putative ecotypes.

Abstract Category

15 Microbial Ecology and Ecological Theory

PS.15.022 MODELING NICHE PARTITIONING OF *SYNECHOCOCCUS* SPECIES IN YELLOWSTONE HOT SPRING MICROBIAL MATS

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We are developing a theory of niche structure for *Synechococcus* species inhabiting microbial mats in the effluent channels of Yellowstone alkaline siliceous hot springs, basing both modeling and microbiological approaches on the physical, chemical and biological realities of this community. Most mathematical speciation models assume that a predetermined Gaussian curve describes how fitness relates to an environmental parameter. In contrast, we developed a procedure that optimizes the shape of the fitness curve subject to given environmental parameters (e.g., temperature fluctuation). Our model output for one parameter (e.g., temperature) was not a Gaussian curve with mean value at the parameter average. Rather, it was a flattened u-shaped curve with maxima at the parameter extremes, possibly resulting from longer exposure times at the extremes than at the average. Given the improbability of bimodal temperature optima in a single organism, these results suggest that two species may be better than one in a fluctuating environment, i.e., fluctuations in environmental conditions may lead to niche partitioning. We have used our model to perform competition experiments between two hypothetical species where the first species has Gaussian fitness and the second species has fitness represented by a sum of two Gaussian curves. We have observed combinations of curve width and separation that allow the second species to outcompete the first, further supporting our niche partitioning theory. We are currently considering two fluctuating environmental parameters and incorporating temperature and light data we have empirically measured in situ into our model. In addition, we have cultivated numerous *Synechococcus* isolates and have characterized them as to species, using *psaA* sequence variation and an evolutionary simulation, Ecotype Simulation. Studies of the isolates' temperature and light adaptations will allow us to test the accuracy of our model results.

Abstract Category
15 Microbial Ecology and Ecological Theory

PS.15.023 TOWARDS THEORETICAL MICROBIAL ECOLOGY: HOW MUCH DO WE BENEFIT FROM BORROWING GENERAL PRINCIPLES OF MACRO-SCALE ECOLOGY?

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It is commonly accepted that modern microbial ecology remains mostly descriptive science with limited ability to predict microbial performance and translate microbiological data (e.g. 16S rRNA survey) into macro-scale perspectives of biogeochemical processes at ecosystem to landscape levels. It is further assumed that ecological theory has been shaped mostly by plant and perhaps animals ecologists, while microbial ecology lags behind. In this presentation, I will analyze the applicability to soil microbial communities of several principles, concepts and 'laws' firmly established in general ecology: the concept of ecosystem and carrying capacity, Liebig's 'Law of Minimum, Shelford's "Tolerance Law", 'Alle principle' etc. It will be shown that some of the listed 'laws' are empirical generalizations of limited theoretical value. Actually they can be derived as particular cases of more general solutions generated by non-linear dynamic theory developed for microbial populations. Modern molecular microbial ecology combined with kinetic experiments in continuous culture gives excellent opportunity to create more refined mathematical description of population dynamics than those used for plants and animals. Simpler life cycle of microbial populations, high growth rates, perfect control of environmental conditions and support by advanced molecular tools gives real chance to improve the bases of general ecological theory by using certain microbial communities as model examples. On the other hand, we will greatly benefit from borrowing mathematical tools and concepts carefully developed in plant and animals ecology to characterize bio-morphological structure of natural populations, such as age-dependent structure, differentiation, genetic heterogeneity, etc. Another important lesson which plant ecologists can teach us is the theory of community dynamics: understanding and prediction of community evolution and ecological succession induced by perturbations

Abstract Category
15 Microbial Ecology and Ecological Theory

PS.15.024 SPATIAL PATTERNS OF BACTERIA SHOW THAT MEMBERS OF HIGHER TAXA SHARE ECOLOGICAL CHARACTERISTICS

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Whether bacteria display spatial patterns of distribution and at which level of taxonomic organisation such patterns can be observed are central questions in microbial ecology. Here we investigated how the total and relative abundances of eight bacterial taxa at the phylum or class level were spatially distributed in a pasture by using quantitative PCR. Geostatistical modelling was used to analyse the spatial patterns of the taxa distributions. To test whether the spatial distributions of the different taxa were related to soil heterogeneity, we performed exploratory analyses of relationships between abundance of the bacterial taxa and key soil properties. The distributions of the relative abundance of most taxa varied by a factor of 2.5 to 6.5 and displayed strong spatial patterns at the field scale with autocorrelation ranging between 2 to 37 m. These spatial patterns were taxon-specific and correlated to soil properties, which indicates that members of a bacterial clade defined at high taxonomical levels shared specific ecological traits in the pasture. Overall, the present study showed spatial patterns of distribution of bacteria both at the meter scale and at high taxonomical levels of organisation. Such spatial patterns allow comprehensive observations and predictions of bacterial occurrence in nature, hence helping in the generation of hypotheses concerning the mechanisms generating and maintaining bacterial diversity. The taxa-specific spatial patterns observed here suggest that, in a given environment, ecological traits are shared at high taxonomic levels within the domain Bacteria. This is a piece of evidence that the 16S rRNA gene tree divisions are not only based on evolutionary theory, but also have an ecological reality

Abstract Category

15 Microbial Ecology and Ecological Theory

PS.15.025 THE NEBRASKA SANDHILLS ALKALI LAKE ECOSYSTEM: FITTING A NULL OR NICHE BASED MECHANISM OF SELECTION

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Understanding the forces that shape microbial community structure and function is an important research topic in microbiology. Contained within the Nebraska Sandhills are numerous small lakes, many of which are highly alkaline. A unique combination of climate, geology, and hydrology has resulted in extreme alkaline-saline and freshwater lakes being in close proximity to each other. Because the lakes are in close proximity, microbes freely exchange by animal movement as well as by wind. Thus, assuming water chemistry is a strong selective force, we hypothesized that community structure would correlate to water chemistry (Niche-based mechanisms). The aim of this study was to survey the diversity of microbes in lakes over space and time by pyrosequencing 16S tag rRNA amplicons as a preliminary investigation into the distribution patterns of microbial taxa. DNA was extracted from 16 samples obtained from different lakes (spatial) and at different times in two seasons (temporal). The samples were amplified with 6-base tags and sequenced using the Roche 454 technology. Sequences were processed through the Ribosomal Database Project pyrosequencing pipeline. Taxa richness, abundance, and co-occurrence between samples were then calculated. Four taxa comprised between 70 - 95% of the most abundant Genera in each sample. Across all 16 samples, there were 166 total unique Genera, of which 31 were abundant (upper quartile abundance) and 135 were rare (less than 0.1% abundance). While there were differences in diversity between samples, these differences did not correlate to pH, alkalinity, salinity, location, or time. The

structure of the lake communities is simple in that there were relatively few abundant Genera. Although our data showed clear differences in community structure between samples, there was no correlation between a presumed selective force and structure. This data suggests that gamma-community structure best fits a null rather than a niche model.

Abstract Category

15 Microbial Ecology and Ecological Theory

PS.15.026 TESTING MACROECOLOGICAL IDEAS USING MICROBIAL COMMUNITIES

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Macroecology aims to determine and explain large-scale patterns in nature. Ideas from macroecology, such as species-area relationships, lie at the heart of conservation and population ecology. Many studies have focused on the nature of the patterns without determining explanations of the mechanisms that produce the patterns. While macroecological-like patterns have been detected in microbial communities most studies are hampered by incomplete sampling of the community and the broad-brush, whole community approach that is often used by microbial ecologists. This is in contrast to ecology, where effective sampling is commonplace and studies are usually focused on taxonomic groups at the family level or lower.

We have avoided these problems by analysing patterns in the distribution of specific microbial populations at the genus level and by exploiting high-throughput sequencing technology to completely sample the community. Using the sulfate-reducing bacterial genus *Desulfobulbus* and the methanogenic archaeal genus *Methanosaeta* as models we have analysed microbial distribution using functional gene markers along an estuarine gradient. Pyrosequence data from 30 sediment samples from 10 sites has been used to determine the distribution patterns of these genera in comparison to traditional microbial ecological analyses. We show clear, distinct distribution patterns for these genera with >98% coverage of the community at all 10 sites. We also use this data to determine what macroecology patterns, such as species-area relationships, heritability of occupancy and nestedness, are present in the data. Excitingly, the ability to experimentally manipulate microbial communities opens up the possibility of testing proposed mechanisms behind macroecology patterns in a way that is not possible with larger organisms. Therefore, microbial ecology could lead the way in the move beyond patterns to mechanisms in macroecology.

Abstract Category

15 Microbial Ecology and Ecological Theory

PS.15.027 THE RELEVANCE OF NEUTRAL THEORY TO MICROBIAL ECOLOGY

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Neutral theory is based on the premise that all individuals are ecologically equivalent. Consequently individual species numbers fluctuate as a result of random birth and death events. In Hubbell's model the distribution of abundances in a community is derived from a combination of this stochastic population dynamics and immigration events from a metacommunity (Hubbell 2001). Neutral theory has been successfully if controversially applied in macrobiology. We will discuss its relevance to microbiology. We will address one of the major objections to neutrality by showing how neutral dynamics can be achieved through trade-offs that allow species to have different life history properties but the same deterministic fitness (Parsons and Quince Theor. Pop. Biol. 2007). This 'quasi-neutrality' generates neutral stochastic dynamics without the assumption of identical individuals. We will illustrate this with the example of bacteria in a chemostat. We go on to use this model to investigate whether environmental fluctuations will swamp the stochastic signature from random birth and death events in this system. We conclude with a discussion of effective population sizes in microbial communities given spatial structure and consequently how neutral theory can explain the dramatic variation in microbial species richness that is observed across environments.

Abstract Category

15 Microbial Ecology and Ecological Theory

PS.15.028 NOT ONLY WHO IS THERE, BUT ALSO WHO IS ACTIVE: HOW COMMUNITIES RESPOND TO ENVIRONMENTAL STRESS?

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Drought and the associated rewetting events are common stressors for microorganisms in soil. Yet little is known regarding how sensitive communities are to such perturbations. In soils, most microorganisms are dormant most of the time, but rewetting events should provide ideal conditions for some groups of microbes to respond and grow quickly. Identifying microbes that are active under these kinds of episodic is an ongoing challenge in microbial ecology. Who grows? How fast? To answer these questions, we characterized bacteria that actively grow following rewetting a dry soil, and following multiple dry/wet cycles. Our study site is a California grassland, with a Mediterranean climate, with long hot summer. We used bromodeoxyuridine (BrdU) to label actively growing cells. BrdU, a thymidine analog, is incorporated into newly synthesized DNA, which is then isolated from total DNA by immunocapture. Incubations were conducted to (1) assess the response of bacterial community 12h, 24h and 48h after a rewetting event, and (2) evaluate the impact of multiple cycles coupled with substrate additions. To evaluate differences between the actively growing and total communities we did initial screening using T-RFLP of 16S rRNA genes; and followed it up with pyrosequencing. Bacteria that can grow rapidly following rewetting are a small group, distinct from the overall community. The response to multiple stress cycles was dependent on substrate availability. Developing the links between community composition and function require

characterizing the composition of communities active under specific conditions and the biological processes they carry out.

Abstract Category

15 Microbial Ecology and Ecological Theory

PS.15.029 TEMPORAL SCALING OF MARINE BACTERIOPLANKTON ASSEMBLAGE COMPOSITION

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Perhaps the simplest descriptor of the biodiversity of an ecosystem is its species richness. However, it can be far from simple to characterize species richness of a system as richness depends not only on sampling methodologies, but the size of the area sampled and the duration of the sampling period. This is especially true of communities as diverse as bacterial communities. The species-area relationship (SAR), the positive relationship between the number of species observed and the size of the area sampled, has received perhaps the most attention of any pattern of diversity. The temporal scaling of species diversity has received considerably less attention than spatial scaling. The species-time relationship (STR), the temporal analogue of the SAR, describes the positive relationship between the number of species observed and the time span over which the community is observed. The STR provides a baseline understanding of the temporal scales over which richness varies in a given area and is central to our understanding of community assembly and colonization dynamics. Here we examine the temporal scaling of marine bacterioplankton monthly for 6 years at multiple depths of the San Pedro Ocean Time Series (SPOT) station in the Southern California Bight. Samples were compared using automated ribosomal intergenic spacer analysis (ARISA) fingerprints. In particular, we find that marine bacterioplankton do exhibit a significant taxa-time relationship. Turnover in community composition over time increases with depth as shown by an increase in the slope of the TTR with depth. Finally, the observed turnovers through time of bacterial communities are relatively low but do not differ from those observed for a range of other taxa.

Abstract Category

15 Microbial Ecology and Ecological Theory

PS.15.030 AQUATIC BACTERIA, LAKES, AND WATER COLUMN OVERTURN: A MODEL MICROBIAL SYSTEM FOR DISTURBANCE ECOLOGY

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Microbes, their processes, and their interactions with each other and with their environments are essential for life. However, a theoretical backbone for microbial ecology is lacking. To help address this, we borrowed the principles of disturbance ecology that were developed in larger-scale systems, and applied them to a model microbial experimental ecosystem. The model

system included lakes, planktonic bacteria, and water column mixing as the disturbance. Mixing is a disturbance because it disrupts physical and chemical gradients known to structure microbial communities in both space and time. We conducted a whole-ecosystem experiment during which we imposed an unprecedented disturbance on microbial communities by artificially mixing a temperate lake during peak summer thermal stratification. We observed real-time environmental responses to the lake mixing using environmental sensors, including profiled water temperature and dissolved oxygen, and analyzed water for carbon, nutrients, and a suite of respiration products. We fingerprinted the bacterial communities using automated ribosomal intergenic spacer analysis and 454 tag-pyrosequencing. Then, we performed multivariate analyses of community composition, diversity, and structure to determine the robustness of the bacterial communities to the mixing disturbance. The artificial mixing radically increased the bottom-lake temperature and exposed the microorganisms to novel physical conditions. Despite the novelty of the post-mixing environment, we found that the communities were not resistant but surprisingly resilient to the disturbance. This reveals an incredible robustness of the lake bacterial communities to unpredictable perturbation. Our results suggest that we may be able to predict microbial community responses to disturbances. This has application for understanding microbial components of global change and human wellness, and for advancing a general ecological theory for microbes.

Abstract Category

15 Microbial Ecology and Ecological Theory

PS.15.031 ANALYSIS OF BACTERIAL METACOMMUNITY ASSEMBLY AND INTERNAL DISPERSAL MECHANISMS

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The metacommunity framework predicts the spatial distribution of community patterns based on the relative importance of environmental factors, dispersal, competition and stochastic processes. However, in microbial ecology there is a debate whether pure spatial factors play any role shaping communities' biogeographic distribution or whether, according to the "everything is everywhere" concept, only species sorting through local conditions is relevant. In this study we assess this question by investigating the factors shaping a rock-pool metacommunity. For this, 50 pools were sampled in an area of approx 5000 m² on the Baltic Sea Coast. Local environmental conditions were identified measuring various abiotic (e.g. salinity, nutrient content) and biotic (e.g. phytoplankton biomass, zooplankton abundance) parameters. For the description of the spatial characteristics, a detailed map was created and spatial statistical tools were applied to the data. Bacterial community structure was determined using both TRFLP and 454 sequencing of the 16S rRNA gene. As salinity (ranging from 0 to 12 psu) was the main factor influencing microbial community composition, the data set will be further used to 1) identify the contribution of different taxa to this pattern, 2) assess the factors influencing the metacommunity structure of halosensitive bacteria, and 3) of halosensitive and halodependent groups in the fresh and saline pools, respectively. In addition, a transplant experiment was performed to examine the mechanisms of internal dispersal between fresh and saline pools. Here tanks were filled with water from two similar pools differing only in salinity, and dialysis bags were used to transfer the communities and the mixes of the two communities to their own and to the alien

environment. Survival and competition between the two communities will be assessed using bacterial abundance and activity data, and community structure determined as described above.

Abstract Category

15 Microbial Ecology and Ecological Theory

PS.15.032 DESPITE LONG TERM COMPOST AMENDMENTS, SEASONAL CHANGES ARE MAIN RUDDERS OF SOIL FUNGAL AND BACTERIAL COMMUNITY IN A VINEYARD

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Organic matter depletion is a serious problem in soil ecosystem. A great consideration has been given to possible utilization of compost in agriculture. Nevertheless organic amendments can influence soil microorganisms which play a pivotal role in sustainability of agricultural system. In a productive Tuscan vineyard soil annually amended since 2001 with 50 Kg ha⁻¹ N, 30 kg ha⁻¹ P, 70 kg ha⁻¹ K (NPK thesis), or 15 Mg ha⁻¹ compost (C thesis), or 25 kg ha⁻¹ N 15 kg ha⁻¹ P 35 Kg ha⁻¹ plus 15 Mg ha⁻¹ compost (C+ ½NPK thesis), bacterial and fungal populations of both bulk soil and rhizosphere of grapevine (*Vitis vinifera*) have been analysed. Differentially treated soils were sampled in three positions along two diverse plants in early summer (July), mid-summer (August), and before harvest (October) and community genetic fingerprints were generated by 16SrDNA and ITSrDNA Multiplex Terminal Fragment Length Polymorphism (M-TRFLP). In bulk soil, multivariate Multi Dimensional Scaling (MDS) ordination method highlighted a significant effect induced by seasonal variations, while compost or NPK treatments showed no significant differences between microbial communities at any time. In grapevine rhizosphere, temporal separation obtained by MDS was even stronger for bacterial population, due to modulation of the rhizosphere effect, while fungal community didn't show any treatment or time effect. Nevertheless, univariate analysis by ecological indices showed an increase in species richness in rhizosphere fungal population long-term fertilized with NPK. Obtained results suggested that soil microbial populations in productive ecosystems may be more sensitive to environmental changes induced by temporal variation and also show a certain degree of resilience to different agricultural practices.

Abstract Category

15 Microbial Ecology and Ecological Theory

PS.15.033 TESTING THE INSURANCE HYPOTHESIS WITH STREAM DENITRIFYING BACTERIA: ARE DIVERSE COMMUNITIES MORE RESILIENT TO DISTURBANCE?

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Ecological theory suggests diverse communities are more resilient to disturbance than species poor communities. This idea, known as the insurance hypothesis, has been tested for artificially assembled, but not naturally occurring microbial communities. We set out to determine whether

denitrification rates of diverse denitrifying bacteria communities from a forested stream are more resilient to disturbance than species poor denitrifying bacteria communities from an urban stream. Denitrification removes harmful excess nitrogen and is, therefore, a critical ecosystem service.

We inoculated microcosms with inoculum from our urban or forested study stream. We used presence-absence data from terminal restriction fragment length polymorphisms of nitrite reductase genes to estimate community diversity. Microcosms were assembled in media containing known amounts of nitrite. There were two experimental treatments: a chemical stress (AgNO₃ and NaCl) and a temperature stress (+10°C) treatment. Microcosms were incubated anaerobically (20 hrs) and nitrite concentrations measured to estimate denitrification rates.

Species poor urban communities removed more nitrite than diverse forested communities (one-way ANOVA, $F(1,90)=7.48$, $p=0.008$). With pollutant stress, species poor communities suffered a reduction in nitrite removal capacity (one-way ANOVA, $F(1,88)=17.11$, $p=8.062 \times 10^{-5}$), while diverse communities were not affected. With temperature stress, species poor and diverse communities removed comparable amounts of nitrite (one-way ANOVA, $F(1,83)=2.77$, $p=0.099$). With both pollutant and temperature stress, species poor communities again suffered a reduction in nitrite removal capacity (one-way ANOVA, $F(1,84)=4.06$, $p=0.047$). These results suggest that, while species poor urban communities show higher levels of denitrification under non-stressful conditions, they may be less resilient to disturbance than diverse forested communities.

Abstract Category

15 Microbial Ecology and Ecological Theory

PS.15.034 NICHE SELECTION OF TILLAGE MEDIATES THE IMPORTANCE OF STOCHASTIC MICROBIAL COMMUNITY ASSEMBLY

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To understand forces driving the pattern of community diversity and composition has long been a central task in ecology but so far no consensus has been reached. Both niche and neutral theories have been proposed to explain the community assembly based on attempts focusing on conspicuous macroorganisms, however, little is known about microorganisms despite their ecological importance. To fill this gap, we analyzed soil samples from agricultural land at KBS LTER experimental site (Hickory Corners, MI, USA) by microarray-based GeoChip, a high-throughput genomics technique, to characterize soil microbial communities at the whole-community-wide scale and the functional gene level to investigate the impact of tillage. Comparing tillage and non-till treatments at high fertilizer level, tillage significantly decreased the community richness (detected gene number) and diversity indexes of Shannon-Weaver and Simpson ($p<0.05$). The detrended correspondence analysis (DCA) for functional gene composition was able to separate tillage and non-till samples clearly. Moreover, tillage samples

clustered tightly but non-till samples were scattered in the DCA profile. By multi response permutation procedure (mrpp), the dissimilarities within tillage and non-till groups were 28.7 and 45.1, respectively, calculated by overall weighted mean of group mean distances. The higher within-group dissimilarity of non-till samples might be attributed to stochastic ecological drift and priority effects. The tillage practice could be regarded as an environmental filter for niche selection to decrease the species pool of regional community in neutral theory and lead to less divergent communities in different locations. These results imply that tillage practices mediate the importance of stochastic processes in soil microbial community assembly. Combined, niche-based processes and stochastic-neutral processes were jointly responsible for community assembly and their relative importance may vary.

Abstract Category

15 Microbial Ecology and Ecological Theory

PS.15.035 TRADE-OFF BETWEEN OXYGEN AND IRON ACQUISITION IN THE BACTERIAL CELLS AT THE AIR-LIQUID INTERFACE

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Many aerobic microorganisms can colonize at the air-liquid interface and form biofilm-like structure, pellicle. Although pellicle formation is considered to provide the fitness advantage for aerobes due to the accessibility for both gaseous (oxygen) and liquid (nutrient and water) phases, contribution of pellicle on the fitness is still unclear. In this study, the fitness effect of pellicle formation was investigated and the physiological characterization of pellicle cells was performed. All experiments were performed with a pellicle-forming facultative aerobe, *Pseudomonas aeruginosa* PAO1 and its derivatives in LB medium at 37 °C under static condition. At first, a pellicle deficient mutant ($\Delta psl\Delta pel$) was constructed. The total productivity (biomass) of the mutant was reduced compared to the wild type in static culture, suggesting the fitness advantage of pellicle formation in static environment. Since the fitness advantage seemed to be caused by the oxygen availability, the effect of an alternative electron acceptor in liquid phase on the pellicle formation was examined. Supplementation of medium with nitrate resulted in the decrease of pellicle mass. On the other hand, supplementation of medium with iron dispelled the negative effect of nitrate on the pellicle formation. Promotion of anaerobiosis by nitrate probably invoked the intraspecies competition for iron between aerobic and anaerobic subpopulations, causing the decline of pellicle formed by aerobic subpopulation. Transcriptome analysis revealed that the iron uptake system was highly upregulated in the pellicle cells. The result indicates that the pellicle cells are under condition of low iron availability and therefore the acquisition of iron would be crucial factor for the growth at the air-liquid interface. Thus, while pellicle formation could provide the fitness advantage for aerobic bacteria, there is a nutritional trade-off between oxygen and iron acquisition in the niche at the air-liquid interface.

Abstract Category

15 Microbial Ecology and Ecological Theory

PS.16.001 FRANKIA-ALDER SYMBIOSIS TOLERANCE TO HEAVY METALS: A PROMISING ASSOCIATION FOR THE REVEGETATION OF CONTAMINATED ECOSYSTEMS

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Frankia are actinobacteria capable of fixing nitrogen as soil saprophytes, as well as within their nitrogen-fixing symbiosis with over 200 plant species (e.g. alders). Alders, a pioneer species distributed worldwide, ameliorate soil composition and structure through the addition of organic matter and nitrogen. In symbiosis, frankiae provide 70% to 100% of the nitrogen needed by the host plant. This endogenous nitrogen supply helps alders grow on contaminated or nutrient-poor soils. Mining and industrialization are major sources of soil contamination. Traditional decontamination methods are often economically unviable when coping with large volumes of contaminated soil, therefore efforts were made to develop phytotechnologies leading to soil decontamination or rehabilitation. Our hypothesis was that *Frankia*-alder symbiosis could tolerate heavy metals and therefore be used in revegetation strategies. To rigorously verify this hypothesis, we developed a hydroponic gnotobiotic culture method to study the impact of heavy metals on symbiosis infectivity and effectivity. Black alders (*Alnus glutinosa* (L.) Gaertn), a species commonly used in mine tailings revegetation, were inoculated with *Frankia alni* strain ACN14a in the presence of heavy metals. We report here the impact of 10 metals (each at one concentration) on the actinorhizal symbiosis. Results showed higher plant yields in inoculated alders and effective nitrogen fixation in all conditions tested. Among the 10 metals, none totally inhibited symbiosis establishment. Some metals (Al, Cd, Mg and Mn) repressed the symbiosis while others (Co, Cr, Pb and Zn) did not significantly affect symbiosis. Finally, Ni and Cu seemed to promote symbiosis formation. Bioaccumulation of metals in shoots was also measured. In conclusion, the *Frankia*-alder symbiosis was tolerant to heavy metals. However, alders were more sensitive than *Frankia*. Future research should turn toward the development of tolerant cultivars.

Abstract Category

16 Microbial Symbioses

PS.16.002 INVESTIGATING BARNACLE-ASSOCIATED BACTERIAL COMMUNITIES WITH A NEW 16S rRNA TAXON-SPECIFIC qPCR TECHNIQUE

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A fundamental community characteristic is diversity, which is a function of taxa richness and relative abundance. The analysis of how diversity evolves in time and space has allowed the development of a theoretical framework to understand community dynamics. However, in comparison to plant and animal ecologists, microbiologists are short of

techniques capable of assessing both taxa richness and abundance, since extrapolating both types of data from SSU rRNA sequencing can produce notable bias.

Our aim was to develop a high-throughput technique that could estimate species richness and higher taxa abundance in natural microbial communities. We accomplished this by implementing a combination of quantitative PCR and denaturing gradient gel electrophoresis. We improved existing 16S rRNA primers specific for the Firmicutes, the Actinobacteria, the Bacteroidetes and for the α and γ subdivisions of Proteobacteria. Rigorous testing of reaction conditions was required to obtain specificity. The qPCR-DGGE method provided reliable information about the structure of microbial communities.

Furthermore, our research focused on the complex interactions between bacteria and invertebrates that play a role in the establishment of marine benthic communities. We applied this method to the study of uncultivable bacteria associated with different developmental stages and body parts of the model barnacle *Balanus amphitrite*. We found that specific bacterial assemblages colonise different organs and that the microbial community changes during barnacle development. These results suggest that bacteria may have unexpected functions in *B. amphitrite* development.

Abstract Category

16 Microbial Symbioses

PS.16.003 MICROBIAL COMMUNITIES ASSOCIATED WITH REEF-BUILDING TROPICAL CORALS IN COMPETITION WITH BENTHIC ALGAE

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Degraded coral reefs are typically dominated by benthic algae, yet we do not fully understand how this shift comes about or even basic characteristics of coral-algal interactions on healthy reefs. Here we examined interactions between corals and algae and found that when corals were in contact with fleshy algae, the coral tissue near the algae showed signs of stress including tissue disruption and mucus release. Oxygen measurements showed that these regions were also hypoxic, suggesting that increased microbial activity may occur along those borders. Conversely, corals in contact with crustose coralline algae showed no hypoxia and coral tissue appeared healthy. We hypothesized that fleshy algae are disrupting the natural coral-associated microbial community by releasing photosynthates that enhance microbial growth, leading to the hypoxia and tissue disruption observed along these borders. To address this, coral and algal tissue was sampled across various coral-algal interactions and bacterial 16S rRNA gene libraries were generated using pyrosequencing. The diversity and richness of microbial communities was higher associated with the various types of algae ($H' = 3.7 - 5.9$; Chao1 = 2600 - 4200) vs. coral tissue ($H' = 2.5 - 3.5$; Chao1 = 400 - 700) with a 97% similarity threshold. Coral tissue was dominated by Proteobacteria (>70%), followed by Firmicutes and Bacteroidetes. Algal-associated microbes were generally composed of a more even assemblage including Proteobacteria, Cyanobacteria, Bacteroidetes, Firmicutes, and Planctomycetes, as well as a wide variety of phyla in low abundance. Microbial diversity was higher at interaction zones than on coral tissue away from algae,

and principal component analysis showed that microbial communities associated with interaction zones grouped apart from those associated with coral zones in some cases, demonstrating that algae are in fact altering the microbial communities associated with corals.

Abstract Category
16 Microbial Symbioses

PS.16.004 RICKETTSIA FELIS INFECTION IN A COMMON HOUSEHOLD INSECT PEST, LIPOSCELIS BOSTRYCHOPHILA (PSOCOPTERA: LIPOSCELIDAE)

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Many *Rickettsia* are well-known pathogens of mammals that are vectored by blood-feeding arthropods. However, recent molecular surveys have uncovered an enormous diversity of *Rickettsia*, including many strains that infect arthropods that do not feed blood. The function of many of these is not known, although some strains that exhibit strictly maternal transmission have been shown to distort host sex ratios towards females, similar to many *Wolbachia*. We characterized a strain of *Rickettsia* that appears to be fixed in the common, cosmopolitan, parthenogenetic household insect pest, the psocid *Liposcelis bostrychophila*. Surprisingly, phylogenetic analysis of five genes showed that this insect is infected with a strain of *Rickettsia felis*, a human pathogen transmitted by fleas that causes serious morbidity and occasional mortality, and that was only identified in the 90s. Using primers that we designed based on *R. felis* in fleas, we also sequenced the plasmid from the *Liposcelis* *Rickettsia*. The plasmids were virtually identical (98% at the nucleotide level), except for a few nonsense mutations. *Liposcelis* are intimately associated with humans and vertebrates, and we therefore speculate that they acquired *R. felis* from fleas. The finding of *R. felis* in a novel host has a number of implications. In particular, as *Liposcelis* do not feed on blood, grow rapidly, and are easily cultivated, they represent a potentially easy system for studying *R. felis*. It will also be useful to determine whether the *Liposcelis* *Rickettsia* can cause disease in vertebrates, and to compare this with pathogenicity induced by the strain in fleas. We also speculate on the role of *R. felis* in *Liposcelis bostrychophila*, which remains a mystery.

Abstract Category
16 Microbial Symbioses

PS.16.005 SNAIL ENDOSYMBIONT TYPE FOLLOWS HYDROTHERMAL VENT CHEMISTRY IN THE EASTERN LAU SPREADING CENTER

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Gastropods of the genus *Alviniconcha* are found at hydrothermal vent fields in the Western Pacific, and have been reported to associate with either γ - or ϵ -*Proteobacterial*

endosymbionts. These symbionts harness energy from the oxidation of chemicals in vent fluid to fix inorganic carbon and are the primary source of nutrition for the host. An extensive sampling effort from a recent expedition to the Eastern Lau Spreading Center (ELSC) has revealed that *Alviniconcha* with symbionts of both types are found at vent fields along the ridge and that the dominance of each type at a vent field relates to the geology and geochemistry of that site. We collected approximately 300 *Alviniconcha* individuals from four vent fields along the spreading center (30-140km apart) which span the north-south transition from fast spreading, basalt-hosted vent fields to slower spreading, andesite-hosted fields. Vent fluids from each field were also analyzed for the abundances of aqueous volatile and non-volatile species. The symbionts of all collected *Alviniconcha* were genotyped using restriction fragment length polymorphism analysis of the 16S rRNA gene. Individuals were found to host one of three symbiont genotypes (two γ - and one ϵ -*Proteobacterial*) and 16S rRNA genes from representative individuals of each type were sequenced. We found that the two northern-most sites (basalt) were dominated by individuals with the ϵ -*Proteobacterial* symbiont, while the two southern sites (andesite) were dominated by individuals hosting one of the two γ -*Proteobacterial* symbionts. This pattern also corresponds to differences in the aqueous chemistry of the vent fluids along the spreading center: in particular, we have measured higher concentrations of hydrogen and hydrogen sulfide in the vent fluids at the northern sites than in the fluids of the southern sites. Thus, vent chemistry may be influencing the dominance of each symbiont type along the ELSC

Abstract Category

16 Microbial Symbioses

PS.16.006 SINGLE-CELL ANALYSIS OF AUTOTROPHIC CARBON FIXATION IN THE SYMBIOTIC COMMUNITY OF THE GUTLESS MARINE WORM *OLAVIUS ALGARVENSIS* USING NANOSIMS

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The marine gutless oligochaete worm *Olavius algarvensis* harbors a phylogenetically and metabolically diverse community of co-occurring bacterial endosymbionts. Based on biochemical and molecular analyses, two symbionts are chemoautotrophic sulfur oxidizers, the Gamma 1 symbiont, common to nearly all gutless oligochaetes, and a secondary symbiont called Gamma 3. Earlier studies showed that the Gamma 1 symbionts store sulfur, which was suggested to serve as energy source for autotrophic carbon fixation. These studies, however, could not resolve if the Gamma 3 symbiont can store sulfur. In this study we followed the autotrophic carbon fixation and carbon transfer in the *O. algarvensis* symbiosis at the single-cell level to identify the autotrophic symbiont population. Live worms were incubated with ^{13}C -labeled inorganic carbon under oxic conditions without an external electron donor for up to 25 h. By combining nanoscale secondary ion mass spectrometry (nanoSIMS) with in situ hybridization we were able to identify the symbionts that incorporated ^{13}C . Only the Gamma 1 symbionts fixed carbon under the conditions used, and we propose that internally stored sulfur served as energy source. Carbon fixation rates of the Gamma 1 symbionts showed a high

variability, indicating physiological differences between cells. Co-occurring symbionts, including the Gamma 3, do not seem to be enriched in ^{13}C after 25 h. This suggests that the Gamma 3 symbionts are not able to store and use sulfur as energy source for autotrophic carbon fixation under the experimental conditions. Worm tissues were considerably enriched after 25 h indicating the transfer of freshly synthesized carbon from the Gamma 1 symbionts to the host. We were thus able to not only deduce symbiotic carbon fixation from bulk analyzes, but also to show single-cell activity within a symbiont community and transfer of symbiotically fixed carbon carbon to host tissues.

Abstract Category

16 Microbial Symbioses

PS.16.007 RPOB AND RPOD GENE FRAGMENTS AS NEW TOOLS IN ASSESSING PHYLOGENETIC DIVERSITY OF GENUS FRANKIA

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Frankia spp. are filamentous actinobacteria capable of fixing nitrogen freely in soil and in symbiotic root nodules formed on more than 200 dicotyledonous plants. Despite the importance of these bacteria, studies are hampered by the difficulty to isolate and cultivate these microorganisms. To date, molecular tools have proven useful to facilitate the identification of potentially new *Frankia* spp. isolates and to assess the diversity amongst these. Previous methods exploited the diversity of *nif* genes, intergenic spacer regions (IGS) or ribosomal RNA genes. Currently, *rpoB* gene, encoding the RNA polymerase β -subunit, is being reported as a more accurate tool compared to gene encoding 16S ribosomal RNA for bacterial identification at species and subspecies level. In this study, segments from *rpoD*, *rpoB* and 16S ribosomal RNA-encoding genes were amplified by PCR from 7 strains of *Frankia* spp. The sequences were compared to corresponding GenBank sequences of strains ACN14a, CcI3, Ean1pec and clustered with ClustalX. Partial *rpoD* gene sequences were comparable to those of 16S rRNA gene segments in regards to their capability to differentiate between strains. Furthermore, as the primers used for *rpoD* gene segment amplification revealed to be specific for *Frankia* spp. with low level of false-positives, this will be a helpful tool in distinguishing between *Frankia* and non-*Frankia* isolates. Also, among the tested methods, the amplification of a 694 bp segment from the *rpoB* gene was the only tool allowing to distinguish AvcI1 from strains ACN10a and ACN14a. Interestingly, ACN10a and ACN14a, two closely related strains isolated from the same geographical point, were identical in all sequences amplified in this study. The sequencing of other gene segments is in progress in order to verify the identity of the strains ACN10a and ACN14a. In the future, we will need to extend our study to larger number of strains in order to verify our present results.

Abstract Category

16 Microbial Symbioses

PS.16.008 SMALL SUBUNIT RRNA (16S) GENE FLUORESCENCE IN-SITU HYBRIDIZATION STUDIES OF THE MICROBIAL COMMUNITY ASSOCIATED WITH THE SHIPWORM *BANKIA SETACEA*

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The shipworms are bivalve marine mollusks in the family Teredinidae, which use wood as shelter and a food source. The gills of shipworms harbor a community of closely related gamma proteobacterial endosymbionts, one of which (*Teredinibacter turnerae*) has the demonstrated capacity to fix N₂ and produce cellulases. Previous studies involving the shipworm species *Lyrodus pedicellatus* have shown endosymbionts localized to bacteriocytes within the gills. However, bacteria associated with the digestive tract, stomach, intestine and caecum, of shipworms have not been extensively investigated. In this study we surveyed the digestive tract and the gill of the shipworm *Bankia setacea* by Fluorescence In-Situ Hybridization (FISH) and laser scanning confocal microscopy utilizing eubacterial and group specific 16s rRNA probes. Bacteria of various morphologies and phylogenetic identities were localized to the digestive system. Spatial distribution of bacterial ribotypes and morphotypes in the gill bacteriocytes and in the digestive tract suggest that these two compartments contain distinct and differentiated microbial communities. This study represents the first in situ exploration of microbial communities in the digestive system of *Bankia setacea*.

Abstract Category

16 Microbial Symbioses

PS.16.009 TOLERANCE OF ALDER AND ITS ACTINORRHIZAL SYMBIONT *FRANKIA* TO ALBERTA OIL SANDS RESIDUES

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The exploitation of Alberta oil sands has disturbed approximately 420 km² of boreal forest in the Athabasca Basin (Canada). Alders, pioneer trees and shrubs actinorrhized by *Frankia*, are readily used in land reclamation and revegetation, especially for sites contaminated by heavy metals. The efficiency they have shown in rehabilitating such sites suggests they could be useful to revegetate tailing sands residues in Alberta. However, the tolerance of *Frankia* and its host plant to tailing sands components needed to be determined.

To do so, *Frankia* strains AvcI1 and ACN14a were grown in BAPS medium supplemented with Na₂SO₄ (0 to 300 mM), NaCl (0 to 750 mM), naphthenic acids (0 to 750 ppm), or an amalgam of these tailing sands compounds ranging from 0 to 2 X the concentrations found on sites. To measure alder tolerance, seeds of the indigenous *Alnus incana* ssp. *rugosa* and *A. viridis* ssp. *crispa* were sterilized, placed on germination media for 4 weeks, and transferred to sterile germination pouches containing Hoagland's 0,1 X.

Two weeks later, contaminants 0 to 2 X the concentrations found on site were added to the pouches. Alders were harvested 15 weeks later, dissected and weighted. In a parallel germination pouch experiment, *Frankia* strain ACN14a was used to inoculate the alder plantlets.

The results indicate that both *Frankia* strains are tolerant to 2.5-fold the concentrations of these tailing sands components. Alders were less resistant, but survived and grew at normal concentrations (1x). However, as indicated by the small amount and weight of nodules, the actinorhizal symbiosis was strongly affected by the presence of TS contaminants, even at 1x concentrations. All nodules present fixed nitrogen, attesting the presence of live and efficient *Frankia*. Taken together, these results indicate that *Frankia*, alders, and their symbiosis tolerate the environmental stressors found in tailing sands from the oil industry and therefore show promise to revegetate such material.

Abstract Category
16 Microbial Symbioses

PS.16.010 DISTINCT MICROBIAL POPULATIONS IN CUTANEOUS SAMPLES FROM SOUTH AMERICAN AMERINDIANS AND NEW YORK CITY RESIDENTS

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The human skin harbors complex residential bacterial communities. Prior studies focused on subjects from developed countries, showing high interindividual variation. We now compare cutaneous bacterial communities of Amerindians in the Venezuelan Amazon, with subjects in New York City (NYC). 72 unilateral forearm skin specimens were studied from Amerindians in Platanillal village near Puerto Ayacucho (age range 2m-80y; Median = 9.0). For comparison, bilateral specimens from 4 NYC adults were studied; from 2 subjects, specimens were obtained >4 years apart. All skin sampling used a uniform swab/buffer technique and DNA extraction (Quiagen DNeasy). Multiplexed V2-targeted 16S rRNA gene pyrosequencing yielded 39,128 high quality sequences from the 84 samples (mean read length 262 nt, mean 466 reads/sample). The results show 22 phyla; 4 [*Firmicutes*, *Actinobacteria*, *Proteobacteria*, *Bacteroidetes*] predominated. On average, Amerindian subjects' bacterial communities were significantly richer in OTUs (defined at 97% sequence similarity) than those from NYC subjects (102 vs. 62 OTUs at 10 iterations of 200 seq/sample; $P < 0.001$; Student's t-test). Three of the most abundant OTUs in NYC subjects (classified as *Propionibacterium* spp.), which had average relative (%) abundances of 9.7, 3.6, and 2.4, were significantly less abundant (0.4, 0.6, and 0.4) in the Amerindian subjects ($P < 0.001$; Bonferroni-corrected ANOVA). By both PCA and UPGMA analyses, the NYC and Amerindian samples clustered distinctly. For the NYC subjects, the most similar communities were from the paired arms, except for

one subject whose left and right arm samples obtained >4 years apart formed independent clusters. Among the Amerindians, there was a deep branching with 30 and 42 samples in two groups, not associated with age or gender, but a trend toward BMI differences. The results provide evidence that ethnicity or lifestyle are associated with the structure of human cutaneous bacterial communities.

Abstract Category
16 Microbial Symbioses

PS.16.011 TRANSCRIPTOME OF DIGESTION-RESISTANT *SALMONELLA* TYPHIMURIUM IN THE *TETRAHYMENA* PHAGOSOME AND ROLE OF ACID TOLERANCE IN VACUOLAR SURVIVAL

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Salmonella enterica Typhimurium remains undigested in the food vacuoles of the common protist, *Tetrahymena*, and is egested as viable cells in its fecal pellets. We investigated the interaction of *S. Typhimurium* with *Tetrahymena* by microarray gene expression profiling. The transcriptome of *S. Typhimurium* in *Tetrahymena* phagosomes revealed that 16% of its genes were altered in expression, many of which reflect a physiological response in the pathogen analogous to that in the vacuole of human macrophages. A great proportion of the upregulated genes have a role in anaerobic metabolism and the use of alternate electron acceptors. Several virulence genes required for survival and replication within human macrophages also had increased expression, including type III secretion system genes and PhoP-regulated genes, such as *pagP/C/K*, *envE*, *mig-14*, *virK* and *mgtB/C*. An MgtC mutant of *S. Typhimurium* did not display decreased viability in *Tetrahymena*, but paradoxically, was maintained at a higher cell density in pellets than the wild-type strain. The expression of *adiA* and *adiY*, which are involved in arginine-dependent acid resistance, also was increased in the protozoan phagosome. An AdiA-AdiY mutant had lower viability after passage through *Tetrahymena*, providing evidence that acid resistance has a role in the survival of the pathogen to digestion by this protist. Furthermore, a higher proportion of *S. Typhimurium* cells within pellets remained viable after exposure to pH 3.4 compared to uningested cells in the same suspension. Our results suggest that *S. Typhimurium* experiences conditions in *Tetrahymena* vacuoles that are similar to those in human macrophages and may have implications for its contamination cycle.

Abstract Category
16 Microbial Symbioses

PS.16.012 GENOME SEQUENCE OF THE OBLIGATE SYMBIONT *BURKHOLDERIA KIRKII*

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The plant symbiont *Burkholderia kirkii* lives in tight association within the leaves of its host, the tropical shrub *Psychotria kirkii* (Rubiaceae). Although the symbiosis is obligatory (neither partner can survive on its own), the nature of the symbiosis has been a mystery for researchers for more than a hundred years. The advent of next generation sequencing technology allowed us to obtain the full genome sequence of *B. kirkii*, and we are now in the process of completing its annotation. The 6Mb genome is encoded on two chromosomes, and is significantly smaller than that of *B. kirkii*'s closest free-living relatives. Moreover, the genome is littered with pseudogenes, indicative of extensive genome reduction. We hope ultimately that the analysis of the genome will reveal the molecular interactions between the symbiotic partners, but genome comparisons have already given us some insights into the forces driving genome evolution in *Burkholderia* species in response to a closed, unique plant-associated lifestyle.

Abstract Category

16 Microbial Symbioses

PS.16.013 WHERE THE ENDOSYMBIONT OF TUBE WORM, LAMELLIBRACHIA SATSUMA, IN GAS SEEP AREA COMES FROM?

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There are two possible pathways of endosymbiont acquisition by the gutless vestimentiferan tubeworms; (1) vertical transmission from parent to progeny or (2) horizontal transmission in which the symbionts are acquired de novo by each generation from the ambient environment. The endosymbionts are seemed to be host-specific, however, the pathway of symbiont acquisition is still unclear. Recently, it was demonstrated that the bacterial symbionts are acquired by entering into the tubeworm larvae through the skin after settlement and proliferate to form the trophosome. In the present study bacterial clones were compared between endosymbiont of vestimentiferan tubeworm and ambient sediment environment in order to elucidate the source of endosymbiont acquisition. Bacterial and archaeal rRNA was PCR-amplified using DNA which extracted from the trophosome of the matured vestimentifera, *Lamellibrachia satsuma* and the surrounding sediment of Kagoshima Bay (depth 112 m). Only one symbiotic bacterial clone, *Gammaproteobacteria*, was detected from the trophosome of *L. satsuma* and the phylogenic distance is quite remote from the clones which were detected from the sediment. The 16S rRNA gene sequence identical to that of the endosymbiont also could not be amplified from the sediments using by specific primer designed based on the 16S rRNA gene sequence of endosymbiont. These results support that the seep vestimentifera, *Lamellibrachia satsuma*, harbors a monospecific bacterial endosymbiont, *Gammaproteobacteria*, and endosymbiont clone is not detected from the surrounding sediment. However, this study demonstrated no supportive evidence for the exclusion of horizontal transmission. Extensive study on the clones from the sediment depending on the season and those from water column is necessary. [Supported by MEGRC]

Abstract Category
16 Microbial Symbioses

PS.16.014 DIVERSIFICATION OF EARTHWORMS AND THEIR MICROBIAL SYMBIONTS

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An estimated 16 families of earthworms occur worldwide, influencing soil structure, nutrient availability, and microbial activities. Our earlier studies established that members of the Lumbricidae harbor *Verminephrobacter* spp., related to the genus *Acidovorax*. The symbiotic status of the other earthworm families remained in question. In this study we assessed occurrence and diversity of nephridial bacteria from 15 earthworm families (79 genera, 208 species) collected from different continents. Individuals were screened by fluorescent in situ hybridization and phylogenies inferred using 16S rRNA gene sequences recovered from symbiont-positive species. In addition, the earthworm host phylogenies were resolved using cytochrome c oxidase subunit I, histone H3, and 28S, 16S, 12S rRNA gene sequences. Although nearly all Lumbricidae members contained nephridial *Verminephrobacter*, none were detected among 64 species outside the Lumbricidae that were examined. Of those 64 species, 19 (representing 6 families) were positive for nephridial bacteria other than *Verminephrobacter*. In addition to the *Verminephrobacter* spp., two or more other bacterial taxa were consistently found within the Lumbricidae. Novel *Flexibacter*-related bacteria were found in over 55% of the Lumbricidae and in five genera outside of Lumbricidae. Other bacterial sequences commonly identified included *Herbaspirillum* spp., members of a Microbacteriaceae species, Alphaproteobacteria from Rhodospirillales (*Azospirillum*) and Rhizobiales (5 genera), *Polaromonas* and *Acidovorax* spp. Colonization patterns suggested that both specific symbioses and possibly transient colonizations of certain types of bacteria occur in the earthworm nephridia.

Abstract Category
16 Microbial Symbioses

PS.16.015 NITROGEN FIXATION IN DRY-WOOD TERMITES: HOST-SPECIFIC NITROGENASE GENE EXPRESSION BY GUT SYMBIONTS

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The nitrogen-poor diet of wood-feeding termites is compensated by the capacity of the gut microbiota to fix atmospheric nitrogen. Nevertheless, the bacteria responsible for this activity are largely unknown. Here we studied expression of nitrogenase genes (*nifH* homologs) in dry-wood termites (Kalotermitidae) - a group of termites known to possess the highest rates of nitrogenase activity in the acetylene reduction assay. Terminal-restriction-fragment length polymorphism (T-RFLP), cloning, sequencing, and phylogenetic analyses of *nifH* homologs and their mRNA transcripts demonstrated that despite a high diversity of nitrogenase genes, only a core set of four homologs is expressed. The patterns of gene expression were specific for the termite species studied and were correlated with the hydrogen partial pressure in the hindgut, measured by microsensors, but were not affected when termites were incubated under external hydrogen. In *Kaloterмес flavicollis* and *Incisiterмес marginipennis*, the most highly expressed genes belonged to the *Treponema*, *Bacteroidales*, and the Proteobacteria-Cyanobacteria group. In *Neotermes castaneus* and *Cryptotermes longicollis*, however, the most abundant transcripts fell into the previously detected termite-gut specific *anfH* group. Recovery of the *anfH* genes from capillary-picked flagellate suspensions of *Devescovina* flagellates provides strong evidence that they belong to "*Candidatus Armantifilum devescovinae*", the ectosymbiont covering the surface of these flagellates, which have coevolved with their host flagellates in dry-wood termites.

Abstract Category

16 Microbial Symbioses

PS.16.016 EXPLORING CORRINOID EXCHANGE IN THE TERMITE GUT MICROBIOTA

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The hindgut of the *Zootermopsis* spp. termite is home to a diverse array of microorganisms which have major environmental impacts on the global carbon cycle and are a potential source for cellulose-degrading enzymes for biofuel production. The termite gut microbiota functions as a web of interconnected metabolisms fueled by the exchange of small molecules such as corrinoids. Corrinoids (vitamin B₁₂ and related molecules) are essential cofactors for gut microbes with major roles in cellulose degradation and those carrying out homoacetogenesis and methanogenesis. Some of these microbes produce their own corrinoids, while others, such as protists, require corrinoids produced by other members of the community. While the role of different forms of corrinoids in microbial communities remains to be determined, preliminary evidence suggests that not all forms of corrinoids are functionally equivalent. Analysis of the corrinoids present in the gut, as well as identification and characterization of corrinoid-utilizing gut microbes will shed light on how corrinoid exchange can influence the make-up of such communities. The corrinoids present in the termite gut were identified as benzimidazolylcobamide and cobalamin using liquid chromatography tandem mass spectrometry (LC/MS/MS). Aerobic and anaerobic culturing conditions were used to isolate corrinoid-utilizing microbes. At present, three corrinoid-producing microbes have been isolated. Organisms which require exogenous corrinoids will also be cultured, and

the forms of corrinoids that support their growth will be characterized. Quantitative PCR will be carried out to determine the relative abundance of each corrinoid-utilizing organism in the community as a whole. The identification and characterization of corrinoid-utilizing organisms in the termite gut will help to elucidate the contribution of corrinoid exchange to the make-up and function of the community.

Abstract Category
16 Microbial Symbioses

PS.16.017 STRUCTURE OF THE HUMAN GASTRIC BACTERIAL COMMUNITY IN RELATION TO *Helicobacter pylori* STATUS

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The human stomach is naturally colonized by *Helicobacter pylori*, which when present, dominates the gastric bacterial community. In this study we aimed to characterize the structure of the bacterial community in the stomach of patients of differing *H. pylori* status. We used a high-density *16S rRNA* gene microarray (PhyloChip) to hybridize *16S rRNA* gene amplicons from gastric biopsy DNA of 10 rural Amerindian patients from Amazonas, Venezuela, and two immigrants to the US (from South Asia and Africa, respectively). *H. pylori* status was determined by PCR amplification of the *H. pylori glmM* gene from gastric biopsies. Out of the 12 patients, 8 (6 of the 10 Amerindians and the 2 non Amerindians) were *H. pylori glmM*-positive. Regardless of *H. pylori* status, the phylochip detected Helicobacteriaceae DNA in all patients, although with lower relative abundance in the patients who were *glmM*-negative. The G2-chip taxonomy analysis of PhyloChip data indicated the presence of 44 bacterial phyla (of which 16 are unclassified by the TOBA taxonomy), in a highly uneven community dominated by only four phyla: Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes. Having positive *H. pylori* status was associated with increased relative abundance of non *Helicobacter* bacteria from the Proteobacteria, Spirochaetes, and Acidobacteria phyla, and with decreased abundance of Actinobacteria, Bacteroidetes and Firmicutes. The PhyloChip detected a high bacterial richness of low abundance phyla, and showed marked differences in the structure of the gastric bacterial community by *H. pylori* status.

Abstract Category
16 Microbial Symbioses

PS.16.018 FLAGELLAR AND TYPE IV PILI MEDIATED MOTILITY IS REQUIRED FOR *VERMINEPHROBACTER EISENIAE* COLONIZATION OF

EARTHWORM NEPHRIDIA.

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Bacterial symbionts colonize the kidney-like nephridial organ of the common composting earthworm, *Eisenia fetida*. Multiple bacterial strains of *Verminephrobacter eiseniae* (VE) and a *Flexibacter* sp. (Fs) have been identified to co-colonize the nephridia. Both symbionts are transferred vertically into the worm egg capsule and colonize the nephridia during embryogenesis. Bacteria in the capsule adhere to and congregate at a pore on the surface of the embryo. Symbionts then move into and colonize ducts of proto-nephridia beginning approximately 10 days into development. Movement then commences into bladder then into the ampulla of the nephridia at around day 14. Typically, VE lines the ampulla walls while the Fs fills the lumen space in adult worms. VE colonizes the bladder of juveniles transiently, and then is cleared as the worm organs mature. To elucidate mechanisms of early colonization and the genes involved, we investigated the requirement for motility during migration and establishment in the nephridia. Worm lines have been cured with antibiotics of VE or both VE and Fs. VE cured embryos are successfully recolonized when capsules are inoculated with $\sim 5 \times 10^7$ cells of VE early in development (0-3 days old). Using directed mutagenesis, we have generated flagellar (*flgK*-) and type IV pilus (*pilBC*-) mutants of VE. Both mutants of VE display movement defects in culture and impaired colonization of the nephridia. The *flgK*- mutant is no longer able to swim in semi-solid plates. Confocal image analysis shows the *flgK*- mutant unable to transition from the bladder to the ampulla. The *pilBC*- mutant no longer displays twitching motility or aggregation in static culture. Additionally, no part of the nephridia was observed to be colonized by the *pilBC*- mutant. Our data suggest an ordered processes of colonization wherein both forms of motility are required for proper colonization of the nephridial ampulla.

Abstract Category
16 Microbial Symbioses

PS.16.019 PROGRESS IN THE STUDY OF THE SECONDARY METABOLOME OF *TEREDINIBACTER TURNERAE*; AN ENDOSYMBIONT OF MARINE SHIPWORMS (FAMILY TEREDINIDAE)

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Shipworms are wood-boring bivalves that belong to the family Teredinidae. Within the gills of these marine mollusk species, exist a group of endosymbiotic bacteria that is thought to play an important role in the nutrition and survival of their host. One of these endosymbionts, *Teredinibacter turnerae*, was isolated almost three decades ago and found to be capable of nitrogen fixation and cellulose digestion. This allows the shipworm to use wood as its sole food source. The secondary metabolome of this

gamma-proteobacterium has never been investigated. Nevertheless, genomic data suggest the presence of interesting metabolites. The genome of *Teredinibacter turnerae* was sequenced recently. Nine gene clusters that are thought to encode the biosynthesis of polyketides and nonribosomal peptides were found, accounting for 7% of the genome. Bioactivity assays in addition to gene expression analysis strongly suggest the presence of compounds that possess antibacterial, antifungal, and cytotoxic activities, in addition to a siderophore. We have isolated one of these, a novel antibacterial metabolite, by bioassay guided fractionation and identified its structure as a novel compound using 1D and 2D NMR experiments.

Abstract Category
16 Microbial Symbioses

PS.16.020 CANDIDATUS ERWINIA DACICOLA A PERSISTENT RESIDENT ENDOSYMBIONT OF THE OLIVE FLY DIGESTIVE SYSTEM

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Intracellular endosymbionts of bacteriocytes that supplement their host's nutritionally incomplete diet have long been considered vertically transmitted, persistent, resident members of the host. In contrast, the digestive microbiota of polyphagous insects is often considered transient. Distinguishing between transiently acquired bacterial associates and persistent, resident bacteria of the digestive tract can be difficult. Using culture-dependent and -independent methods, several bacterial species have been found associated with the tephritid olive fly, *Bactrocera oleae*. *Candidatus* *Erwinia dacicola* has been proposed to be a persistent endosymbiont of the olive fly based on limited sampling. We surveyed a total of 10 New and Old World olive fly populations for *Ca. E. dacicola* using species-specific PCR primers. *Ca. E. dacicola* was present in all olive fly populations, in the majority of individuals in each population, and in all life stages. The olive fly recently naturalized in the United States (US), yet *Ca. E. dacicola* was retained in all US populations and in over 91% of the sampled males and females in all surveyed years. Olive flies reared on olives in the laboratory for less than two years had frequencies of *Ca. E. dacicola* similar to that of wild populations; however, flies reared on artificial diets in the laboratory for several decades rarely had the endosymbiont. The relative abundance of the endosymbiont varied across development stages. *Ca. E. dacicola* was most common in adults and larvae. Among adult flies, the endosymbiont was more common in ovipositing females than mated males or virgin females. The endosymbiont was more common in 2-day old flies that had a chance to feed as compared to recently eclosed adults. Our findings that *Ca. E. dacicola* was found in the majority of individuals across all populations and life stages suggest that this bacterium is a persistent, resident endosymbiont of the digestive system of the polyphagous olive fly

Abstract Category
16 Microbial Symbioses

PS.16.021 LINKING SYMBIONT ABUNDANCE IN HYDROTHERMAL VENT MUSSELS TO THE AVAILABILITY OF GEOFUELS

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The deep-sea mussel *Bathymodiolus* dominates the biomass of hydrothermal vents on the Mid-Atlantic Ridge. It lives in a nutritional symbiosis with sulfur- and methane-oxidizing bacteria that depend on the availability of geofuels such as sulfide and methane from the vent fluids for their own biomass and productivity as well as that of their host. It is assumed that temporal and spatial gradients in vent fluid flow affect symbiont abundance and distribution, but to date, this relationship is only poorly understood. We examined the dynamics of this symbiosis experimentally by removing *Bathymodiolus* mussels from active venting to a site at the same depth (3000 m) but with no access to sulfide or methane. We quantified symbiont abundance by combining a morphological approach using 3-dimensional fluorescence *in situ* hybridization (3D-FISH) with a molecular approach using quantitative real time PCR (q-PCR). A comparison of both methods showed similar results, allowing us to use the high-throughput q-PCR method to analyze large numbers of individuals.

Our results showed that after only one day of removal from vent fluids, symbiont metabolic activity was greatly reduced. However, symbiont abundance only decreased after seven days (by about 30%), with a 60% decrease observed after 10 days. These results indicate that there is a short term response of days to one week in which symbionts remain intact and abundant but their activity decreases when energy sources are no longer available. Thereafter, symbiont numbers decrease dramatically, either through autolysis or host digestion, or a combination of both. We are currently examining these processes in more detail to better understand how symbiont activity and abundance are regulated at shorter and longer time scales in response to changes in energy sources.

Abstract Category

16 Microbial Symbioses

PS.16.022 MICROBIAL DIVERSITY OF THE GILLS AND DIGESTIVE SYSTEM OF LIGNOCELLULOSE-DEGRADING SHIPWORM SPECIES

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Marine bivalves of the family *Teredinidae* (shipworms) have prolific capacity to consume lignocellulose (wood). Two separately compartmentalized microbial communities may contribute to this capacity, an endosymbiotic bacterial community in the gills, and a previously unexplored microbial community in the gut. Here we characterize these communities and assess their potential contribution to lignocellulose degradation. Community DNA was extracted and 16s rRNA gene clone libraries were

generated independently from gills and guts of seven and five specimens of *Bankia setacea*, respectively. A single 16S clone library was prepared from *Lydrodus pedicellatus* gill. Inserts were sequenced (469 gill clones, 218 gut clones, ~1.3 kbp, Sanger method, 1X coverage,) and analyzed phylogenetically along with previously published symbiont and free-living bacterial sequences. All gill-associated sequences were identified as gamma-proteobacteria and were inferred to represent a single closely related monophyletic lineage. Phylogenetic analyses suggest that this lineage is comprised of three branches differentiated according to host taxonomy; two associated with *B. setacea* and one with *L. pedicellatus*. Using 98% sequence identity to estimate ribotype boundaries, ~23 ribotypes were identified in *B. setacea* gills; ~64 were predicted by the Chao1 estimator. Ribotype abundance and frequency in clone libraries were strongly skewed and varied widely among individual specimens. Gut-associated bacterial sequences of *B. setacea* are comparatively diverse, representing ~49 ribotypes (200 predicted by Chao1) and seven phyla. Ribotype abundance is highly skewed, with one *Bacteroidetes* ribotype comprising over 50% of clones. FISH using taxon- and ribotype-specific oligonucleotide probes indicates specific spatial distribution of bacterial ribotypes in both gill and gut compartments, suggesting distinct functional and physiological roles for co-occurring ribotypes.

Abstract Category

16 Microbial Symbioses

PS.16.023 ARBUSCULAR MYCORRHIZAL SYMBIOSIS IMPROVES SALT-STRESS TOLERANCE IN PLANTS

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Salinization of soil is a serious land degradation problem and is increasing steadily in many parts of the world, particularly in arid and semi-arid areas. Increased salinization of arable land is expected to have devastating global effects, resulting in 30% land loss within the next 25 years and up to 50% by the middle of 21st century. Plants growing in saline conditions are generally subjected to low osmotic potentials, toxic effects of specific ions such as Na⁺ and Cl⁻, which disrupt the structure of enzymes, damage cell organelles, disrupt metabolic processes and built nutrient imbalance. Arbuscular mycorrhizal fungi are ubiquitous soil microorganisms that establish mutual symbiosis with the majority of higher plants, providing a direct physical link between soil and plant roots. They are known to exist in saline environments and to modulate plant growth by enhancing nutrients availability and by improving plant tolerance to environmental stresses. A study was conducted to investigate the influence of arbuscular mycorrhizal fungi and salt-stress on growth and productivity of plants, and the mechanism/s employed by arbuscular mycorrhizal fungi to improve salt-stress tolerance in mycorrhizal plants. Salt stress was imposed using 0, 50, 100 and 200 mM of NaCl. *Acacia* and *Trigonella* spp. were used as test plants, inoculated with arbuscular mycorrhiza culture at the time of transplantation. Arbuscular mycorrhiza showed a profound effect on the growth, biomass

and mineral nutrition of these test plants at all salinity levels. Improved nutrition particularly P seems to be very likely mechanisms to improve salt stress tolerance. However, higher chlorophyll content, proline, total sugar, K:Na ratio, anti-oxidants accumulation, photosynthetic efficiency and root hydraulic conductance could be other related strategies employed by mycorrhizal fungi to improve salt-stress tolerance in these plants.

Abstract Category
16 Microbial Symbioses

PS.16.024 CONSTRUCTION OF BACTERIAL AGENT BASED ON ECOLOGICAL THEORIES AND ITS APPLICATION IN LOW-TEMPERATURE MUNICIPAL WASTEWATER TREATMENT

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Functionally stable wastewater treatment bioreactors had stable microbial community structures under normal operating conditions and the microbial community was able to adapt in response to perturbations. In resolving the issues of long start-up period of newly-built municipal wastewater treatment plants and their unstable performances at low temperatures, low-temperature bacterial agent was constructed based on ecological theories. First, specialized consortia were enriched by repeating selection and batch cultivation with target pollutants. Each of which was composed of several bacteria with the same function. Consortia for fat and oil degrading, protein degrading, carbohydrate degrading, nitrification and flocculation were constructed separately. Under normal conditions, only one bacterium among the consortium functioned principally while others were less important; when the environmental conditions changed, other species replaced as redundancy to sustain the relative stability and proper function of the microbial community. The specialized consortia were further domesticated at varied temperatures (5-30 °C) to enlarge their ecological amplitude. Before application, specialized consortia were primarily activated with real municipal wastewater for self-adaptation to desired environments. The field application showed that three types of biological processes were successfully started within 15 days and stable and efficient daily performances were obtained. In conclusion, knowledge about wastewater characteristics and ecological balance metabolisms was the basis for successful construction of bacterial agent.

Abstract Category
16 Microbial Symbioses

PS.16.025 SPECTRUM OF IN-VITRO ACTIVITY OF CHONDRAMYCES APICULATUS AND PYXICOCCUS FALLAX AGAINST SELECTED PATHOGENIC BACTERIA AND YEAST

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The hunt for bioactive compounds in microbial cultures continues since penicillin was discovered by Alexander Fleming. Well-known good antibiotic producers are the prokaryotic *Streptomyces*, *Bacillus* and *Pseudomonas*, and the eukaryotic mold *Penicillium* and *Aspergillus*. Recently, another group of prokaryotes for novel secondary metabolites was recognized, namely, the Myxobacteria, commonly referred to as the gliding-fruiting bacteria. Two of several novel secondary metabolites by myxobacteria are now in the stage for industrial development namely the soraphen as fungicide and epothilon as cytotoxin for antitumor application.

Pure cultures of one Philippine strain each of the myxobacteria *Chondromyces apiculatus* and *Pyxiccoccus fallax* were characterized on their growth and developmental stage. Thin Layer Chromatography and Bioautography were conducted to test the metabolites extract of *C. apiculatus* and *Pyxiccoccus fallax*.

The rotavaporated methanolic extracts yielded six possible compounds based on their Rf values in TLC chromatograms namely: (Rf1) chondromides, (Rf3) melithiazol, (Rf4) nanochellins, and (Rf6) epothilons. Rf values for the other components of *P. fallax*, Rf2 and Rf5, do not fit for now those of the other known metabolites. However, only the possible epothilon from *P. fallax* exhibited limited growth inhibition of test pathogenic microorganisms both in the bioassay by the Kirby-Bauer method and bioautography. In plate culture, *P. fallax* and *Cladosporium sp.* exhibited mutual antagonism, a phenomenon heretofore unreported in the literature between myxobacterium and a fungus.

The yeast *Candida albicans* and the bacteria *Pseudomonas aeruginosa* and *Bacillus cereus* var. *mycoides* were inhibited but not strains of *Escherichia coli*, *Staphylococcus aureus*, *Bordetella brochiseptica*, and *Nocardia sp*

Abstract Category

16 Microbial Symbioses

PS.16.026 THE SIDEROPHORE BIOSYNTHESIS GENE CLUSTER FROM THE NITROGEN FIXING, SHIPWORM ENDOSYMBIONT *TEREDINIBACTER TURNERAE*

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Teredinibacter turnerae is an intracellular endosymbiont of wood-boring marine bivalves of the family Teredinidae (shipworms). Many shipworms utilize wood as their sole source of particulate food, as well as a form of shelter. Although rich in carbon, a diet primarily of wood is deficient in nitrogen. Therefore, a source of nitrogen must be

provided for elsewhere. The shipworm's endosymbiotic microbial community has been shown to provide the host with fixed nitrogen to support the nutrition of the host. Genomic and experimental analysis of *T. turnerae* reveals capabilities of nitrogen fixation, along with numerous secondary metabolite pathways. One of these secondary metabolite pathways contains genes for siderophore biosynthesis and iron transport. Here we present an analysis of this gene cluster. The genes responsible for siderophore biosynthesis are homologous to those responsible for the biosynthesis of enterobactin, a catechol siderophore, and the domain organization of these genes is similar to that of vanchrobactin biosynthesis, a marine catechol siderophore. The siderophore produced by *T. turnerae* has structural features similar to that of vanchrobactin. Gene expression analysis shows an up-regulation of genes in this cluster when cultures of *T. turnerae* are grown in iron-limiting conditions. This work examines a strategy of *T. turnerae* to acquire iron, which is integral to the process of nitrogen fixation. A diazotrophic lifestyle imposes higher demands for iron, as iron is a required co-factor in the nitrogen fixation process. *T. turnerae*'s ability to acquire iron in support of nitrogen fixation may provide a benefit to its host and is an example of one role for this bacterium in the symbiosis.

Abstract Category

16 Microbial Symbioses

PS.16.027 EXPLORING THE POTENTIAL OF BIOSYNTHETIC GENE CLUSTERS IN SPONGE ASSOCIATED FUNGI FROM RED SEA, EGYPT

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Red Sea sponges harbor diverse and abundant microbial communities. Type I polyketide synthases (PKS-I) and Nonribosomal peptide synthetases (NRPS) are biosynthetic systems often involved in the synthesis of biologically active compounds. We explore the potential of putative PKS and NRPS of fungi associated Red Sea fauna. Fungal isolates were cultured from Red Sea sponges collected near Sharm El Sheikh using mycological techniques. Fungal cultures were identified using 18S rRNA gene and antimicrobial activity were determined using test microorganisms *E. coli*, *C. albicans*, *S. aureus* and *B. subtilis*. Degenerative primer pairs target KS, junction between KS and A-T, or MT domains were used in touchdown PCR. The PCR products were gel electrophoresed, bands were excised from the gel and purified. Purified PCR products were cloned, and random colonies were picked and the plasmid carrying the insert was purified and sequenced. Similarity search were performed using BLASTX, and Maximum parsimony phylogenetic trees were constructed using retrieved sequences from Genbank. Out of 26 fungal isolates having antimicrobial activity, one fungal isolate identified as *Aspergillus nomenas* was used in this study. Thirteen PKS and one NRPS genes were detected using PCR, judged by expected PCR product size. A BLAST analysis showed that the putative PKS KS fragments exhibit the highest similarity with a wide range of KS fragments of fungi. For example 70% and 92%, respectively similarity to the KS fragments of WA type *Aspergillus parasiticus* and 6-MSAS type of *Aspergillus ochraceus*. Meanwhile, the putative NRPS fragments exhibited sequence similarity (50%) to bassianolide producing

NRPS A domain of *Xylaria* sp. The results indicate that sponge associated fungi has a wide array of PKSs as well as NRPS. Both PKS and NRPS found in this fungus might be responsible for the biosynthesis of natural compounds that provide the host defense against pathogens and/or competition with other microbes.

Abstract Category
16 Microbial Symbioses

PS.16.028 ANAEROBIC DEGRADATION OF BENZOATE BY A DEFINED PHOTOTROPHIC COCULTURE

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Benzoate is poorly degraded in anaerobic environments. Anaerobic benzoate degradation has been found in a limited species of sulfate- or nitrate-reducing and anoxygenic phototrophic bacteria. We have been examining the ecology of anoxygenic phototrophs utilizing benzoate in marine environments. During the screening process for marine phototrophs, we found cooperative benzoate degradation by a defined coculture consisting of a purple phototrophic bacterium and two non-phototrophs.

Marine sediment taken from Tokyo bay was cultivated in media containing benzoate as the sole carbon source under anaerobic conditions. After repetitive subcultures, benzoate-dependent growth was observed in an enrichment culture under illumination. This culture showed typical spectral properties for purple phototrophic bacteria. Sequencing analysis of 16S rRNA genes from the enrichment culture indicated equivalent populations of three species closely related to *Marichromatium* sp., *Shewanella* sp., and *Marinobacterium* sp. We successfully isolated these bacteria, but no single strain utilized benzoate anaerobically. The isolate related to *Marichromatium* sp. showed the same spectral properties with that of the original enrichment culture. Growth of this purple phototrophic bacterium was suppressed by 16 mM benzoate. However, significant growth was observed on benzoate under illumination when the phototroph was grown in coculture with the two other bacteria. This defined phototrophic co-culture required thiosulfate for its growth on benzoate.

This study is the first report of the cooperative utilization of benzoate under anaerobic conditions by purple phototrophic bacteria and non-phototrophs. We propose that fermentative degradation of benzoate by one or both of the non-phototrophs removed the suppressive effect of benzoate on the phototroph while simultaneously providing the phototroph with carbon.

Abstract Category
16 Microbial Symbioses

PS.16.029 MICROBIAL ECOLOGY FOR PEST CONTROL: THE FRUIT FLIES' EXAMPLE

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Fruit flies such as *Ceratitis capitata* -the medfly- and *Bactrocera oleae* -the olive fly- inflict great losses to agricultural production. Both flies harbor large bacterial communities. The fruit substrates used by the two species are greatly different: medfly larvae feed on diverse fruits while the olive fly larvae grow exclusively in olive fruits. Both flies bear large bacterial communities that appear to be radically different in composition and function.

To relate the gut microbiota to functions, fly fitness and environment, in order to identify critical features in the flies' relationships with their gut microbes. The comparison between the two pests may also yield general principles, and this knowledge can be applied for controlling these insects.

The medfly microbiota was extensively analyzed using culture-dependent, culture-independent and biochemical methods. The role of the olive fly gut bacteria was analyzed using differential diets. The impact on fly fitness of their gut mutualists was investigated in mating and egg-laying experiments.

The medfly microbiota is dominated by diverse populations of enterobacteria, the structure of which change during ontogeny. These populations are found in the gut lumen, are highly active, fix nitrogen in-situ and degrade pectin in-fructa, contributing nitrogen and carbon to the fly's diet. One bacterial species appears to dominate the olive fly gut. In both flies, the elimination of the microbiota had a significant impact on fitness. Consequently, the competitiveness of sterile medfly males used in the sterile insect technique was improved by inoculation of specific gut bacteria.

In both flies, the gut microbiota affects fitness, possibly because of, but not exclusively, nutritional benefits. By effectively linking data from the microbial community, functional analyses and the fly's environment, pest management can be improved.

Abstract Category
16 Microbial Symbioses

PS.16.030 THE SYMBIOTIC GUT MICROBIOTA OF TERMITES AND COCKROACHES: ARE THERE EVOLUTIONARY PATTERNS IN THE DICTYOPTERAN LINEAGE?

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Previous studies on the intestinal microbiota of termites have identified numerous clusters of bacteria that seem to occur exclusively in termite guts. Some of these termite-specific clusters are also affiliated with sequences originating from their closest relatives, the cockroaches. However, the microbial diversity in cockroach guts has not been studied in any detail, and it is therefore not clear whether this exclusiveness reflects properties of the particular niches in the dictyopteran gut (e.g., habitat preferences or diet-related factors) or whether these are even evolutionary patterns (i.e., elements of the gut

microbiota are cospeciating with their dictyopteran hosts). To address these points, we investigated the diversity of the bacterial gut microbiota in numerous representatives of the dictyopteran lineage using 454 GS FLX Titanium pyrosequencing. Total DNA was extracted from the microbe-packed hindguts of 27 insect species, comprising 10 termites, 13 cockroaches, 1 mantid, and 3 insect species outside the *Dictyoptera*. The 16S rRNA genes were sequenced after PCR amplification with a modified primer set targeting the V3 and V4 regions (ca. 450 bp) to exploit the full capacity of the Titanium technology; the tagged primers split the read capacity of each run, resulting in 3,000 - 15,000 reads per sample. We are presently processing the sequences using a pipeline combining BLAST and distance matrix analysis. OTU assignment, statistical and phylogenetic analyses are being performed using MOTHUR, R, and ARB software. Preliminary results indicate that the gut microbiota of termites and cockroaches contains phylotypes typical of the gut environment in general, and particular lineages are apparently cospeciating with their dictyopteran host. The presence of bacterial groups occurring exclusively in soil-feeding species suggests a participation of these groups in the degradation of soil organic matter.

Abstract Category

16 Microbial Symbioses

PS.16.031 FUNCTIONAL SEARCH FOR CELLULOSE DECOMPOSITION PROTEINS IN THE TERMITE SYMBIOTIC SYSTEM

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Termites play an important role in litter decomposition and recycling in forest ecosystems. Plant biomass is a complex (lignocellulose) that consists of several polymers (lignin, hemi-cellulose and cellulose). The termite symbiotic system fulfills many functions in dissolving this complex and decomposing each polymer for the host. Prokaryotic and protistan gut symbionts efficiently support lignocellulose degradation but little is known about this process. To identify genes of previous unknown function that contribute to the role of the host in maintaining this symbiotic relationship with microorganisms, an expressed sequence tag analysis of the termite symbiotic community from several host species was previously performed. To narrow down candidate proteins from this work we performed a controlled 10-day experiment to monitor the decomposition process. We fed the lower termite *Hodotermopsis sjostedti* cellulose and performed daily multi-omics analyses of symbiont community, proteome, and metabolome and performed correlational analysis with this information. Relative abundance of typical protists was determined by microscope and denaturing gradient gel electrophoresis, protein abundance ratios were determined by 2D gel electrophoresis, and the abundance ratio of metabolites was determined by 1H nuclear magnetic resonance. In this presentation we will report our most recent results. Although the unculturable nature of the symbiont species makes it difficult to reveal the functions of these proteins, it is important as some may have new functions related to biomass decomposition. We discuss identified candidate proteins and their function during cellulose decomposition.

Such knowledge improves our understanding of an efficient biorecycling system and will benefit applied microbiology and biotechnology.

Abstract Category
16 Microbial Symbioses

PS.16.032 COMPLEX ARRAY OF ENDOBIANTS IN *PETALOMONAS SPHAGNOPHILA*, A LARGE HETEROTROPHIC EUGLENID PROTIST FROM SPHAGNUM-DOMINATED PEATLANDS

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Petalomonas sphagnophila is a poorly studied plastid-lacking euglenid flagellate living in *Sphagnum*-dominated peatlands. Here we present a broad ranging microscopic, molecular, and microspectrophotometric analysis of uncultured *P. sphagnophila* collected from four field locations in Nova Scotia, Canada. Consistent with its morphological characteristics, 18S rDNA phylogenies indicate that *P. sphagnophila* is specifically related to *Petalomonas cantuscygni*, the only other *Petalomonas* species sequenced to date. One of the peculiar characteristics of *P. sphagnophila* is the presence of several green-pigmented particles ~5 µm in diameter in its cytoplasm, which a previously published study had suggested were cyanobacterial endosymbionts. New data presented herein, however, suggest that the green intracellular body may not be a cyanobacterium, but rather an uncharacterized prokaryote yet to be identified by molecular sequencing. 16S rDNA library sequencing and fluorescence *in situ* hybridizations show that *P. sphagnophila* also harbors several other endobionts including bacteria that represent five novel genus-level groups (one firmicute and four different proteobacteria). 16S rDNA phylogenies suggest that three of these endobionts are related to obligate intracellular bacteria such as Rickettsiales and *Coxiella*, while the others are related to the *Daphnia* pathogen *Spirobacillus cienkowskii* or belong to the Thermoactinomycetaceae. Transmission electron microscopy, 16S rDNA library sequencing, and a battery of PCR experiments show that the presence of the five *P. sphagnophila* endobionts varies markedly among the four geographic collections and even among individuals collected from the same location but at different times. Our study adds significantly to the growing evidence for complex and dynamic protist-bacterial associations in nature.

Abstract Category
16 Microbial Symbioses

PS.16.033 DEVELOPMENT OF AXENIC CULTURE OF THE ARBUSCULAR MYCORRHIZAL FUNGI *Gigaspora decipiens* UNDER ROOT ORGAN CULTURE WITH Ri T-DNA TRANSFORMED CARROT ROOT

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The present work describes the first time Axenic culture establishment of arbuscular mycorrhizal fungi *Gigaspora decipiens* with Ri - transformed carrot roots. The Arbuscular Mycorrhizal Fungi are obligatorily symbiont, which is a characteristic that is to be expected in highly compatible symbioses with more than 80 % of land plants. The fungi are most beneficial microbes for plant growth promotion and nutrient uptake. Therefore development of axenic cultures are carrying an immense importance for bulk inoculum production of arbuscular Mycorrhizal Fungi. Single-spore isolates of *Gigaspora decipiens* grown with Ri T-DNA transformed carrot root-organ culture in monoxenic system, which includes culture establishment, mycelial proliferation and auxiliary cells formation following spore production. Mycelial growth and colonization with new host in fresh minimal media plates were also achieved. Spore viability and germination percentage of surface sterilized spores were checked on 0.3% Phytigel solidified minimal media plates followed by data analysis of spore germination and optimisation of spore decontamination protocol. Mycelial proliferation and auxiliary cells formation started within a week of infection with roots. Growth rate of extra radical mycelia, intra radical mycelia and auxiliary cells were found very high comparison to *Glomus* species. Auxiliary cells numbers were recorded per experimental unit and its continuation of production during the life cycle of AMF. Mycelial biomass is very high and thickness of primary, secondary and tertiary hyphae is varies, lower order hyphae bears spores. Day of sporulation started and how long it continued was also recorded. Number of Spores varies plate to plate, sporulation continued longer than as compare to other Glomalean fungi. Sub-culturing was also achieved in three to four months old cultures. This phenomenon not previously reported in *Gigaspora* culture, to the best of our knowledge.

Abstract Category
16 Microbial Symbioses

PS.16.034 SPATIAL AND TEMPORAL ANALYSIS OF MICROBIAL DIVERSITY ASSOCIATED WITH THE MEDITERRANEAN GORGONIAN *PARAMURICEA CLAVATA*

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In Northwestern Mediterranean Sea, recurrent mass mortalities have been observed affecting benthic macroinvertebrate species during climatic anomalies in summer. One of the most affected species was the red gorgonian *Paramuricea clavata*, and the infectious pathogen *Vibrio coralliilyticus* was implicated in disease in this organism. As the composition and dynamics of the natural microbial communities living in association with temperate Mediterranean gorgonians are unknown, we attempted to establish a baseline for diversity and abundance of the bacterial community of *P. clavata*. In this

study, we used both terminal-restriction fragment length polymorphism (T-RFLP) and clone library analysis to assess the spatio-temporal changes of bacterial assemblages associated with the gorgonian. We found a clear difference between the bacterial communities during summer and winter, while T-RFLP profiles were highly similar between *P. clavata* populations separated by hundreds of kilometers. Sequencing data from 16S rDNA clone libraries demonstrated the presence of distinct phylogenetic taxa during summer and winter, with an increased diversity in warm season. Monitoring of bacterial assemblage variability during a longer period of time is currently in process to assess if the seasonal pattern observed between summer and winter is repeated during successive years. In the context of recurrent gorgonian diseases and mortalities, this picture of the bacterial communities structure will be helpful to monitor the effects of thermal anomalies on the resident microbial flora. Acknowledgments: This research was funded by the French ANR (National Research Agency) project Medchange. The authors also thank the "Fondation Total" for financial support.

Abstract Category
16 Microbial Symbioses

PS.16.035 THE ARCHITECTURE OF ORCHID MYCORRHIZAL NETWORKS IN THE GENUS ORCHIS

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Most orchid species rely on mycorrhizae to complete their life cycle. Despite a growing body of literature on identifying orchid mycorrhizal associations, the nature and specificity of the association between orchid species and mycorrhizal fungi remains largely an open question. Nonetheless, better insights in these obligate plant-fungus associations are indispensable for orchid biology and conservation of orchid populations. The aim of this study was to investigate the mycorrhizal diversity, and in particular mycorrhizal relationships within the terrestrial orchid genus *Orchis*.

An internal transcribed spacer (ITS)-based DNA array was developed from extensive clone library sequence datasets, enabling rapid and simultaneous detection of a wide range of basidiomycetous mycorrhizal fungi, and was used to investigate orchid mycorrhizal associations in sixteen species of the genus *Orchis*.

Phylogenetic analysis revealed that the majority of *Orchis* mycorrhizal fungi are members of the Tulasnellaceae, but in some plants members of the Thelephoraceae, Cortinariaceae, Russulaceae, Sebacinaceae and Ceratobasidiaceae were also found. A low degree of specificity was observed, with five orchid species associating with more than 5 different fungal partners. In most species, individual plants associated with more than one

fungus simultaneously, and in some cases associations with ≥ 3 mycorrhizal fungi at the same time were identified. Nestedness analysis showed that orchid mycorrhizal associations were significantly nested, suggesting asymmetric specialization and a dense core of interactions created by symmetric interactions between generalist species.

Our results add support to the growing literature that multiple associations may be common among orchids. Low specificity and preference for a widespread mycorrhizae may partly explain the wide distribution of the investigated species.

Abstract Category

16 Microbial Symbioses

PS.16.036 MICROORGANISMS ARE NOT RESPONSIBLE FOR THE DISEASE-LIKE SYNDROME AFFECTING THE MARINE SPONGE *IANTHELLA BASTA*

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A disease-like syndrome is currently affecting a large percentage of *Ianthella basta* over a large geographical area, with 43% and 66% of surveyed sponges displaying symptoms in the Palm Islands and Torres Strait, respectively. Symptoms of the syndrome include discolored, necrotic spots leading to tissue degradation, exposure of the skeletal fibers and disruption of the choanocyte chambers. To ascertain the role of microbes in the disease process, a comprehensive comparison of bacteria, viruses, fungi and other eukaryotes was performed in healthy and diseased sponges. All techniques indicated a very low diversity of microbes with both healthy and diseased sponge communities dominated by three strains: an *Alphaproteobacteria*, a *Gammaproteobacteria* and a Group I Crenarchaea. Bacterial cultivation, community analysis by DGGE (bacteria and eukarya), sequencing of 16S rRNA clone libraries (bacteria and archaea) and direct visual assessment by electron microscopy failed to reveal any putative pathogens. In addition, infection assays could not establish the disease in healthy sponges even after direct physical contact with affected tissue. These results provide substantial evidence that microbes are not responsible for the formation of brown spot lesions and necrosis in *I. basta*. Future research will investigate the role of environmental factors and auto-immune dysfunction in the onset of brown spot lesions and necrosis. Preliminary findings suggest that elevated sea water temperatures have little effect on *I. basta*, with similar microbial community compositions between treatments.

Abstract Category

16 Microbial Symbioses

PS.16.037 EFFECT OF THE USE OF PROBIOTICS AND PREBIOTICS ON *ARTEMIA FRANCISCANA* MICROBIOTA

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The probiotics and prebiotics have been proved being a viable alternative to improve the performance in aquaculture production. Their use promotes improvements in the water quality and the increase in the yield of the target organism. However, little is known about the effect on the autochthonous microbiota during the use of probiotics, although is hypothesized that reduce the incidence of the potential pathogenic bacteria by the production of antibacterial substances. In order to assess the processes that occur in the autochthonous microbiota, *Artemia* nauplii were cultured on an artificial diet supplemented with prebiotics and the probiotic bacteria *Exiguobacterium* sp. and *Bacillus subtilis*. The changes in the culturable bacteria were analyzed by the by ERIC-PCR. It was observed that the probiotics remain in the *Artemia* microbiota without an apparent effect on the autochthonous bacteria, in spite of their ability to produce antimicrobial substances. Different strains of *Bacillus subtilis* were found in the cultures of *Artemia*; including in the controls where *Bacillus subtilis* was not incorporated. Naturally some probiotic bacteria occurs as part of the native microbiota requiring biostimulation for their adequate performance.

Abstract Category
16 Microbial Symbioses

PS.16.038 SECONDARY METABOLITE PRODUCTION BY MICROBES CULTIVATED FROM THE SPONGE *LEIODERMATIUM* SPP. POSSESSING SIGNATURE TYPE I POLYKETIDE SYNTHASE GENES

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Leiodermatolide is a potent antimitotic agent isolated from marine sponges of the genus *Leiodermatium*. These sponges are found in deep-water environments of South Florida and host a diverse microbial population. Since leiodermatolide possesses structural features which bear a strong resemblance to microbial Type I polyketides, we have hypothesized that leiodermatolide is produced by a member of the sponge's microbial community, rather than by the sponge itself.

To test this hypothesis we used PCR to screen microbial isolates from *Leiodermatium* for three signature Type I PKS genes: the ketosynthase (KS), the O-carbamoyl transferase (OCT), and the HMG-CoA synthase (HMG). This allowed us to identify those microbes which have the genetic potential for leiodermatolide biosynthesis. The resulting subset of microbes was then fermented, extracted and analyzed for the presence of leiodermatolide and related compounds using LC-MS, HPLC and NMR techniques.

R10% of the microbes cultivated from *Leiodermatium* were found to contain both KS and OCT genes. Fermentation and analysis of these microbes identified extracts that showed

the LC-MS spectra expected for leiodermatolide. Three isolates were further cultured in rich (SYZ) and poor (10% Marine Broth) media. After 7 days, the cultures were harvested, extracted and chemically analyzed. For each microbe, LC-MS data revealed a fragmentation pattern matching that of leiodermatolide. In addition, multiple, potentially novel, compounds were observed by HPLC.

The data obtained from these experiments show that leiodermatolide is produced by a member of the symbiotic microbial community associated with this sponge and has brought us closer to the discovery of a potential microbial producer of leiodermatolide. This will facilitate the development of a sustainable biological production method which is critical in enabling advanced testing of this novel anti-cancer compound.

Abstract Category

16 Microbial Symbioses

**PS.16.039 BACTERIAL COMMUNITIES ASSOCIATED WITH
DENDROCTONUS VALENS AND *D. RHIZOPHAGUS*
(CURCULIONIDAE:SCOLYTINAE) THROUGH THEIR LIFE CYCLES**

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Dendroctonus species carry on largely their life cycles in galleries excavated in conifers trees (Fam: Pinaceae), where they feed of phloem, a nitrogen-poor substrate. Nitrogen-use efficiency is increasing by uric acid recycling bacteria. During the process of invading the tree and initiating the gallery, they are exposed to many toxic terpenes (e.g. α -pinene, mircene). *Dendroctonus valens* and *D. rhizophagus* are two bark beetles phylogenetically related, whose life cycles and colonization strategies are different. In this study, gut bacterial communities from different life stages of these two beetles were compared. Insect guts obtained under sterile conditions were used to extract metagenomic DNA, and for the isolation of bacteria in media with α -pinene or uric acid as sole carbon sources without nitrogen source. DGGE profiles of V3 region of 16S rRNA were generated to compare bacterial communities. 16S rRNA gene sequences were used to identify bacteria by a phylogenetic approach. Additionally *nifD* and *nifH* genes were amplified from some isolates and gut metagenomic DNA. Finally, acetylene reduction assays (ARA) were performed from bacterial strains growing with different carbohydrates. The diversity of bacteria associated with larvae, pupae and adults of *D. valens* and *D. rhizophagus* gut was relatively low. *Kokuria*, *Microtunatus*, *Rahnella*, *Serratia*, *Pantoea* and *Pseudomonas* were found. No bacteria were detected in eggs using culture-dependent and independent techniques. DGGE profiles showed limited differences among the insect stages and species. Some isolates grew with α -pinene as sole carbon source, others isolates were capable of using uric acid as a sole nitrogen and/or carbon source. ARA results indicated that the carbon source used to grow the

bacteria affected the nitrogen-fixing activity of the tested strains. Bacteria of bark beetles guts seem to be symbiotic by catabolizing α -pinene, uric acid recycling and by fixing nitrogen.

Abstract Category
16 Microbial Symbioses

PS.16.040 DEFORESTATION RESULTS IN DECREASED TAXONOMIC AND PHYLOGENETIC DIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI IN AMAZONIA

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Land use change is one of the greatest threats to biodiversity worldwide, but the response of microbial diversity to land use change is not well understood, particularly in tropical ecosystems. We examined the impact of deforestation on the diversity of arbuscular mycorrhizal fungi (AMF), plant symbionts found in association with most terrestrial plant, within the Amazon Basin using a chronosequence of land use types, including primary forest, pasture, and secondary forest. We examined the AMF community using high through-put sequencing (454) and quantified AMF richness using both taxonomic and phylogenetic measures. Loss of aboveground plant diversity resulted in decreased AMF species richness. In addition, phylogenetic richness was significantly lower in human-modified sites than predicted based on the regional species pool, suggesting that land use change leads to non-random species losses within AMF communities.

Abstract Category
16 Microbial Symbioses

PS.16.041 GENOME WIDE SELECTION OF STRESS RESPONSE IN A VIBRIO-SEPIOLID SQUID MUTUALISM: WHAT EXPERIMENTAL EVOLUTION CAN TELL US

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The dynamic mutualism between *Vibrio* bacteria and bobtail squids (Cephalopoda: Sepiolidae) has provided a window for observing the mechanisms by which free-living symbiotic *Vibrios* are subject to the selection pressures of both environment and host simultaneously. Abiotic factors (i.e., temperature, salinity) certainly have an effect on the competitive dominance of *Vibrio* symbionts that colonize different species of squid host, with secondary colonization events by invading non-native *V. fischeri* occurring. To determine whether a successful infection by a non-native *Vibrio* in a different squid host has specific fitness consequences associated with this switch, we experimentally evolved an isogenic strain of Hawaiian *V. fischeri* ES114 (*V. fischeri* JRM200) into a novel squid host from Australia, *Euprymna tasmanica* over 500 generations in 24 different lineages.

Since a "frozen fossil record" of symbionts can be generated, the evolving non-native clone was monitored every 100 generations to determine if its competitive ability had improved relative to native strains of *V. fischeri*. In addition, the relative competitive ability of the derived non-native strain was compared to the ancestral *V. fischeri* ES114 to determine if fitness was improved through adaptation. Non-native *V. fischeri* JRM200 demonstrated rapid adaptation to *E. tasmanica* within 500 generations by serial passage providing evidence that competitive dominance can be neutralized with phenomena such as founder effects due to tight genetic bottlenecks. Further examination of a multiple 500th generation clones by whole genome sequencing has provided intriguing evidence that specific genes are targeted when selection to a novel host is driving bacterial speciation, granting non-native Vibrios an opportunity to establish foothold beachheads in foreign *Euprymna* hosts.

Abstract Category
16 Microbial Symbioses

PS.16.042 LIGHT/ELECTRICITY CONVERSION BY A DEFINED MIXED CULTURE OF *CHLAMYDOMONAS* AND *GEOBACTER*

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A microbial solar cell (MSC) is a device that converts light energy into electricity with the aid of photosynthetic microorganisms. We have recently reported MSCs using natural microbial communities that formed biofilms on anodes (1). Community analyses suggested that the biofilm community was comprised of microalgae and anode-respiring bacteria. Although that study has suggested a novel strategy for developing MSCs, interactions among these microbes are not sufficiently elucidated, and it was considered difficult to find methods to improve the power-conversion efficiency of MSCs with unidentified natural communities. In the present study, we examined biological light/electricity conversion systems containing a defined coculture of model microbes (a photosynthetic microalga, and an iron-reducing bacterium) whose genomes had been sequenced.

A single-chamber MSC reactor was equipped with an air cathode and a graphite-felt anode, filled with an inorganic medium, and inoculated with *Chlamydomonas reinhardtii* and *Geobacter sulfurreducens*. The MSC reactors were incubated in the light and dark, and voltages across an external resistor set between the anode and cathode were monitored. After the voltage output became stable, performances of the MSCs were evaluated by measuring polarization curves and cyclic voltammograms using a potentiostat.

The *Chlamydomonas/Geobacter* MSC generated light-dependent currents with higher efficiencies than natural-community MSCs. Cyclic voltammetry showed a *Geobacter*-

dependent redox wave, suggesting that *Geobacter* generated electricity by using organics produced by the photosynthetic activity of *Chlamydomonas*.

It was demonstrated that MSC can be constructed using a coculture of a phototroph and an anode-respiring heterotroph. Genomic approaches will be used to understand how they interact with each other for the light/electricity conversion.

(1) Nishio et al. Appl. Microbiol. Biotechnol. In press.

Abstract Category
16 Microbial Symbioses

PS.16.043 CELLULAR ADAPTATIONS OF SYMBIOTICALLY COMPETENT *NOSTOC PUNCTIFORME* PCC 73102 TO HETEROTROPHY

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'Heterotrophic competence' is an essential character of the restricted number of nitrogen fixing cyanobacteria holding 'symbiotic competence'. Adaptations in the symbiotically competent *Nostoc punctiforme* strain PCC 73102 to chemoheterotrophy (growth on 5 mM fructose in the dark) were studied with focus on cell division and cell differentiation, before and after induction of hormogonia (motile plant colonization unit). Morphological development and transcription patterns of selected genes related to cell division, *ftsZ*, to heterocyst differentiation, *hetR*, and to nitrogen fixation, *nifH*, were followed. Our data reveal that heterotrophy significantly affects cell volume, heterocyst frequency (up to 2-fold), and nitrogen fixation (up to 8-fold). High *ftsZ* transcription levels preceded enhanced *hetR* expression and heterocyst differentiation. Furthermore, the initial life stage is important in determining the physiological response to heterotrophy. The data define our general knowledge about the great physiological adaptability of the cosmopolitan genus *Nostoc*, including as plant symbionts.

Abstract Category
16 Microbial Symbioses

PS.16.044 CHARACTERIZATION OF THE ENDOGLUCANASES GHF7 AND GHF45 FROM A SYMBIOTIC PROTIST OF THE LOWER TERMITE, *RETICULITERMES SPERATUS*

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Cellulose, the main structural component of plant cell walls, is the most abundant carbohydrate polymer in nature. The enzymatic hydrolysis of plant saccharides appears to be one of the promising ways to produce renewable energy. Termites are super-abundant soil animals and play an important role in the process of litter decomposition in tropical

terrestrial ecosystems. The relationship between termites and their symbiont protists is one of the most famous examples of symbiosis. Recently we constructed a cDNA library from a mixed population of protists in the hindgut of the termite, *R. speratus*, to elucidate the mechanism of lignocellulose degradation by protists. The endoglucanases of glycoside hydrolase family (GHF) 7 and 45, respectively, encoded by two transcripts isolated from the symbiotic protist of the termite *R. speratus*, were expressed in *Aspergillus oryzae* and found to be functional proteins. Interestingly, these purified GHFs have a relatively higher specific activity (603 and 786 micromol min⁻¹ mg⁻¹ protein, respectively) and V(max) value (769.6 and 833.0 unit/mg protein, respectively) than previously reported for the GHF7 endoglucanase of *Trichoderma reesei*. They also have the similar K(m) value (1.97 and 2.58 mg/ml, respectively) as *Clostridium cellulolyticum* enzymes that contain a cellulose-binding module, a property indicative of high affinity to substrate, although no cellulose-binding module is found within either. Thin-layer chromatography analysis revealed that the GHF7 enzyme preferentially hydrolyzes the beta-1,4-cellulosic linkage of cellodextrins into cellobiose and glucose.

Abstract Category

16 Microbial Symbioses

PS.16.045 FACTORS AFFECTING THE STRENGTH OF CYTOPLASMIC INCOMPATIBILITY INDUCED BY THE INHERITED SYMBIONT *CARDINIUM* IN A PARASITIC WASP

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Insects commonly harbor bacterial symbionts that are transmitted from mothers to their offspring, often in the egg cytoplasm. To increase the frequency of infected females, many maternally inherited symbionts manipulate their hosts' reproduction. Perhaps the most widespread form of manipulation is cytoplasmic incompatibility (CI), whereby matings between uninfected females and infected males produce virtually no offspring. Thus far, two unrelated symbionts, *Wolbachia* (Alphaproteobacteria) and *Cardinium* (Bacteroidetes), are known to induce CI in a wide range of arthropods. Although the mechanism of CI is not yet known, there is great interest in using CI symbionts to control insect pests and vectors of disease, as infected individuals can quickly spread and replace uninfected ones. One of the major outstanding questions in studies of CI is: why is there so much variation in CI strength? While many factors have been shown to affect the strength of CI induced by *Wolbachia*, including male age, development time, and bacterial density, little is known about determinants of CI induced by *Cardinium*. In *Encarsia pergandiella* (Hymenoptera: Aphelinidae), a parasitic wasp that attacks whiteflies, crosses between *Cardinium*-infected males and uninfected females can result in weak to very strong levels of incompatibility. We performed a number of experiments aimed at understanding this variation. Only male development time had a major effect on CI expression, while male age, body size, *Cardinium* densities, and developing in a previously parasitized host had no effect. Interestingly, the effect of development time was opposite to a previous report of CI *Wolbachia* variation in *Drosophila melanogaster*.

Male *Encarsia* that took longer to develop expressed much stronger CI than their faster-developing brothers.

Abstract Category
16 Microbial Symbioses

PS.16.046 ADAPTIVE EVOLUTION VIA SYMBIOSIS: RAPID SPREAD OF A DEFENSIVE SYMBIONT IN *DROSOPHILA*

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There is a growing awareness that many organisms harbor microbial symbionts that provide protection against natural enemies. We have identified a novel defensive symbiosis in the mycophagous fly *Drosophila neotestacea*, and show that this is its primary mode of defense against a virulent nematode parasite. We show that *D. neotestacea* harbors an inherited *Spiroplasma* symbiont that protects it against the sterilizing effects of the parasitic nematode, *Howardula aoronymphium*, both in the lab and in the wild. Flies are parasitized at high frequencies in nature, and until recently, almost all infections resulted in complete sterility. While the mechanism of resistance to sterility is not yet known, nematodes in *Spiroplasma*-positive flies are half as small as in *Spiroplasma*-negative ones. This is the first reported case of natural symbiont-mediated defense against nematodes, which are among the most diverse and widespread macroparasites known. This is also the first report of *Spiroplasma* acting as a mutualist. Although *Spiroplasma* are widespread invertebrate symbionts, their role is mostly unknown. *Spiroplasma* infections appear to be spreading rapidly in *D. neotestacea*. Almost all infected flies collected in New York in the 80s were sterile, suggesting that *Spiroplasma* infection was rare or absent. To confirm this, a sample of museum-preserved specimens was screened for *Spiroplasma* and was shown to be uninfected. There is a dramatic continent-wide cline for *Spiroplasma*, with infections at high frequency in eastern N. America but absent in British Columbia. We are actively monitoring infection spread across the Rocky Mountains. Finally, sequencing of mitochondrial haplotypes in infected and uninfected flies is also consistent with spread. This study demonstrates the profound and potentially rapid effects of defensive symbionts, which are increasingly recognized as major players in the ecology of species interactions.

Abstract Category
16 Microbial Symbioses

PS.16.047 FROM SPONTANEOUS TO INOCULANT SYMBIOTIC NODULATION OF LEGUME TREES: THE EXAMPLE OF *ACACIA MANGIUM*

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Acacia mangium is a legume tree native of Australasia. It has been introduced in many tropical countries especially in the context of industrial plantations. It has the exceptional capacity to establish symbioses with three types of microorganisms: nitrogen-fixing bacteria, arbuscular and ecto-mycorrhizal fungi, enabling a great productivity and adaptability. In this context, many field trials were established to test the effects of controlled inoculation with selected symbiotic bacteria versus natural colonization with indigenous strains. In introduction areas, *A. mangium* spontaneously nodulate with local and often ineffective bacteria. When inoculated, the persistence of inoculants, their impact on local biodiversity and possible genetic recombination with local strains have to be explored. The aim of this study was to describe the genetic diversity of bacteria spontaneously nodulating *A. mangium* in Brazil and to evaluate, by molecular tracing, the durability of selected strains used as inoculants. Three different sites, several hundred kilometres distant, were studied, each with inoculated and non inoculated plots. About one hundred strains were isolated and sequenced on three housekeeping (*glnB*, *dnaK* and *recA*) and one symbiotic (*nodA*) genes. We showed that, whatever the site and the treatment, *A. mangium* is nodulated by bacteria of the genera *Bradyrhizobium* and *Mesorhizobium*. We observed a significant genetic differentiation not only among the three sites, but also between inoculated and non-inoculated plots in each site. The two inoculated strains were never detected, even few months after inoculation, be they in nursery or in plantation, raising the question of the usefulness of such artificial inoculation. At last, *Mesorhizobium* strains, display a genetic diversity within the housekeeping genes, shared exactly the same symbiotic sequence gene, opening the door to the possible transfer of the symbiotic island from unknown symbiotic strain.

Abstract Category

16 Microbial Symbioses

PS.16.048 TEMPORAL ANALYSIS OF DIVERSITY AMONG SYMBIOTIC VIBRIO FISCHERI STRAINS

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The symbiotic association between the bacterial species *Vibrio fischeri* and its squid host, *Euprymna tasmanica* (Mollusca: Cephalopoda), is a well-known and extensively studied example of mutualistic symbiosis. Bobtail squid are benthic organisms found in shallow waters throughout the world. Several *V. fischeri* strains inhabit these waters before many environmentally infect the light organ of their squid host during the first stage of the symbiotic life cycle. Previous studies have analyzed bacterial diversity found within *Euprymna* light organs on a geographical scale, but few, if any, have observed diversity on a temporal scale. This study aims at determining the diversity of *V. fischeri* strains by creating a haplotype network from sequenced data obtained on a yearly or biennial basis of a single population of *Euprymna-Vibrio* pairs. We primarily focused on the variation

observed in bacterial diversity found in *E. tasmanica* over a 10-year period. The haplotype network and subsequent diversity analysis show interesting patterns of multiple strains present every year, as well as a few strains predominating over the entire 10-year period. Results of this work are aimed at comparing temporal diversity patterns between and among various locations to determine whether "host generalists" are more commonly found among sepiolid populations that span wider geographic boundaries compared to those found in narrow ranging habitats that exhibit higher specificity.

Abstract Category
16 Microbial Symbioses

PS.16.049 POTENTIAL FOR PLANT GROWTH PROMOTION AND GENETIC DIVERSITY OF RHIZOBIA ISOLATED FROM *PISUM SATIVUM* FROM THE INDIAN TRANS-HIMALAYAS

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The bacteria capable of eliciting nitrogen-fixing nodules in leguminous plants belong to at least 15 genera and 50 species collectively known as rhizobia. Screening and characterization of pea rhizobia is reported from Lahaul and Spiti regions in the Indian trans-Himalayas for the selection of potential strains. One hundred twenty rhizobia isolated on Yeast Extract Mannitol agar from pea root-nodules were screened for nitrogenase activity by acetylene reduction assay, root nodulation in pea grown in vermiculite, production of IAA-like auxins in tryptophan presence, phosphate solubilization on modified Pikovskaya agar and siderophore production on Chrome azurol S agar. Twenty seven potential isolates varied in acetylene reduction (0.8-3.5 n moles C₂H₄ produced/h/mg nodule fresh weight), nodulation (24-38 nodules per plant), IAA-like auxins production (2.85-26.7 µg/ml), siderophore production (0.5-63% units), ACC deaminase activity (68-2549 α-ketobutyrate nM/h/mg protein) and tri-calcium phosphate solubilization (108-309 µg/ml). Production of only carboxylate siderophores was recorded. Fourteen isolates exhibited significant increase in pea yield in microplot evaluation. Amplified ribosomal restriction analysis and 16S rRNA gene sequencing revealed the presence of *Rhizobium leguminosarum* bv. *viceae* in the two regions. The phylogenetic analyses based on *nodC*, *nifH*, *atpD* and *recA* genes divided the strains into two major groups in accordance to their geographical distribution. Geographical variation was also observed in fatty acid-profiles for the two regions with the detection of 18:1w9c only in the strains from Lahaul region. Work has led to the selection of strains with multiple plant growth promoting attributes as the candidate strains for application as microbial inoculants and revealed biogeographic distinction among the strains of Lahaul and Spiti regions.

Abstract Category
16 Microbial Symbioses

PS.16.050 ECOLOGICAL AND MORPHOLOGICAL PREFERENCES OF CYANOBACTERIAL PHOTOBIONTS IN, CYANOLICHENS OF BIODIVERSITY HOTSPOTS OF INDIA

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Lichen, one of the best symbiotic composite of a fungus (mycobiont) and an algae or cyanobacteria (photobiont), is capable of inhabiting nearly all of the terrestrial habitats. The morphology of lichens is strongly influenced by the occurrence of photobionts. Cyanobacterial photobiont either alone (bipartite) or along with a green algae (tripartite) as primary photobiont constitutes a separate functional group of lichens. In India three regions- The Himalaya, Indo-Burma and Western Ghats are identified as biodiversity hotspots. The Himalayan region harbors maximum diversity of lichens followed by Western Ghats and Indo-Burma. A total of about 1500 samples of 162 cyanolichen species of the three biodiversity hotspots of India, lodged in lichen herbarium of NBRI (LWG), were analysed microscopically for their photobionts. Cyanobiont selectivity for lichen growth forms and ecological preferences of habitats were analysed using multivariate analysis. Within 162 species examined three filamentous cyanobacteria with heterocysts- Nostoc, Scytonema and Stigonema and three unicellular forms- Anacystis, Chroococcus and Xanthocapsa were identified. Nostoc followed by Scytonema were the dominant cyanobacteria. Majority of cyanobacteria formed bipartite associations with only two cyanobionts (Nostoc and Stigonema) formed tripartite association. All the six cyanobacteria showed selective preference towards foliose lichen growth form, followed by squamulose crustose and fruticose. Corticolous (on bark) habitat was preferred by Nostoc and Scytonema where as Anacystis, Chroococcus and Xanthocapsa preferred saxicolous (on rock) habitat. Altitudinal distribution showed that all the cyanobacterial photobionts preferred subtropical (700-1500 mt.) to temperate (1500-3500 mt.) regions, clearly avoiding alpine habitats. The preferences of cyanobacteria to lichen growth forms and habitats can be attributed to efficient adaptability these forms and habitats provide to symbiotic ecophysiology.

Abstract Category

16 Microbial Symbioses

PS.16.051 INFLUENCE OF DMSP AVAILABILITY ON CORAL ASSOCIATED BACTERIA

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Marine bacteria play a central role in the degradation of dimethylsulfoniopropionate (DMSP) to dimethylsulfide (DMS), two key compounds in the global sulfur cycle. DMS is particularly important in climate regulation, through its role in cloud formation. Reef

building corals largely contribute to the production of DMSP and DMS through their symbiotic association with dinoflagellates. DMSP and DMS also potentially play a role in structuring coral-associated bacterial communities with a large overlap between the bacterial communities associated with corals and those capable of metabolising these two sulfur compounds. The ability of coral associated bacteria to degrade DMSP, including *Roseobacter* and *Spongiobacter* species isolated from two abundant Great Barrier Reef corals, *Montipora aequituberculata* and *Acropora millepora*, was investigated. The metabolic pathways and breakdown products of DMSP metabolism was identified using novel spectroscopy-based approach that allowed real-time characterisations. In addition, the *dmdA* gene involved in the first step of DMSP breakdown was identified in bacterial isolates, enrichment cultures and samples taken directly from corals to determine a broader spectrum of coral-associated bacteria possessing these metabolic capabilities. Finally, the effects of thermal stress on coral DMSP production and its consequences for associated bacterial communities were examined through measurements of DMSP concentrations, changes in bacterial community diversity and in functional metabolic genes such as *dmdA*. Together, these multidisciplinary approaches allow us to increase our current understanding of coral-microbial associations and the effect of natural stressors on coral symbioses, with important implications for coral health and the resilience of coral reefs.

Abstract Category

16 Microbial Symbioses

PS.16.052 SYMBIODINIUM-BACTERIAL INTERACTIONS

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Many dinoflagellates, including the coral endosymbiont (*Symbiodinium* spp.) cannot be grown in culture axenically, despite efforts to remove bacterial associates. To date, a thorough assessment of bacteria present in *Symbiodinium* cultures has not been initiated. This study aims to characterize bacterial associates of *Symbiodinium* and explores the potential for *Symbiodinium*-bacterial interactions. Culture-based methods, clone library analysis, T-RFLP analysis and DGGE analysis were used to identify bacteria in association with ten cultures of *Symbiodinium* representing six different *Symbiodinium* clades. Bacteria in common among different *Symbiodinium* cultures include members of the *Roseobacter* clade, the CFB group and *Cytophaga* species, suggesting that specific bacterial groups may be necessary for the growth of *Symbiodinium* spp. in culture. Ongoing efforts aim to identify functional roles of bacterial mutualists of *Symbiodinium* spp., both in culture and within the coral host.

Abstract Category

16 Microbial Symbioses

**PS.16.053 GAMMA- AND EPSILONPROTEOBACTERIAL
ECTOSYMBIONTS OF A SHALLOW-WATER MARINE WORM ARE**

CLOSELY RELATED TO DEEP-SEA HYDROTHERMAL VENT ECTOSYMBIONTS

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Tubificoides benedii is a small oligochaete worm that lives in eutrophic oxygen-limited and sulfide-rich coastal sediments. In the tidal mudflats of the North Sea, the tail ends of these worms are colonized by a dense "fur" of filamentous bacteria, particularly during the summer and autumn when sulfide concentrations are high. In some of the filaments the basal cells have penetrated the cuticle and are anchored within it, suggesting a specific association. To identify these ectosymbionts and assess their metabolic potential we used comparative sequence analysis of the 16S rRNA gene and genes involved in autotrophy and sulfur oxidation, as well as fluorescence in situ hybridization. We found that two phylotypes dominate the filamentous community: one is a Gammaproteobacterium attached to the exterior of the worm and the other is an Epsilonproteobacterium which penetrates into the cuticle. Phylogenetic analyses revealed that both ectosymbionts belong to clades that consist nearly exclusively of bacteria associated with deep-sea invertebrates from hydrothermal vents such as the shrimp *Rimicaris exoculata*, the crab *Shinkaia crosnieri* and the barnacle *Vulcanolepas oshehai*. The presence of genes for autotrophic carbon fixation (*cbbL* and *cbbM*) and sulfur metabolism (*aprA*) indicate the chemosynthetic potential of the epibiotic community of *T. benedii*. Such close relationships between symbionts from shallow-water and deep-sea marine hosts that are not closely related to each other are unusual, and indicate that biogeography and host affiliation did not play a role in these associations. Instead, similarities between the dynamic environments of vents and organic-rich mudflats with their strong fluctuations in reductants and oxidants may have been the driving force behind the establishment and evolution of these symbioses.

Abstract Category

16 Microbial Symbioses

PS.16.054 COMBINED MORPHOLOGICAL, MOLECULAR AND BIOCHEMICAL CHARACTERIZATION OF ARBUSCULAR MYCORRHIZAL FUNGUS (GLOMUS sp.) GROWN UNDER MONOEXENIC CONDITION

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Arbuscular mycorrhizal fungi are important symbionts of plants that improve plants nutrient uptake and in turn helps in plant growth promotion. Genetic analysis of arbuscular mycorrhizal fungi has been a complicated task because of its difficulties in isolation and cultivation from contaminate free system and heterogeneity of rDNA sequence within single arbuscular mycorrhizal spore. This diversity allows us to find reliable methods for genotyping of arbuscular mycorrhiza fungi. Eight different strains of

Glomus collected from centre for mycorrhizal culture collection, TERI New Delhi, India were grown in a monoexenic condition. A comparative characterization of each strain was done using spore fatty acid methyl ester profile, partial sequencing of SSU-ITS and LSU rRNA gene in combination with morphological examination of arbuscular mycorrhizal spores. Neighbor joining tree obtained from SSU-ITS rDNA sequences was broadly similar to the neighbor joining tree obtained from LSU rDNA sequences. Cladistic analysis was performed on fatty acid methyl ester profile profiles using unweighted pair group method with arithmetic mean and result were compared to a neighbor joining of rDNA sequence within same species. We suggest a combination of the morphological, biochemical and molecular (Sequencing of highly variable D1-D2 region of LSU and ITS rRNA gene) method could employed for phylogenetic analysis and species level resolution of Glomeromycota. PCR probes were designed from D1-D2 of LSU and ITS region of rRNA gene to evaluate the quality of arbuscular mycorrhiza produced on a large scale and to track the selected arbuscular mycorrhiza after inoculation into the field.

Abstract Category

16 Microbial Symbioses

PS.16.055 ANALYSIS OF MICROBIAL COMMUNITIES ASSOCIATED WITH THE COFFEE BERRY BORER (*HYPOTHENEMUS HAMPEI*; COLEOPTERA: CURCULIONIDAE)

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The coffee berry borer is the most important pest of coffee worldwide. It feeds on coffee seeds, a food source containing the alkaloid caffeine, known to be toxic to many insects and fungi. It is possible the internal microbial communities of these beetles allow them to overcome the toxic effects of caffeine. The microbial communities of coffee berry borers, as well as the ecological factors that influence these communities, are poorly understood. Male, female and immature coffee berry borers were collected from multiple locations in a number of countries (Puerto Rico and Kenya) from both beans with lower caffeine content (*Coffea arabica*) and beans with higher caffeine content (*C. canephora*) as well as both shade and sun-grown coffee. In order to distinguish between external/internal microbes some beetles were surface sterilized in a sodium hypochlorite solution, while controls were rinsed in sterile water prior to physical homogenization, enzymatic lysis and DNA extraction (modified QiaAmp micro kit, Qiagen). Bacterial 16S rRNA genes were PCR amplified using barcoded primers ligated to 454 adaptors for pooled 454-pyrosequencing. Physical homogenization using BioMasher disposable homogenizers resulted in more PCR amplifiable 16S rRNA genes compared to standard grinding, and DNA yields from surface sterilized insects were significantly lower than non-sterilized specimens. Preliminary data analysis for 454 pyrosequencing of pooled PCR amplicons shows a community mostly comprised of *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*

and *Firmicutes*. Further phylogenetic analyses will determine the partitioning of variance in community structure among sampling locations, specimen gender/growth stage and caffeine concentration in the seed. Determining the microbial components of the coffee berry borer and the ecological factors influencing their relative abundances are critical first steps towards understanding the ecology and niche specialization of this important pest.

Abstract Category
16 Microbial Symbioses

**PS.16.056 GUT BACTERIAL DIVERSITY IN THE OMNIVOROUS
COCKROACH *SHELFORDELLA LATERALIS***

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The symbiotic gut microbiota of termites is essential for the digestion of wood or other lignocellulosic diets. Surprisingly little is known about the dense bacterial communities colonizing the guts of their closest phylogenetic neighbors, the cockroaches. Previous studies, typically employing highly biased culture-dependent techniques, have focused only on cockroaches as a pest species and their guts as reservoirs for pathogens. Here, we present the first cultivation-independent characterization of the gut microbiota of an omnivorous cockroach, *Shelfordella lateralis*, which belongs to the family *Blattidae*, the sister group to termites. A clone library of bacterial 16S rRNA genes in the hindgut was constructed, and dominant clones were identified as members of *Bacteroidetes*, *Firmicutes* (mainly *Clostridia*) and δ -*Proteobacteria*. *Fibrobacteres* and *Spirochaetes*, the major cellulolytic groups in higher termites were absent. Nevertheless, many of the clones clustered with sequences previously obtained from the termite gut, some of them belonging to lineages of *Bacteroidetes* and *Elusimicrobia* (formerly Termite group 1) that comprise endosymbionts of termite gut flagellates. Our results show that the cockroach gut contains a highly diverse and complex bacterial community that is reflective of the close phylogenetic relationship between cockroaches and termites.

Abstract Category
16 Microbial Symbioses

**PS.16.057 DIVERSITY AND SPECIFICITY OF CHLOROFLEXI BACTERIA
IN SPONGES**

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Sponges are associated with dense and diverse microbial communities and many of these microbes seem to be sponge specific as they are not detected in other environments. Sponge specific microbes are identified based on their 16S rRNA gene sequences that fall together with other sponge derived sequences into monophyletic sequence clusters.

Members of the Chloroflexi are often detected in sponges and usually comprise a large fraction of 16S rRNA gene clone libraries. To provide an updated overview of the diversity and specificity of Chloroflexi bacteria in sponges we performed an extensive phylogenetic analysis. We identified 579 Chloroflexi-affiliated 16S rRNA gene sequences from sponges in GenBank and contributed another 206 sequences from various New Zealand sponges. A comparison of neighbor-joining, maximum parsimony and maximum likelihood trees revealed 29 sponge specific clusters with number of sequences ranging from 3 to >100 and representing 2-12 different sponge species. Four of these clusters also contain up to three sequences from corals. An additional sponge super-cluster was identified that consists of 252 sequences from 20 sponge species, 3 coral sequences and a single non-sponge, non-coral sequence. Overall, 58% of sponge derived sequences fell into sponge specific clusters (90% including the sponge super-cluster) and these clusters were equally distributed among the Chloroflexi subgroups, with the exception of class Chloroflexi that did not contain any sponge derived sequence. These data show that Chloroflexi bacteria in sponges are highly diverse but also form highly specific associations with their hosts. To further elucidate the ecology of Chloroflexi bacteria in sponges we are currently using a range of additional molecular tools such as Denaturing Gradient Gel Electrophoresis and Fluorescence In Situ Hybridization and are investigating abundance and community composition of these prominent but elusive members of the sponge-specific community.

Abstract Category

16 Microbial Symbioses

PS.16.058 THE ROLE OF REDOX-SENSING AND CELL-CELL SIGNALING IN LUMINESCENCE INDUCTION IN THE SQUID SYMBIONT *VIBRIO FISCHERI*

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Vibrio fischeri is a bioluminescent marine bacterium that forms a symbiotic association with the squid *Euprymna scolopes*. Luminescence is required for *V. fischeri* to fully colonize the host and is important for the squid's nocturnal behavior. Shortly after colonizing the juvenile squid, *V. fischeri* induces the *lux* operon responsible for luminescence, however the mechanism of this regulation is unknown. Luminescence is controlled in part by an autoinducer pheromone leading to cell-density dependent "quorum sensing"; however, high cell density in culture is not sufficient to induce symbiotic levels of luminescence. Previous work has shown that the redox-sensing two-component system ArcAB directly represses *lux* in *V. fischeri*. A mutation in *arcA* leads to increased autoinducer production and bright luminescence in culture. Moreover, inactivation of the Arc system could largely account for the induction of symbiotic luminescence. We hypothesize that ArcAB may sense changes in the cells' redox state to derepress *lux* expression during colonization. By manipulating culture conditions, we show that *V. fischeri* ArcAB appears to respond to the availability of respiratory substrates, as it does in *E. coli*. Using co-culture and plate assays, we also show that excess autoinducer produced in ArcA-derepressed cells can induce luminescence in

nearby ArcA-repressed cells potentially communicating both cell density and environmental conditions. In combination with redox-sensing, this form of cell-cell signaling may play an important role in luminescence induction in the squid. Additionally, we propose using a redox-insensitive ArcB allele to test the hypothesis that ArcAB derepresses luminescence during symbiotic initiation.

Abstract Category
16 Microbial Symbioses

PS.16.059 BACTERIAL COMMUNITY DIVERSITY AND DYNAMICS IN EARLY LIFE STAGES OF THE CARIBBEAN CORAL *PORITES ASTREOIDES*

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High bacterial diversity in corals makes it challenging to identify the extent to which coral-bacterial associations are taxon-specific. However, characterization of the diversity in early life stages may be more tractable. Using the early developmental stages of the coral *Porites astreoides*, we identify consistent, specific bacterial-coral associations, determine when those associations are initiated, and explore functions that bacteria may play in larval ecology. Larvae were collected in the field, maintained in native seawater, and collected at different time points for molecular analysis, microscopy, and settlement assays. FISH (fluorescence in situ hybridization) imaging shows that *P. astreoides* vertically transmits bacteria via its larvae, and bacteria are associated with the ectoderm layer during swimming stages. After settlement, bacteria are present around the newly forming septae of the developing polyps. Clone libraries and terminal restriction fragment length polymorphism (TRFLP) analysis suggest that at least two species, belonging to the genera *Roseobacter* and *Marinobacter*, are detectable throughout early development of *P. astreoides*, and in *P. astreoides* specimens spanning three locations in the Caribbean and four years of collection. The addition of bacterial strains isolated from corals, including a *Roseobacter* strain, significantly encourages *P. astreoides* larval settlement. FISH imaging was used to localize the bacteria in the settlement assay samples and to determine whether biofilm formation and composition drives larval settlement. FISH also was used to assess whether the bacterial communities associated with juvenile coral recruits reflect the composition and relative abundances of the bacterial strains added to the surrounding seawater. These results reveal new insights into how corals transmit, acquire, and maintain bacterial communities that play a role in larval ecology.

Abstract Category
16 Microbial Symbioses

PS.16.060 "SPONGE-SPECIFIC" BACTERIA ARE WIDESPREAD (BUT RARE) IN DIVERSE MARINE ENVIRONMENTS

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Marine sponges form associations with a diverse range of microbes, yet it is the apparent specificity of these associations that is most striking. Numerous studies have reported the existence of monophyletic sponge-specific 16S rRNA sequence clusters, representing bacteria found in sponges but not detected in other environments such as seawater. In a recent 16S rRNA gene tag pyrosequencing study (Webster et al. 2010, Environ. Microbiol.) we revealed the presence of sequences affiliated with "sponge-specific" clusters in seawater samples. Whilst this finding suggests that "sponge-specific" bacteria can in fact reside outside of sponge hosts, the seawater was collected from near to sponges and thus a sponge origin for these bacteria could not be ruled out. To clarify this fundamental issue in marine symbiont biology, we screened >9 million 16S rRNA gene pyrotags generated under the auspices of the International Census of Marine Microbes (ICoMM). These sequences were derived from a total of 512 temperate, tropical and polar samples and included seawater, sediment, hydrothermal vent and coral samples. We detected 49 of 72 previously described sponge-specific clusters in seawater or other non-sponge samples, albeit generally at extremely low abundances (e.g. 1 sequence read out of 25,000 for a given sample). Those that were present in higher numbers included clusters within the *Alphaproteobacteria* (c. 13% of sequence reads in one seawater sample), *Gammaproteobacteria*, and a lineage of uncertain affiliation related to the *Planctomycetes-Verrucomicrobia-Chlamydiae* superphylum. Sequences representing the candidate phylum "*Poribacteria*", previously considered to be restricted to sponges, were detected in 42 seawater and 40 sediment samples. While the presence of an organism does not imply that it is active *in situ*, our results do suggest that many of the so-called sponge-specific bacteria occur more widely outside of sponge hosts than previously thought.

Abstract Category

16 Microbial Symbioses

PS.16.061 INVESTIGATING THE COCKROACH GUT MICROBIOTA USING A GNOTOBIOTIC MODEL

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Insects harbour a diverse and metabolically active microbial community within their intestinal tracts. In termites the gut community functions as an efficient bioreactor, enabling the host to survive on lignocellulosic diets such as wood or humus. While the composition of this community is known, the interactions between the gut microbiota and host are not well understood and the role of the microbiota in host development is uncertain. These host interactions and microbial functions can be studied using gnotobiotic models. However termites are unsuitable for this approach due to the elaborate social system required for their reproduction. Cockroaches are the closest phylogenetic neighbours of termites and have a non-social nature. Here we present a gnotobiotic model of the cockroach *Shelfordella lateralis*. Gnotobiotic cockroaches were

used to determine whether the gut microbiota contributes to host development by measuring immune response and assessing histological changes in comparison to conventionally raised cockroaches. Phenoloxidase (PO) activity was assayed in both gnotobiotic and conventional cockroaches and no significant difference in levels was found at either 1 week or 1 month after hatching. This indicates that the gut microbiota may not affect activity of innate immune components such as PO. Inoculation of gnotobiotic cockroaches with a GFP-labelled *Enterobacter* species isolated from the conventional host showed that it predominately colonised the hindgut region in the zone between the epithelium and the lumen. The non-social nature and the non-specialist diet of the cockroach *Shelfordella lateralis* make it a viable gnotobiotic model for understanding digestive symbiosis and host development.

Abstract Category

16 Microbial Symbioses

PS.16.062 INVESTIGATING THE STRUCTURE AND FUNCTION OF THE NEMATOSTELLA VECTENSIS MICROBIOME

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The starlet sea anemone *Nematostella vectensis* is emerging as an increasingly important model system for the study of developmental biology, evolution, and ecology. To understand whether microbe-host interactions influence the physiology of *N. vectensis* we have sought to identify potential microbial symbionts that are stably associated with the anemone and may confer some ecological benefit. Molecular phylogenetic characterization of microbes associated with the anemone collected from three separate salt marsh environments revealed a microbial flora with variable diversity (mean chao1 23 to 1653). Four microbial 16S rRNA ribotypes were found to be associated with *N. vectensis* at multiple sites and/or sampling times: a novel ribotype within the epsilon-proteobacterial order *Campylobacterales* sharing <85% identity with cultivated strains, two γ -Proteobacterial ribotypes sharing 99% identity with *Endozoicomonas elysicola* and 99% identity with *Pseudomonas pseudoalcaligenes*, respectively) and a *Spirochaete* sharing 92% identity with a coral associated clone. Quantification of *N. vectensis* associated bacterial cells by fluorescent *in situ* hybridization (FISH) with the general bacterial probe EUB338 indicated that both field-collected and laboratory-acclimated *N. vectensis* harbored approximately 2×10^6 bacteria per specimen where bacteria were specifically localized to aggregates in the *N. vectensis* mesentery tissues. FISH with group specific probes revealed a change in the predominance of cell types in wild and laboratory acclimated *N. vectensis* where *Campylobacterales* constituted 40% (s.d. 4%) of total bacteria in aggregates of wild anemones and 19% (s.d. 3%) in laboratory-acclimated animals. We hypothesize that these mesentery-associated bacterial aggregates may harbor bacterial symbionts and we have developed a novel microbial metatranscriptomics approach to explore the nature of these interactions.

Abstract Category
16 Microbial Symbioses

PS.16.063 COMPARATIVE METAGENOMIC ANALYSIS OF THE NON-CYANOBACTERIAL SYMBIANTS OF TWO *PELTIGERA* LICHENS

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Background and aims Traditionally, lichens are considered bipartite symbiotic organisms, comprising a fungal mycobiont and an algal or cyano photobiont. Recently, a more complex picture has emerged, as non-cyanobacterial bacteria have been shown to be present in the lichen thallus and postulated to carry out significant roles in the growth and environmental adaptation of these hardy organisms. In the present study, we characterized the total symbiomes of two closely related lichen species, *Peltigera membranacea* and *P. malacea*, with emphasis on potential physiologic roles. **Methods** Total DNA from field samples of the two lichens has been sequenced using a combination of 454 and Solexa approaches. We have screened the data for sequences of presumptive bacterial origin, excluding the cyanobacterial photobionts, and determined the likely composition of the non-cyanobacterial symbiome. We have also probed the data for selected enzyme sequences involved in roles such as nitrogen fixation, polysaccharide degradation, and quorum sensing. **Results** In both lichen species, about 60% of non-cyanobacterial 16S-rDNA sequence reads were found to belong to the *Proteobacteria*, nearly all in the orders *Rhizobiales*, *Rhodospirillales*, *Sphingomonadales* and *Burkholderiales*. The second largest group was the *Actinobacteria*, comprising 20% of the non-cyanobacterial microbiota of *P. malacea* and 13% of *P. membranacea*. Nearly all actinobacterial sequence reads belonged to the *Frankineae*. The *P. membranacea* symbiome also contains a substantial population of *Sphingobacteria* absent in *P. malacea*. The symbiomes contain genes encoding several distinct types of nitrogenases, secreted glucanases, and several other enzymes of interest. **Conclusions** The non-cyanobacterial symbiomes of *P. membranacea* and *P. malacea* are dominated by *Alphaproteobacteria* and *Actinobacteria* and carry out diverse functions in the lichen symbiotic association, including nitrogen fixation.

Abstract Category
16 Microbial Symbioses

PS.16.064 CHARACTERIZATION OF THE NON-PHOTOTROPHIC BACTERIAL SYMBIOMES OF ICELANDIC SEASHORE LICHENS

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Background and aims Traditionally, lichens are considered bipartite symbiotic organisms, comprising a fungal mycobiont and an algal or cyanobacterial photobiont. Recently, a more complex picture has emerged, as non-cyanobacterial bacteria have been shown to be present in the lichen thallus and postulated to carry out significant roles in the growth and environmental adaptation of these hardy organisms. In the present study, we aim to characterize the total symbiomes of four species of seashore lichens. **Methods** Bacteria were isolated from field samples of the four lichens (*Verrucaria ceuthocarpa*, *Hydropunctaria maura*, *Lecanora helicopsis* and *Caloplaca verruculifera*) and characterized in terms of Gram-reaction, cell and colony morphology, biodegradative and several other biochemical characteristics, and partial 16S rRNA gene sequence. Total DNA was also extracted from the field samples and subjected to PCR using 16S rDNA-specific primers for analysis by DGGE and generation of clone libraries for amplified ribosomal DNA restriction analysis and sequencing of representative clones. **Results** We have isolated 168 culturable bacterial strains that form 21 distinct colony types on MA medium. Most strains are psychrophilic or borderline psychrophilic, and halotolerant to halophilic. Sequence-based identification of representative strains is currently ongoing with several strains assigned to the phyla *Actinobacteria*, *Proteobacteria*, *Firmicutes* and *Bacteroidetes*. **Conclusions** We find the composition of the culturable associate microbiota to be markedly lichen species-specific, even to the point of being observable by colony morphology. Thus, the culturable associate microbiota of *C. verruculifera* is dominated by oxidase-negative cocobacilli that form bright yellow, matte, flat colonies whereas the *H. maura* microbiota is dominated by oxidase-positive cocci that form orange, shiny, highly convex colonies.

Abstract Category

16 Microbial Symbioses

PS.16.065 SYMBIONT TRANSMISSION IN BATHYMODIOLIN MUSSELS FROM DEEP-SEA HYDROTHERMAL VENTS

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Bathymodiolin mussels are endemic to marine habitats such as hydrothermal vents, which are rich in reduced compounds. They harbor chemosynthetic bacteria in their gills, which gain energy from oxidizing reduced sulfur compounds (thiotrophic symbionts) and methane (methanotrophic symbionts). Previous studies have shown that neither female nor male gametes possess symbionts, suggesting a horizontal mode of transmission in which symbionts are acquired from the environment. Interestingly, newly settled juveniles are reported to harbor bacteria. This may mean that larvae are either infected with bacteria from their dispersal sites or that freshly settled juveniles acquire their symbionts from their surroundings. To determine if the symbionts exist in the environment, glass slides were placed near hydrothermal vents to catch any free-floating microbes. 16S ribosomal RNA gene clone libraries were constructed from this sample and screened using symbiont-specific primers. Initial results indicate that the

methanotrophs are present while it remains uncertain for the thiotrophs. Further studies of additional genes will determine if strains in the environment are the same as those in the mussel.

To elucidate the mechanism of symbiont acquisition, we analyzed via fluorescence *in situ* hybridization bathymodiolin mussels collected from Southern Mid-Atlantic Ridge vent sites. Semi-thin sections of entire juveniles (3 ± 2 mm in shell length) were analyzed. In addition to imaging symbiont and host cells with phylotype-specific probes, key markers of cellular processes, including cell division and cell motility, were tagged with fluorescently labeled antibodies. Results show that all parts of the gills, except the posteriorly located budding zone, harbor symbionts. This suggests that newly formed gills are initially sterile and are only later infected. Because the gills grow continuously throughout a mussel's life, symbiont acquisition appears to be a life-long process.

Abstract Category
16 Microbial Symbioses

PS.16.066 THE INFLUENCE OF ARBUSCULAR MYCORRHIZAL FUNGI ON CAESIUM ACCUMULATION BY PLANTS - DETERMINED BY EXTERNAL CAESIUM SUPPLY?

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Radiocaesium (Cs) contamination of soils is a worldwide problem that has arisen from human activities, such as accidents at nuclear power plants and radioactive fallout from nuclear weapons tests. The contamination of soils by Cs is of serious concern because of the long half-lives of the radionuclides ($^{134}\text{Cs} = 2$ years, $^{137}\text{Cs} = 30$ years) and the emission of harmful β and γ radiation during decay. Radiocaesium enters the food chain through vegetation because plants acquire Cs from the rhizosphere and Cs is transported symplastically to the xylem. Because of the chemical similarity of Cs and potassium (K), K transport proteins contribute to Cs uptake by roots and the delivery of Cs to the shoot. The outward-rectifying K and voltage-insensitive cation channels as well as high-affinity K/H symporters from *Arabidopsis* are all permeable to Cs.

Arbuscular mycorrhizal (AM) fungi play an important role in plant nutrition and have an impact on the gene expression of plant channels and transporters. Since AM fungi deliver nutrients to plant roots it has been suggested that they might also affect Cs uptake by plants.

To investigate the influence of AM fungi on Cs accumulation by plants, *Medicago truncatula* was grown in association with *Glomus* sp. under *in vitro* conditions on medium containing 50 μM Cs. Under K-deficient conditions, a colonization rate of around 10% did not change K or Cs concentrations in plant tissues. Results obtained from pot experiments where mycorrhizal *M. truncatula* plants were exposed to increasing Cs supply showed that high external Cs supply reduced the formation of the symbiosis. Although the accumulation of Cs by *M. truncatula* was not affected by AM fungi, these fungi might still change the expression of genes encoding K channels and transporters.

Therefore, Cs driven differences in the gene expression of mycorrhizal and non mycorrhizal plants will be discussed.

Abstract Category
16 Microbial Symbioses

PS.16.067 SEVERAL UNNAMED *ENSIFER* GENOSPECIES ISOLATED FROM ROOT NODULES OF ACACIA SP. AND SESBANIA TREES GROWING AT ECOLOGICALLY DIVERSE SITES IN ETHIOPIA

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Multipurpose woody legumes are an integral part of most agroecosystems in sub-Saharan Africa, providing fuel (firewood & charcoal), food, fodder, timber (poles, furniture, carvings etc.) as well as improving soil fertility and stability. Species of *Acacia* are the most common in arid and semi-arid areas, and 69 species out of the 130 taxa in Africa are indigenous to Ethiopia. In earlier investigations we surveyed natural nodulation of provenances of acacias (*A. abyssinica*, *A. seyal*, *A. tortilis*) and *S. sesban*, isolated root nodule bacteria (from ecologically diverse locations in Ethiopia). Based on different phenotypic and genotypic methods we characterized four unnamed genospecies in the genus *Mesorhizobium*. In the present study we conducted multilocus sequences analysis on 71 strains belonging to the genus *Ensifer*. Phylogenetic analysis, based on nearly full-length 16S rRNA gene, grouped these strains into 10 distinct clusters separated from all 15 currently recognized *Ensifer* species. Except few strains which closely related to *E. gramanticus* (a newly described species from infra arid region in Tunisia), most of our strains (59) clustered into nine distinct branches, representing genospecies I, III - VII, IX - XI. Some of these unnamed genospecies (I, III, IX) further divided into a number of sub-groups, thus indicating the possibility of having several genomovars within each of these genospecies. In most of the cases, tree topologies based on six different house-keeping genes (*recA*, *gyrB*, *rpoB*, *atpD* *gap*, and *pnp*) were consistent and in agreement with that of 16S rRNA. Similarly, symbiosis-related genes *nodC* and *nifH* were generally congruent and similar to those shown by the core genes, suggesting that these *Acacia* and *Sesbania* symbionts have a long history of separate evolution within *Ensifer*. Our findings emphasize the need to focus on previously unexplored biogeographical regions to unearth as yet unidentified rhizobia.

Abstract Category
16 Microbial Symbioses

PS.16.068 HOST ECOLOGY AND PHYSIOLOGY STRUCTURE MICROBIAL SYMBIONT POPULATIONS IN THE *VIBRIO FISCHERI*/*EUPRYMNA SCOLOPES* MUTUALISM ON OAHU, HAWAII.

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The symbiosis between *E. scolopes* and *V. fischeri* serves as a common model for marine host/symbiont associations. Ecologically, two distinct populations of *E. scolopes* have been identified on Oahu, HI. In previous work, a significant association between *V. fischeri* and host population structures has been found on Oahu. We have begun analyzing the microbial population's composition and structure at symbiosis initiation to address the following: (how) does host developmental physiology influence symbiont population ecology; (how) does local host population ecology influence symbiont population ecology?

Three approaches were used to study the above questions. First: the host's physical and developmental limitations on founding symbiont population structure within the light organ were quantitatively analyzed. Second, the relationship among ecologically distinct symbionts and identifiable sub-populations within the *V. fischeri* clade was studied via phylogenetic reconstruction. Finally, experimental competitions assessed the role that host population biology (via selection) might play in initiating symbiont population dynamics.

Phylogenetic results demonstrate that Oahu symbionts can be divided into two major groups: a well-supported, monophyletic clade (Group I) containing symbionts found predominantly in one host population on Oahu; a paraphyletic group (Group II) containing symbionts found predominantly in the other host population on Oahu. When directly competed in juvenile squid from the former host population above, Group I symbionts were more frequently found in clonal populations in host light organs when compared with Group II symbionts. Confocal microscopy of symbiosis initiation suggests that host selection for Group I vs. Group II symbionts may be related to a specific juvenile light-organ feature: a physical constriction of the light-organ entryway designated the 'bottleneck'.

Abstract Category

16 Microbial Symbioses

PS.16.069 CONTRIBUTION OF A GENOMIC ISLAND IN *DESULFOVIBRIO VULGARIS* HILDENBOROUGH IN PROMOTING RESISTANCE TO OXYGEN AND NITRITE STRESS

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A genomic island (GEI) in the sulfate-reducing bacterium *Desulfovibrio vulgaris* Hildenborough (DvH) can be spontaneously deleted from the genome by a site-specific recombinase that acts on direct repeats at the GEIs extremities, resulting in GEI⁻ cells. In pathogenic organisms, GEIs usually harbour genes that contribute to survival in the targeted host. Their role in environmental bacteria is largely unknown. The role of the DvH GEI in resistance to oxygen or nitrite stress is examined here. Although a strict anaerobe, DvH is capable of surviving in microaerophilic conditions, using both oxygen defense and oxygen respiratory proteins such as rubredoxin:oxygen oxidoreductase (Roo). DvH can also overcome nitrite stress by hybrid cluster proteins (Hcps), which function in a pathway that reduces polysulfide back to sulfide. Polysulfide, formed by the

chemical reaction of nitrite and sulfide, is inhibitory to SRB. The GEI encodes genes for Roo1 and Hcp1, promoting survival under oxygen and nitrite stress, respectively. DvH has additional genes (*roo2* and *hcp2*) elsewhere in the genome. Deletions of *roo2* and *hcp2* were made in the past. However the constructed mutant strains also lost the island and thus lacked either both *roo* or both *hcp* genes. To determine the function of the GEI-encoded *roo1* and *hcp1*, mutant strains in which *roo2* and/or *hcp2* were deleted, while retaining the GEI, were constructed. Anaerotaxis of the mutant strains has been monitored using a soft agar tube assay. Following inoculation, the tubes were exposed to air for 20 minutes. Observing the position of cells as bands of turbidity relative to the air-agar interface indicated that the Roo2 (GEI⁺) strain was more oxygen tolerant than the Roo2 (GEI⁻) strain, as it moved closer to the air-agar interface. Tolerance to nitrite will be assessed in a similar manner, using nitrite overlays instead of exposure to air

Abstract Category
16 Microbial Symbioses

PS.16.070 LOW TEMPERATURE ADAPTATION AFFECTS COMPETITIVE ABILITY OF SYMBIOTIC VIBRIO FISCHERI IN THE SQUID-VIBRIO SYMBIOSIS

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The bobtail squid *Euprymna tasmanica* forms an environmentally-transmitted mutualistic symbiosis with the gram-negative bacterium *Vibrio fischeri*. Symbiotic *V. fischeri* are acquired after hatching from the surrounding seawater and therefore can exist in a free-living state or a symbiotic state within the squid. Previous evidence has demonstrated that abiotic factors have a major influence in determining whether the symbiosis is successful, and selects for specific *V. fischeri* that are adapted for the particular habitat where squids reside. Therefore, the goal of this study was to test whether temperature adapted *V. fischeri* were better able to compete for entry into the host light organ compared to those from other habitats. Native and non-native symbiont strains of *V. fischeri* were adapted to low temperatures over the course of 4 months in vitro. Adapted strains were competed against wild-type native strains in vivo and screened for colonization success 48 hours post-infection. Adapted strains out-competed the native strain at the novel temperature in all cases, indicating adaptation to environmental temperature confers a competitive advantage for light organ colonization. The same result was observed for the adapted non-native strain (Hawaiian host-derived), which out-competed the native wild-type strain at low temperature. These results suggest the relative importance of the environment and the ability of a symbiotic bacterium to adapt to abiotic changes, and can be a major factor driving the population structure of the squid-*Vibrio* symbiosis.

Abstract Category
16 Microbial Symbioses

PS.17.001 INVESTIGATING THE ROLE OF MARINE YEAST, *CANDIDA* spp. ON THE INDUCTION OF FLOCCULATION OF FOUR MICROALGAL SPECIES

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A marine yeast isolated from a mangrove area was found to induce flocculation of four aquaculture important microalgae species, *Chaetoceros muelleri* IMOCC, *Nannochloropsis* spp. IMOCC, *Tetraselmis suecica* IMOCC and *Isochrysis* spp. IMOCC. The marine yeast, *Candida* spp. IMOCC, were screened and identified for its ability to induce flocculation of all four algal species. Flocculation studies were conducted in a Celstir bioreactor set-up and using light and confocal laser scanning microscope floc structure, size, and compactness were studied. Cell counts and aggregation factors were analyzed using a CaSy cell counter. Induction of flocculation by the yeast was shown to be stable in the presence of a carbon source (42 moles of C). Floc diameter was observed to increase to as much as 1000-1200 µm from day 7th to 14th after induction (Fig 1). *Isochrysis* spp. shows the biggest floc size. After the 14th day, there is a general decline in floc size for all algal species. Floc aggregation factor ranges from 2.267 to 3.05. Microbial induction of algae to flocculate has been proven to be possible with the isolation of an exopolymeric substance secreting marine yeast. Floc stability can be achieved with addition of a carbon source and floc size and density depends on the type of algae that are induced to flocculate

Abstract Category

17 Marine Microbiology

PS.17.002 MICROBIAL INVENTORY RESEARCH ACROSS DIVERSE AQUATIC LTERS (MIRADA-LTERS): A LOOK AT MICROBIAL DIVERSITY FROM THE POLES TO THE TROPICS

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Microorganisms help shape Earth's habitability by orchestrating the major carbon transformations, biogeochemical cycles, and input into atmospheric composition. Marine microbes in particular account for the majority of the biomass in the ocean and yet our understanding of their diversity and biogeography remains fragmentary. Advances in high-throughput DNA sequencing allow us to address questions of microbial diversity and biogeography at scales never before imaginable. The MIRADA project has completed a microbial biodiversity survey and inventory across all 13 of the major aquatic Long Term Ecological Research (LTER) sites in the National Science Foundation's United States LTER Program. Eight LTERS are marine and cover a range of diverse environments from the polar Antarctic Palmer Station site to the tropical coral reef of Moorea. The long-term objective of our study is to document and describe

baseline diversity and relative abundance data for both common and rare members of microbial communities and to relate this diversity to the underlying physical and chemical environment. In total, we have generated over 3.5 million bacterial, archaeal and eukaryotic pyrotags combined from 8 replicated samples per LTER. Our massively-parallel pyrosequencing of small-subunit ribosomal RNA gene hypervariable regions allow for the discovery of novel microbial diversity in the bacterial, archaeal and eukaryal domains of life, enable us to examine whether or not biogeographical patterns exist for microbial populations and facilitate the exploration of co-occurrence patterns across domains. Our initial results confirm the ubiquity of the "Rare Biosphere" - many diverse taxa in a community dominated by few major groups. Alpha, beta and gamma diversity analyses underway will allow us to address a suite of ecological questions at local, regional and global scales. We conclude that our approach will serve as a model for future microbial inventories that are global in scope.

Abstract Category

17 Marine Microbiology

**PS.17.003 AMMONIUM AND NITRATE SALTS IN SEAWATER:
SIMULTANEOUS REMOVAL BY BACTERIA**

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Recent research has revealed that many heterotrophs are able to nitrify NH_4^+ into NO_2^- or NO_3^- under aerobic conditions. Some organisms denitrify from NO_3^- aerobically. Anammox bacteria anaerobically generate N_2 from NH_4^+ and NO_2^- . On the other hand, in the assimilatory removal of NH_4^+ and NO_3^- , the two nitrogen compounds are incorporated into microbial cells as nitrogen sources. The microorganisms mentioned above are of interest for wastewater treatment because they are available for single-stage nitrogen removal. We reported a variety of heterotrophic microorganisms utilizing both NH_4^+ and NO_3^- simultaneously. For example, *Pseudomonas aeruginosa* NBRC 12689 was able to utilize and remove 5 mg/ml of NH_4NO_3 completely at 30°C. Another isolated bacterium, *Bacillus licheniformis* T-7-2, removed 1 mg/ml of NH_4NO_3 completely at 30 and 50°C. In the process of nitrogen removal by the two bacteria, denitrification was observed.

However, these organisms did not grow in seawater containing inorganic salts at a high concentration. The effective removal of NH_4^+ and NO_3^- from seawater in fisheries is an urgent subject in order to cultivate fishes healthy and without damage. We designed an isolation medium containing sea sand to maintain the pH value of the medium, and isolated and identified two bacteria that could remove both NH_4^+ and NO_3^- simultaneously from seawater. *Bacillus megaterium* 3118-1 removed 1 mg/ml (360 ppm as N) of NH_4NO_3 completely within 32 h at 30°C by adjusting the amount of sea sand. *Vibrio alginolyticus* 2812-3 removed 1 mg/ml of NH_4NO_3 completely within 24 h at 30°C by adding Fe^{2+} and Sn^{2+} to the medium. During the incubation, the *Vibrio* strain could reduce 40% of the total nitrogen initially added to the medium, which was probably

denitrified.

Since these organisms could grow well in seawater and utilize both NH_4^+ and NO_3^- in the readily available medium containing sea sand, they will be applied for treating seawater containing NH_4^+ and NO_3^- in fisheries.

Abstract Category

17 Marine Microbiology

PS.17.004 MUTATIONS IN HYPERVARIABLE GENOMIC REGIONS IN MARINE CYANOBACTERIA CONFER RESISTANCE TO CYANOPHAGES

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Viruses are important mortality agents of microorganisms in the oceans. The continuous coexistence of high densities of antagonistic viruses and their hosts in the oceans may be due in part to the appearance of resistant host strains. Using the ecologically important marine cyanobacteria we are investigating the evolutionary impact of resistance to viruses on cyanobacterial genomes. *Prochlorococcus* MED4 cells were challenged with the host specific T7-like podovirus P-SSP7 to select for resistant cells. A total of 56 independent resistant strains were isolated. We identified mutations in 6 different genes of unknown function that confer resistance to P-SSP7 by resequencing 7 resistant strains. Most of these genes are not conserved across cyanobacteria and have low sequence identity to those homologues that are found in the databases. These genes are located within a single cluster in a genomic island - a region of high gene diversity between closely related cyanobacteria. PCR screening of additional resistant strains revealed a total of 40 different mutations in these same genes. Adsorption of the phage to the cell surface was impaired in at least 3 of the resistant strains. Additional *Prochlorococcus* strains belonging to another clade and resistant to podoviruses were isolated and fully resequenced. Mutations in these strains were found mostly (but not exclusively) within the equivalent genomic island. Many of these mutations seem to be associated with the cell surface genes based on their annotation. Two of the genes appear to have been laterally transferred from other bacteria as determined from phylogenetic analysis. Our findings suggest that phage pressure may lead to increased sequence diversity in cell-surface related genes as well as enhanced diversity in gene content in hypervariable genomic islands. Adsorption of podoviruses to non-conserved proteins in cyanobacteria may also explain their high degree of host specificity.

Abstract Category

17 Marine Microbiology

PS.17.005 INTACT LADDERANE LIPIDS AS BIOMARKERS FOR VIABLE ANAMMOX BACTERIA IN MARINE SEDIMENTS: AN ANALYTICAL APPROACH USING LC-ESI-MS

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Anammox (anaerobic ammonium oxidation) constitute an important pathway in the global marine nitrogen cycle, and locally more than 75 % of total N₂ production may be attributed to this reaction. Anammox bacteria have several unique features, including the anammoxosome, an organelle-resembling membraneous compartment. The membrane lipids of the anammoxosome contain sequential structures of four-membered aliphatic cyclobutane rings arranged like a "staircase" (i.e. "ladderane" lipids). Observations indicate that ladderane lipids are only confined to the anammox bacteria, leading to their potential use as environmental biomarkers in e.g. sediment deposits. We have developed an analytical protocol for the extraction and detection of a specific intact membrane-forming ether-ester phosphocholine ladderane lipid using liquid-liquid extraction, solid phase extraction, and reversed phase LC-ESI-MS. In this study, ladderane lipids were used as biomarkers to confirm and compare the presence of viable anammox bacteria in marine sediments with no or significant anammox activity. Results are presented from the ladderane lipid MS-analysis (m/z = 816 and 820) in direct comparison to anammox activity (^N amendments), molecular diagnostic tools (FISH and q-PCR), and pore water distributions (O₂ and nutrients).

Abstract Category
17 Marine Microbiology

PS.17.006 COMPARATIVE GENOMICS OF *PHAEOCYSTIS GLOBOSA* TRANSCRIPT PROFILES DURING VIRAL INFECTION AND OTHER DEFINED CONDITIONS

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The chlorophyll-c containing haptophyte algal genus *Phaeocystis*, found worldwide from the Arctic to the Antarctic, is a major player in the ecology and biogeochemistry of pelagic environments. A member of this genus, *Phaeocystis globosa*, is currently being sequenced by JGI. In concert with this genome project, we are conducting a 454 FLX-based transcript sequencing project in order to understand how gene expression in this organism is affected under various stress conditions. These conditions include viral infection, CO₂ enrichment and limitation, and nitrogen limitation. Results from infecting *P. globosa* with a (as yet unsequenced) nucleocytoplasmic large DNA virus (NCLDV) indicates significant up regulation (as compared to a control) of light harvesting proteins in the early stages of infection, consistent with the hypothesis of viral-induced stress. We also detected a significant down regulation in the infected samples of Sphingolipid delta(4)-desaturase/C4-hydroxylase DES2. Sphingolipids are known to be involved in programmed cell death in uninfected host cells. We have also performed other analyses on this data. We have analyzed our transcripts with our APIS (Automated Phylogenetic

Inference System), a pipeline that automated the construction and interpretation of phylogenetic trees, which confirms that the majority of the transcripts clade with *Emiliana huxleyi*, the closest sequenced relative of *P. globosa*.

Abstract Category
17 Marine Microbiology

PS.17.007 A COMPARATIVE STUDY ON BIODEGRADATION RATES OF NAPHTHALENE AT FOUR INCUBATION TEMPERATURES IN TEMPERATE AND ARCTIC MARINE MICROCOSMS

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Despite a high number of laboratory and field experiments, it still remains unclear how the origin of the microbial community or the incubation temperature affects biodegradation rates of petroleum hydrocarbons. Low temperature slows biological breakdown, according to the general assumption degradation rates decrease 50 % at a 10°C temperature drop. Studies, however, showed that the temperature of the habitat (temperate or arctic) may not have as significant effect on biodegradation rates as previously thought. Our aim was to study whether observed degradation rates are a result of the difference in dominating community members at selected incubation temperatures, or they are due to a growth kinetic effect on the same populations. Naphthalene was selected as sole carbon source (15 mg/L) and two sets of microcosm experiments were incubated at four temperatures (0.5, 4, 8 and 15°C). The seawater inocula originated from a temperate (Stavanger, Norway) and from an Arctic (Kongsfjorden, Spitsbergen) source. Half-life of naphthalene was calculated from biological oxygen consumption data which was measured online. At suitable intervals, random bottles were sacrificed for gas chromatography analysis of naphthalene, and DNA was extracted for microbial community profiling. Taxonomical diversity was examined by denaturing gradient gel electrophoresis of amplified 16S ribosomal RNA gene fragments. Observed biodegradation rates followed a strictly decreasing exponential trend when plotted against temperature but microbial community profiles showed only slight differences depending on the incubation temperature. Different dominating populations appeared in the Arctic compared to temperate degrading communities but no clear-cut conclusions can be drawn regarding the degradation rates of the two seawater inocula. Our results suggest that the rate of biodegradation varies according to a growth kinetic effect of temperature and according to the dominating degrading populations.

Abstract Category
17 Marine Microbiology

PS.17.008 METAGENOMIC ANALYSIS OF SPECIFIC PROCHLOROCOCCUS POPULATIONS USING CELL SORTING AND MULTIPLE DISPLACEMENT AMPLIFICATION

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Prochlorococcus is the most abundant photosynthetic organism in oligotrophic waters and therefore is responsible for a significant portion of the earth's primary production. Due to its high abundance and wide distribution, many physiologically and genetically distinct lineages of *Prochlorococcus* are commonly found in the ocean. Here, we describe the development of method for metagenomic sequencing of *Prochlorococcus* populations using cell sorting and whole genome amplification (WGA). We employed WGA via multiple displacement amplification to provide a broad view of the genetic composition of the population. This study will provide insight on the genomic diversity of *Prochlorococcus*. We collected four liters of seawater from 25m depth at the Bermuda Atlantic Time-Series station. Secondly, we filter-concentrated the unpreserved cells and sorted 5E7 *Prochlorococcus* cells based on forward scatter and chlorophyll content. The resulting cells were filtered onto a polycarbonate filter and subsequently lysed using a combination of heat and acid/base treatments. We then amplified the genomic DNA using multiple displacement amplification with phi29 DNA polymerase. Each sample was sequenced using Illumina high-throughput sequencing to determine novel variants of nutrient assimilation genes associated with *Prochlorococcus*. To examine the extent of amplification bias, we also amplified genomic DNA from 1E3 to 1E8 *E.coli* cells and compared the sequence coverage to unamplified *E.coli* DNA.

The population specific *Prochlorococcus* metagenomic dataset will be analyzed for the presence and diversity of genes involved in phosphate and nitrogen assimilation. This includes different variants of alkaline phosphatase and newly discovered nitrate assimilation genes in *Prochlorococcus*. This project will expand our current understanding of the genome diversity and ecology of *Prochlorococcus* in the ocean.

Abstract Category

17 Marine Microbiology

PS.17.009 ECOLOGY AND EVOLUTION OF THE BACTERIAL COMMUNITY OF THE CORAL HOLOBIONT

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Corals form the basis of one of the most complex marine ecosystems: Coral Reefs. Corals are host to representatives from all three kingdoms of Life - Bacteria, Archaea and Eukarya - as well as viruses. This dynamic, mutualistic assemblage of organisms is referred to as the coral holobiont. The best characterized relationship of this community is between photosynthetic algae of the genus *Symbiodinium* and corals. While recent studies indicate that the bacterial community of corals is highly diverse and specific, the ecological significance and genomic makeup of entire bacterial communities harbored by corals is largely unexplored. We used massively parallel pyrosequencing of 16S small ribosomal subunit fragments amplified by PCR to characterize the coral associated bacteria of the scleractinian coral *Galaxea fascicularis*. Samples were taken from three

sites over a geographically large area: The Gulf of Aden off Djibouti, and San Brandon Island (Mauritius) and Mayotte Island, east and west of Madagascar, respectively. Each site has been sampled at depth of 5, 10 and 15 meters. This sampling strategy allows comparisons of the bacterial assemblage in the same species in geographically and thus ecologically distinct areas, while also analyzing local variability along a depth gradient. This enables us to examine the differences among corals of different ocean systems on the background of the variations that are present even in one location. We can thus discern whether the conditions that change in one location with depth, such as temperature, or the geographically different settings between sites, such as ecosystem composition and water chemistry, cause a greater change in the bacterial population in the same coral species. In conclusion, our study aims to answer the question whether the composition of the bacterial population associated with corals is specific to one ecotype, or varies according to conditions (depth) within one site.

Abstract Category

17 Marine Microbiology

PS.17.010 ANNUAL CHANGES IN THE HETEROTROPHIC BACTERIAL COMMUNITY STRUCTURE AND ACTIVITY LEVELS OF SURFACE WATERS IN THE WESTERN ENGLISH CHANNEL

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In order to facilitate conservation of biological diversity we require comprehensive knowledge of the microbial ecology of an ecosystem. As the vast majority of microbes are not readily culturable, we must use molecular tools to investigate their diversity and function in marine ecosystems. However, it is still difficult to understand their role in ecological processes. Here we present a study to characterize changes in the diversity and metabolic activity of the heterotrophic bacterial community over an annual cycle in the Western English Channel. For this purpose, surface water samples were collected from the sampling station, L4, every week for a year. The respiration rate of the heterotrophic microbial community was determined from the decrease in dissolved oxygen content of the <0.8 µm size-fraction of the sea water. Additionally, the active cells in the bacterial community were identified using bromodeoxyuridine (BrdU) incubations, followed by immunocapture of the labelled DNA. We are currently analysing captured DNA using 16S rRNA amplicon and shotgun metagenomic pyrosequencing to determine the diversity and functional profile of the active bacteria in order to characterize annual changes in the active bacterial community. This dataset sits within the larger framework of the Western English Channel bacterial diversity time series (2003-2009) and the seasonal metagenomic and metatranscriptomic studies associated with this site. We demonstrate that respiration rates are characterised by specific microbial diversity and function at different times of the year, highlighting seasonal fluctuation in this ecosystem.

Abstract Category
17 Marine Microbiology

PS.17.011 EAT, GLOW AND BE EATEN: PROPAGATION OF BIOLUMINESCENT BACTERIA IN THE OCEAN

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The benefits of bioluminescence for marine bacteria are uncertain. Over 30 years ago Hastings and Nealson proposed that bioluminescence augments the propagation and dispersal of bacteria by attracting fish to consume the luminous material; here we test this hypothesis for the first time. We show that (a) luminescence in *Photobacterium leiognathi* attracts zooplankton; (b) that zooplankton individuals contacting a culture of this bacterium turn luminous; (c) this, in turn, renders the zooplankton highly vulnerable to predation by nocturnal fish, which show a clear preference for luminous zooplankton individuals; (d) *P. leiognathi* survives passage through the fish guts, which presumably leads to its enhanced dispersal in the ocean. Our findings support the hypothesis that the use of bioluminescence as a bait can be highly beneficial for marine bacteria, especially in food-deprived environments such as the deep sea.

Abstract Category
17 Marine Microbiology

PS.17.012 SEASONAL FLUCTUATIONS IN THE ABUNDANCE OF NITRATE UTILIZING *PROCHLOROCOCCUS* IN TWO OPEN OCEAN ECOSYSTEMS

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Prochlorococcus is an abundant oxygenic phototrophic bacterium that forms the base of the marine food web in tropical and subtropical ocean gyres. Until recently, none of the of strains in isolation could use nitrate, a surprising observation given that nitrate is a major source of nitrogen in marine environments. Genome sequences from 12 *Prochlorococcus* isolates supported this conclusion, revealing the absence of genes encoding nitrate assimilation proteins. However, a recent analysis of marine metagenomic data revealed that the ability to assimilate nitrate is associated with microdiverse lineages within several known *Prochlorococcus* groups (Martiny, Kathuria, Berube. 2009). Although widely distributed among the *Prochlorococcus* clades, little is known about the factors governing retention of this trait. The aim of our work is to map the distribution and abundance of the *Prochlorococcus* gene that encodes an assimilatory nitrate reductase (*narB*). We developed a qPCR assay to quantify gene copy numbers of two types of *Prochlorococcus narB* with distinct GC contents. This assay was used to determine the distribution and abundance of putatively low-light adapted

Prochlorococcus narB genes at the Hawai'i Ocean Time-series and the Bermuda Atlantic Time-series, study sites where the distribution and abundance of several *Prochlorococcus* groups have been previously characterized (Malmstrom *et al.*, in press). As expected, these *narB* gene copies were only detected when low-light adapted *Prochlorococcus* groups were also abundant. However, only a few of these *Prochlorococcus* blooms had significant fractions of *narB* containing cells. These data suggest that unidentified factors may be selecting for the presence or absence of nitrate assimilation capabilities in some of the low-light adapted populations. Further work will seek to determine which environmental parameters are the most important selective pressures controlling the ability of *Prochlorococcus* to assimilate nitrate.

Abstract Category
17 Marine Microbiology

**PS.17.013 *PSEUDOVIBRIO DENITRIFICANS* - A METABOLICALLY
VERSATILE ALPHAPROTEOBACTERIUM BELONGING TO THE FAMILY
RHODOBACTERACEAE**

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Pseudovibrio denitrificans has initially been described in the year 2004 as a marine, facultatively anaerobic bacterium capable of denitrification and fermentation. It is closely related to the Roseobacter clade, which is highly abundant in marine environments. In this study, we investigated the physiology of a new *Pseudovibrio denitrificans* strain, which was isolated from a *Beggiatoa* culture. The complete genome of the isolate has been sequenced and, to our surprise, the data indicate a metabolically versatile life style including the capability of CO oxidation, degradation of aromatic compounds and oxidation of inorganic sulfur compounds. In addition, the genomic data also indicate a high number of transport proteins including ATP transporter for peptides, branched chain amino acids, sugars as well as putrescine and spermidine. Moreover, quorum sensing genes and methyl-accepting chemotaxis proteins are present in the genome, which, in connection with its motility, allows the bacterium to react to gradients of substrates and physiological adaptation to conditions of different substrate availability. Additionally, growth experiments show the capability to survive and grow under extremely oligotrophic conditions. In contrast to the current state of knowledge, our data indicate that *Pseudovibrio denitrificans* is a metabolically versatile bacterium capable of a generalistic, lithoheterotrophic life style under eutrophic and oligotrophic conditions. The broad range of physiological traits of the *Pseudovibrio denitrificans* strain resemble the physiology of many species from the Roseobacter clade, which is known for its high abundance, ubiquitous distribution and its important role for the marine environment. Considering the close phylogenetic and physiological relationship to the Roseobacter clade, the *Pseudovibrio* genus might play an important role in the marine bacterial community and have a significant impact on the marine carbon, nitrogen and sulfur cycling.

Abstract Category
17 Marine Microbiology

PS.17.014 EXPRESSION OF TWO CARBON METABOLISM GENES IDENTIFIED THROUGH TRANSCRIPTOMIC ANALYSIS OF ESTUARINE BACTERIAL COMMUNITIES OF SAPELO ISLAND, GEORGIA, USA

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Coastal DOC is a complex mixture of compounds, dominated by structural biomolecules derived from lignin, cellulose, and soil/sediment organic matter, and much of the available carbon is refractory in nature. Thus far it has been impossible to discern which specific molecules in this material bacteria utilize as substrates. The Sapelo Island Microbial Observatory (SIMO) has created transcriptomic libraries, seasonally and diurnally, from one estuarine location that is significantly marsh-influenced, and periodically riverine-influenced. We have taken advantage of this resource and identified two unique carbon metabolism genes that are involved in processes related to refractory carbon. The first is 4-carboxymuconolactone decarboxylase, *pcaC*. This gene is involved in the protocatechuate branch of the β ketoadipate pathway used in the breakdown of aromatics from lignin. The second target identified is taurine dioxygenase, *tauD*. Taurine is found in vertebrates, many marine creatures, algae and bacteria, but it is absent in plants. In two transcriptomic libraries 50 and 56 different *tauD* were identified from a variety of bacterial species, and 85 and 42 *pcaC* respectively. Transcription of these two targets was monitored in bacterial RNA samples collected quarterly (both day and night) at the SIMO site using quantitative reverse-transcription polymerase chain reaction (RTq-PCR). Primer development based on transcriptomic data of these unique targets proved very challenging due to highly divergent sequences. We narrowed our focus to specific groups. *pcaC* primers target Rhodobacterales. *tauD* primer sets separately target α and β proteobacteria groups. Statistical analyses were conducted to examine the correlation of gene expression to a variety of environmental parameters such as nutrients, temperature, salinity, and DOC concentration. In this study we demonstrate the expression of two unique carbon metabolism genes discovered through transcriptomic analysis.

Abstract Category
17 Marine Microbiology

PS.17.015 THE FIRST AUSTRALIAN MARINE MICROBIAL OBSERVATORY - THE PORT HACKING TIME SERIES, NSW

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We report the findings from the first year of a new pelagic microbial observatory based in South Eastern Australian coastal waters, eight miles off-shore from Sydney. Our study

site, the Port Hacking National Reference Station (34° 05'S, 151° 13'E), is one of the longest ongoing oceanographic monitoring sites in the world, providing a continuous record of around 50 years' oceanographic data. In collaboration with the Department of Environment, Climate Change and Water, NSW, and the Integrated Marine Observing System, we initiated a microbial observatory dedicated to long-term characterization of shifts in coastal pelagic microbial community structure. This is a warm temperate region where surface waters range between 16-24°C. The major oceanographic impact at this site is the seasonal southward flow of the East Australia Current (EAC) which maintains a ~6°C thermal stratification for much of the year, strongly inhibiting vertical mixing in the water column. However, in late autumn and winter when the EAC is weaker, this stratification collapses, allowing increases in surface nutrient concentrations due to increased circulation with the nutrient rich bottom waters. The continental shelf is narrow, extending 30-50 km offshore. As a result, thermal stratification may break down, and surface nutrients may be enriched, at other times of the year due to intrusions of cold, nutrient rich continental slope waters driven onto the shelf by eddies pinched off the EAC. These seasonal cycles and intrusion events have been reported to drive the abundance and distribution of some macro-planktonic species. Samples are taken monthly from six depths at each of two sites set 1.6 nautical miles apart: The Port Hacking 100m node (0, 10, 20, 50, 75, 100 m depths), and the Port Hacking 50 m node (0, 10, 20, 30, 40, 50 m). We correlate monthly microbial composition with a comprehensive suite of oceanographic data and identify seasonal cycling and patchiness in planktonic communities.

Abstract Category
17 Marine Microbiology

PS.17.016 UNEXPECTED FUNGAL COMMUNITIES IN DEEP-SEA HYDROTHERMAL ECOSYSTEMS : DIVERSITY, ECOLOGICAL ROLE AND GROWTH UNDER ELEVATED HYDROSTATIC PRESSURE

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Over the last years, the diversity of microbial eukaryotes in deep-sea hydrothermal vent ecosystems was investigated using PCR amplification of SSU ribosomal RNA genes and sequence analysis. Numerous papers revealed the presence of fungi and some nucleotidic sequences were novel. However, the occurrence of fungal communities (filamentous fungi and yeasts) at deep-sea hydrothermal vents remains an underexplored topic.

Consequently, our group initiated a research program and focused on isolation of fungi from samples collected at deep-sea hydrothermal vents of the Mid-Atlantic Ridge and the East Pacific Rise. It allowed (i) to describe deep-sea filamentous fungi and yeasts, their physiological characteristics which suggest isolation of endemic and ubiquitous species and their variable adaptations to elevated hydrostatic pressures ; (ii) to reveal distribution

heterogeneities between hydrothermal sites and frequent association with dominant animal communities (*Bathymodiolus azoricus* and *Rimicaris exoculata*) ; (iii) to compare culturable fungal communities with data from amplified rDNAs of hydrothermal samples ; (iv) to assess the presence of yeasts in deep-sea water surrounding endemic animal communities with in situ hybridizations and (v) to describe a new species in the *Candida* genus, submitted as *Candida marinus*.

This research describes for the first time the presence of filamentous fungi at deep-sea hydrothermal vents and enlarges our view of the microbial diversity in these extreme ecosystems using both culture-dependent and culture-independent methods. It finally suggests future works to study fungal communities in marine environment.

Abstract Category

17 Marine Microbiology

PS.17.017 CANDIDA MARINUS SP. NOV., A NOVEL YEAST ISOLATED FROM A MID-ATLANTIC RIDGE HYDROTHERMAL VENT

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Recently, a survey was realized in order to describe the yeast communities occurring at deep-sea hydrothermal vents (Burgaud *et al.*, 2010). We isolated yeasts from different types of substrates, from seawater to deep-sea endemic animals and even deep-sea corals. Our strain collection mainly consists of commonly observed yeasts as *Rhodotorula mucilaginosa* and *Debaryomyces hansenii* but some correspond to already known marine endemic strains as *Leucosporidium scottii*, *Rhodospiridium diobovatum* and *Candida atlantica*. Previously, several new yeast species were isolated from deep-sea samples. These new deep-sea species were affiliated to *Kluyveromyces* (Nagahama *et al.*, 1999), *Cryptococcus* (Nagahama *et al.*, 2003), *Rhodotorula* (Nagahama *et al.*, 2001, 2003, 2006) and *Dipodascus genus* (Nagahama *et al.*, 2008).

Regarding our yeast culture collection isolated from deep-sea vents, sequence analysis of the D1/D2 domain of the 26S rDNA has shown that one strain was a good candidate to represent a new species in the *Candida* genus. Thus strain Mo39, isolated from an unidentified deep-sea coral sample near *Rainbow* hydrothermal vent, is considered as a new species based on rDNA nucleotidic sequences (small subunit, large subunit, internal transcribed spacers and 5.8S). Sequences similarities indicated that strain Mo39 has for closest neighbors *Candida atlantica* and *Candida atmosphaerica*, for which morphological and physiological comparisons have been processed. Strain Mo39 display an optimal growth at 3% seasalts indicating that this isolate is a true marine yeast. Sequences similarities between strain Mo39 and related species and its adaptation to marine conditions revealed that strain Mo39 represent a hitherto unknown species. This strain is described as *Candida marinus* sp. nov. To our knowledge, this is the first description of a new micro-eucaryotal species from a deep-sea hydrothermal ecosystem.

Abstract Category
17 Marine Microbiology

PS.17.018 DEEP-SEA YEASTS UNDER PRESSURE : GROWTH AND MORPHOLOGICAL PLASTICITY

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Since few years there is increasing and clear evidence that fungi are present in the deep-sea and more specifically in the deep-sea hydrothermal vents. One paper based on culture-independent method assessed that yeast forms dominate fungal diversity in the deep-oceans (Bass *et al.*, 2007) and confirmed the high rate of yeast isolation in hydrothermal vents (Gadanho & Sampaio, 2005; Burgaud *et al.*, 2010). But whether these organisms are specifically adapted to deep-sea habitats can only be confirmed with genomic and/or culture-based analyses.

Recently, a survey was realized in order to isolate and describe the yeast communities occurring at deep-sea hydrothermal vents (Burgaud *et al.*, 2010). Regarding yeast response under elevated hydrostatic pressure, our yeast culture collection was divided in two groups (i) piezosensitive yeasts, whose growth is sensitive to elevated pressure and (ii) piezotolerant yeasts, capable of growth at both atmospheric pressure and high pressure. In addition, all the yeasts cultured were able to grow under hydrostatic pressure that confirmed their adaptations to deep-sea conditions.

At atmospheric pressure all strains grow as unicellular budding yeasts. Under hydrostatic pressure four strains have grown as pseudohyphae and only one as true hyphae. These specific morphological formations were analyzed with optical and scanning electron microscopy under increasing hydrostatic pressure conditions. Complementary works have shown that these yeasts display an active ribosomal activity and nuclear divisions. For the yeast that grow as true hyphae under hydrostatic pressure, our results clearly indicates a dimorphism induce by hydrostatic pressure.

Although *Escherichia coli* are known to become filamentous with several nuclei under elevated hydrostatic pressure, this is the first report of a yeast filamentation induced by hydrostatic pressure. Moreover and contrary to *E. coli* one yeast display a real dimorphism with structured filaments.

Abstract Category
17 Marine Microbiology

PS.17.019 ROLE OF BIOTURBATING ACTIVITIES ON MICROBIAL COMMUNITY STRUCTURE AND HYDROCARBON DEGRADATION

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The fate of hydrocarbons depends on both abiotic and biotic processes that mainly involve microorganisms. Burrowing macrofauna plays a role in the transfer of nutrients and contaminants to the sediments, affecting thus the fate of contaminants. In addition, these organisms can modify the microbial metabolisms. With a multidisciplinary approach, we propose to determine the role of bioturbating fauna on the fate of pollutants and to estimate the effects of bioturbation and crude oil on microbial activities.

Microcosms were performed with pristine sediment, miming the tide cycles. Four conditions were applied: sediments with oil pollution, sediments with *Nereis diversicolor*, sediments with oil pollution and bioturbation, and control sediments. Samplings were performed over 270 days for chemical and microbiological analysis. Hydrocarbon pollution and burrowing impacts on Bacteria community structures were investigated by T-RFLP and 16S rRNA gene library analyses. The fate of hydrocarbon compounds in muddy sediments was followed by GC-MS. The mobilization of vanadium was also followed. Bioturbation activity was quantified by using luminophores. Integrons were followed in order to evaluate their implication in the adaptation to petroleum compounds.

The fate of hydrocarbons present in the upper layer of sediment (0-2cm) was similar irrespective of the addition of *N. diversicolor*. This result is correlated with the content of vanadium that was not detected after 90 days in the oiled conditions. However, a greater bioturbating activity was recorded in polluted condition with *N. diversicolor*. This is consistent with the dynamic of active bacterial community. Different structure organization between polluted conditions among the initial sampling and 270 days were observed. Similarly, the diversity of integron was impacted by the pollution. All these results highlight the macrofauna effect on the microbial community in presence of oil.

Abstract Category

17 Marine Microbiology

PS.17.020 ARCHAEAL AND BACTERIAL COMMUNITIES CHANGES IN OIL POLLUTED AND BIOTURBATED SEDIMENTS

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The coastal zones are exposed to pollutant injury such as oil spills and accumulate various pollutants arising from continental hydrosystems. Hydrocarbons may be attacked by abiotic and biotic process, as microbial degradation. By their bioturbating activity, burrowing macrofauna has a major position in the functioning of marine ecosystems. They can affect microbial communities by modifying the chemical and physical sediment properties and provide nutrients and oxygen to microorganisms. We investigated the effects of bioturbation and crude oil on bacterial and archaeal activities in sediment. Microcosms miming the tidal cycles with natural seawater were performed with pristine sediment. Four conditions were applied: bioturbation (*Nereis diversicolor*), crude oil pollution, oil pollution plus bioturbation, and a control. Samplings were done over 9 months for chemical and microbiological analyses. Bacterial and archaeal communities structures were followed at genomic and transcriptomic levels by T-RFLP and 16S rRNA transcripts library analyses. The fate of hydrocarbon compounds in sediments was followed by GC-MS. The reworking activity modified the depth repartition of hydrocarbon in the sediment without enhancing the removal of hydrocarbon compounds. This reworking activity affected the structure of microbial communities. T-RFLP fingerprints based on 16S rRNA transcripts showed that archaeal communities structures were affected by bioturbating activity irrespective of oil contamination, whereas bacterial communities were mostly impacted by oil. In addition, the microbial diversity was increased in presence of *N. diversicolor* with or without oil. 16S rRNA transcripts libraries showed a "gamma shift" in presence of *N. diversicolor* that was not observed in presence of oil. In such conditions hydrocarbonoclastic microorganisms were selected. These results indicated that the selection of microorganisms by bioturbation did not affect the hydrocarbonoclastic capacities.

Abstract Category

17 Marine Microbiology

PS.17.021 ARE ABUNDANT BACTERIA MORE ACTIVE THAN RARE BACTERIA IN THE OCEAN?

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One of the major questions microbial ecologists face with the advent of molecular tools is: Are all those bacteria that we detect using 16S rRNA gene surveys active? Our research goal is to combine 16S rRNA gene tag pyrosequencing, qPCR of ribosomal RNA and other measures of bacterial activity to address this important question. We hypothesize that in dynamic marine systems abundant bacteria are always active and rare bacteria have variable activity levels.

To address this hypothesis we examined two measures of activity of the bacterial communities from the Sargasso Sea and a eutrophic marine site off the coast of Delaware (MOPE-FB). One activity measure is the ratio of rRNA to rRNA genes estimated from tag pyrosequencing of the entire community. The second is the incorporation of

bromodeoxyuridine by actively replicating bacteria into DNA. Selected abundant and rare ribotypes were further analyzed by qPCR to determine their rRNA to rRNA gene copy ratios.

Analyses of rRNA and rRNA gene frequencies in three years of monthly tag data from the MOPE-FB site indicated that some populations, e.g., two major SAR11 subclades as identified by a 0.03 distance cutoff, were abundant and active in different seasons, whereas others, e.g., *Oceanospirillum*, varied greatly in abundance (rRNA genes) with no apparent seasonal pattern. Distinct differences were also observed in their rRNA/rRNA gene ratios, suggesting that phylogenetically highly related bacteria are not equally active. Some rarer bacteria, e.g., another SAR11 subclade, had rRNA/rRNA gene ratios that were more than one standard deviation from the mean, whereas others had undetectable rRNA levels.

Abundant bacteria seem to be active, rarer bacteria more variable. Both phylotype and seasonal differences contribute to rRNA/rRNA gene ratio variation. These results will be compared to bromodeoxyuridine-incorporating bacteria and Sargasso Sea data.

Abstract Category

17 Marine Microbiology

PS.17.022 SPATIAL DISTRIBUTION OF BACTERIOPLANKTON LINEAGES ALONG A MERIDIONAL TRANSECT OF THE PACIFIC OCEAN

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The Pacific Equatorial Divergence Zone is characterized by macronutrient concentrations in excess of the requirement for new primary production, and has been widely studied due its importance to the global carbon cycle. Understanding the planktonic bacterial community structure in this habitat is an important key to understanding nutrient cycling, carbon flux, and trophodynamics. As part of the "Biogeochemistry of the Upper ocean: Latitudinal Assessment" cruise, molecular methods were used to investigate the relationship between bacterial community structure and the surrounding environment in the Equatorial Pacific Ocean between Suva, Fiji and Honolulu, Hawaii in April 2007 during non-El Nino conditions. At nine latitudes, water column depth profiles spanning the surface to 1000 m were sampled for nucleic acids, inorganic nutrients, conductivity, temperature, oxygen, direct cell counts, and photo-pigments. Terminal restriction fragment length polymorphism and cloning and sequencing of 16S rRNA genes were used to characterize the bacterial community. Patterns in bacterial community structure were correlated with the surrounding environment, and helped explain the distributions of several abundant bacterial lineages. Surface ocean bacterial communities from 3.0°S to

9.0°N latitude were more closely related to each other than to communities sampled in the gyres to the north or south. Niche partitioning was observed between some of the dominant lineages; marine *Synechococcus* and the alphaproteobacterial lineage SAR11 subclade IA were abundant in the equatorial surface ocean, while *Prochlorococcus* dominated the subtropical gyres. The phylum *Proteobacteria* dominated the mesopelagic, including such ubiquitous lineages as SAR11 subclade II, deltaproteobacterial lineages related to the nitrite oxidizing genus *Nitrospina* and the SAR324 cluster, and relatives of the uncultured gammaproteobacterial lineage ARCTIC96BD-19, among others.

Abstract Category
17 Marine Microbiology

PS.17.023 RELATIVE COMPETITIVE ADVANTAGES IN PROCHLOROCOCCUS ECOTYPES AS A FUNCTION OF TEMPERATURE

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Prochlorococcus is an open ocean cyanobacterium, thought to be globally significant in nutrient cycling. Genetically and physiologically distinct "ecotypes" of *Prochlorococcus* populate the world's subtropical and tropical oceans. A few key ecotypes comprise the majority of these populations, with the dominant ecotype(s) varying as a function of depth and latitude. The mechanisms underlying the specific distributions of the ecotypes remain poorly understood, but temperature is believed to play a key role in latitudinal partitioning. Quantitative PCR (Q-PCR) was used to assess ecotypic abundances across a transect spanning the Pacific Ocean from 20°N to 37°S latitude. A major region of interest for the transect was the Western Pacific Warm Pool (WPWP), the warmest body of water (30°C+) in the open ocean. Our field data indicated that these areas of elevated temperatures are dominated by the eMIT9312 ecotype, known in prior studies of Atlantic Ocean populations to predominate in warm waters. Fitness trends were assessed in mixed culture competition studies between strains of the eMIT9312 and MED4 ecotypes, and correlate strongly with field QPCR measurements along the WPWP transect. Studies of the WPWP and other regions that experience oceanic temperature fluxes may provide an important indication of future trends in *Prochlorococcus* population dynamics, as a consequence of shifting oceanic temperatures.

Abstract Category
17 Marine Microbiology

PS.17.024 MICROBIAL DIVERSITY OF SEDIMENTS AT GAS SEEP AREA OF KAGOSHIMA BAY AS DETERMINED BY PYROSEQUENCING

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Bacterial community structure was compared between two different sediments based on the 16S rRNA gene sequence analysis by pyrosequencing method. The sediment samples were collected from tube worm, *Lamelibrachia satsuma* inhabited area (MEGC0044) and caldera region (MEGC0046), which are located at shallow water of marine hydrothermal vent area in Kagoshima Bay, Japan. The phylotypes affiliated into the class δ -, ϵ - and γ -*Proteobacteria*, and *Flavobacteria* are predominant in both sediments but the proportion was different. Other phylotypes, *Acidobacteria*, *Chloroflexi*, *Planctomycetes*, and the candidate division WS3, also could be detected by more than 1%. The predominance of δ - and ϵ -*proteobacteria* represents distinct characteristics compared with the bacterial community structure previously reported from other cold seeps or hydrothermal vent areas where the γ -*Proteobacteria* is dominant. The proportion of ϵ -*proteobacteria* at MEGC0044 (28.6%) was much higher than that of δ -*proteobacteria* (14.8%) but vice versa at MEGC0046 by 13.5 and 20.6%, respectively. The results imply that the sulfur oxidizing is major biogeochemical process at MEGC0044, however, MEGC0046 is dominant by the sulfur reducing activity. The difference of major biogeochemical process between two sediments may result in the environmental factors (e.g sulfur concentration). [Supported by MEGRC]

Abstract Category
17 Marine Microbiology

PS.17.025 DO MICROBE-MEDIATED PROCESSES INFLUENCE THE SUCCESS OF SEAGRASS RESTORATION?

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There is evidence that seagrasses and sediment microbial communities interact to influence the success of seagrass restoration projects. Seagrasses produce large amounts of organic matter, and have the ability to release oxygen from the tips of their roots, creating suitable niches for a wide variety of microorganisms. In turn, these organisms alter the environment for the plants with which they coexist. We hypothesize that seagrass restoration affects local sediment biogeochemistry, including sulfate reduction, nitrification and denitrification. In order to test this hypothesis, we studied the dynamics of the microbial community during the initial stages of a seagrass restoration project, by measuring microbial diversity in the restored bed and in adjacent bare areas. The microbial community was characterized by PCR amplification of genes from Bacteria (*AmoA*, *NirS*, *NirK*, *DSR*) and Archaea (*AmoA*) in samples taken at 3 different depths in the sediment, and subsequent analysis by denaturing gradient gel electrophoresis. Microbial assemblages were compared among cores and related to potential nitrification rates and depth profiles of sulfide, nitrate, nitrite, ammonia, dissolved organic nitrogen, dissolved inorganic carbon and dissolved organic carbon in the pore water. The microbial community changed over the month-long sampling period, both in the transplanted bed and the adjacent bare areas. For specific groups, the composition of the microbial community differed markedly between transplanted seagrass and bare sediment. These differences became less pronounced when the restored bed started to decline. Death of

the seagrass corresponded to changes in the biochemistry of the sediment, suggesting a role of microbes in seagrass survival. Our findings indicate that successful seagrass restoration projects can alter the composition of the microbial community, and indirectly influence biogeochemical cycles at the restored sites.

Abstract Category
17 Marine Microbiology

**PS.17.026 NEW DEBLOCKING AMINOPEPTIDASES FROM
HYPERTHERMOPHILIC ARCHAEON *THERMOCOCCUS ONNURINEUS* NA1**

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It has been reported that one of the hyperthermostable deblocking aminopeptidase (DAP) from *Thermococcus onnurineus* NA1 (TNA1_DAP) exhibits hydrolytic activity toward short peptides and acyl-peptides. In the genome database of *T. onnurineus* NA1, three new open reading frames homologous to the DAP of *T. onnurineus* NA1 were found. The deduced amino acid sequences were similar to DAPs of *Pyrococcus furiosus* and *Pyrococcus horikoshii*, a member of peptidase family M42. The three new genes for the proteins were cloned and overexpressed in *Escherichia coli*. Molecular masses assessed by SDS-PAGE were 38 kDa, 37 kDa and 36 kDa respectively, and exhibited aminopeptidase activity, including deblocking activity. One of the purified DAPs showed the optimum activity at 90°C and its half-life (t_{1/2}) was 2.5 hr. It was confirmed that *T. onnurineus* NA1 has four similar aminopeptidases with deblocking activity and that these enzymes appear to play important roles in hydrolyzing small and acyl-peptides in *T. onnurineus* cells.

Abstract Category
17 Marine Microbiology

**PS.17.027 PROFILING BACTERIAL COMMUNITIES ASSOCIATED WITH
YELLOW BAND DISEASED CORALS**

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Coral reefs are one of the most productive and biologically diverse ecosystems in the world; unfortunately, they are also highly endangered. Corals have symbiotic algae and other associated microbial organisms, which together comprise the coral holobiont. As an integral part of the coral holobiont, bacteria are used as indicators of coral health. Human population growth and environmental change have been noted to contribute to coral mortality and more recently disease events. In the Caribbean Sea, Yellow Band Disease

(YBD) is widespread and is known to affect several coral species including *Montastraea* spp., which are dominant reef building species in this region. In this study, a dual approach of high-density 16S rRNA gene microarrays and 454-tag sequencing were used to investigate the abundance and diversity of bacteria associated with *M. faveolata* displaying phenotypic signs of YBD. Using this high-throughput approach, relative microbial abundances were compared to profile diseased colonies. Using these techniques we are aiming to 1) survey the coral-associated microbial community, 2) aid the discovery of novel bacteria, and 3) identify disease-causing pathogen candidates. The results of this work unveil levels of magnitude of coral-associated bacteria not yet revealed and refine our understanding of bacteria associated with *M. faveolata* under healthy and diseased conditions.

Abstract Category

17 Marine Microbiology

PS.17.028 CULTIVATED VERSUS CULTIVATION-INDEPENDENT DIVERSITY OF LABYRINTHULOMYCETES IN ESTUARINE SEDIMENT

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Labyrinthulomycetes are ubiquitous, abundant and diverse marine fungus-like osmoheterotrophic protists. Although heterotrophic bacteria are generally thought to be responsible for the decomposition of organic matter in the oceans, labyrinthulomycetes may also play an important role, especially in the remineralization of particulate organic matter. Molecular methods have revealed the presence of many novel types of labyrinthulomycetes, and we are working to cultivate some of these organisms. In this study, we test the hypothesis that frequently observed ephemeral isolates, those that do not survive in culture beyond four transfers, may include novel labyrinthulomycetes. The surface one centimeter of a sediment sample from Great South Bay (NY, USA) was collected, mixed well, diluted and inoculated onto agar-solidified media containing one of several organic carbon sources (horse serum, slurries of *Ulva*, diatoms, krill, or *Artemia*, or a combination of yeast extract, glucose, and peptone). Part of each initial colony was collected for 18S rDNA sequence analysis, while part was transferred to fresh media of the same composition and characterized morphologically through repeated transfers. For comparison, total community DNA was extracted from the original sediment sample and PCR amplified with seven pairs of primers designed to recognize all known labyrinthulomycetes. Different types of labyrinthulomycetes dominated the culture collection compared to the sequenced PCR products. Of 44 labyrinthulomycete isolates, only 28 survived through four transfers onto fresh media. Isolates most closely related to previously cultivated labyrinthulomycetes survived better than isolates most closely related to 18S rDNA sequences known only from direct sequencing of environmental samples. Our results support the hypothesis that at least some labyrinthulomycetes represented only by environmental sequences are able to grow in culture, but only for a short time.

Abstract Category
17 Marine Microbiology

PS.17.029 TEMPORAL AND SPATIAL CHANGE IN THE MICROBIAL COMMUNITY ASSOCIATED WITH BROWN TIDE IN A COASTAL LAGOON, GREAT SOUTH BAY, LONG ISLAND, NY, USA

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Resolving the factors that govern the spatial and temporal distributions of organisms is a fundamental problem in microbial ecology. Understanding how microbial communities are distributed in the ocean and why certain distribution patterns exist is necessary to help predict how marine ecosystems will respond to environmental change. This study focuses on patterns of planktonic microbial community structure within a coastal embayment, Great South Bay along the south shore of Long Island, NY. Samples were collected in 2008 weekly or biweekly from early May to early October. A 10-station transect was covered with 0.5 mile between stations. 2 liters of surface water was collected and 100 ml was filtered through a 0.2 μm polycarbonate membrane. Total DNA was extracted and terminal restriction fragment length polymorphism (TRFLP) of 18S and 16S rDNA amplicons was used to visualize changes in community structure. Cloning and sequencing were also performed to identify the major terminal restriction fragments (TRFs). 2008 featured the most widespread and intense brown tide observed in Great South Bay for more than a decade. For 18S rDNA, one major TRF (174B) matched that predicted for the brown tide species *Aureococcus anophagefferens*. It was present for most of the sampling time with four distinct peaks of about 40% of total peak area. *Chaetoceros*, dinoflagellates and chlorophytes dominated the eukaryotic community when *A. anophagefferens* did not. For 16S rDNA, TRF 297Y matched that predicted for the plastid of *A. anophagefferens* and followed the temporal variation of TRF 174B. TRF 475Y had a reciprocal distribution pattern to 297Y and represented other picophytoplankton. Multidimensional statistical analyses showed that temporal variation in eukaryotic and prokaryotic communities was greater than spatial variation in Great South Bay, and that similar patterns were found whether TRFs from *A. anophagefferens* were included or excluded from the analysis.

Abstract Category
17 Marine Microbiology

PS.17.030 USING QPCR AND T-RFLP TO ASSESS THE TEMPORAL PATTERNS OF LABYRINTHULOMYCETE COMMUNITY STRUCTURE IN A QPX (QUAHOG PARASITE UNKNOWN) EPIZOOTIC AREA

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Labyrinthulomycetes (labyrinthulids, thraustochytrids and aplanochytrids) are ubiquitous marine protists that appear to play an important role in the mineralization of particle organic matter in the ocean. However, their significance as a component of the marine ecosystem has been underappreciated, mainly due to the limited knowledge of some basic questions such as their abundance and diversity. In this study, we used an 18S rRNA gene-based quantitative real-time PCR assay with primers Laby-A and Laby-Y to quantify the abundance of labyrinthulomycetes in sediment and seawater samples collected between April and September, 2006, from several sites in Raritan Bay (New York) where epizootic QPX (a thraustochytrid pathogen of hard clam) disease has been found since 2002. Labyrinthulomycetes were estimated to range from 2 to 75 cells mg⁻¹ in 21 of 29 sediment samples, and from 3 to 56 cells ml⁻¹ in 20 of 30 seawater samples. 18S rRNA gene-based terminal restriction fragment length polymorphism (T-RFLP) with primers Laby-A and Laby-Y was used to examine the diversity of labyrinthulomycetes in the same environmental samples. Nonmetric multidimensional scaling (NMS) analysis of T-RFLP profiles revealed temporal trends in labyrinthulomycete community structure in both sediment and seawater samples, although the pattern was more distinct in seawater. Likewise, multi-response permutation procedure (MRPP) tests showed significant differences among sediment and seawater samples collected from different seasons. NMS and MRPP results also indicated that the composition of labyrinthulomycete communities in sediment was significantly different from those in seawater samples. Labyrinthulomycete community structure was not related to the prevalence of QPX disease in clams collected along with the sediment and seawater samples.

Abstract Category

17 Marine Microbiology

PS.17.031 AN "OPEN SOURCE" SAMPLE FILTRATION AND ARCHIVING INSTRUMENT FOR MARINE MICROBIAL ECOLOGY

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Autonomous systems that make molecular measurements remain out of reach of the majority of aquatic researchers due to their inherent operational complexity, high production/running costs, limited analytical capability and proprietary nature. Therefore the likelihood that in their current stage of development they will be able to be broadly adopted by most researchers. However, marine microbial ecologists urgently need autonomous platforms that are capable of taking ambient water samples and storing them for subsequent molecular biological analysis. To address this, we are developing a small autonomous in situ sampling and archival device. The Sample Filtration and Archiving (SaFA) system can be deployed in the aquatic environment where it will automatically collect and filter between 20-30 user defined time-stamped water samples of between 200-500 ml. Preservation of the genetic material in the filtered material is performed by the addition of a stabilization buffer, making it available for downstream interrogation in

the laboratory using a range of molecular biological techniques upon retrieval. The instrument will enable autonomous sample collection and archiving at high temporal resolution, for subsequent laboratory analysis. Currently we have shown that we can stabilize samples for up to 3 days without appreciable degradation of the DNA. The system will enable increased in situ sampling regimes without the need for personnel to be deployed in the field, particularly during dangerous or inconvenient sampling periods. To enable broad integration into the aquatic sciences the instrument will be open source, all information relating to the construction and operation of the instrument will be download-able. We are also focusing on using commercially available parts. The aim of making the instrument freely available is that the system will evolve as users provide feedback to other users with application specific information.

Abstract Category

17 Marine Microbiology

PS.17.032 NUTRIENTS AND OVERFISHING IMPACT BACTERIAL AND SYMBIODINIUM COMMUNITIES ON REEF CORALS

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Global climate change, overfishing, and eutrophication contribute to coral reef decline and increased macroalgal abundance in tropical seas. Few studies address how these stressors affect coral-associated microbial communities (e.g., Bacteria, or the algal endosymbiont genus *Symbiodinium*), despite the influence of these taxa on coral health. To explore the mechanisms that contribute to coral-depauperate reefs, we quantified changes in coral-associated bacterial and *Symbiodinium* communities following top-down (herbivorous fish exclusion) and bottom-up (inorganic nutrient addition) treatments in a multi-year field experiment. We monitored benthic cover, coral and algal growth rates, and fish diversity/abundance, as well as Bacteria and *Symbiodinium* communities on 3 coral species every 1-2 months. Bacterial diversity and abundance were assessed with T-RFLPs of 16S rDNA and flow cytometry. *Symbiodinium* clade diversity and relative abundance were determined using RT-PCR. Results suggest nutrient availability and herbivore pressure affect bacterial communities over time. Bacterial abundance increased following treatment initiation except in control (uncaged, ambient nutrients) colonies. Bacterial diversity increased in uncaged treatments compared to corals in treatments where herbivores were excluded. Numerous scars from parrotfish bites were observed on uncaged corals. Predation may therefore be a mechanism that introduces novel Bacteria and/or spreads diseases among corals. The diversity of *Symbiodinium* clades present and their relative abundances also changed over time, likely in response to ambient temperature shifts and/or plot treatments. Since coral disease epizootics are common and since algae, nutrients, and elevated temperature are hypothesized to be mediators of these diseases, this multi-scale experiment provides important insights into the stability of reef coral-microbe symbioses and thus, the trajectories of coral reefs.

Abstract Category
17 Marine Microbiology

PS.17.033 MICROBIAL ASSOCIATIONS ACROSS THE NORTH PACIFIC GYRE AND AMAZON RIVER PLUME

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While marine microbes are recognized as important drivers of ecosystem processes, key elements of their basic ecology are still not understood. We examined distributions of bacterial communities across broad stretches of the Pacific Ocean Central Gyre as well as the Atlantic Ocean near the Amazon River Plume, and studied concurrence among bacterial taxa and environmental parameters. Samples were collected on four cruises over two years. Measurements included physical oceanographic parameters, concentrations of dissolved nutrients, metals and pigments, counts of bacteria, viruses and other microorganisms, and microbial process rate measurements. We determined bacterial community structure with a community fingerprinting technique (Automated Intergenic Spacer Analysis) and clone libraries, and analyzed the results with the goal of understanding the distributions of planktonic bacteria over large spatial scales, and the causes thereof. We used association networks to visualize positive and negative co-occurrence patterns among taxa and environmental measurements. Prominent within these networks were near-species level taxa within the SAR11, Roseobacter, Actinobacter and *Synechococcus* clades as well as several strains of *Prochlorococcus*. We used Canonical Correspondence Analysis to identify environmental factors, including physical conditions and dissolved phosphorus in the Pacific, and light intensity and low density river-water in the Atlantic, that had particularly strong effects on some taxa. Investigating microbial associations is allowing us to identify new patterns that will help us to explain spatial variation in bacterial community structure.

Abstract Category
17 Marine Microbiology

PS.17.034 PROTEOMIC PROFILES OF *AUREOCOCCUS ANOPHAGEFFERENS* CCMP 1784 DURING NITROGEN REPLETE, NITROGEN LIMITED, AND UREA-SUPPLEMENTED GROWTH

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Aureococcus anophagefferens is a eukaryotic picoplankton that causes harmful algal blooms along the northeastern coast of the United States. While induction of algal blooms typically correlates with the input of dissolved inorganic nitrogen, *A. anophagefferens* blooms during periods of low inorganic nitrogen, likely due to its unique ability to utilize organic nitrogen. In order to elucidate the physiological processes that instigate *A. anophagefferens* algal blooms, proteomes were profiled from nitrogen replete, nitrogen limited, and organic nitrogen cultures using mass spectrometry-based shotgun proteomics. *A. anophagefferens* CCMP 1784 was grown in brown-tide medium with nitrate, urea, or no nitrogen source. Cell pellets were lysed, denatured, and reduced prior to trypsin digestion. Peptides were measured using 24-hour two-dimensional liquid chromatography tandem mass spectrometry runs (2D-LC-MS/MS) and identified from a protein database by SEQUEST software. Protein expression was quantified using label-free data, analyzed using the Poisson regression model. A total of 2,621 non-redundant proteins were confidently identified across all 2D-LC-MS/MS runs. Of these identifications, 1,177 were common to all growth conditions, while 128 were unique to nitrogen limitation, 290 to nitrogen replete, and 310 to urea growth. Differential expression across growth conditions was determined for 391 proteins. While a urea transporter was identified, it was not deemed differentially expressed. KOG category analysis revealed similar expression patterns among growth conditions with exceptions including cytoskeleton proteins, which increased in expression under nitrogen limitation. These proteome profiles will next be compared to those measured directly from environmental samples collected during a brown tide bloom to provide information regarding the nitrogen status of the brown tide as well as other physiological comparisons with cultured isolates.

Abstract Category

17 Marine Microbiology

PS.17.035 NUTRIENT LIMITED BACTERIA DO NOT RESPOND TO CASCADING EFFECTS ELICITED BY A GELATINOUS TOP-PREDATOR IN A BALTIC SEA FOOD WEB

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Increasing biomasses of jellyfish in marine waters presumably have major implications for the structure and function of local planktonic food webs; however, current data are scarce as most studies have focused on immediate effects on zooplankton and fish larvae. We examined the cascading impact of the invasive ctenophore *Mnemiopsis leidyi* on plankton in the brackish Baltic Sea, with special emphasis on bacterioplankton. We compared short-term effects of *M. leidyi* adults and larvae, respectively, with controls without added *M. leidyi* in 200 L mesocosms during 7 days in August 2009 (4 replicates of each). Strong grazing by *M. leidyi* significantly reduced the mesozooplankton biomass

(mainly cladocerans, *Bosmina* sp.), but no differences in chlorophyll *a* (chl *a*) were observed between treatments and controls. While chl *a* generally increased towards the end of the experiment, bacterial abundance and production (H^3 -thymidine incorporation) decreased with no or minor differences between treatments and controls. Similarly, only minor differences in bacterial ectoenzymatic activities (glucosidase and proteinase) and community structure, as analyzed by 16S rRNA gene amplification and denaturing gradient gel electrophoresis, were observed between treatments and controls. Undetectable levels of phosphate and a gradual accumulation of dissolved organic carbon over the course of the experiment suggested that bacterial substrate utilization was limited by phosphorus availability. The experiment demonstrates that under phosphate limited conditions in the Baltic Sea, short-term grazing by *M. leidy* affects the higher levels in the food web, while the trophic cascade does not affect bacterioplankton growth or composition.

Abstract Category
17 Marine Microbiology

PS.17.036 PYROTAG SEQUENCING OF THREE MICROBIAL DOMAINS IN A SUBTROPICAL SHALLOW ESTUARY NEAR SAPELO ISLAND, GEORGIA, USA

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As part of MIRADA-LTER, an NSF-funded pyrotag sequencing project inventorying microbial diversity of the three domains of life at 13 of the aquatic Long Term Ecological Research (LTER) sites, over 300,000 sequences were generated from four locations in an estuary on the Georgia coast. Samples were collected across a tidal cycle in a freshwater site (Altamaha River), coastal-influenced site (Sapelo Sound), marsh-influenced site (Doboy Sound), and a human-impacted site (Tolomato Development) in replicate. In the Bacteria, the V6 region of the SSU rDNA marker was sequenced, generating over 128,182 reads at an average of 8,011 reads per sample. The V6 region was also sequenced in the Archaea, with fewer total (57,902) and average (3618) reads generated, while the V9 region was sequenced in the Eukarya with 116,411 total reads and an average of 7275 reads per sample. Community composition in each of the domains was analyzed along with 22 ancillary environmental parameters to determine which factors are most related to community structure, and also which taxonomic groups drive the signature community of each environment sampled. We made the following observations: (1) Community composition; Sapelo Sound and Doboy Sound were highly similar, the Altamaha River site was the most divergent, and the Tolomato Development site was intermediate (2) Diversity; Tolomato Development had significantly lower bacterial diversity than all of the other sites with 2-3 fold higher numbers of bacterial cells (3) Environmental parameters; Statistical analyses reveal that the combined parameters of salinity, NO₂ /NO₃ concentration, and bacterial cell number are highly correlated (93%) with differences in community composition. This initial characterization of a *Spartina* marsh-dominated estuarine microbial community lays the groundwork to

explore the previously undiscovered diversity of Bacteria, Archaea, and particularly microbial Eukarya.

Abstract Category
17 Marine Microbiology

PS.17.037 SIMILARITIES BETWEEN THE SUBSURFACE OF TIDAL FLAT SEDIMENTS AND THE MARINE DEEP BIOSPHERE

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Subsurface sediments of tidal flats and the marine deep biosphere share several features. Although on different scales, geochemical profiles and microbial successions follow the same trends. Microbial activities are governed by the availability of electron acceptors and the quality of electron donors. A comparison of geochemical settings and microbiological features indicated high similarities of the upper 5 meters of North Sea tidal-flat sediments and the upper hundred meters of deep-sea sediments. Similar microbial community compositions were found at deep and shallow sites and the microbial abundance was elevated at sulfate-methane transition zones.

Furthermore, tidal flats offer the possibility to study the vertical zonation of microbial communities that thrive under different energetical constraints in close proximity. The activity of sulfate reducers and methanogens varies with enhanced turn over rates at tidal flat margins. The highest activities are found close to the low water line and gradually decrease across the margins within the first tens of meters. This is due to the high availability of fresh organic matter and electron acceptors in margin sediments induced by high sedimentation rates and/or pore water flow. In contrast, the central parts of tidal flat are microbially less active. Here, microbial and biogeochemical profiles are vertically stretched and form a transition between the shallow subsurface and the marine deep biosphere. This transitional state was suggested after the analysis of a 20 meter-long core from the same tidal-flat area.

Our findings lead to the conclusion that we are presently unable to draw a cutting line between the shallow and the deep subsurface. Rather, it appears to exist only one "subsurface biosphere" with gradual differences. Therefore, tidal flats serve as an excellent model to test novel techniques and perform microbiological experiments before applying them to much deeper and older samples.

Abstract Category
17 Marine Microbiology

PS.17.038 DEVELOPMENT OF ENVIRONMENTAL MULTI-OMICS FOR PLANKTONIC MICROBIAL COMMUNITIES

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Environmental metabolomics is a promising field that to date has seen limited application to the microbial world. One difficulty has been the inadequacy of methods suitable for microbes, particularly in planktonic systems. We are developing an integrated multi-omics approach for such communities by incorporating community-level metabolome data with data from other omics levels to better understand microbial responses to environmental and experimental changes. Using water from highly eutrophic Tokyo Bay, several series of 2-10 liter microcosms with different starting conditions have been used to develop the protocols required for such an approach. These methods were then applied to a proof-of-concept study exploring the suitability of natural planktonic communities for simultaneous bioremediation and biomass production. Here we report the development of our methods for metabolite extraction from planktonic cells and the surrounding liquid, and the results of combined metabolomics (^1H nuclear magnetic resonance of extracted metabolites), genomics (denaturing gradient gel electrophoresis and sequencing of 16S and 18S rRNA loci), and traditional microbiological approaches (cell counts, fluorescent staining, microscopic observation and culture isolation) for these experiments. We also discuss the suitability and potential advantages of using natural plankton assemblages from eutrophic water for biomass production. We conclude that multi-omics data for microbial communities can be readily extracted from microcosm and natural systems. This offers to expand applied and ecological research of microbial interactions and dynamics.

Abstract Category

17 Marine Microbiology

PS.17.039 METAGENOMIC INVESTIGATION OF EXTRACELLULAR AND MYTOMYCIN-C INDUCED SSDNA AND RNA-CONTAINING VIRUSES FROM DEEP-SEA HYDROTHERMAL VENTS

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Prior investigations of viruses inhabiting deep-sea hydrothermal vent environments indicate that they are abundant and dynamic components of vent microbial communities. Furthermore, hydrothermal vents support substantial populations of temperate viruses and a high incidence of lysogenic hosts. However, to date, all studies of viruses inhabiting hydrothermal vents have focused on those with dsDNA genomes. Here we discuss the purification, fractionation and library construction strategies for ssDNA and RNA viruses present at deep-sea hydrothermal vents in preparation for 454 pyrosequencing. Extracellular viruses and potential lysogens were collected from deep-sea hydrothermal vents located along the East Pacific Rise and Guaymas Basin in the Gulf of California using an in-situ large volume water sampling device. Temperate viruses were induced from lysogenic hosts by incubation with Mitomycin-C. Following rigorous purification of virus particles, total viral nucleic acids were isolated and fractionated on a hydroxyapatite chromatography column using increasing concentrations of phosphate buffer to elute

specific nucleic acid types. Single-stranded DNA and RNA genomes were converted to dsDNA using sequential *E. coli* DNA polymerase I treatment and RT-PCR respectively. A modified linker amplified shotgun library construction approach was used to generate quantities of dsDNA sufficient for 454 pyrosequencing. One extracellular and one induced ssDNA and RNA viral metagenome from each sampling site (n=8) were sequenced at the Broad Institute as a part of the Marine Phage Sequencing Project sponsored by the Gordon and Betty Moore Foundation. Comparison of viral metagenomic data across nucleic acid types and virus "lifestyles" will facilitate a more complete analysis of the total gene complement and diversity of vent viral communities.

Abstract Category
17 Marine Microbiology

PS.17.040 DIFFERENTIAL TRANSFER OF MARINE PLANKTONIC BACTERIA TO THE ATMOSPHERE

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In Surface Ocean, breaking waves ejects aerosols containing marine bacteria, which is the most important source of bacteria to the atmosphere. However, little is known about the ability of different bacteria to adhere to aerosols. Bacteria in the atmosphere are expected to have a strong impact on earth climate, since they contribute to cloud formation and albedo. Thus, detailed knowledge about the diversity of atmospheric bacteria, and how their features affect atmosphere chemistry, is desirable. We carried out three experiments in the Northeast Atlantic where seawater was enclosed in a tank where a water jet stream generated aerosols. This allowed characterization of the bacterial community composition in aerosols compared to seawater using 16S rDNA clone libraries. Substantial differences were observed between seawater and aerosol samples both at the level of major groups and species level. Alphaproteobacteria largely dominated the seawater bacterial community (54%) but only accounted for 9% of bacteria in aerosols. Notably, members of the ubiquitous marine SAR11 clade were the most abundant group in seawater (28%), but none were found in aerosols. In contrast, Cyanobacteria were highly enriched in aerosols accounting for 31% of the OTUs, compared to 14% in seawater. Similarly, Gammaproteobacteria and Firmicutes were enriched in the aerosols. The most abundant species in aerosol were *Pseudomonas* sp. (57 sequences) that also had one sequence represented in the seawater samples. Moreover, sequences belonging to genera harboring known pathogens, e.g. *Treponema*, *Vibrio*, and *Bacillus*, were only detected in aerosols. Overall, there was only a 5.4% overlap of sequences detected in both seawater and aerosols. This suggests key differences in the transfer efficiency of different microorganisms from seawater to the atmosphere. Understanding the role of bacteria in the atmosphere is an important challenge for the future that demands further research.

Abstract Category
17 Marine Microbiology

PS.17.041 MICROBIAL INDICATORS OF HOST SPECIES, HABITAT, AND DISEASE IN THE RELIC CORAL GENUS *MUSSISMILIA*

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We have recently carried out a study of microbial community composition in corals of the genus *Mussismilia* from Abrolhos Bank, Brazil. These corals are threatened by a disease with symptoms similar to the coral White Plague (WP). We have used 454 pyrosequencing of bacterial 16S rRNA gene V6 hypervariable regions to examine the hypothesis that coral microbiome structure is influenced by coral species, reef site, and disease/health status. 170,802 V6 tags were generated from bacteria associated with 14 coral individuals and 2 water samples. 7929 coral- and 1314 water-associated bacterial sequence types were observed based on 97% sequence identity. Chao1 estimates of microbial diversity ranged from 526 to 4925 for each coral individual. Surprisingly, Unifrac and Bray-Curtis analysis did not reveal trends in microbial community structure correlated to coral genotype or reef site. However, WP afflicted corals and water samples harbored microbial communities that were distinct from healthy corals. Analysis of the distribution of microbial OTUs revealed 15 specifically associated with *Mussismilia braziliensis* from both reefs (n=6) that were notably absent from *M. braziliensis* with White Plague. Among these a novel OTU (Phylum Chlamydiae) was abundant among all *M. braziliensis*. All 14 *Mussismilia* corals shared a common set of 3 OTUs (a *Bacteroidetes*, *Proteobacteria* and an *alpha-Proteobacteria*). In addition, OTUs closely related to the agent of WP disease (*Thalassomonas* spp.) were found in both diseased and healthy corals, suggesting that this pathogen may be opportunistic. Notably, sulfate reducing bacteria comprised ~25% of sequences associated with diseased coral. To investigate the activities of microbes associated with healthy and diseased corals we have developed a novel metatranscriptomic approach which has provided the first glimpse into the functional role of microbes in healthy and diseased corals.

Abstract Category

17 Marine Microbiology

PS.17.042 DYNAMICS OF AEROBIC ANOXYGENIC PHOTOTROPHIC COMMUNITIES IN THE WESTERN MEDITERRANEAN

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Aerobic anoxygenic phototrophs (AAPs) are a ubiquitous part of the marine microbial communities. We studied their abundance and dynamics in the Blanes Bay Microbial Observatory (Western Mediterranean). There, AAPs accounted for 3-8% of the total prokaryotes. Based on diel changes of bacteriochlorophyll a the AAP community grew at rates 1.1 to 1.5 day⁻¹, which is faster than the rates reported for heterotrophic bacteria in such environments. We further studied this phenomenon by top-down manipulation experiments and compared the growth rates of AAP to total heterotrophic bacteria.

During the experiments AAPs displayed growth rates of 1.6-2.0 day⁻¹ whereas the average growth rate for total heterotrophic bacteria was approximately 1.1-1.6 day⁻¹. The reduction of grazing pressure in the experimental treatments led to a significant increase of the percentage of AAP bacteria compared to the control. This indicates that grazing is the major source of the AAP mortality and that they are under stronger grazer pressure than other bacteria.

Abstract Category
17 Marine Microbiology

**PS.17.043 MICROBIAL COMMUNITY STRUCTURE OF MARINE
HYDROTHERMAL VENT DEPOSITS SHAPED BY HOST-ROCK
COMPOSITION.**

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Diverse microbial communities are known to thrive on the outside of high-temperature vent deposits formed in deep-sea hydrothermal environments. In this study, vent deposits were collected from basalt-hosted (mafic) vent fields (Lucky Strike - 37° 17' N, 32° 16.3' W and TAG - 26° 08' N, 44° 49.6' W) and a peridotite-hosted (ultramafic) vent field (Rainbow - 36° 13' N, 33° 54.1' W) along the Mid-Atlantic Ridge. Differences in host rock underlying the vent fields result in contrasting fluid chemistries, most notably in hydrogen concentrations. We hypothesized that due to elevated hydrogen concentrations generated in ultramafic systems, we would observe differences in overall community composition and/or structure between the deposits from the different vent fields. Several culture-independent techniques, including multiplexed barcoded pyrosequencing of the 16S rRNA gene, functional gene surveys, and clone libraries were used to examine the microbial community structure of numerous deposits from each field. Using taxon- and phylogenetic-based analyses of pyrosequencing amplicons, we show statistically significant differences in the microbial community structure between basalt-hosted and peridotite-hosted vent fields. Differences in community structure can be attributed to community membership rather than organism abundances and is exemplified by the notable absence of methanogens at the basalt-hosted vent fields. Additionally, within the vent-ubiquitous archaeal and bacterial lineages such as the *Desulfurococcaceae* and *Epsilonproteobacteria*, each field supported distinct populations. Geologic processes, such as differences in host-rock, help to shape the functional diversity of microbial communities in hydrothermal vent deposits along the Mid-Atlantic Ridge. This study also provides evidence that the phylogenetic diversity of each vent field is distinct.

Abstract Category
17 Marine Microbiology

PS.17.044 SPATIAL AND TEMPORAL VARIABILITY OF BACTERIOPLANKTON COMMUNITIES ACROSS A RIVER TO OCEAN GRADIENT ASSESSED WITH TAG PYROSEQUENCING

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Bacterioplankton communities vary across a river to ocean gradient with estuaries as an intermediate environment where freshwater and marine bacteria mix. Spatial and temporal variability, as well as taxonomic composition, were assessed for bacterioplankton communities of the Columbia River, estuary, and adjacent coastal ocean using the high throughput method of tag pyrosequencing. A total of 331 water samples, collected from the river, estuary, and coastal ocean in 2007 and 2008, were sequenced in a single pyrosequencing run using a unique barcoded primer for each sample. A total of 344,500 PCR-amplified bacterial 16S rRNA gene sequences were obtained. An initial analysis of a subset of samples (n=255) found bacterioplankton communities separated across both salinity and depth gradients into six distinct groups: Columbia River, estuary, Columbia River plume, coastal surface/chlorophyll maximum, coastal bottom (depth<150 m), and coastal deep (depth>300 m). The great spatial variation of the dataset, however, overwhelmed any larger seasonal trends as temporal variation was only observed within similar environment types including the estuary, plume, coastal surface/chlorophyll maximum, and coastal bottom groups. Overall taxonomic composition consisted of typical marine bacteria phyla including *Bacterioidetes*, and *Alpha* and *Gamma Proteobacteria*, with influence from *Actinobacteria* and *Beta Proteobacteria* in lower salinity samples. Understanding how and why bacterioplankton communities change across a river to ocean gradient allows for better prediction of how these communities, and ecosystems as a whole, might be shaped by future environmental change.

Abstract Category

17 Marine Microbiology

PS.17.045 PHYLOGEOGRAPHY AND SECONDARY METABOLITE PRODUCTION IN THE MARINE ACTINOMYCETE SALINISPORA PACIFICA

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The marine actinomycete genus *Salinispora* is composed of three closely related species. Two are formally described (*S. arenicola* and *S. tropica*) [1], while '*S. pacifica*' has been proposed as the third [2]. '*S. pacifica*' is the most diverse of the species and has been cultured from 7 global locations providing a chance to address the role of geographic isolation in species diversification. Neither 16S rRNA nor internally transcribed spacer sequences revealed geographically distinct populations of '*S. pacifica*', suggesting high rates of connectivity between populations. Nevertheless, sub-species level correlations were made between phylotype and secondary metabolite production. In one case, a

biosynthetic pathway appears to have been inherited from a common ancestor of '*S. pacifica*' and *S. tropica*; with divergence between these two sister taxa leading to the production of structurally related but distinct compounds. In the second case, a clade within '*S. pacifica*' shares a biosynthetic pathway with *S. arenicola* suggesting the pathway was acquired through horizontal gene transfer (HGT). Phylogenetic analyses of the relevant biosynthetic genes were used to test hypotheses about the evolutionary history of secondary metabolite biosynthetic pathways. The results indicate that both HGT and vertical inheritance have contributed to the current patterns of secondary metabolite distribution within the '*S. pacifica*' clade.

[1] Maldonado, LA, et al. 2005. *S. arenicola* gen. nov., sp. nov. and *S. tropica* sp. nov., obligate marine actinomycetes belonging to the family Micromonosporaceae. *Int J Syst Evol Microbiol.* 55:1759-1766

[2] Jensen, PR & Mafnas, C. 2006. Biogeography of the marine actinomycete *Salinispora*. *Environ Microbiol.* 11:1881-8

[3] Cho, JC & Tiedje, JM. 2000. Biogeography and Degree of Endemicity of Fluorescent *Pseudomonas* Strains in Soil. *Appl. Environ. Microbiol.* 66: 5448-5456

Abstract Category

17 Marine Microbiology

PS.17.046 EFFECTS OF *MARINOBACTER ADHAERENS* ON POLYMER EXUDATION BY *THALASSIOSIRA WEISSFLOGII* GROWN AT DIFFERENT N:P RATIOS

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To better understand exopolymer accumulation, phytoplankton aggregation, and sinking fluxes of organic matter in the ocean, an in-depth investigation of the contribution of algae-interacting bacterial species and their effects in respect to algal exopolymer production under differing environmental conditions is required. To evaluate the physiological response of the marine diatom *Thalassiosira weissflogii* to different nutrient conditions in vitro and the impact of associated bacteria, such as *Marinobacter adhaerens*, on algal exudation, the chemical composition and amount of released algal exudates were analyzed. Batch cultures of *T. weissflogii* were grown at nutrient balanced (N:P=16) as well as nitrogen- (N:P=1) or phosphorus- (N:P=95) deficient conditions. To test whether *M. adhaerens* affects algal growth and exudation in dependence of N:P ratios, results of axenic cultures were compared with those inoculated with *M. adhaerens*. Effects of the bacterium on dynamics of dissolved organic carbon, amino acid composition, and transparent exopolymer particle (TEP) formation greatly depended on N:P ratio. At balanced N:P ratio, presence of *M. adhaerens* had positive effects on *T. weissflogii* growth and stimulated TEP production. Under nutrient limited conditions, however, polysaccharide secretion by *T. weissflogii* was enhanced regardless whether *M.*

adhaerens was present or not. Furthermore, bacterial effects on remineralization of organic matter, TEP formation, and conversion of dissolved to particulate organic matter were complex and variable processes. In conclusion, mechanistic understanding of bacteria-algae interactions and their consequences on oceanic organic matter and nutrient cycling requires careful interpretation in an environmental context.

Abstract Category
17 Marine Microbiology

PS.17.047 PROTEORHODOPSIN CONFERS LIGHT-ENHANCED SURVIVAL DURING STARVATION IN MARINE BACTERIA

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Proteorhodopsins are globally abundant bacterial photoproteins found in the photic zone of the ocean. Although their function as proton pumps with energy-yielding potential has been established, the ecological role of proteorhodopsins remains largely unexplored. We report the presence and function of proteorhodopsin in a member of the genus *Vibrio*, discovered through whole genome analysis. Phylogenetic analysis suggests the *Vibrio* sp. strain AND4 obtained proteorhodopsin through lateral gene transfer, which could potentially have modified the ecology of this marine bacterium. Here we demonstrate an improved long-term survival of AND4 when starved in seawater exposed to light rather than held in darkness, and mutational analysis is used to directly link proteorhodopsin to this enhanced survival. Thus, proteorhodopsin phototrophy confers a fitness advantage to its native host, representing a novel mechanism for bacterioplankton to endure frequent periods of resource scarcity in the ocean's surface.

Abstract Category
17 Marine Microbiology

PS.17.048 COMPARISONS OF THE PLANKTONIC FLAVOBACTERIA COMMUNITY FROM TWO COASTAL SYSTEMS: RIO DE LA PLATA ESTUARY AND LAGOON OF VENICE

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Coastal lagoons and estuaries harbour a large microbial diversity that is influenced by complex hydrological regimes. Members of the class *Flavobacteria* are one of the most

abundant picoplankton in coastal systems. They are also phylogenetically diverse; and therefore it is relevant to understand their population dynamics along those highly variable coastal gradients. In the present study we analyzed the *Flavobacteria* community, by 16S rRNA gene clone libraries and by fluorescence *in situ* hybridization (FISH), in two coastal sites: the Río de la Plata estuary (Uruguay) and the southern basin of the Lagoon of Venice. The abundance of total *Flavobacteria* was significantly high in the Lagoon of Venice (up to 60% of total picoplankton) and in the river-ocean mixing zone of the Río de la Plata (25%) where chlorophyll and organic matter was the highest. Flavobacterial clade specific FISH probes gave further evidence for this distribution pattern: the abundance of all the evaluated clades was also higher in the mixing zone. About half of the flavobacterial clades, at a 95% 16S rRNA gene sequence identity, were present in both distinct coastal sites. However, at a higher threshold exclusive phylotypes in each system were distinguished. We also identified the presence of clades that have been defined from open ocean sites indicating that they are ubiquitous phylotypes. Our analysis revealed that members of the genus *Polaribacter* accounted for a significant fraction of the flavobacterial community in the Lagoon of Venice and in the Río de la Plata estuary. A detailed phylogenetic analysis indicated that there are distinct closely related temperate and polar *Polaribacter* clades. The *Polaribacter* sequences retrieved from temperate coastal sites comprised a clearly separated clade within the genus. Our results highlight the relevance of *Flavobacteria* in coastal systems and provide evidences about the existence of widespread flavobacteial clades in the marine realm.

Abstract Category

17 Marine Microbiology

PS.17.049 IDENTIFICATION AND CHARACTERIZATION OF CULTURED FUNGI ASSOCIATED WITH HEALTHY COLONIES OF THE COLD-WATER CORAL *LOPHELIA PERTUSA*

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The coral holobiont consists of the cumulative genomes of the coral host and all associated microbiota, including bacteria, archaea, and microeukaryotes. Determining the eukaryotic members of the microbial community using culture-independent techniques has been difficult because 18S rDNA primers are often overwhelmed by the abundance of coral genetic material in DNA extractions. Fungi are recognized members of the coral holobiont, found associated with tissue and skeleton in healthy and diseased shallow-water corals. Culture-based methods were employed in this study to isolate fungi, allowing further investigation and characterization of these under-sampled holobiont members.

Branches of the cold-water coral *Lophelia pertusa* were collected by submersible from two sites in the western Atlantic at depths greater than 400 m. Individual coral samples were kept isolated and insulated during ascent. Immediately after collection, a homogenate of the coral tissue, skeleton, and mucus was plated onto Orange Serum Agar, a low-pH media. Plates were maintained at 4°C to mimic *in situ* temperatures and

allowed to grow for several months. Visible colonies were picked and streaked to isolation on glycerol artificial seawater agar (GASWA). Light microscopy of the cultures showed that they were non-filamentous, budding cells, although the ploidy of the cells or stage in sexual reproduction is unknown. Isolates were further characterized by 18S rDNA amplification and sequencing. The sequences revealed a closely related cluster of basidiomycete fungi obtained from different coral colonies at both sites. These are the first fungi to be cultured and characterized from an apparently healthy deep-sea coral. To date, the only reported fungi in deep-sea corals are endolithic fungi associated with dead coral skeleton. It remains to be determined what symbiotic role these fungi play in the coral holobiont.

Abstract Category
17 Marine Microbiology

**PS.17.050 ARE POLY-PHOSPHATE ACCUMULATING BACTERIA
PREDOMINANT IN SEDIMENTS SUBJECTED TO COMMUTATIVELY
CHANGING REDOX CONDITIONS?**

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Phosphorus in the form of poly-phosphate (Poly-P) can be accumulated by a wide range of microorganisms. The ability of some bacterial taxa to accumulate this poly-P is well known in the treatment of highly phosphate-polluted waters. The conditions applied to treat these waters allow to avoiding further eutrophication of the ecosystems caused by excessive release of phosphorus to the environment. At present there is a major group of bacteria known for their ability to accumulate high weight polymers of poly-P in these waste-water treatment facilities, namely *Candidatus Accumulibacter* and related. However, little is known about these microorganisms or others capable of the same trade being present in other habitats. The fact that *Candidatus Accumulibacter* has only been found in waste-water treatment plants (and their surroundings) raises the questions on why these bacteria excel over other poly-phosphate producing microorganisms and what other bacteria may reproduce this success in other habitats given the appropriate conditions. The idea that all species are present everywhere but only those microorganisms that are better adapted to the ecosystem are able to develop in higher numbers is here applied to the study of poly-P accumulating bacteria. To this purpose, we reproduced the same changing redox conditions used in waste-water treatment plants specialized in phosphorus removal to bacterial communities extracted from marine sediment. The extracted communities were cultivated in bioreactors specially designed for this kind of studies. The communities and their development as well as key genes for the production of polyphosphates were studied at the ecosystem, community, and single cell level using state-of-the-art techniques.

Abstract Category
17 Marine Microbiology

PS.17.051 A NEW DEBROMINATING BACTERIUM FROM MARINE SEDIMENT

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Microorganisms play an important transforming role in the natural halogen cycle. The marine environment, including sponges, is a rich source of diverse organohalides. To better understand the microbial role and the fate of organohalogens in the environment we are characterizing the microbes associated with sponges and their vicinity. To date, only a handful of debrominating microbes that have been isolated. *Desulfoluna spongiiphila* is an anaerobic debrominating microorganism that appears to have a widespread distribution within diverse marine sponges and adjacent sediments according to microbial community analyses performed previously. In this study we sought to identify and characterize novel debrominating microbes associated with sponges in addition to microorganisms in the sponge vicinity. To accomplish this we established enrichment cultures to isolate anaerobic bacteria from geographically disparate marine sponges and their surrounding sediments. Microcosms were amended with 2,6-dibromophenol as a model compound to test for reductive debromination. Dehalogenating activity was assayed with 2,6-dibromophenol as an electron acceptor and a short chain fatty acids mixture as electron donors and analyzed. Cultures that continued to display debromination activity at the highest dilution were analyzed by 16S rRNA gene terminal restriction fragment polymorphism to determine the complexity of the enrichment and when pure isolated were obtained. From a sediment sample adjacent to a sponge we enriched a debrominating culture from which we isolated a *Chlorobium* sp. The strain, designated TS7, is a green colored rod-shaped bacterium approximately 2 µm in length that debrominates 2,6-dibromophenol to phenol under anaerobic conditions. From a study of this new microorganism we aim to further elucidate what role it may play in fate of natural and anthropogenic organohalogens in the

Abstract Category

17 Marine Microbiology

PS.17.052 INSIGHTS INTO DIVERSITY, FUNCTIONING, AND TEMPORAL DYNAMICS OF A MICROBIAL COMMUNITY IN THE NORTH SEA

Hammer, T*¹; Teeling, H¹; Huang, S¹; Weber, M¹; Waldmann, J¹; Klindworth, A¹; Klockow, C¹; Kassabgy, M¹; Fuchs, B¹; Wichels, A²; Gerdtts, G²; Amann, R¹; Gloeckner, F¹

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In the last two decades, sequencing and imaging technologies have greatly advanced our understanding of the diversity of microbes in the marine ecosystem, but linking community structure with biogeochemically relevant processes and the physicochemical environment remains a challenge. For example, it is unknown how functions such as polymer degradation, light utilization, or carbon fixation are distributed among the

various groups comprising the heterotrophic bacteria, and how responsive the expression of these functions is to environmental variability.

We have taken an integrative approach to characterizing the bacterioplankton fraction (0.2 μm -3 μm) of a coastal ecosystem in the North Sea. From January to November 2009, surface water was sampled biweekly and subjected to nutrient and chemical analysis, cell quantification, and fluorescence *in situ* hybridization. Samples representing summer, winter, and phases of a spring algal bloom were chosen for metagenomic, metatranscriptomic, and 16S ribosomal tag pyrosequencing.

Sequence-based methods and FISH indicated a bacterial community dominated by *Alphaproteobacteria*, *Gammaproteobacteria*, and *Bacteroidetes*; however, both total cell numbers and the relative abundances of individual taxa varied widely in concert with environmental fluctuations over time. In spring, changes in nutrient levels and a concomitant bloom of *Rhizosolenia* algae were followed by a striking increase in *Flavobacteria*. Preliminary analyses of the post-bloom sample revealed functions shared by all major members of the community, such as TonB-dependent transporters and gliding motility, and others that are taxon-specific, such as sulfur oxidation, photosynthesis, and toxin production.

Our results demonstrate that an integrative approach enables the tracking of shifts in microbial community composition over variable temporal scales, while relating these changes to physicochemical conditions and the presence and expression of ecological functions.

Abstract Category
17 Marine Microbiology

PS.17.053 SYNTHETIC MICROPLASTICS FUNCTION AS HOTSPOTS FOR THE FORMATION OF MICROBIAL BIOFILMS IN COASTAL SEDIMENTS

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Synthetic thermoplastics constitute the most abundant anthropogenic pollutants in the Earth's oceans. Recently microplastics (fragments of ≤ 5 mm) have been highlighted as emerging pollutants in coastal sediments. Although microplastics are known to absorb organic contaminants and to become ingested by animals, their interactions with, and impacts upon microorganisms are unknown.

This study characterized the ecological interactions between microorganisms and microplastics in coastal sediments. The research aimed to identify the dominant microbial

taxa attaching to plastics, and to determine how the structure of plastic-associated biofilms varies over time and across different types of sediment.

Fourteen-day laboratory microcosms were established using sediments from the Humber Estuary (United Kingdom). The microcosms were spiked with polyethylene as a model polymer and used to characterize biofilms at the plastic-sediment interphase. The coverage and composition of the biofilms were analyzed by DAPI staining and fluorescence *in situ* hybridization, and via terminal restriction fragment length polymorphism analysis and sequencing of ribosomal RNA genes.

Biofilms formed at the plastic-sediment interphase within one day of exposure to sediment. Moreover, their coverage and complexity increased over time. Bacterial colonization of the plastic was followed by archaeal and picoeukaryal colonization within seven days. Microbial consortia associated with plastics constituted subsets of the taxonomic pools within the seabed. The prominence of gamma-proteobacteria within these consortia was confirmed by gene sequencing.

This study provides the first evidence for the potential of microplastics to facilitate the rapid formation of biofilms whose composition and activities may differ from those in bulk sediments. This evidence has opened an avenue for microbial research into the ecological impacts of microplastics, and into the biodegradation of plastic-associated contaminants.

Abstract Category
17 Marine Microbiology

PS.17.054 ANAMMOX AND DENITRIFICATION IN THE CAPE FEAR RIVER: COMPARATIVE ANALYSIS OF STABLE ISOTOPE RATE MEASUREMENTS AND MOLECULAR QUANTIFICATION

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Anaerobic ammonium oxidation (anammox) and denitrification are two main microbial processes capable of removing fixed nitrogen by conversion into a gaseous species. Both microbial processes are known to occur in anoxic sediments of estuaries, yet little is known about the spatial and temporal variations of their rates along the estuarine gradients. In order to gain a better understanding of anammox and denitrification in estuarine ecosystems, we conducted two seasonal samplings to collect sediments across transects at fifteen stations along the Cape Fear River Estuary, which is heavily impacted from agricultural and industrial nitrogen inputs. Sediment slurry incubation experiments were conducted with ¹⁵NO₃⁻ tracer and resultant N₂ gases with different isotope composition were analyzed on an Isotope Ratio Mass Spectrometer. Quantitative PCR of hydrazine oxidase (*hzxA/hzoB*) and nitrous oxide reductase (*nosZ*) genes was developed and optimized to measure the abundance of anammox and denitrifying bacteria in sediments, respectively. Denitrification was found to be the dominant process in all

samples analyzed, with anammox accounting for an average of 6.5% of N₂ production (ranging from 1% to 19%). There was no clear pattern in terms of activities along the salinity gradient of the estuary, and potential N₂ production rates were similar between the summer and winter. Higher N₂ production was measured in sediments from the banks than in the channel, yet this was not the case throughout the entire estuary. Q-PCR analyses of *hzoA/hzoB* and *nosZ* genes will estimate the abundance of anammox and denitrifying bacteria which will be correlated with the rate measurements. Thus, this study provides a refined view of potential N₂ production capacity in the sediments of the Cape Fear River based on an extensive sampling regime coupled with molecular and stable isotope analyses.

Abstract Category

17 Marine Microbiology

PS.17.055 *TRICHODESMIUM THIEBAUTII* COLONIES AT THE BERMUDA ATLANTIC TIME SERIES STATION ARE ASSOCIATED WITH DISTINCTIVE EPIBIONT POPULATIONS

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Scientific interest in *Trichodesmium* has been fueled by its prominent role in the marine nitrogen cycle, however, it is often overlooked that *Trichodesmium* colonies are only one member of a complex consortia composed of bacteria, microzooplankton, and metazoa. Associated bacteria, in addition to improving the efficiency of nitrogen fixation, may also dictate the longevity of *Trichodesmium* blooms, and the fate of the carbon and nitrogen fixed by *Trichodesmium*. In this manner, the attached bacteria are themselves important players in the marine carbon cycle and of biogeochemical significance in their own right. In this study, we aimed to phylogenetically classify the communities associated with two morphological types of *Trichodesmium* colonies, tufts and puffs, employing a culture-independent small subunit ribosomal RNA gene sequence-based clone library survey. Samples were obtained at the Bermuda Atlantic Time Series (BATS) site in the Sargasso Sea and data was compared with previously published clone libraries constructed from seawater at the same site. Communities associated with *Trichodesmium* are distinct with respect to seawater and with respect to one another: No overlapping OTUs at the 97 percent similarity threshold were identified between puff and tuft samples, possibly indicating a role for heterotrophic bacteria in determining the colony morphology and perhaps different ecosystem roles for tufts and puffs in the oceans. Overall, these data suggest that *Trichodesmium* possess an epibiotic microbial community distinctly different from that observed in bacterial picoplankton (interestingly, no archaeal or eukaryotic SSU rRNA gene sequences were detected). Moreover, *Trichodesmium* epibiotic communities display drastically lower diversity than bacterioplankton, supporting the idea that these assemblages are microbial hot spots in marine oligotrophic surface water environments.

Abstract Category
17 Marine Microbiology

PS.17.056 SINGLE CELL ENVIRONMENTAL MICROBIOLOGY WITH SECONDARY ION MASS SPECTROMETRY

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We describe a unique method for coupling microorganisms' phylogenetic identity and function in soil, water, sediment or other ecosystems. By combining a stable isotope pulse-chase experiment with in situ hybridization using a halogen labeled oligonucleotide probe, metabolically active cells can at the same time be phylogenetically identified [1-3]. A NanoSIMS (Secondary Ion Mass Spectrometer) instrument with 50nm lateral resolution was used to obtain the following in one single measurement: 1) phylogenetic identity via the visualization of oligonucleotide probe hybridization signal in single cells (similar to FISH but replacing fluorescent oligonucleotide by an isotopically or elementally labeled probe), 2) a quantitative measurement of the metabolic activity of individual cells by using stable isotope labeling following a pulse-chase experiment. SIMS imaging uses the sputtering and ionization of surface atoms by a beam of primary ions scanned across a sample surface. Ejected ions are mass filtered in a magnetic sector. After introduction to the technique and recent applications including cyanobacterial cultures and hypersaline mats and seafloor microbes [4], we introduce a new low energy (< 100eV) reactive ion pre-sputtering, allowing to enhance the ionization yield by orders of magnitude with only limited sputtering of the sample for very surface measurements (ex: cell membranes). [1] Simultaneous analysis of microbial identity and function using NanoSIMS, Tianlun Li et al., Environ. Microbiology (2007). [2] Linking phylogeny with metabolic activity of single microbial cells using FISH-NanoSIMS. Behrens, S., T. Loesekann et. al. (2008). Applied and Environmental Biology, 74:3143-3150. [3] A single-cell view on the ecophysiology of anaerobic phototrophic bacteria, N. Musat et al. , PNAS, Nov 18, 2008, vol. 105, no. 46. [4] Seafloor microbe data by courtesy of Dr. Yuki Morono, Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Kochi, Japan.

Abstract Category
17 Marine Microbiology

PS.17.057 MOLECULAR CHARACTERIZATION OF BACTERIAL COMMUNITY ASSOCIATED WITH ABALONE HALIOTIS RUFESCENS FARMED IN THE NORTH OF CHILE.

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Like all marine organisms, shellfish live in an ecosystem with high numbers of bacteria, among which opportunistic pathogens may be frequent. Bacteria may colonise the integument, gut and hemolymph of mollusk as abalone. The use of probiotics is becoming popular in aquaculture. It has been reported that probiotics are preparations of microbial cells or cell components, containing live and/or dead bacteria, which confer a health benefit on the host by and improvements in nutrition. Most probiotics tested for use in aquaculture have been preparations of Gram-negative bacteria such as *Vibrio* and *Pseudomonas*; Gram-positive bacteria such as *Bacillus* and lactic acid bacteria; yeasts such as *Saccharomyces* and *Debaryomyces*; and microalgae such as *Tetraselmis*. Here we show the molecular characterization of microbial community associated with the abalone gut and the effect of bacterial strains used as probiotics. *Macrocystis integrifolia* was pre-conditioned with the addition of some selected bacterial strains and their effect on the weight and size was evaluated after 30 days trial. The change on the microbial community associated to the abalone gut was examined, before and after feeding with the pre-conditioned macroalgae by amplification of 16SrRNA and DGGE. A significant mean improvement in size and weight of *H. rufescens* (9,3% and 19,7% respectively) was provided by the selected strains. The gut microbial richness was characterized, with five dominant genera *Vibrio* (27,3%), *Pseudomonas* (13,0%), *Alteromonas* (12,8%), *Agarivorans* (8,9%) y *Cytophaga* (7,1%). The other genera had less than 4%: *Pseudoalteromonas* (3,6 %); 3,3% (*Bacillus* and *Photobacterium*); 3,1% (*Halomonas*); 2,3% (*Formosa*); 1,5% (*Marinomonas*). These selected strains are good candidates for their use as probiotic supplement in abalone farming.

Abstract Category

17 Marine Microbiology

PS.17.058 COMMON DISTRIBUTION OF HALOARCHAEA IN MARINE ENVIRONMENTS

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Haloarchaea are the extremely halophilic and aerobic members of *Archaea*, classified within the family *Halobacteriaceae*. It has been regarded that they are unable to live in marine environments because of low salinity. However, recent analyses of 16S rRNA genes have indicated their presence in non-hypersaline environments. The purpose of this study was to show the common distribution of haloarchaea in marine environments using culturing methods. Seawater samples obtained from various environments were filtrated and incubated on high-sodium chloride agar plates. Altogether 68 strains with red or pink-color were isolated from Arabian Sea, Japan Sea, Lake Saroma (blackish-water) in Hokkaido, Japan, Sagami Bay and the aquarium of shark at the Ocean Research Institute, the University of Tokyo. Isolates were broadly divided into nine genera, *Halococcus*,

Halogranum, *Halorubrum*, *Haloferax*, *Halalkalinicoccus*, *Halovivax*, *Halostagnicola*, *Natrinema* and one strain from novel lineage, which is closely related to Marine Group IV, an indigenous archaeal group in the ocean. The results indicate that haloarchaea belonging to variety of phylogenetic group are commonly present in marine environments. The evolutionary process, distribution and ecological significance of halophilic archaea need reevaluation.

Abstract Category
17 Marine Microbiology

PS.17.059 DIVERSITY OF CADMIUM CONTAINING CARBONIC ANHYDRASES FROM DIATOMS IN A HEAVY METAL CONTAMINATED ENVIRONMENT

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The enzyme carbonic anhydrase plays an important role in the cellular preparation of photosynthesis by converting bicarbonate to CO₂ which is then used for carbon fixation. Carbonic anhydrase is particularly important for marine microalgae as the pH of the ocean dictates that the vast majority of dissolved carbon in the ocean is not available without conversion. Marine phytoplankton are known to contain a form of carbonic anhydrase which replaces its catalytic zinc atom with cadmium, an element normally highly toxic to cells. Here we have cloned and sequenced genes coding for cadmium containing carbonic anhydrases from a marine environment that is heavily contaminated by heavy metals. Phylogenetic analysis of these genes and comparison with sequences from an environment with undetectable heavy metal concentrations have provided an example of how continuously high metal concentrations have influenced the diversity of this gene and how human activity has impacted the base of the marine food web at the molecular level.

Abstract Category
17 Marine Microbiology

PS.17.060 HIGH DIVERSITY OF MICROPLANKTON SURROUNDING A DEEP-WATER CORAL REEF AND LINKS WITH AUTOTROPHY

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The free-living microbial plankton community (Archaea, Bacteria, Eukarya) of deep-water coral reefs are poorly understood although of importance to the reef. Microplankton were here directly compared for two sites at 350 m depth surrounding a cold water coral reef and a cold seep on the north Atlantic continental margin of the

fishing ground Haltenbanken of Norway. Sequence analysis of small subunit rRNA gene clones from the plankton revealed the presence of the Crenarchaeote *Nitrosopumilus maritimus*, the Proteobacterium *Pelagibacter ubique*, the protist lineage Alveolata and the dinoflagellate lineage Syndiniales, along with many other less abundant phyla. The reef plankton sample contained the highest microbial diversity and included a distinct community of microeukaryotes and members of the photosynthetic Cyanobacteria, Chlorophyta and Cryptophyta, which were undetected in the seep sample. Potentially seep-fuelled plankton was targeted by investigating methanotrophy. We detected functional gene sequences related to the methanotroph *Methylococcus capsulatus* (*pmoA*) and to the ammonium oxidiser *Nitrosospira* (*amoA*) in this environment. The highly diverse microplankton identified represents food for the many animals that inhabit or interact with this reef system at Haltenbanken.

Abstract Category

17 Marine Microbiology

PS.17.061 ROLES OF AAPB IN CARBON AND ENERGY CYCLING IN THE OCEAN: EFFECTIVE PHOTOTROPHY, HIGH CARBON FLUX AND TRANSFORMATION EFFICIENCY

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Aerobic anoxygenic phototrophic bacteria (AAPB) are bacteriochlorophyll a (BChl_a)-containing bacteria which have been proved to be ubiquitous in marine environments. Yet, controversial observations were reported on their roles in the ecosystem, such as the low abundance v.s. the high growth rate, and the light independency in lab cultures v.s. the confined distribution within the ocean euphotic zone. Based on extensive investigations in the Pacific, Atlantic and Indian Oceans and the China Seas, as well as eco-physiological studies in the lab, we have shown that 1) the high growth rate of AAPB (2-3time higher than non-AAPB) is benefited from association with phytoplankton as well as the supplemental BChl_a-based phototrophy (Jiao et al., 2007). Phylogenetic and statistical analysis of *pufM* sequences from the field revealed the existence of dim AAPB with relatively low GC and GC3 contents the twilight zone (Zeng & Jiao 2009). Genome sequencing of an AAPB strain JL354 even revealed, for the first time, the coexistence of two different photosynthetic operons in a single strain (Jiao et al., 2010). 2) The low AAPB abundance (1-15% of total microbes) is the result of their high mortality due to large cell size (2 time larger than non-AAPB) caused high grazing pressure and high viral infection rate (20-30% higher than on non-AAPB). The first AAPB-phage has been isolated and named RDJL-1 whose DNA is refractory to common restriction enzymes (Zhang and Jiao , 2009), providing an evidence for the effective viral lysis of AAPB in the field. The abundance-specific carbon flux through AAPB is 3-5 folds that through non-AAPB, contributing more to the "microbial carbon pump" (Jiao et al., 2010). The phototrophy in AAPB could reduce respiratory organic carbon consumption of up to 5% of the primary production, and would be critical for a marine region to be a sink or a source of atmospheric CO₂ (Jiao et al., 2010).

Abstract Category
17 Marine Microbiology

PS.17.062 INSIGHTS INTO THE BIO-OXIDATION AND BIO-REDUCTION OF ARSENIC IN CONTAMINATED MARINE SEDIMENTS

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Arsenic (As) occurs naturally and anthropogenically in the environment and chronic exposure to this element is directly linked to human health problems. Recently, a study has revealed high As concentrations along the South Marseille littoral (France) and an excess of carcinogenic effect level through mussel consumption. If the marine cycle of As is well described in the water column, only scarce data exist on its transport from the sediment to the water column. The role of bacteria on arsenic speciation (arsenite (AsIII) oxidation, arsenate (AsV) reduction) and thus on its mobility, is now widely acknowledged. However, the bacterial cycle of As in marine sediments remains largely unknown. Within the ASEDMAR project, supported by the French National Research Agency (reference 2008 CESA-003), the final aim being the elaboration of a model integrating biogeochemistry, bioavailability and toxicity of As in marine sediments, our task is to study the impact of the bacterial compartment on the sequestration and mobilization of arsenic, taking its chemical forms into account that will directly influence its mobility and bioavailability. Sediments were sampled from two seaports, one being polluted with As by several industrial activities, and the other one as a reference for low arsenic content. Sediment microcosms in defined media were used for the determination of kinetics of potential AsIII oxidation and AsV reduction. Molecular methods based on the functional genes *aoxB* (AsIII oxidation) and *arrA* (AsV respiration) were applied in order to evaluate their diversity. As we recently developed the DGGE fingerprinting technique on *aoxB* genes, the *aoxB*-carrying community was studied in more details. Altogether, our results showed a very active bacterial community against As in the marine sediments, where active AsIII oxidizers and AsV reducers co-exist at both locations. Our results give insights into a likely transfer of As from the sediment toward the water column.

Abstract Category
17 Marine Microbiology

PS.17.063 ESTUARINE 'BLOOMS' OF A HETEROTROPHIC FLAGELLATE AND THE FATE OF THE RIVER'S SPRING PRIMARY PRODUCTION

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The Columbia River estuary, which divides Oregon and Washington (USA), is typically classified as a detritus-driven system due to light limitation of phytoplankton growth. Much of the microbial activity is associated with particles. While the particle-associated bacterial community has been examined in the Columbia River estuary, major consumers

of bacteria in the system are not well characterized. Molecular analysis of microbial eukaryotic assemblages indicates that during April the heterotrophic flagellate, *Katablepharis*, dominates the estuary, comprising over 50% of 18S rDNA clone libraries in both 2007 and 2008. No other eukaryotic microbe during the two-year time series was found at such high relative abundance. Other events occurring in the spring, such as an upriver diatom bloom and spring runoff, deliver organic matter to the estuary that could fuel its presence. *Katablepharis* is a 5 µm heterotrophic protist that is known to be both a primary consumer and bacterivore associated with particles in other freshwater and marine systems. To our knowledge, this is the first report of *Katablepharis* as the dominant member of an estuarine protist population. To better resolve the spatial and temporal distribution of *Katablepharis* in the Columbia River estuary, a specific molecular probe for *Katablepharis* was developed based on its internal transcribed spacer 1 (ITS 1) region of rDNA, and both qPCR and RT-qPCR were performed on water samples collected at different locations and depths throughout the estuary from February to July 2010. Due to its high abundance, *Katablepharis* could play an important, previously unseen role in the fate of spring phytoplankton organic matter. Furthermore, the fact that *Katablepharis* displays a repeatable pattern of occurrence indicates that changes in the distribution of this microorganism could potentially be used to track environmental shifts in the Columbia River estuary ecosystem.

Abstract Category

17 Marine Microbiology

PS.17.064 SULFATE REDUCTION ACTIVITIES AND DISTRIBUTION OF DISSIMILATORY SULFITE REDUCTASE GENES IN THE SEISMOGENIC ZONE OF NANKAI TROUGH

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Dissimilatory sulfate reduction is a key biogeochemical process in marine subsurface sediments, retrieving energy from organic matter using sulfate as the electron acceptor. The present work reports the abundance, activity and community structure of sulfate-reducing prokaryotes (SRP) along transect of the Nankai Trough Seismogenic Zone beneath the ocean off the southwest coast of Japan. Sediments were cored from six representative sites during the IODP Expeditions 315 and 316. The abundance of SRP was studied by combining fluorescence microscopy, automated image analysis, and quantitative PCR of dissimilatory sulfite reductase genes (*dsrA*), dissimilatory adenosine-5' phosphosulfate reductase genes (*apsA*) and 16S rRNA genes. Potential sulfate reduction rates (SRR) were measured by incubating sediment samples with ³⁵SO₄²⁻ radiotracer at 5°C for 100 days, and measuring the potential SRRs by the cold-chromium distillation method. The dissimilatory sulfite reductase genes (*dsrB*) were profiled using denaturing gradient gel electrophoresis of *dsrB* genes mediated by PCR and multiple

displacement amplification of whole genomes. SRP were observed as deep as 818 meters below sea floor. SRP numbers were generally highest (up to about 10^5 SRB cells/cm³) in the shallow sediments, but elevated counts were obtained also from depths close to megasplay-fault zone. Potential SSRs were low apart from in some shallow sediments, where 10-1500 pmol/cm³/day of SSRs were observed. The potential seafloor SRP community was highly diverse, and comprised of 21 phylotypes (with < 95% similarity) representing families *Desulfoarculaceae*, *Desulfobacteraceae*, *Desulfobulbaceae*, *Desulfobacteraceae*, *Desulfovibrionaceae*, *Syntrophobacteraceae*, and *Peptococcaceae*. In conclusion, numbers and activity of SRP were generally highest in shallow sediments of the sulfate reduction zone, but a number of SRP phylotypes were spatially detected in the deep-biosphere, even at very low concentrations of sulfate.

Abstract Category

17 Marine Microbiology

PS.17.065 BENZYL SUCCINATE SYNTHASE GENE ABUNDANCE IN THE MARMARA SEA SEDIMENTS

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Although Marmara Sea has been extremely polluted with hydrocarbons, the level of benzene, toluene, ethylbenzene, and the isomers of xylene (BTEX) in this environment has not been determined yet. BTEX are priority environmental pollutants and can be removed by means of bioremediation as long as BTEX degrading microorganisms are abundant in the polluted environment. Benzylsuccinate synthase (bssA) is responsible for anaerobic toluene and xylene degradation and bssA gene can be targeted to quantify BTEX degraders in environmental samples. Quantitative real-time PCR (Q-PCR) have been widely used for the quantification of gene abundances. In this study, we used the Q-PCR primers targeting the widest range of physiological and phylogenetical group of microorganisms carrying the bssA gene to monitor abundance of BTEX degraders in sediments from the most polluted regions of the Marmara Sea (10 different locations). The bssA gene abundance changed in a range of 2.4×10^{10} - 7×10^{12} during the 2 years monitoring period. The bssA was abundant where total petroleum hydrocarbon (TPH) levels are high, and BTEX % in TPH was negatively correlated to the relative abundance of the bssA in the total microbial community (3-40 %). Our results revealed that the Marmara Sea Sediments seems to be a promising candidate for further investigation of microbial BTEX degradation under anaerobic/anoxic conditions.

Abstract Category

17 Marine Microbiology

PS.17.066 COMPARATIVE ANALYSIS OF MICROBIAL COMMUNITY DEPENDING ON THE CELL SIZE FROM THE BATHYPELAGIC LAYER OF

ULLEUNG-BASIN, KOREA

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The size of microorganisms varies considerably. At the smallest end of this size spectrum are the ultramicrobacteria(UMB) which has a cell volume of less than $0.1 \mu\text{m}^3$, irrespective of growth conditions. UMB are highly abundant in marine environments and they play important roles in the global nutrient cycle and in the marine food chains. In the present study the microbial diversity in the bathypelagic layer of Ulleung-basin was compared by the size fraction which is larger than $0.2 \mu\text{m}$ (non-UMB) or smaller than $0.2 \mu\text{m}$ (UMB). A total 420 clones of archaeal group and 303 clones of bacterial group were analyzed by 16S rRNA gene target sequence. All archaeal clones belong to *Crenarchaeota* and *Euryarchaeota* and a majority of both fractions was affiliated with *Crenarchaeota* marine group I by 82-85%. Bacterial clones were derived from 5 major lineages, *Alpha-*, *Beta-*, *Gamma-* and *Delta-Proteobacteria* and *Chloroflexales*. *Gammaproteobacteria* represents a dominant bacterial group in the fraction of non-UMB and UMB by 85% and 78%, respectively. Most clone of UMB is grouped into the uncultured group and these results support UMB possess high frequency of unique microbial community compared to non-UMB. [Supported by MEGRC]

Abstract Category

17 Marine Microbiology

PS.17.067 SPATIAL COMPARISON OF BACTERIAL DIVERSITY DEPENDING ON THE SIZE OF MICROBIAL CELL AT EAST-SEA, KOREA

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Most study has been conducted on marine microbes of which cell size is larger than $0.2 \mu\text{m}$ while there is few information about bacteria smaller than $0.2 \mu\text{m}$ (Ultramicrobacteria). Compared to a group of large bacteria, smaller one has advantage to uptake nutrients resulting from the high ratio of surface to volume and also might be less subject to grazing pressure by predators. It results in that UMB become abundant and play a key role of the biogeochemical cycle of organic and inorganic matters in marine environment. Bacterial diversity was analyzed depending on the size of bacteria from two different marine environments (bathypelagic and surface layer). Approximately 200 liters of each sea water sample were successively filtered through 0.2 and $0.1 \mu\text{m}$ -pore size filters. Genomic DNA was extracted from filters and the phylogenetic analysis of bacterial clones was conducted. Bacterial diversity of the surface seawater affiliated into 4 major lineages *α -*, *γ -* and *ϵ -Proteobacteria* and *Firmicutes*, and uncultured *alphaproteobacterium* F4C31 represented dominant clone up to 64%. This clone belongs to the *Roseobacter* clade in which most groups of marine environment possess aerobic and phototrophic features. In the bathypelagic seawater, bacterial community was classified into 5 major lineages, *α -*, *β -*, *γ -* and *δ -Proteobacteria*, and *Chloroflexales*. *Halomonas meridiana* (*γ -Proteobacteria*) which is able to grow in the broad range of salt and frequently founded from deep-sea sediments and hydrothermal vents was dominated

in the range of 67 and 90%. The bacterial community depending on the cell size did not show any significant differences at the level of species, however, most clone of UMB was affiliated with uncultured bacterium representing high similarity. It likely seems that UMB comprises of unique microbial community which is hardly cultivated. To confirm this premise, the isolation of UMB was conducted by high through-put cultivation (HTC) method.[Supported by MEGRC]

Abstract Category
17 Marine Microbiology

PS.17.068 THE MICROBIOME ASSOCIATED WITH HEALTHY AND YELLOW BAND DISEASED *MONTASTRAEA FAVEOLATA*, AN IMPORTANT REEF-BUILDING CORAL IN THE CARIBBEAN

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Coral-associated microbial communities are important components of the coral holobiont that influence coral health and disease. Zooxanthellae, or symbiotic algae of the genus *Symbiodinium*, provide the coral holobiont with essential nutrients. Similarly, bacteria and archaea are thought to play an important role in the biogeochemical cycling of the coral holobiont. During disease these communities can be disrupted, affecting their role in maintaining a healthy ecosystem. We hypothesize that the bacterial and archaeal communities will shift between healthy and diseased *Montastraea faveolata* colonies in order to compensate for the reduced production known to occur in the zooxanthellae community during yellow band disease. Therefore, we assessed the community structure of the zooxanthellae, bacteria and archaea associated with three healthy and three yellow band diseased *M. faveolata* colonies using mucus and tissue slurry samples. Community members were sequenced by amplifying the ribosomal RNA gene with community specific primers. In all samples, clades B and D were the predominant zooxanthellae community members identified, while clade A was detected more sporadically. Interestingly, the zooxanthellae community structure differed between healthy and diseased colonies with the thermal tolerant clade D being most abundant in the healthy mucus samples. The majority of the bacterial community members were from the Phyla Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, Nitrospira, Proteobacteria, and Tenericutes. Greater bacterial diversity was observed in the yellow band diseased samples compared to their healthy counterparts. Euryarchaeota and crenarchaeota were detected in the archaeal community. This is the first coral microbiome study to examine the zooxanthellar, bacterial and archaeal communities simultaneously from individual coral samples, providing evidence of changes in the coral microbiome during yellow band disease.

Abstract Category
17 Marine Microbiology

PS.17.069 EVALUATION OF MARINE MICROBIAL DIVERSITY AND BIOREMEDIATION TECHNIQUES CONCERNING OIL SPILL AROUND JAPAN ISLANDS

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To evaluate marine microbial community changes under oil spill and bioremediation processes, fundamental information about marine microbes must be required. Surface seawater samples were collected from six coasts around Japan islands in both summer and winter seasons for a couple of years. Direct counts of microbes ranged from 10^5 to 10^6 cells ml^{-1} , showing the highest in Tokyo Bay through all the course of sampling. Phylogenetic analyses based on 16S rRNA gene indicated the common occurrence of phylotypes related to Cytophaga-Flavobacter-Bacteroides, *Roseobacter*, SAR11, and SAR86, whereas some phylotypes were characterized by the sampling site and season. In bioremediation experiments, the addition of oil leads to a dramatic community change from the above cosmopolitan groups to alpha- and gamma-proteobacterial ones, suggesting bacterial community succession for oil adaptation. This was confirmed by the occurrence of phylotypes related to known hydrocarbon degraders, while most of the recorded phylotypes represented unique phylogenetic lineages. For rapid and reliable evaluation of oil degradation, several image analysis techniques were compared using different types of analyzers, microplates, and petroleum oils. To date, meaningful decreases in both LED reflection light and fluorescence image intensities of crude oils, as pollutants, have been shown in combination of inorganic nutrients and some bacterial species that we have observed during the survey. We concluded that the study on dynamic change of natural marine bacterial ecosystem is essential to develop new and safe bioaugmentation techniques for marine oil spill, as well as reliable bio-monitoring techniques.

Abstract Category

17 Marine Microbiology

PS.17.070 CHANGES IN MICROBIAL COMMUNITY COMPOSITION ACROSS GEOCHEMICAL ZONES IN A COASTAL MARINE SEDIMENT CORE SPANNING 8000 YEARS OF HOLOCENE DEPOSITION

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Marine sediment-dwelling microbes are key players in the global carbon and sulfur cycles yet their identity and the mechanisms governing their distribution remain unknown at large. The objective of the presented study was therefore to relate microbial community structure to distinct geochemical zones in a deep sediment core, and to resolve to which extent deeply buried microbes represent a subset of the surface

community, i.e. to identify actively growing vs. slowly dying community members. We examined the microbial community composition and geochemistry at 30-cm intervals in 11-m long sediment cores from Aarhus Bay (Denmark). The cores covered the entire Holocene period of sedimentation back to 8000 years before present and reached terrestrial soils from the early interglacial at the base of the Holocene deposits (≥ 10 m depth below the seafloor). Porewater concentration profiles of sulfate, sulfide, dissolved iron and methane suggest five distinct geochemical zones: (i) the top 1 cm of oxic/suboxic sediment, (ii) a sulfate reduction zone, (iii) a sulfate-methane transition zone at 2 m depth, (iv) a low sulfate methanogenic zone and (v) a low-sulfide zone that is rich in dissolved iron and has micromolar concentrations of sulfate below 8 m depth. Peaks in qPCR-based enumerations of bacterial and archaeal 16S rRNA gene copies were associated with the sulfate reduction and methanogenic zones, indicating elevated microbial activity in these zones. According to amplified ribosomal intergenic spacer fingerprinting, the individual geochemical zones harbored similar bacterial communities across their vertical extension with a distinct composition as compared to the other zones. Detailed analysis of the microbial community composition within each of the five zones was performed by massively parallel sequencing of 16S rRNA gene PCR amplicons. Together these data provide insights into the geochemical factors, which control the sediment microbial community.

Abstract Category

17 Marine Microbiology

**PS.17.071 HALOTAXIS OF CYANOBACTERIA IN AN INTERTIDAL
HYPERHALINE MICROBIAL MAT**

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An intertidal hypersaline cyanobacterial mat from Abu Dhabi (United Arab Emirates) exhibited a reversible change in its surface color within several hours upon changes in salinity of the overlying water. The mat surface was orange-reddish at salinities above 15‰ and turned dark green at lower salinities. We investigated this phenomenon using a polyphasic approach that included denaturing gradient gel electrophoresis (DGGE), direct light and epifluorescence microscopy, high performance liquid chromatography (HPLC), hyperspectral imaging, absorption spectroscopy and oxygen microsensor measurements. Additionally, the dynamics of salinity inside the mat were modelled, and measured directly in the pore water of mat sections. Filaments of *Microcoleus chthonoplastes*, identified based on 16S rRNA sequencing and morphology, were found to migrate up and down when salinity was decreased below or increased above 15‰, respectively, causing the color change of the mat uppermost layer. Migration occurred in light and in the dark, and could be induced by different salts, not only NaCl. The influence of salinity-dependent and independent physico-chemical parameters, such as water availability, oxygen solubility, H₂S, gravity and light, was excluded, indicating that the observed migration was most likely due to a direct response to salt stress. In conclusion, we

propose to term this salinity-driven cyanobacterial migration as "halotaxis", a process that might play a vital role in the survival of cyanobacteria in environments exposed to continuous salinity fluctuations such as intertidal flats.

Abstract Category
17 Marine Microbiology

PS.17.072 ANALYSIS OF TWO GROUPS OF NOVEL PICOEUKARYOTES AT THE SAN PEDRO OCEAN TIME SERIES USING A FOSMID LIBRARY

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Culture-independent molecular studies have facilitated the discovery of tremendous diversity and new taxa of microorganisms. However, among microbes, characterization of functional diversity and novel gene discovery in protists (single-celled eukaryotes) lags behind the archaea and bacteria, largely due to the former's larger genome size and complexity. Protists play major roles in oceanic food webs and in marine biogeochemical cycles. Among them, two diverse, abundant and widely distributed groups could prove significant: novel alveolates (NA) and marine stramenopiles (MAST). The organisms that belong to these groups have not been successfully cultured; their potential ecological and physiological roles are unknown but their occurrence in diverse environmental conditions suggest different life strategies and resource specializations. The purpose of this study is to use a fosmid library to link a phylogenetic marker gene with flanking functional genes. For this study, we collected 50 L of pico-size fractionated seawater from the San Pedro Ocean Time-series, off the coast of Southern California (USA) and generated a fosmid library, which contained 103, 680 clones with an average insert length of 36 Kb. Screening this library for the 18S rRNA gene gave us 136 positive clones. Clones were Sanger-sequenced and classified at the phylum level. Sequences within the within the Stramenopiles and Alveolates were further classified into clades. Of the 136 clones sequenced, 29 clones fall within Stramenopiles, and 10 clones belong to the Alveolates. Novel MAST groups that were represented in our fosmid library were MAST-1, 4, 7 and 8; NA Group 1 Clade I, Group II, Clades 6, 7, and 10 and 11 were also present. Thirty-one clones were chosen for 454 FLX sequencing. The resulting reads were assembled and analyzed. In this study, we were able to generate a large fosmid library that begins to unveil linked gene information in an ecologically important group of microbes.

Abstract Category
17 Marine Microbiology

PS.17.073 DIVERSITY AND COMMUNITY STRUCTURES OF PHYTOPLANKTON DURING SPRING SEASON IN SAGAMI BAY ANALYZED BY 18S RIBOSOMAL DNA CLONE ANALYSIS

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Some phytoplankton species are difficult to identify by microscopic observation. However, small subunit ribosomal RNA gene (SSU rDNA) clone analysis has been widely used for taxonomic classification and biodiversity analysis of prokaryotes because of its reliability and accuracy. We applied this method combining newly designed PCR primers to reveal short term change of phytoplankton community structure in spring season. Field sampling was carried out monthly from April to June 2006. 20 L of seawater was filtered through 180 µm nylon-mesh and phytoplankton cells were collected on 10 µm membrane filters and fixed with Lugol's solution. Mixed DNA was extracted and used for phytoplankton 18S rDNA amplification. Amplicons were cloned into pT7 Blue vector and introduced into *E. coli* cells. 18S rDNA clones were re-amplified and separated into groups by RFLP analysis. Representative clones were sequenced and identified by BLAST search. In April, phytoplankton community consisted of mainly 19 and 11 phylotypes of diatom and dinoflagellate, respectively. Diatom species gradually increased monthly and reached 40 phylotypes in June. In contrast, dinoflagellate dramatically increased to 56 phylotypes in May and was reduced to 13 phylotypes in June. The genus *Thalassiosira* and genus *Gymnodinium* were mostly dominant in April and May, respectively. In June, the members of the family Skeletonemataceae dominated. We detected more than 60 phytoplankton species through the analysis. This score was much higher than the number of species detected by microscopic observation. Biodiversity and community structures of phytoplankton in spring season in Sagami Bay were revealed by molecular biological approach. Although we should consider some biases in the quantitative population analysis, biodiversity of phytoplankton in a blooming season was much better clarified.

Abstract Category
17 Marine Microbiology

PS.17.074 HIGH ANAEROBIC MICROBIAL HYDROCARBON DEGRADATION ACTIVITIES OF THE MARMARA SEA SEDIMENTS

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Marmara Sea has been extremely polluted with hydrocarbons. Because the Marmara Sea Sediments (MSS) were anoxic, a bioremediation strategy based on anaerobic hydrocarbon degradation is the best alternative as long as oil-degrading microorganisms are abundant and active in the sediments. Benzylsuccinate synthase (bssA) and alkylsuccinate synthase (assA) gene transcripts can be targeted in order to assess activity of aromatic and aliphatic hydrocarbon degraders respectively. Quantitative real-time PCR (Q-PCR) primers for assA have not been reported yet. The previously reported Q-PCR primers for bssA were able to target narrow range of microorganisms. In this study, the

most comprehensive Q-PCR primers for *bssA* and the first Q-PCR primers for *assA* were designed to monitor activity of anaerobic hydrocarbon degraders in the sediments from the most polluted regions of the Marmara Sea (10 different locations). The *bssA* and *assA* mRNA levels changed in ranges of 4.2×10^9 - 7.8×10^{11} and 4.8×10^9 - 8.9×10^{11} mRNA/cm⁻³ sediment respectively during the 2 years monitoring period. mRNA levels were positively correlated to N and P content of the sediment porewaters ($p < 0.05$, $r > 0.8$, $n = 46$). These results showed that prokaryotic biomass were actively degrading hydrocarbons under anoxic/anaerobic conditions in the sediments. Further laboratory scale hydrocarbon degradation microcosms which were inoculated using the MSS under denitrifying and methanogenic conditions verified that the MSS microorganisms were able to degrade wide range of aliphatic and aromatic hydrocarbons. Microcosm studies also revealed that it was possible to increase hydrocarbon degradation potential of the MSS ~10x by N-P amendment. Our study is the first that reports marine benthic microorganisms can actively degrade wide range of hydrocarbons under anaerobic conditions which may eventually lead to less human intervened and more economical field-scale bioremediation applications

Abstract Category

17 Marine Microbiology

PS.17.075 SPATIALLY DIFFERING BACTERIAL COMMUNITIES IN THE NORTHERN BALTIC SEA

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The Baltic Sea is a large, shallow and strongly stratified brackish water basin. It suffers from eutrophication, cyanobacterial blooms and oxygen depletion, all of which pose a threat to local marine environment and microbial communities. In this study, the diversity and community structure of the northern Baltic Sea bacterial communities in the water column were studied by 454-sequencing. Based on the 36 000 sequences obtained, the bacterial communities were one order of magnitude less diverse than those in recently studied oceanic habitats. Patchiness and strong stratification were clearly detectable, since less than 1% of OTUs were shared among all the samples. The community composition showed more uniform nature in same horizontal water layers at different sampling sites than vertically within one site. This can be explained by different environmental conditions, i.e. different temperature, salinity and oxygen concentration. Taxonomic affiliations revealed a total of 23 bacterial classes and 169 genera, and five percent of the sequences remained unclassified. Different classes of Proteobacteria constituted the majority of the sequences, but Flavobacteria and Actinobacteria were also abundant in all samples. The bacterial genera present were unevenly distributed vertically and horizontally. However, *Pseudomonas*, the most abundant genus in the whole data, was found in all sampling sites and it was the dominant group in surface waters.

Cyanobacteria constituted less than two percent of the sequences and potentially toxic cyanobacterial genera were practically absent during the sampling seasons. In conclusion, the northern Baltic Sea bacterial communities cannot be described as diverse but largely uncharacterised at species level. Our observations indicated that patchiness and spatial variation are characteristic for the bacterial communities in the area, and the stratification of temperature, salinity and oxygen concentration affect the community structure.

Abstract Category
17 Marine Microbiology

PS.17.076 BACTERIAL COMMUNITY DIVERSITY OF THE ENDEMIC BRAZILIAN REEF CORAL *MUSSISMILIA HISPIDA*

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*The South Atlantic reef coral *Mussismilia hispida* play a key role in marine life. This endemic organism of Bahia State in Brazil is one of the main reef builders, yet it has been under serious threat in the last years. Our aim was to characterize the bacterial community diversity associated to mucus of the coral *Mussismilia hispida*. To that end four 16S rDNA libraries were constructed and 400 clones from each library were analyzed from two healthy colonies, one diseased colony and the surrounding sea water. Nine bacterial phyla were identified in healthy *M. hispida*, with a dominance of Proteobacteria, Actinobacteria, Acidobacteria, Lentisphaerae and Nitrospira. In contrast, diseased *M. hispida* had a predominance of one single species of Bacteroidetes, corresponding to more than 70% of the sequences. Rarefaction curves using evolutionary distance of 1% showed a greater decrease in bacterial diversity in the diseased *Mussismilia hispida*, with a reduction of almost 85% in OTUs in comparison to healthy colonies. J-Libshuff analyses show that significant p-values obtained were < 0.0001, demonstrating that the four libraries are significantly different. In addition, the sympatric corals *Mussismilia hispida* and *Mussismilia braziliensis* appear to have different bacterial community compositions according to Principal Component Analysis (PCA) and Lineage Specific Analysis. Moreover, lineages that contribute to the differences between these two endemic corals were identified as α -Proteobacteria, Bacteroidetes and Firmicutes. The results obtained in this study suggest host-microbe co-evolution in *Mussismilia* and it was the first study on the diversity of the microbiota of the endemic and endangered of extinction Brazilian coral *Mussismilia hispida* from Abrolhos bank. Financial sponsor: FAPDF, FAPERJ and CNPq*

Abstract Category
17 Marine Microbiology

PS.17.077 COMPARATIVE GENOMIC ANALYSIS OF ANTARCTIC MARINE GI CRENARCHAEOTA ENVIRONMENTAL GENOME FRAGMENTS

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The Archaea kingdom Crenarchaeota is an abundant microbial group in the ocean where they can play important roles in carbon and ammonia assimilation. By analyzing the metagenome of surface Antarctic Peninsula seawater collected in austral winter, end sequences from 21 crenarchaeal fosmids (WF) related to the marine chemolithoautotrophic nitrifier *Nitrosopumilus maritimus* SCM1 (NM), were sequenced. In a preliminary assembly fosmid sequences ranging between 9493-44827 bp, formed larger contiguous DNA fragments up to 137,620 bp. To get a better understanding of the similarity between the WF and other marine crenarchaeota genome sequences, genes encoded in the Antarctic crenarchaeotal fosmids (1080) were annotated, and then compared to 1797, 2017 and 44 predicted gene products from NM, *Cenarchaeum symbiosum* (CS) (a crenarchaeotal sponge symbiont), and to the Antarctic crenarchaeotal fosmid 74A4, respectively. The results reveal a low level of synteny in the gene organization of the fosmids as compared to the NM genome. By comparing the four datasets using the program CD-HIT with a 40% shared identity cut-off, it was observed that 16, 34, 30, and 11% of predicted gene products from WF, CS, NM and 74A4, respectively, were unique. From a total of 2701 clusters, 15% of the clusters contained the same WF and CS predicted gene products whereas 18% of the clusters contained both WF and NM predicted gene products, suggesting a higher relationship between WF and NM. The unified core of the three data sets included 18.5% of all clusters comprising ~ half of the WF genes. The average GC content of the Antarctic fosmids (31.3% G+C) was also more similar to the G+C content of the NM genome (34% G+C) rather than the CS genome (57.7%). Differences in the genome structure from the fosmids and metabolic capabilities from this Antarctic seawater community with focus on nitrogen and carbon cycles and cold adaptive signatures are the target of future studies.

Abstract Category

17 Marine Microbiology

PS.17.078 CHARACTERIZATION OF AN ANTI VIBRIO PARAHAEMOLYTICUS SUBSTANCE PRODUCED BY A MARINE VIBRIO

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Background and aims: The pandemic bacterium *V. parahaemolyticus* is responsible for gastroenteric diseases caused by the ingestion of contaminated marine organisms. There are still problems to control the proliferation of *V. parahaemolyticus* and depuration of marine contaminated mollusks when outbreaks of this bacterium happen. The aim of this work is to identify products excreted by a marine inhibiting bacterium of *V. parahaemolyticus*.

Methods: The identification of antibacterial substances of a marine bacterium (C33)

against the pathogenic *V. parahaemolyticus* was performed by means of the method of double layer. It was done an extraction and a sequencing of the gene 16S rRNA of the strain C33, its growth curve was estimated and parallel and extraction was carried out by organic solvents to estimate the optimal of production time. The active products were analyzed by Infrared Spectroscopy, Nuclear Magnetic Resonance and High Performance Liquid Chromatography for its structural elucidation. Equally they were separated by size putting on Müller-Hinton plates bags of dialysis of 1, 3.5, 7.5 and 10 KDa, with the culture of the bacterium inside, after 24 hours the bags were removed and on the agar it was sowed *V. parahaemolyticus*. Finally the sensibility to the temperature was estimated using the same previous procedure but with bags of 10 KDa and after 24 hours the agar was exposed for 1 hour to 100°C, plating again and inoculating *V. parahaemolyticus*. Results: The bacterium C33 belongs to the genre Vibrio, its products inhibit the growth of *V. parahaemolyticus*, it is a secondary metabolite, minor to 10 KDa, thermostable, bacteriostatic and organic. The Infrared Spectroscopy shows OH/ Carbonyl presence. Conclusion: The antibacterial molecule could be used as biocontrol against *V. parahaemolyticus* and make a contribution at clinical level and in the acuicola industry. Acknowledgments: Financial support came from the Fund to Foment Scientific and Technological Development.

Abstract Category
17 Marine Microbiology

PS.17.079 PHYLOGENETIC DIVERSITY OF DDDP, A NOVEL GENE RESPONSIBLE FOR BACTERIAL DMS PRODUCTION, IN MANGROVE SOILS OF CHINA REVEALED BY CULTURE-INDEPENDENT PCR

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The phylogenetic diversity of *dddP*, a recently identified functional gene responsible for bacteria convert dimethylsulfoniopropionate (DMSP) into dimethyl sulfide (DMS), in the mangrove soils of China was investigated by culture-independent PCR.

A pair of appropriate primers was designed and validated by PCR-sequencing with reference strains which were known possessing *dddP* gene. The metagenomic DNA was isolated and purified from four mangrove soil samples, which were taken from Wenchang, Sanya, Danzhou and Beihai mangrove ecosystems in China respectively. Therefore, four *dddP* clone libraries were constructed and 50 randomly selected clones were sequenced for each library. By using neighbor-joining method, a phylogenetic tree was constructed basing on the cloned *dddP* sequences and together with data available in NCBI. It was found that the cloned *dddP* in four libraries generally covered all currently reported cultured bacteria which contain *dddP* gene. But for most of cloned *dddP*, their phylogenies were not affiliated with any cultured Ddd⁺ bacteria, which indicated the highly diverse and novel DMS-producing bacteria existed in mangrove soils. The *dddP* clones could be grouped into 8 clades, and 4 of them were clustered as independent groups which represented hitherto unidentified novel *dddP*-typed Ddd⁺ bacteria. In

addition, it was noted that each clade comprised clones retrieved from different libraries, suggesting that the diversity and distribution of *dddP* gene in mangrove soils were poorly associated with the geographical origins. The expression analysis of *dddP* gene in the mangrove soils above is undergoing by total RNA isolation and reverse transcriptase PCR approach.

Our result advances understanding of mangrove soil bacteria and their role in the biogeochemical cycling of organic sulfur.

Abstract Category

17 Marine Microbiology

**PS.17.080 SPATIAL VARIATIONS OF HETEROTROPHIC
NANOFLAGELLATES MAST-1 TO -4 IN THE EAST CHINA SEA DURING
SPRING AND SUMMER**

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Heterotrophic nanoflagellates (HNF) are not only important grazers of picoplankton but also nutrient remineralizers in aquatic ecosystems. Recent studies have revealed MAST-1 to -4 (marine stramenopiles) account for a substantial proportion of the HNF in the world oceans. However, little is known about the distribution of these MAST groups in the western Pacific Ocean, and the relationship between MAST groups and environmental factors and food resources. Here we studied the spatial and temporal variations of MAST-1 to -4 (-1A, -1B, -1C, -2 and -4) concentrations in surface waters of the East China Sea (ECS).

Two cruises were conducted in the ECS in spring (May) and summer (July), 2009. Abundances of picoplankton (bacteria, *Synechococcus* and photosynthetic picoeukaryotes) were counted by a flow cytometer (FACS Aria, Becton Dickinson). The abundances of MAST groups were determined by FISH using the CY3-labeled 18S rDNA probes.

Our data indicated that MAST-4 was the most dominant group within the MASTs, and contributed to 10% and 6% of HNF in spring and summer, respectively. MAST-1 contributed to 1% of HNF in spring and 2% in summer. MAST-2 was always below detection limit in subtropical ECS. MAST-1C was more abundant than MAST-1A and MAST-1B with the average was 15 ± 2.5 cells ml⁻¹ in spring and 40 ± 14.9 cells ml⁻¹ in summer. The average abundance of MAST-4 was 187 ± 28.2 cells ml⁻¹ in spring and 272 ± 62.5 cells ml⁻¹ in summer. High abundance of MAST-4 (>1000 cells ml⁻¹) was observed along the edge of Changjiang Plume (salinity < 31 psu) in summer. In addition, both abundance and cell size of MAST-4 showed a significant positive correlation with *Synechococcus* abundance ($r=0.56$, $p<0.001$ *** and $r=0.23$, $p<0.05$ *, respectively),

which implied that *Synechococcus* might be an excellent food source to enhance the MAST-4 abundance.

Abstract Category
17 Marine Microbiology

PS.17.081 OIL DEGRADING BACTERIA IN THE BALTIC SEA

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The Baltic Sea is one of the largest brackish water bodies in the world. Its coastline is shared by nine industrialised countries which all use the Baltic as an important route of transportation of freight. Petroleum hydrocarbons (PHCs) belong to the most widespread contaminants entering water systems around the world. Microbes, which have a high capacity of metabolic adaptation have developed various mechanisms for using PHCs as sources of carbon and energy. Although a considerable amount of research has been carried out on biodegradation of the oil in the oceans and in terrestrial environments, there are only few studies on biodegradation of oil in the Baltic Sea. The objective of our study is to characterize oil degrading microbial communities in coastal waters in the Finnish Archipelago. Microcosm experiments were carried out to study the dynamics of indigenous oil degrading bacterial communities of the Baltic Sea. Genetic profiles of microbial communities were achieved using bacterial 16S rRNA gene PCR, Terminal Restriction Fragment Analysis (T-RFLP) and Denaturing Gradient Gel Electrophoresis (DGGE). Based on the community profiles, samples were selected for clone library construction and sequencing to determination of the major bacterial groups. Enumeration of bacteria was done using flow cytometer analysis. The results showed that distinct bacterial communities developed in the diesel-exposed microcosms compared to the clean controls. Further, it was possible to conclude that the bacterial communities from the previously contaminated sites responded to the oil pollution faster than the community from a non-polluted site. The sequencing of diesel exposed communities showed that the dominating bacterial phylotypes resembled oil degraders found in rhizosphere and soil environments, whereas typical marine oil degraders were missing.

Abstract Category
17 Marine Microbiology

PS.17.082 LINKING SPATIAL AND TEMPORAL VARIATIONS OF N₂ PRODUCTION TO ANAMMOX AND DENITRIFYING COMMUNITY DYNAMICS IN THE NEW RIVER ESTUARY

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Estuaries are extremely important ecosystems with the highest biological productivities on earth. Excess N loading from anthropogenic activities is one of the greatest threats to

the ecological integrity of estuaries. The New River Estuary (NRE), North Carolina, USA is an estuary experiencing periodic eutrophication as a result of excess nutrient loading from non point sources within the watershed. Denitrification and anammox primarily control the intensity and duration of estuarine eutrophication by converting fixed N to N₂ gas. We proposed to estimate potential N removal capacity and identify key players of N₂ production in this ecosystem. ¹⁵N tracer techniques were used to measure spatial and temporal variations of N₂ production by anammox and denitrification in estuarine sediments along transects of 12 stations. Molecular analysis of nitrous oxide reductase (*nosZ*) and hydrazine oxidase (*hzxA/hzxB*) genes was conducted to examine community structures of denitrifying and anammox bacteria, respectively. T-RFLP and phylogenetic analyses of both genes were used to compare community structures and diversity of both N₂ producers over spatial and temporal gradients in the NRE. Potential N₂ production rates varied spatially and seasonally with highest rates in the headwaters during winter. Denitrification was the major N₂ production pathway while anammox contributed to 0 to 15% of total N₂ production. *Candidatus Scalindua* spp. were found to be dominant anammox bacteria in the NRE. Community structure of anammox and denitrifying bacteria will be further examined to find out if there is a correlation with their activities over time and space. Furthermore, multivariate analysis will be conducted to define key biogeochemical parameters influencing N₂ producing community dynamics and activities in the NRE. Thus this study will provide the importance of community structure to the ecosystem function as well as estimate potential N removal capacity in an estuarine ecosystem.

Abstract Category
17 Marine Microbiology

**PS.17.083 COMPOSITION OF DISSOLVED ORGANIC MATTER
RELEASED BY PHOTOSYNTHETIC ORGANISMS**

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Marine photosynthetic microorganisms play a key role in the global carbon cycle because they convert inorganic carbon into organic carbon compounds. Despite the global importance of this process, we know little about the molecular composition and production mechanisms of photosynthetically-derived dissolved organic matter in marine ecosystems. We used a combination of laboratory cultures and field samples to define the impact of photosynthetic organisms on the molecular-level composition of dissolved organic matter in the marine environment. First, we used ultrahigh resolution mass spectrometry to assess the molecular composition of dissolved organic matter released by marine phytoplankton (*Thalassiosira* and *Synechococcus*) in different growth stages. Our mass spectrometry techniques provide mass accuracies sufficient to assign elemental formulas to the thousands of organic compounds that were detected and identified. Second, we used the same methodology to track temporal and spatial variability in phytoplankton-derived organic matter in a coastal environment. The comparison of these two data sets provides an unparalleled view on the molecular-level impact of

photosynthesis on dissolved organic matter in marine ecosystems. Since this material provides growth substrates for the microbial loop, variability in its molecular-level composition influences the expression and activity of metabolic pathways by heterotrophic microorganisms.

Abstract Category
17 Marine Microbiology

PS.17.084 COMPARATIVE METAGENOMICS OF BATHYPELAGIC PLANKTON AND BOTTOM SEDIMENT FROM THE SEA OF MARMARA

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To extend early comparative metagenomic analyses of the deep-sea, we produced direct 454 pyrosequence data from bathypelagic plankton (1,000 m depth) and bottom sediment of the Marmara Sea, the gateway between the Mediterranean and the Black Sea. Data from both SSU rRNA gene libraries for archaea, bacteria and eukaryotes, and direct pyrosequencing indicated that Gamma- and Alphaproteobacteria, followed by Bacteroidetes, dominated the bacterial fraction in Marmara deep-sea plankton, whereas Planctomycetes, Delta and Gammaproteobacteria were the most abundant groups in a high bacterial-diversity sediment. Group I Crenarchaeota /Thaumarchaeota dominated the plankton sample, with Group II and III Euryarchaeota also present. Although eukaryotes were highly diverse in SSU rRNA gene libraries, with Group I (Duboscquellida) and II (Syndiniales) alveolates and Radiozoa dominating plankton libraries and Opisthokonta and Alveolates sediment libraries, detectable eukaryotic sequences were scarce in pyrosequence data. Archaeal amo genes were abundant in Marmara deep plankton, suggesting that Thaumarchaeota are ammonia oxidizers, while genes involved in sulfate reduction, carbon monoxide oxidation, anammox and sulfatases were over-represented in Marmara sediment. Genome recruitment analyses showed that *Alteromonas macleodii* "surface ecotype", *Pelagibacter ubique* and *Nitrosopumilus maritimus* were the most represented genomes in 1,000 m-deep plankton. We compared the Marmara metagenomes with other deep-sea metagenomes (ALOHA deep-sea plankton, whale carcasses, Peru subsurface), as well as ALOHA surface plankton and soil metagenomes. Marmara deep plankton clustered with ALOHA deep plankton and whale carcasses, which is likely due to the suboxic conditions reigning in the deep Marmara water column. The Marmara sediment clustered with soil, highlighting the common ecological role of both types of microbial communities in the ultimate degradation of organic matter.

Abstract Category
17 Marine Microbiology

PS.17.085 TRACKING HEAT-LOVERS IN THE COLD: TOWARDS A GLOBAL PHYLOGEOGRAPHY OF THERMOPHILIC SPORE-FORMING BACTERIA IN MARINE SEDIMENTS

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Spores of thermophilic bacteria have been repeatedly found in cold and temperate marine sediments, yet their sources and distribution pathways remain unclear. While these spores are apparently dormant in situ, they readily germinate upon sediment incubation at high temperatures. Passive deposition through sedimentation has been proposed for supplying a physiologically diverse community of thermophilic Firmicutes spores to Arctic fjord sediments (Hubert et al. 2009. *Science* 325: 1541-1544.). The spores presumably originate from warm anaerobic habitats and are passively dispersed through water currents and via sedimentation. Due to their environmental persistence but inability to grow and evolve under unfavorable conditions, and because they can be easily selected for in laboratory germination experiments, thermophilic spores can be a valuable model and bioindicator for studying biogeography and dispersal of microbial cells in the oceans. In this project, the diversity and distribution of thermophilic spores in marine sediments were assessed on global and regional scales. Sediment samples from various locations around the world, including a regional Arctic sample set from the Svalbard archipelago, were amended with organic substrates and incubated at 50°C under anoxic conditions. Shifts in bacterial community composition and activity owing to germination and growth of thermophilic spores were monitored by measuring sulfate reduction rates and by using massively parallel sequencing of 16S rRNA gene PCR products. 25 out of 39 sediments showed considerable sulfate reduction after incubation for 120 hours at 50°C, indicating that spores of thermophilic sulfate-reducing bacteria are widely distributed but are not necessarily ubiquitous. 16S rRNA gene sequences of thermophilic spores persisting in these sediments are being analyzed to reveal patterns in biogeography, providing valuable insights into the extent and limits of microbial dispersal in the marine environment.

Abstract Category

17 Marine Microbiology

PS.17.086 IDENTIFICATION OF POTENTIAL BIOMARKER GENES TO DETECT PHOSPHORUS LIMITATION CONDITIONS IN DIATOMS

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Due to recent publication of the genomes of two diatoms species *Phaeodactylum tricorutum* and *Thalassiosira pseudonana*, the research focus is drawn on the molecular mechanisms which enable diatoms population to successfully adapt to different

ecosystems. In fact, the diatoms are often characterized as a genetic "melting pot" due to the acquisition of genes of different ancestors and by horizontal gene transfer during their evolutionary history. While we have a general understanding of changes in nutrient concentrations that contribute to regulation in diatoms genes, little is known about regulation of phosphorus reduction within diatom assemblages. Here, we focus on the characterization of potential genes involved in P-uptake during phosphorus limitation and on the physiological status of cells. Single species of batch and continuous cultures of diatoms *Phaeodactylum tricornutum* UTCC 162, *Thalassiosira pseudonana* CCMP 1013 have been cultivated with and without phosphorus supply. We measured (1) photosynthesis activity (2) alkaline phosphatase activity, specific enzyme involved in the hydrolyze of organic phosphorus into inorganic phosphorus, detected via the molecular probe ELF® and which is only synthesized during phosphorus shortage and (3) several potential genes involved in P-uptake: e.g. genes coding for P-transporters and a P-binding proteins. In total 12 potential genes in phosphorus nutrient uptake for *T. pseudonana* and 4 potential genes for *P. tricornutum* are being tested in this experiment using quantitative PCR. We chose 3 housekeeping genes TBP, ELF1a and RPS and they all showed a relatively constant expression in different condition. Our analyses of cultures showed that photosynthesis activity decreased in phosphate limitation condition. We are currently analyzing which biomarkers genes among the selected genes are suitable in regulation under phosphorus limitation.

Abstract Category

17 Marine Microbiology

PS.17.087 TEMPORAL DYNAMICS OF *PROCHLOROCOCCUS* ECOTYPES IN THE ATLANTIC AND PACIFIC OCEANS

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To better understand the temporal and spatial dynamics of *Prochlorococcus* populations, and how these populations co-vary with the physical environment, we followed monthly changes in the abundance of five ecotypes - two high-light adapted and three low-light adapted - over a five-year period in coordination with the Bermuda Atlantic Time Series (BATS) and Hawaii Ocean Time-series (HOT) programs. Ecotype abundance displayed weak seasonal fluctuations at HOT and strong seasonal fluctuations at BATS.

Furthermore, stable 'layered' depth distributions, where different *Prochlorococcus* ecotypes reached maximum abundance at different depths, were maintained consistently for five years at HOT. Layered distributions were also observed at BATS, although winter deep mixing events disrupted these patterns each year and produced large variations in ecotype abundance. Interestingly, the layered ecotype distributions were regularly reestablished each year after deep mixing subsided at BATS. In addition, *Prochlorococcus* ecotypes each responded differently to the strong seasonal changes in light, temperature, and mixing at BATS, resulting in a reproducible annual succession of

ecotype blooms. Patterns of ecotype abundance, in combination with physiological assays of cultured isolates, confirmed that the low-light adapted eNATL could be distinguished from other low-light adapted ecotypes based on its ability to withstand temporary exposure to high intensity light, a characteristic stress of the surface mixed layer. Finally, total *Prochlorococcus* and *Synechococcus* dynamics were compared with similar time series data collected a decade earlier at each location. The two data sets were remarkably similar - testimony to the resilience of these complex dynamic systems on decadal time scales.

Abstract Category
17 Marine Microbiology

PS.17.088 ISOLATION OF A NOVEL SULFUR OXIDIZING BACTERIUM

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Members of the marine gamma-proteobacterial sulfur oxidizer (GSO) cluster thrive in oxygen minimum zones (OMZs), where they have been implicated in sulphide detoxification and denitrification. Environmental genomic data from the North Pacific indicate that these chemolithoautotrophic organisms contain genes for carbon fixation, sulfide oxidation, and nitrate reduction, suggesting that they have important roles in marine carbon, nitrogen, and sulfur cycles. We used a high-throughput dilution to extinction approach to isolate the first cultured representative from the GSO cluster. We inoculated 384 cultures containing natural seawater media and media amended with a microbial cell lysate. 35% of the cultures were positive for growth after three week incubations. We obtained more isolates and observed greater diversity in seawater media amended with cell lysates relative to unamended controls. Five cultures contained 16S rRNA gene sequences most closely related to ACRTIC96BD-19 clones from the GSO cluster. Phylogenetic analyses of our type strain (GSO-PS1) indicate that our isolates are closely related to clones from Saanich Inlet and the Benguela upwelling region. Preliminary growth experiments indicate that GSO-PS1 can grow aerobically, has a doubling time of approximately 18 hours, and reaches a maximum cell density of approximately 2.5×10^5 cells/ml. Physiological studies of GSO-PS1 will advance efforts to understand the role of this important group of organisms in marine biogeochemical cycles.

Abstract Category
17 Marine Microbiology

PS.17.089 THE ROLE OF BACTERIOPHAGE INTERACTIONS DURING THE INTENSIVE AQUACULTURE PRODUCTION

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The bacteriophages are the most abundant biological particles in nature; they affect the abundance of bacteria and the flow of matter and energy in the marine environment, also they are important players in the bacterial evolution. However, their effect in the intensive aquaculture systems is unknown. In this study we evaluated the presence of bacteriophages during the intensive rearing of shrimp larvae and its effect on: 1) the modulation of the metabolic capabilities of indigenous bacteria (2) the modification of the virulence of some bacterial species and (3) the performance of probiotic bacteria. We found that in the intensive shrimp culture tanks there is an abundant bacteriophage population closely associated with the shrimps, which includes both lytic and lysogenic phages, specific for most of the isolated bacteria. Some isolated phages induce significant changes in the bacterial ability to use some resources. Also, we isolate phages that increase the virulence of *Vibrio parahaemolyticus* and *Vibrio alginolyticus*; which in part explains the uncertain role of these bacteria into the aquaculture systems. Finally, we isolated some lytic phages that specifically recognize the probiotic bacteria. It explains why sometimes the probiotics fails to improve the aquaculture production. The results from this study therefore support the need to modify the classic paradigm where the changes in the bacterial populations are explained only through the environmental changes.

Abstract Category
17 Marine Microbiology

PS.17.090 THE DISTRIBUTION OF CHEMICALS OF CONCERN IN RECREATIONAL WATERS DOES NOT CORRELATE WITH COMMONLY USED MICROBIAL INDICATORS OF WATER QUALITY

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The beach waters of Presque Isle State Park are used by millions of recreational swimmers annually, yet little work has been done to determine if biotic and/or abiotic factors are present in these waters with the potential to damage aquatic wildlife and compromise human health. Towards this end, we undertook a study in the summer of 2009 to test for four chemicals of concern that are emerging in recreational waters, which includes fluoxetine (anti-depressant), triclosan (anti-bacterial), diuron (herbicide), and ethynylestradiol (synthetic hormone). Using ELISA based assays, we detected the presence of all four chemicals in all water sampled. The concentrations of these chemicals were at or just below what is known to be harmful for aquatic wildlife. Further analysis indicates no relationship between the concentration of these chemicals in a water sample to fecal indicator microbes which are traditionally used to assess water quality. Moreover, we find that these chemicals can directly influence bacterial and phage populations, thus altering the microbial balance in this freshwater ecosystem. The results of this study and its implication to beach water management will be presented.

Abstract Category
17 Marine Microbiology

PS.17.091 SEASONAL MICROBIAL COMMUNITY DYNAMICS WITHIN THE MOOREA CORAL REEF LONG TERM ECOLOGICAL RESEARCH SITE

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The Moorea Coral Reef Long Term Ecological Research (LTER) Site (17.50 S, 149.83 W) is comprised of the fringe of coral reefs and lagoons that surround the volcanic island of Moorea in the society islands of French Polynesia. As part of the Microbial Inventory Research Across Diverse Aquatic LTERs biodiversity inventory project, we conducted a survey of the bacterial, archaeal and eukaryotic communities along a transect from inshore to the open ocean to study the effect of the island and reef on the microbial populations and their geochemical environment. In collaboration with the University of California at Santa Barbara and the Sea Education Association, we conducted comparisons of microbial populations and their physical and chemical environment to assess the impacts of anthropogenic inputs on near- and offshore microbial community structure. These included the identification of landscape-scale patterns of diversity and richness from near-shore, lagoon and offshore microbial communities within all three domains, signature communities representative of specific geographic and geochemical milieu, and the co-occurrence of specific groups within the ecosystem. Microbial populations from 8 sites along our transect were assessed in two seasons by sequencing a total of >530,000 V6 (bacterial and archaeal) and >66,000 V9 (eukaryotic) small-subunit ribosomal RNA gene sequence tags using 454 pyrosequencing technology. In initial beta-diversity analyses, we found that comparisons of communities grouped into Operational Taxonomic Units (OTUs) and compared by Bray-Curtis dissimilarity were far more effective in resolving differences in community diversity than community taxonomic comparisons. We found a strong correlation between geographical region and microbial community structure, including several bacterial groups that persist in geographical niches across seasons, and the dominance of few OTUs accompanied by a large number of diverse, less-abundant taxa.

Abstract Category

17 Marine Microbiology

PS.17.092 ULTRASTRUCTURAL CHARACTERIZATION OF BLACK BAND DISEASE INFECTING CORALS OF THE *MONTASTRAEA ANNULARIS* SPP. COMPLEX

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Coral diseases are considered to be a significant factor in the decline of coral reefs worldwide. Of these, black band disease (BBD) is thought to be one of the more ecologically relevant. Though discovered in 1973, the primary pathogens of BBD and their sources are still being debated. While the mechanisms governing BBD pathobiology are slowly being elucidated, little is known about pathogenesis and the progression of this

polymicrobial disease. The purpose of the current study was to use scanning (SEM) and transmission (TEM) electron microscopy to build upon current understanding of the infection process by examining infected fragments of the *Montastraea annularis* spp. complex. Gliding, filamentous cyanobacteria dominated the BBD mat and several morphotypes were apparent. Many were actively boring at the time of fixation. Extensive boring was observed in the exposed coral skeleton behind the band. Cyanobacteria were observed to penetrate coral tissue from within the skeleton and to have glided through the mesoglea, separating coral epidermis and gastrodermis. Cyanobacterial filaments migrated out of bundles of sloughed off epidermal tissue ahead of the forward edge of the band. Filaments migrating into the oral disk of polyps indicated a possible mechanism of the initial colonization of the coral skeleton by cyanobacteria. Degraded gastrodermis with clusters of actively dividing zooxanthellae were also observed. Twisted cyanobacterial filaments, characteristic of negative-tactic responses, were prevalent. Some evidence of boring was present in apparently healthy (control) coral fragments, however no cyanobacteria were detected. Our results suggest that the BBD microbial mat may initiate from cyanobacteria within the skeleton of apparently healthy corals, as it is known to be a significant repository of boring cyanobacteria in shallow waters. The migration patterns suggest mechanisms by which BBD migrates across and through coral tissue and skeleton.

Abstract Category
17 Marine Microbiology

PS.17.093 SUPPLY AND DEMAND OF METHANOL IN THE MARINE ENVIRONMENT: METHANOL PRODUCTION FROM PHYTOPLANKTON AND ITS CONSUMPTION BY EPIBIOTIC BACTERIA

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Methanol is a major atmospheric gas on our planet and an important carbon and energy source for abundant methylotrophic bacteria. Although well studied in terrestrial ecosystems, marine sources of methanol are currently unknown. By employing Purge and Trap Mass Spectrometry/Gas Chromatography (P&T-GC/MS) we have discovered that cyanobacteria of the genus *Trichodesmium*, dominant in tropical and subtropical oligotrophic waters, are capable of producing methanol in the range of 1 - 10 micromolar in a culture containing $1 - 5 \times 10^4$ cells/ml (about 0.02 - 1 picomoles methanol per cell). Interestingly, the highest amounts of methanol were measured during early log phase growth. Cultivation-based approaches of *Trichodesmium* community epibionts have yielded pure cultures of *Methylophaga marina* as well as other facultative methylotrophic bacteria. An ongoing comparative metagenomic project of wild *Trichodesmium* communities is yielding additional insight into the flux of methanol, and potentially other single-carbon molecules, and will be discussed. Additionally, preliminary P&T-GC/MS survey results suggest methanol production is a common trait among cyanobacteria and other phytoplankton, suggesting methanol could play a broad role in the relationship between host and epibiont. This current work will provide a foundation of how methanol

is produced and consumed in the marine environment, an important and underexplored phenomenon in water-column biogeochemical cycling.

Abstract Category
17 Marine Microbiology

PS.17.094 ABUNDANCE AND DIVERSITY OF EUKARYOTIC MICROBES IN THE DEEP ATLANTIC INCLUDING THE ROMANCHE FRACTURE ZONE

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We know relatively little about the abundance and ecological role of eukaryotic microbes in the deep sea. Enumeration is complicated by the fact that some eukaryotes are as small as some prokaryotes and may easily be missed in counts using traditional fluorochromes. On the other hand, using fluorescence in situ hybridization (FISH) with universal probes may exclude organisms with unknown sequences and abundances may thus be underestimated. Employing a combination of DAPI staining and catalyzed reporter deposition fluorescence in situ hybridization, we distinguished a variety of morphological types of eukaryotic microbes present in the deep tropical and subtropical north Atlantic. Samples were also taken on a transect across the tropical Atlantic, and collected as deep as 7000 meters in the Romanche Fracture Zone, the deepest location in the Midatlantic Ridge. Using FISH probes for some broad taxonomic groups revealed that the relative distribution of organisms from FISH counts are different from what was expected from published 18S rRNA gene clone libraries. Kinetoplastids and fungi, for example, were more abundant in the FISH counts than expected from previous studies. Alveolates, on the other hand, were present but much less prevalent than expected. Eukaryote abundance data were compared with other biological, chemical, and hydrographic data collected simultaneously, including macroscopic particles (>500 μm).

Abstract Category
17 Marine Microbiology

PS.17.095 GENOMIC SEQUENCE OF AMMONIA-OXIDIZING ARCHAEA FROM LOW-SALINITY SEDIMENTS IN THE SAN FRANCISCO BAY ESTUARY

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Mesophilic Crenarchaea are among the most abundant microorganisms on Earth and may significantly impact the global carbon and nitrogen cycles. We sequenced the genome of ammonia-oxidizing archaea (AOA) in an enrichment culture from low-salinity sediments in San Francisco Bay, using a combined single-cell and metagenomic approach. The genome is ~1.77 Mb with >2100 genes and a G+C content of 32%. Across the entire genome, the average nucleotide identity to *Nitrosopumilus maritimus* is <80%,

suggesting this AOA represents a new species or perhaps genus of mesophilic Crenarchaeota. The low salinity AOA appear to have an ammonia oxidation pathway distinct from AOB. The genome lacks homologues for hydroxylamine oxidoreductase or cytochromes *c₅₅₂* and *c₅₅₄*, which are also missing in genomes of *Cenarchaeum symbiosum* and *Nitrosopumilus maritimus*. The genome codes for numerous copper-binding proteins, including multicopper oxidases and small blue copper-containing proteins, which are likely important components of the electron transfer system. The AOA appear to use the 3-hydroxypropionate/4-hydroxybutyrate pathway for autotrophic carbon fixation. In contrast to other described AOA, these low-salinity AOA may be motile based on electron microscopy evidence of flagella, as well as numerous motility-associated genes in the genome (including Flp pilus assembly, flagellar assembly, and chemotaxis genes). Cultivation and genomic sequencing of environmentally relevant AOA allows for in-depth studies on the physiological and metabolic potential of this novel group of organisms that will ultimately advance our understanding of the global carbon and nitrogen cycles.

Abstract Category
17 Marine Microbiology

PS.17.096 TRANSCRIPT PROFILING OF A LARGE PHYTOPLANKTON BLOOM IN PUGET SOUND

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Puget Sound is a diverse ecosystem with a wide variety of marine and estuarine habitats on a small spatial scale that sees frequent harmful algal blooms, particularly in early fall. We conducted a metatranscriptome sequencing in Puget Sound, focusing on eukaryotic microbes, particularly eukaryotic phytoplankton, in the early fall of 2007 when diatoms and dinoflagellates blooms are known to occur frequently. Using 454 sequencing of cDNA populations sampled from five different sites representing the major ecological niches, we generated an average of 200,000 environmental cDNA reads from each site. To examine the microbial diversity among the sampling sites, we used a phylogenomic approach to predict the taxonomic affiliation and functional and structural annotation. Our data show a gradient in the composition of the phytoplankton blooms from diatoms to dinoflagellates in the south-north direction. We observed a major shift in the transcript profile from Great Bend, which was dominated by diatoms, to Hein Bank, which was dominated by dinoflagellates. During the sampling time at Trip Junction, in eastern Puget Sound, we did not detect any phytoplankton blooms and the collected phytoplankton cells appeared stressed. Indeed, our data indicate only limited traces of dinoflagellates and diatoms transcripts in this region. However, we identified a large population of marine copepods transcripts, mostly encoding motor-proteins such as myosin and tropomyosin. Interestingly, in the same region, we found an up-regulation of genes transcribed by double-stranded DNA viruses. Likely, the presence of viruses and a large population of grazers led to the termination of the phytoplankton blooms prior to our sampling. Here,

we will present our results highlighting the dynamics of the phytoplankton blooms in the Puget Sound and the impact of environments stressors and the interactions among the microbial populations on the dynamics of these blooms.

Abstract Category
17 Marine Microbiology

PS.17.097 COMMON COMMUNITIES FOUND IN MICROBIAL MATS FROM UNDERSEA VOLCANO HYDROTHERMAL VENTS

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Much interest has been generated by the discoveries of biodiversity associated with undersea volcanoes. These hydrothermal vent systems host a remarkably diverse range of unusual microbial habitats, from black smokers rich in hydrogen sulfide to cooler, diffuse iron-rich vent sites. As such, these hydrothermal vents potentially represent hotspots of microbial diversity, yet our understanding of these ecosystems is still limited. Here we discuss our recent work on the detection and dissection of these microbial communities and the observation that specific community types may be indicative of specific geochemical scenarios, such as those associated with iron and sulfur cycling. These observations are based on the metabolisms predicted by phylogenetic characterizations exhibited by the dominant populations (or ecotypes) found within these microbial communities as compared to the closest related isolate found in culture. Therefore, these studies combine the use of both cultivation-dependent and -independent analyses. Cultivation-independent studies were primarily completed using cloning and sequencing techniques targeting small subunit ribosomal genes (SSU rDNAs) along with similar molecular tools like Terminal-Restriction Fragment Length Polymorphism (T-RFLP) and Quantitative-PCR (Q-PCR), which allow for the determination and discrimination of phylotypes. We conclude that the newly discovered *Zetaproteobacteria* and the *Epsilonproteobacteria* are the most common chemoautotrophic members of hydrothermal habitats associated with undersea volcanoes.

Abstract Category
17 Marine Microbiology

PS.17.098 THE DISTRIBUTION OF EXTRACELLULAR CELLULASE ACTIVITY IN MARINE EUKARYOTES, THRAUSTOCHYTRIDS

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Thraustochytrids (kingdom Chromista, class Labyrinthulomycetes, family Thraustochytriaceae) are heterotrophic unicellular protists and occur in marine and

estuarine waters. Thraustochytrids have the ability to decompose marine detritus by means of extracellular hydrolytic enzymes. Cellulase production by thraustochytrids is particularly important in the process of degradation of refractory leaf litters. To evaluate the role of thraustochytrids in the process of degradation of cellulosic materials in the marine ecosystem, it is essential to know their degradative capability to cellulose. The cellulolytic enzyme activity of 19 thraustochytrid strains representing 9 genera was determined using carboxymethyl cellulose (CMC) as a substrate. Thraustochytrids were grown in a liquid medium for 5 days. Wells were cut in the 0.2% CMC agar plates and cell-free culture supernatant was introduced into the well. After incubation, the plates were stained with 0.1% Congo red solution, which stains the undigested CMC. The increment of the clear zone diameter was recorded. CMC hydrolysis was observed for 14 out of the 19 strains examined; these belonged to the genera *Aplanochytrium*, *Botryochytrium*, *Oblongichytrium*, *Palietchytrium*, *Schizochytrium*, *Sicyoidochytrium*, *Thraustochytrium* and *Ulkenia*. On the other hand, cellulolytic enzyme activity was not detected in any strains belonging to the genus *Aurantiochytrium*. As demonstrated in this study, the ecological importance of thraustochytrids in marine food webs lies in their cellulolytic ability as well as in their food value for higher trophic levels. Because thraustochytrids typically have the ability to accumulate a high amount of polyunsaturated fatty acids (PUFA) in the cells, thraustochytrids as a food source may contribute to the transfer of PUFA towards higher trophic levels in marine food webs, particularly phytodetritus-based food webs.

Abstract Category

17 Marine Microbiology

**PS.17.099 SIGNIFICANCE OF HETEROTROPHIC BACTERIAL
PRODUCTIVITY IN CARBON FLUX TO DEEPER LAYERS IN THE BAY OF
BENGAL**

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The highly stratified and shallow mixed layers (<50m) in the northern Bay of Bengal (BoB) with low or no nutrient injections into the surface lead to lowered autotrophic/chlorophyll production compared to similar tropical basins. This stratification is brought about by the lowered surface-salinities due to large influx of freshwater and weak wind forcings that cannot induce upwelling. From a comprehensive study on the biology of BoB that covered different seasons, we observed higher biomasses of bacterioplankton and meso-zooplankton in the regions of localised cold core eddies in particular. The seasonal ranges of bacterioplankton carbon biomass in the top 120m column were similar in the open: central Bay (CB: 104-677 mg C m⁻²) and western Bay (WB: 95-662 mg C m⁻²) regions. The near-coastal WB is supposedly receiving substantial inputs of allochthonous heterotrophic bacteria from the many rivers discharging into it. This input appears far more important in the food web dynamics of the coastal BoB. Bacterial productivity was about similar in its pattern to that of abundance and, ranged from 76 to 220 mg C m⁻² in WB and, 70-248 mg C m⁻² in CB. It

accounted for 43% of primary production in WB and, 56% in CB indicating high BP:PP in both regions. With lower abundance of microzooplankton in the study area, it appears that heterotrophic bacteria play a central role in trophodynamics aiding the nourishment of mesozooplankton as well as in the overall biological productivity in the oligotrophic regions of BoB. Since both abundance and production of bacteria were high in the deeper waters (~1000m) and, there is sizable mesozooplankton biomass, we postulate that there are greater amounts of carbon fluxes into the deep ($\geq 5 \text{ mg C m}^{-2} \text{ d}^{-1}$ at 1000m) and that the sizable biomasses of mesozooplankton in the deep are sustained by heterotrophic bacterial productivity processes.

Abstract Category
17 Marine Microbiology

PS.17.100 A NOVEL MARINE AMMONIA-OXIDIZING CRENARCHAEOTE 'CANDIDATUS NITROSOPUMILUS SP. NM25' ENRICHED UNDER HIGH AMMONIUM CONCENTRATION

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Molecular ecological approaches based on the ammonia monooxygenase alpha subunit (*amoA*) gene have revealed the predominance of ammonia-oxidizing archaea among ammonia-oxidizing prokaryotes in the ocean. In addition, ammonia-oxidizing archaea are generally cultivated at a low ammonium concentration less than 2 mM. The aim of this study is to establish an enrichment culture of a marine ammonia-oxidizing archaeon at a high ammonium concentration. Ammonia-oxidizing enrichment was established at approximately 15 mM ammonium from coastal sand of an eelgrass zone. Fluorescence *in situ* hybridization and denaturing gradient gel electrophoresis analyses revealed that ammonium was oxidized to nitrite by a single population of *Crenarchaeote* '*Candidatus Nitrosopumilus* sp. NM25' in the enrichment culture. Sequence analysis of the 16S rRNA gene revealed that the NM25 was most closely related to '*Candidatus Nitrosopumilus maritimus*' SCM1 with a similarity of 98.5%. Moreover, sequence analysis of the *amoA* gene revealed that the NM25 was less closely related to known cultivable ammonia-oxidizing archaea (<95%) and environmental clones (<97%). These results demonstrated that the ammonia-oxidizing archaeon '*Candidatus Nitrosopumilus* sp. NM25' was a novel species within the *Crenarchaeota* Group 1.1a., suggesting that ammonia-oxidizing archaea distribute not only in low ammonia-containing environments but also in high ammonia-containing environments.

Abstract Category
17 Marine Microbiology

PS.17.101 GENE EXPRESSION PROFILES FOR A MARINE ROSEOBACTER GROWN WITH DIFFERENT PHOSPHORUS SOURCES

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The Roseobacter lineage is one of the most abundant marine bacterial groups in coastal oceans. The numerical and ecological significance of this lineage makes its members, many of which are readily amenable to maintenance in lab cultures, ideal model systems for understanding the physiological capabilities and ecosystem contributions of coastal marine bacteria. From several previous analyses of Global Ocean Sampling data, it appears that roseobacters in the wild harbor a diverse array of genes for metabolizing phosphorus compounds. In this study, we sought to identify those genes/pathways in the heterotrophic Roseobacter *Ruegeria pomeroyi* strain DSS-3. Chemostat cultures of *R. pomeroyi* were maintained under nitrogen-limiting conditions, a situation typically observed in coastal marine systems. Compounds representing three of the major phosphorus classes in marine systems (orthophosphates, phosphonates, and phosphomonoesters) were added as a sole phosphorus source in triplicate chemostats. *R. pomeroyi* gene expression patterns during growth with the three different phosphorus sources were compared with custom oligonucleotide whole-genome microarrays. Surprisingly, several hundred genes exhibited differential expression under each of the phosphorus regimes, suggesting phosphorus source availability may have ramifications for many physiological activities of coastal ocean roseobacters. Gene pathways potentially involved in the transport and metabolism of the phosphonate and phosphoester compounds were identified and included several genes on the *R. pomeroyi* megaplasmid as well as those not previously recognized to be involved in phosphorus metabolism.

Abstract Category
17 Marine Microbiology

PS.17.102 ELUCIDATION OF MATTER AND ENERGY PATHWAYS IN AQUATIC FOOD WEBS

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Bacteria mediate the transfer and recycling of matter and energy throughout aquatic environments. Their activity is constrained by nutrient availability and predator activity; specifically mortality caused by microzooplankton and viruses. Microzooplankton grazing transfers bacterial matter and energy to higher trophic levels whilst viral lysis recycles matter and energy in the microbial loop, therefore the dominant mortality agent exerts a selective influence on community composition and growth thus mediating biogeochemical cycling. As aquatic communities contain diverse assemblages of bacteria in varying states of activity and function there is a need to quantify the impact of grazing and lysis on individual bacterial populations. Dilution experiments were used to quantify total bacterial mortality and the mortality of sub-populations, as defined by flow cytometry. In the system studied viral lysis was the dominant mortality agent, whilst larger bacteria were generally more susceptible to microzooplankton grazing than viral lysis. We also found that each constituent of the bacterial community exhibits a specific mortality pattern, with viral lysis and microzooplankton contributing differently to the

mortality of each bacterial sub-population. This demonstrates that movement of energy and matter is influenced by the bacterial community present at the time and the overall impact of bacterial mortality on aquatic systems is more complex than initially envisioned. This research further defines matter and energy pathways, determines how mortality impacts upon a whole system and enhances understanding of the role of microbial structure and function in identifying fluxes of matter and energy in aquatic systems.

Abstract Category
17 Marine Microbiology

PS.17.103 ISOLATION OF A MARINE BACTERIOPHAGE INFECTING STRAIN IMCC1322 OF THE SAR116 CLADE AND ITS COMPLETE GENOME SEQUENCE

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The SAR116 clade in the Alphaproteobacteria comprises approximately 5% of the total bacterial community in the ocean surface. Here we report isolation and the genome sequence of a bacteriophage that infects a representative strain of the SAR116 clade, strain IMCC1322. IMCC1322phage#1 was recovered from a surface seawater sample collected from the East Sea where the host was isolated, and the viral genome was analyzed using whole genome shot gun sequencing. IMCC1322phage#1 had double-stranded DNA genome of 55,282 bp and it harbored modules of genes for replication machinery and structural proteins. In addition the genome contained putative genes for Rieske (2Fe-2S) domain protein, phosphoglycerate mutase, L-gulonon-gamma-lactone oxidase, and transcriptional regulators. Based on DNA polymerase phylogeny and transmission electron microscopy, it is suggested that IMCC1322phage#1 belong to an unassigned Podoviridae group, distantly related to other marine bacteriophage isolates such as Roseophages SIO1, DSS3phi2 and EE36phi1.

Abstract Category
17 Marine Microbiology

PS.17.104 AUTOMATED SAMPLE COLLECTION AND METATRANSCRIPTOMIC ANALYSIS OF MARINE COMMUNITIES USING THE ENVIRONMENTAL SAMPLE PROCESSOR

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Community transcript sequencing, or metatranscriptomics, can be used to describe environmental gene expression and the response of microbial populations to environmental stimuli. Such studies are well suited to the use of automated sampling systems. Using the Environmental Sample Processor (ESP) platform, we have developed

a protocol for automated collection and fixation of marine microbes that generates samples suitable for metatranscriptomic analysis. As proof of concept, the ESP was used to collect and fix a surface water sample, which was then retained on the instrument during a 29 day deployment at Monterey Bay (~10°C). This sample, along with a sample collected in parallel by vacuum filtration and flash frozen, was analyzed by metatranscriptomics. Both samples yielded comparable transcript abundance profiles when sequenced using the FLX genome sequencing system (Roche). We used the ESP platform to examine short-term variability in transcript abundance profiles from Monterey Bay surface waters. A moored ESP collected samples for metatranscriptomic analysis at four time points on June 2, 2009. Taxonomic analysis of sequenced transcripts revealed distinct microbial populations associated with the distinct water masses sampled at each time point. Additionally, individual microbial groups showed changes in transcript abundance that likely represent responses to changing conditions. For example, two transcripts associated with anoxygenic CO₂ fixation in *Polaribacter* showed increased abundance (relative to other *Polaribacter* transcripts) at 10am vs. 5am, matching published observations of increased CO₂ fixation in light-exposed *Polaribacter* sp. MED152 cultures. Our results indicate that the ESP platform can successfully collect and store samples suitable for metatranscriptomic studies, thereby providing a new, high-resolution tool for observation of in situ changes in microbial gene expression and community composition.

Abstract Category

17 Marine Microbiology

PS.17.105 THE BIOGEOGRAPHY OF *SYNECHOCOCCUS* BASED ON THE NARB GENE AND CHANGES IN NARB GENE EXPRESSION IN EXPERIMENTS WITH COASTAL ISOLATE CC9311.

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Marine *Synechococcus* are small (typically <2µm in cell diameter) unicellular cyanobacteria common to coastal and open-ocean waters. A large diversity of *Synechococcus* exists in the marine environment, yet their roles in N cycling are not well characterized. The *narB* gene (encoding for assimilatory nitrate reductase) was used to examine the biogeography and potentially the activity of nitrate-assimilating *Synechococcus*. *narB* qPCR (quantitative PCR) assays were developed to target 7 *Synechococcus* sub-populations and applied to depth profile samples from biologically different regions of the California Current System (CCS). The *narB* sub-populations had distinct distributions from each another with high abundances occurring in waters of low, intermediate or high nutrient concentrations. This distribution of *narB* sub-populations suggests that the populations differ in physiology. Ultimately, *narB* gene expression could be used to examine the relative roles of these populations in nitrate assimilation. *narB* gene expression (examined by reverse transcription qPCR, RT qPCR) was investigated in a culture of *Synechococcus* isolate CC9311.. The relationship between *narB* mRNA levels and growth conditions (light and N source) in *Synechococcus* isolate

CC9311 cells grown on nitrate-replete media exhibited a diel pattern similar to expression of *cbbL* (encoding for the large RuBisCO subunit) over a 12hr light, 12hr dark period. A 10 fold up-regulation of the *narB* gene was observed in CC9311 cells grown on nitrate relative to CC9311 cells grown on ammonium. Up-regulation of *narB* gene expression also occurred under N deprivation. Results are important for determining sampling strategies for gene expression in the environment. Probing for *narB* expression in the natural environment will be most effective when sampling times are kept consistent and a genetic indicator of N deprivation (e.g. *ntcA* or *cbbL*) is examined.

Abstract Category

17 Marine Microbiology

PS.17.106 ARCHAEAL AND BACTERIAL DIVERSITY ISOLATED FROM A MARINE SOLAR SALTERN OF THE YELLOW SEA NEAR GOMSO BAY, KOREA

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The population density and diversity of Archaea and bacteria from a marine solar saltern in Korea were analyzed compared by polymerase chain reaction (PCR)-based molecular phylogenetic techniques and cultivation approaches. The samples originated from a crystallization pond of solar saltern of Gomso bay in Korea and collected in summer 2009. The salinity of samples were determined with a refractometer. The samples were serially diluted and were plated onto Marine media and incubated at $25 \pm 2^\circ\text{C}$ for 14 days. The population densities of aerobic heterotrophic bacteria were in the range of $3.5 \pm 1.0 \times 10^3$ cfu ml⁻¹ during the sampling period. For the total DNA extraction of archaea and bacteria, samples were filtrated through 0.2 μm and 0.45 μm membrane filters, respectively. The 16S rDNAs were amplified by using oligonucleotide primer complementary to 16S rDNA and purified PCR products were cloned into pGEM-T vector. And 100 clones of Archaeal group and 100 clones of Bacterial group were selected and analyzed by 16S rRNA gene target sequence. In the case of Archaea, all sequences recovered from environmental DNA grouped within the *Halobacteriaceae*. The identified sequences fell into the genera *Halorubrum* (37%), *Haloarcula* (23%) and *Halobacterium* (2%). In contrast, among identified clones (88 isolates) of Bacteria, 39 isolates belong to *Gamma-proteobacteria* group, 19 isolates belong to *Alpha-proteobacteria* group, 5 isolates belong to *Firmicutes* group and other bacteria belong to *Actinobacteria*, *Beta-proteobacteria* and *Bacteroidetes* group, respectively, and 22 isolates were unclassified.

Abstract Category

17 Marine Microbiology

PS.17.107 METAGENOMIC MONITORING OF MICROBIAL COMMUNITY SHIFTS AND DIOXYGENASE GENES IN A KOREA TIDAL MUDFLAT

CONTAMINATED WITH CRUDE OIL SPILL

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On December, 2007, Uihang tidal mudflat area, west coastline of South Korea, was seriously impacted by a huge spill of crude oil. In this study we attempted to monitor microbial community shifts and dioxygenase genes in respond to the degradation of crude oil in Uihang tidal mudflat. To capture the diversity of microbial populations in the tidal mud flat, titanium pyrosequencing was used. The numbers of sequenced 16S rRNA gene amplicons were 16,585 (the 0th month), 15,596 (the 4th month), 11,701 (the 6th month), 17,166 (the 13th month), and 12,516 (the 16th month). The concentrations of TPH (total petroleum hydrocarbons) continuously decreased in the tidal mud flat sediment. The following n-alkane analysis indicated that the n-alkane composition was significantly shifted by the time, i.e., longer chain alkanes (C28-C34) were significantly degraded to shorter chain alkanes (C12-C28). It was found that the microbial diversity was significantly decreased at the 4th month due to the oil spill, and that the diversity was gradually getting recovered after the 6th month. A further analysis on the bacterial populations revealed that marine petroleum-degradative populations (*Alcanivorax*, *Cycloclasticus*, and *Thalassolituus*) significantly grew at the 4th month. The pyrosequencing results from the dioxygenase amplicons revealed that significantly higher amounts of aromatic dioxygenase genes were detected at the 4th month. MG-RAST analysis showed that those were related to benzoate degradation, benzoate catabolism, and phenylpropanoid compound degradation. These suggest that the indigenous marine petroleum-degrading populations played a key role in detoxifying the petroleum-contaminated tidal mudflat sediment. The microbial detoxification provided positive effects in recovering the microbial diversity; however, the community structure was not recovered to the original status.

Abstract Category

17 Marine Microbiology

PS.17.108 MICROBIAL COMMUNITIES IN THE COASTAL MARINE SEDIMENT INFLUENCED BY MELTWATER RUNOFF DURING DEGLACIATION IN SVALBARD, ARCTIC CIRCLE

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Increases in global temperatures cause enhanced glacier melting in Arctic area, which transports nutrients, microorganisms and soil particles to coastal area. We studied the effect of meltwater runoff on the microbial communities of coastal marine sediment along a transect of Temelfjorden in Svalbard. As close to the glacier-proximal zone, the

sediment properties were changed gradually which is strongly corresponding with the microbial community changes as analyzed by 16S rRNA gene PCR-denaturing gradient gel electrophoresis. Diversity indices based on the analysis of 16S rRNA gene clone libraries suggested that microbial diversity was higher in the glacier-proximal zone (station JM188) directly affected by the runoffs than the outer fjord (station JM176) distant from direct runoffs. Further, differences in microbial community composition between the station JM188 and JM176 were evident. Although the crenarchaeotic group I.1a (CGI.1a) was the only abundant group in the station JM176 (62%), CGI.1a was decreased to 32% and other archaeal groups (MCG and MBGB) typically observed in subsurface seafloor and presumed to be heterotrophic became proportionally abundant in the station JM188. Clones of alpha-Proteobacteria and Flavobacteria lineages were dominant (60%) in JM188. In contrast, clones of delta-Proteobacteria (mostly sulfate-reducers) were dominant (32%) in the station JM176. Our results suggest that aerobic heterotrophic microorganisms are abundant in the station JM188. Due to the low primary productivity in water column and high sedimentation rate of soil particles, the sediment of melting glacier-proximal basin harbors unique microbial communities.

Abstract Category

17 Marine Microbiology

PS.17.109 BACTERIAL COMMUNITIES ASSOCIATED WITH DIGESTED PIGGERY EFFLUENT AS A PRIMARY FOOD SOURCE FOR THE BATCH CULTURE OF THE ZOOPLANKTON, MOINA AUSTRALIENSIS

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The use of wastewater grown zooplankton is gaining global attention to wastewater treatment. However, zooplankton culturing is problematic and a decrease in zooplankton abundance is noted with increase in pH due to high phytoplankton activity and raised un-ionised ammonia (UAN) concentrations. The replacement of algae with bacteria as the primary food source could help address the problem associated with high pH induced UAN levels. In this study, a native cladoceran planktonic invertebrate, *Moina australiensis* was cultured on digested piggery wastewater containing bacteria. The purpose of study was to test the viability of digested piggery effluent and its associated bacterial populations as an alternative food source for the commercial production of *M. australiensis*.

The suitable effluent total ammonia nitrogen concentration (TAN) for the culture of *M. australiensis*; six levels set 10mg/l-1 to 60mg/l-1 were studied. Microbial profiling and quantification of the associated bacterial populations in the wastewater culture media and within the digestive tract of *M. australiensis* was conducted by denaturing gradient gel electrophoresis (DGGE) and real-time PCR.

The highest total *M. australiensis* biomass production of 0.94 ± 0.47 g and the rate of population increase (*r*) of 0.15 ± 0.08 was recorded in the 30mg l⁻¹ TAN concentration treatment with the lowest 'r' values and decreased biomass production with increasing TAN concentration levels. DGGE and sequence analysis showed 97-98% identity of β , γ -Proteobacteria while, real-time PCR indicated 22.0% relative abundance of γ -Proteobacteria within *M. australiensis* revealing feeding specificity of *M. australiensis* towards γ -Proteobacteria.

Increase the understanding of the biomass that could be used for maximum pathogen reduction in wastewater by introducing a suitable number of *M. australiensis* at optimum nutrient conditions resulting in improved water quality.

Abstract Category
17 Marine Microbiology

PS.17.110 IMPACT OF LONG-TERM FERTILIZATION ON COMMUNITY STRUCTURE OF AMMONIA OXIDIZERS IN THE GREAT SIPPEWISSETT MARSH, CAPE COD, MASSACHUSETTS, USA

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Ammonia-oxidizing microbes play an important role in the nitrogen cycle, and may be particularly important in nitrogen-sensitive habitats such as salt marshes, yet many questions remain about their ecology and their response to environmental perturbations. We investigated the effects of long-term nitrogen fertilization on community structure of ammonia oxidizing Archaea and Betaproteobacteria in the Great Sippewissett Marsh, Cape Cod, MA. Sediment samples were collected in July 2009 from replicate plots that have received low, medium, and high levels of fertilizer biweekly during the growing season since 1974. Additional untreated plots were included as controls. Sequences encoding the ammonia monooxygenase genes (*amoA*) from Archaea revealed distinct communities in control and high-fertilization plots, with no shared sequences between the plots. Additionally, richness of archaeal ammonia oxidizers was significantly higher in control plots relative to high-fertilization plots. Conversely, *amoA* sequences from Betaproteobacteria were more similar between control and high-fertilization plots. Terminal restriction fragment length polymorphism analysis of archaeal and betaproteobacterial *amoA* genes, however, suggest distinct communities at control and high-fertilization plots for both archaeal and betaproteobacterial ammonia oxidizers. Ammonia-oxidizing communities from low- and medium-fertilization plots generally overlapped with communities from control and high-fertilization plots. Real-time PCR data of *amoA* genes indicate increased abundance of archaeal and betaproteobacterial ammonia oxidizers in high-fertilization plots relative to control plots in the upper marsh, but no difference in abundance was detected in the lower marsh. Our data indicate significant impacts of long-term fertilization on ammonia-oxidizing communities and provide important insights into the ecophysiology of ammonia-oxidizing Archaea and Betaproteobacteria in salt marshes.

Abstract Category
17 Marine Microbiology

PS.17.111 TESTING SPECIES BOUNDARIES IN MARINE ACTINOMYCETES

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Salinispora is the first formally described, obligate marine actinomycete genus. It is comprised of three species all of which share >99% 16S rRNA gene identity. Previous comparisons of two complete genome sequences from *S. tropica* and *S. arenicola* revealed that most of the differences between the species were concentrated in genomic islands. These islands contained a large number of secondary metabolite and prophage genes with many lines of evidence supporting acquisition via horizontal gene transfer. This study seeks to understand the effects of location and species boundaries on the abundance and composition of genomic islands in six *Salinispora* strains. The strains include representatives of *S. arenicola* from Palau, Fiji, and the Bahamas, *S. pacifica* from Palau and Fiji, and *S. tropica* from the Bahamas. Pairwise comparisons of these six genomes provide the first evidence that genomic islands are species specific and that strains of the same species, even when recovered from distant collection sites, maintain fewer genomic islands than strains of different species recovered from the same collection site. Thus, genes acquired from a local gene pool do not appear to have a major effect on the architecture and composition of genomic islands. It is interesting to note that mobile genetic elements represent the majority of location specific genes. Also, as would be expected if species boundaries are maintained over broad geographical distances, highly similar genes share, on average, 97% within species similarity but only 91% similarity between species. Differences between *Salinispora* species continue to be dominated by genes associated with secondary metabolism and mobile genetic elements further emphasizing the functional importance of these traits and their potential role in ecological divergence.

Abstract Category
17 Marine Microbiology

PS.17.112 ARB & SILVA: ADVANCES IN TOOLS AND DATABASES FOR RIBOSOMAL RNA SEQUENCE ANALYSIS

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The adoption of the ribosomal RNA (rRNA) gene as a phylogenetic marker has revolutionized our view on the biological world. Its ubiquitous use has led to a wealth of over 1.4 million small and large subunit (SSU and LSU) rRNA sequences available

today. While the sequencing depth makes rRNA a solid basis for methods building upon it, the sheer amount of data also poses a great challenge. For over 15 years, the software suite ARB has been supporting scientists in their phylogenetic work. ARB as well as its associated, manually curated databases, have been continuously maintained, enhanced and extended. Today, it is probably the most prominent and comprehensive bioinformatic tool in its field. With the recent major version upgrade to ARB 5.0 usability, speed and scalability were significantly improved. Since 2007, the provisioning of ARB with sequence data is done by the SILVA project. Semi-annually, datasets are released that comprise all publicly available LSU and SSU sequences. The sequences are aligned using the high-throughput aligner SINA, specifically developed for this purpose. All sequences are checked for anomalies and carry a rich set of contextual information, as well as classifications for several taxonomies and the latest validly described nomenclature. With SILVA release 100, the phylogenetic classification of bacterial and archaeal sequences in the Ref datasets has been substantially revised. The SILVA web interface has been redesigned to provide improved online database navigation and simplified downloading of custom subsets of the available sequences.

Abstract Category

17 Marine Microbiology

PS.17.113 GENOME EVOLUTION IN THE SAR11 CLADE OF MARINE BACTERIOPLANKTON

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The SAR11 clade of Alphaproteobacteria is ubiquitous in the global ocean, and is one of the most abundant heterotrophs on Earth. While the accumulation of neutral variation is one likely explanation for the high genetic heterogeneity observed in natural SAR11 populations, ecotypic differentiation is another: some subgroups can be distinguished by evolutionary divergence concomitant with distinct characteristics such as environmental distribution. Previously, the whole genome from one of the first cultivated members of the SAR11 clade isolated from coastal waters of the Northeast Pacific (strain HTCC1062) was characterized. It was unusual in that it was extremely small (1.31 Mbp), included the smallest number of predicted genes and shortest intergenic spacers of any genome from a free-living microorganism, and contained virtually no nonfunctional or redundant DNA. Despite this streamlining, the HTCC1062 genome encoded for nearly all of the core functions of proteobacterial cells, included complete anapleurotic pathways for most cellular constituents, and included nearly complete pathways for amino acid biosynthesis and all but a few cofactors. In this study, six additional genomes from members of the SAR11 clade were compared with HTCC1062 in order to characterize the SAR11 pan-genome and identify the core genetic repertoire that distinguishes SAR11, including two additional strains from the temperate coastal Northeast Pacific, three from the subtropical North Pacific, and one from the Sargasso Sea in the Atlantic Ocean. Despite significant sequence variation (e.g. 16S rRNA gene identities of <88%), the SAR11 genomes were

uniformly small (1.28-1.46 Mbp) and of low %GC (25.0-32.0). Consistent with predictions based on genome streamlining, the SAR11 pan-genome was dominated by genes shared by all members of the clade. Though small, the variable fraction contained genes predicted to contribute to ecological differentiation among SAR11 lineages.

Abstract Category
17 Marine Microbiology

PS.17.114 THE NITROGENASE GENE POOL IN SURFACE WATERS OF THE GLOBAL OCEAN IS DOMINATED BY GENES OF NON-CYANOBACTERIA

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Biological nitrogen (N₂) fixation plays a critical role for primary production and carbon and nitrogen fluxes in the oceans. Cyanobacteria are thought to be the main N₂ fixers (diazotrophs), but recent molecular analyses indicate that non-cyanobacterial diazotrophs are also present and active in the oceans. Data are however sparse, and knowledge on the composition and diversity of diazotrophs in the global ocean is lacking. Using 454 pyrosequencing, a total of 117,440 nitrogenase (*nifH*) sequences encoding 10,099 unique proteins were obtained from twelve surface samples (ten DNA samples and two RNA samples) collected at ten oceanic locations world-wide. The analyses provide a unique overview of composition and diversity of diazotrophic communities in the global ocean and document a striking dominance of non-cyanobacteria, especially of Proteobacteria. Surprisingly, six of the samples contained sequences related to anaerobic N₂ fixers, which are so far thought to be uncommon in the surface ocean. A sample from the Arctic, a region previously unexplored for *nifH*, was dominated by these sequences. Although unicellular cyanobacteria were most prevalent in the two transcript samples, significant transcription of proteobacterial *nifH* was also shown (34% of sequences in one sample). We conclude that non-cyanobacteria account for a substantial part of the global marine *nifH* gene pool and that these genes are at least occasionally expressed. While an equally high importance in oceanic N₂ fixation is not implicit, the drawn conclusion certainly merits further study of the role of these bacteria in N cycling at local, regional and global scales.

Abstract Category
17 Marine Microbiology

PS.17.115 MARINE BACTERIAL COMMUNITIES ALONG THE SÃO PAULO COAST IN BRAZIL

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The oceans harbor a tremendous diversity of marine microbes. Different functional groups of bacteria, archaea, and protists emerge from this diversity to dominate various habitats and drive globally important biogeochemical cycles. Marine microbes are intimately connected to essentially every biological system and oceanic process found in the marine environment. Thus, a community perspective is essential for understanding the distribution and function of microorganisms in Earth's oceans. Advances in DNA sequencing technology now allow previously uncultured organisms to be probed in their natural environment, which has already revealed an almost unimaginable level of microbial diversity. Sequencing of cultured marine microbes and metagenomic surveys of natural environments are beginning to reveal genes related to community interactions. In the present study, viable marine bacteria were quantified from seawater samples collected over a period of twenty months at two São Sebastião sites, on the São Paulo coast, using Marine Agar and spread plate method. A total of 168 isolates of viable marine bacteria were submitted to 16S r-RNA amplification using conserved primers (27F and 1041R). Subsequently, the PCR products were purified and the 16S r-RNA regions sequenced using the 1041R primer. Our results showed that most of these isolates belonged to the genus *Vibrio*, which appeared in all collection months, except December 2006. In addition to the *Vibrios*, eleven other taxa were found — all were Gram-negative bacteria. The most frequent among these eleven taxa were members of the genus *Photobacterium*, *Pseudomonas* and *Yangia*. From these data we observed that the region studied presented a little part of the biodiversity viable bacteria of thousands that exist there. However studies are in progress to compare this biodiversity with the total DNA diversity.

Abstract Category
17 Marine Microbiology

PS.17.116 PHYLOGENETIC DIVERSITY OF CHITINOLYTIC BACTERIA ISOLATED FROM PLANKTON SAMPLES COLLECTED IN THREE MARINE ECOSYSTEMS OF SÃO PAULO STATE, BRAZIL

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Chitinolytic bacteria play an important role in the chitin recycling process. They can be found free-living, associated with the chitinous exoskeleton of animals or associated with particulate detritus in the water column of marine ecosystems. The aim of this study was to evaluate the diversity of chitinolytic bacteria isolated from plankton samples collected from marine environments with different levels of anthropic activity. A total of 263 chitinolytic bacteria were isolated from plankton samples collected in three sites of the Brazilian Coast: "Ubatuba" (low anthropic activity), "Canal de São Sebastião" (medium anthropic activity) and "Baixada Santista" (high anthropic activity). Chitinolytic activity was verified by the presence of clear zones (halo) around the colonies on plates containing minimal medium and colloidal chitin (MMCC). It was selected at least one chitinolytic bacteria in each sample, which showed the largest halo of chitin degradation in MMCC agar. Additionally, were also selected chitinolytic bacteria that exhibited

distinct morphological characteristics of most bacteria isolated in MMCC agar. Thus, a total of 87 chitinolytic bacteria were submitted to partial sequencing of 16S rDNA gene. Plankton samples collected at "Canal de São Sebastião" and "Ubatuba" showed a wide diversity covering 19 genera belonging to three phylum: *Proteobacteria*, *Actinobacteria* and *Firmicutes*. Most isolates from "Baixada Santista" (81%) belonged to the phylum *Proteobacteria*, with high incidence of *Aeromonas* sp. (78%), and 21% consisted of members of the phylum *Firmicutes* (*Paenibacillus* sp. and *Bacillus* sp.). The present work showed that the phylogenetic diversity of chitinolytic bacteria was influenced by the level of fecal contamination (anthropic activity) present in each environment. The highest diversity of chitinolytic bacteria was found in the environment with low and medium anthropic activity.

Abstract Category

17 Marine Microbiology

PS.17.117 ADAPTATION OF SULFATE-REDUCING BACTERIA TO AMBIENT SEDIMENT TEMPERATURES IN POLAR, TEMPERATE, AND TROPICAL MARINE ENVIRONMENTS

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Temperature is an important factor regulating the rate of biological processes and is likely to exert a selective pressure in the environment. The temperature response of carbon mineralization via bacterial sulfate reduction of polar, temperate and tropical marine sediments was studied in thermal-gradient incubation experiments by measuring sulfate reduction rates (SRR) using ³⁵S-sulfate. Sediment slurries were incubated in a thermal gradient between -10°C and +50°C to cover the physiological temperature range of the active sulfate-reducing bacteria (SRB) and the resulting temperature response profiles were used to characterize the competitiveness of SRB, in terms of relative SRR at in situ temperatures, the temperature dependence for energy metabolism of SRB and the correlation of cardinal temperatures of sulfate reduction and sediment temperatures. In polar regions, only temperatures close to the freezing point of the sediment limit the rates of sulfate reduction. In these environments SRB exhibited high metabolic rates of ca 10-17% of maximal potential rates at the in-situ temperature of 0°C. Similar relative SRR in temperate and tropical sediments were only observed at 15-20°C. These observations imply psychrophilic adaptation of polar SRB and the predominance of mesophilic SRB in warmer latitudes. Further examination of the temperature dependency for sulfate reduction using Arrhenius plots revealed that tropical sediments exhibited a more limited metabolic regulation at temperatures below 8-18°C and optimal temperature conditions for sulfate reduction closer to their ambient temperatures. Together, the inspection of the temperature responses for metabolic activity of SRB in marine sediments showed that temperature adaptations of SRB form a continuum with respect to their environmental

latitude, which implies the potential of environmental temperatures for the selection of adaptive physiologies and for evolutionary divergence of microbiota

Abstract Category
17 Marine Microbiology

PS.17.118 INVESTIGATING THE DYNAMICS OF BIOGEOCHEMICALLY-IMPORTANT MICROBES IN MONTEREY BAY WITH THE ENVIRONMENTAL SAMPLE PROCESSOR

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Marine microbial dynamics in coastal zones are controlled by a variety of factors including ocean circulation and river runoff. In Monterey Bay these processes supply limiting nitrogen, which fuels the growth of phytoplankton species including the cyanobacterial species *Synechococcus*, and upwelling events transport ammonia-oxidizing *Crenarchaea* to surface waters. In order to gain an understanding of the interplay between physical, chemical and microbial processes, the Environmental Sample Processor (a robotic instrument which detects genes in situ and relays the data to shore) was deployed at Monterey Bay coastal station M0 over the course of several months in 2009 and 2010. *Synechococcus* populations were monitored via qPCR using a RuBisCO (cbbL), and two assimilatory nitrate reductase probes (narB, group B and E), while *Crenarchaea* were quantified with a 16S ribosomal RNA probe and one specific for the ammonia monooxygenase gene (*amoA*). Spring 2009 was characterized by intense upwelling and although *Crenarchaea* abundances correlated well with nitrate concentration, *Synechococcus* sp. only began to bloom later in the deployment when upwelling ceased, suggesting that during upwelling smaller phytoplankton are outcompeted for nutrients by larger eukaryotic species. By contrast, the fall season was fairly well stratified throughout the deployment and blooms of diatoms and dinoflagellates dominated this sampling period. *Crenarchaea* abundances remained high in the surface waters and *Synechococcus* abundances fluctuated dramatically over the course of the deployment, despite long periods of high stratification. It seems that the fall conditions represent a more steady state, where nutrient supplies are high enough to induce blooms of eukaryotic phytoplankton that can coexist with both *Synechococcus* and *Crenarchaea* for extended periods. We are currently exploring the role of different nitrogen species in fueling the growth of each of these populations.

Abstract Category
17 Marine Microbiology

PS.17.119 VERTICAL DISTRIBUTION OF CULTURED MICROBIAL METABOLIC GROUPS IN TIDAL SEDIMENTS OF THE SEVERN ESTUARY

(UK)

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Marine sediments are referred to as stratified systems characterised by the vertical sequence of electron acceptors available, although particularly in coastal sediments the situation can be more complex with several biogeochemical processes potentially co-occurring (e.g. metal and sulphate reduction). Biogeochemical activities and fluxes have been extensively studied, so have microbial communities using molecular biological techniques. However, for the majority of the detected organisms present in these sediments it is unknown which metabolic pathways they can actually use. In order to get a better insight into the vertical distribution of various metabolic groups and their relation to the geochemical gradients (e.g. SO_4^{2-} , CH_4 , Mn^{2+} , Fe^{2+} , NH_4^+ , volatile fatty acids) most-probable-number counts (MPN) were prepared with sediments from the Severn estuary (UK). A range of different substrate combinations targeting various organoheterotrophic and chemolithotrophic groups (e.g. fermenters, sulphate, nitrate, iron and manganese reducers) was used and MPN series were analyzed chemically and by molecular biological methods (PCR-DGGE, 16S rRNA gene analysis) to elucidate the metabolic activities and phylogenetic identities of the enriched microorganisms. Although MPN counts generally decreased with depth, it appeared that the different substrate combinations peaked (<0.01 to 1.8% of total cell count) at different depths and that they could be correlated to specific geochemical conditions. In addition, several MPN series also yielded unspecific growth. For example methanogens were found in relatively high numbers in almost all MPN series for heterotrophic microorganisms and apparently co-occur with sulfate, iron and manganese reducers. Although our study does not resolve which processes actually occur in the sediment, it has helped to detect specific populations and by analysis of the metabolic products to reveal how they may interact.

Abstract Category

17 Marine Microbiology

PS.17.120 PHYTOPLANKTON HETEROTROPHIC ACTIVITY: EFFECTS OF ULTRAVIOLET RADIATION IN THE ARCTIC AND ANTARCTICA

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Ultraviolet radiation (UVR) is well recognized as an environmental stressor of organisms in aquatic ecosystems. UVR, and specially its shortest wavelength fraction (UVBR, 280-320 nm) causes damage on biomolecules and can also induce changes in dissolved organic matter thus modifying its bioavailability. Several studies have shown that UVR affects bacterial and primary production, but hitherto no study has addressed its effects on

algal heterotrophic activity. We assess the effect of UVR exposure on the uptake of radioactive leucine and dimethylsulfoniopropionate (DMSP) by natural phytoplankton assemblages in Arctic and Antarctic waters. We used a single-cell approach involving microautoradiography and image analysis of samples exposed to three different treatments: full sunlight spectrum (280-700 nm), photosynthetically active radiation (PAR)+UVAR (320-700 nm) and darkness. Pennate and centric diatoms, and autotrophic dinoflagellates and flagellates were examined for their substrate uptake. Our results indicate high algal heterotrophic activities during Arctic and Antarctic summer. Exposure to full sunlight radiation generally inhibited leucine uptake compared to the dark treatment, whereas the response of DMSP incorporation was more variable. In Arctic waters, where DMSP concentrations were high due to a bloom of the DMSP-producer *Phaeocystis globosa*, most of the algae were strongly labeled for DMSP and either stimulation due to PAR+UVA or photoinhibition were detected depending on the studied groups. In Antarctica, however, where lower DMSP concentrations occurred, DMSP uptake was lower and generally stimulated by light, showing no inhibition by UVR. Our results suggest a significant role of polar phytoplankton in the use of labile dissolved organic matter and further confirm the central role that UVR plays in regulating trophic fluxes in the pelagic ocean.

Abstract Category

17 Marine Microbiology

PS.17.121 METHANOTROPHIC COMMUNITY DETECTED BY DNA-SIP AT A BRAZILIAN MANGROVE AREA POLLUTED WITH PETROLEUM DERIVATES

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Methanotrophic bacteria are known for their ability of using methane as the only source of carbon and energy. Brazilian mangroves present large potential for methane production, are highly productive and their microbial community is little studied. In this work, we utilize DNA-Stable Isotope Probing technique associated with DGGE and genomic libraries to link phylogeny to function of methanotrophs from a mangrove chronically polluted with petroleum derivatives. Surface sediment samples were collected at a mangrove area in Bertioga, São Paulo, Brazil. Total DNA was extracted from the sediment and genomic libraries of the 16S rRNA gene and the functional gene *pmoA* were carried out. For SIP experiment, 10 culture flasks were prepared with 5g of sediment each, 30mL of nitrate minimal salt medium, and enriched with 8% of methane (five of them with labeled methane, $^{13}\text{CH}_4$). Every time methane consumption reached about 95%, atmosphere in the flasks was renewed. One flask enriched with labeled and another with unlabeled methane were sacrificed as consumption reached a total of 0.2, 0.7, 1.4, 1.7 and 2.2mmol/flask (t1, t2, t3, t4 and t5). The content of the flasks was centrifuged and DNA extracted from the pellets was submitted to ultracentrifugation and then fractionated into 20 fractions. DGGE was performed with the fractions of t2 and t5. Sediment clone library revealed the presence of bacteria related with mangroves, CH_4

oxidation and hydrocarbon pollution. In SIP experiment, methane consumption started soon after inoculation and there was no difference between the rates for labeled and unlabeled CH₄ enrichments. Labeled DNA recovery started at t₂. DGGE profile showed differences in the community structure considering the light and heavy fractions of DNA for t₂ and t₅. DGGE profiles of all incubation times will help us choose interesting fractions to perform analysis of active methanotrophs throughout genomic libraries and to understand better methane consumption dynamics.

Abstract Category
17 Marine Microbiology

**PS.17.122 MICROBIAL DEGRADATION OF OIL IN MUDFLAT
MESOCOSMS**

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Intertidal mudflats are ecologically important and productive ecosystems supporting diverse wildlife and shell-fisheries. Consequently, it is imperative to determine the effects of oil pollution on these vital habitats. This study was therefore initiated to investigate the responses of a mudflat ecosystem to crude oil pollution. Cores of mudflat sediment from the Colne Estuary, Essex, UK were maintained in simulated tidal mesocosms established in quadruplicate. The mesocosms contained cores contaminated with weathered Forties crude oil and control non-oiled cores. Over 28 days, 0-2 mm and 2-4 mm sections of the cores were sampled at six time points. Hydrocarbon-enrichment cultures from the cores were established and a succession of hydrocarbonoclastic bacteria involved in the initial and later stages of biodegradation was isolated after one and three weeks of incubation. In the tetradecane enrichment cultures for example, *Vibrio* sp. and *Rhodococcus* sp., seemingly absent at the early incubation stage, were isolated at the late stage. A new *Dyella*-related genus was also isolated from the pristane enrichment cultures at the late incubation stage. Changes in microbial community composition as determined by Terminal Restriction Fragment Length Polymorphism analysis of the 16S rRNA genes were influenced equally by incubation time and oil addition. A rapid decrease in both Total Petroleum Hydrocarbon and Total Nitrogen concentrations within 6 days of incubation indicates the presence of an oil-acclimated community. Further studies are underway to identify the individual microbes and the main genes expressed during hydrocarbon degradation.

Abstract Category
17 Marine Microbiology

**PS.17.123 DRIVERS OF BACTERIAL DIVERSITY IN PERMEABLE
CARBONATE AND SILICATE REEF SANDS**

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Permeable sandy sediments, with their high abundance of associated microbes, play an important role in the recycling of organic material and nutrients in oligotrophic warm-water coral reef ecosystems. Previous studies on two adjacent sand types of different mineralogy in a coral reef of the Northern Gulf of Aqaba, Red Sea, demonstrated that biogenous carbonate sands react with significantly higher sedimentary oxygen uptake to the supply of natural particular organic matter than terrigenous silicate sands. This finding was hypothesized to be caused by differences in the community structure of sand-associated bacterial assemblages. In a comparative study using molecular DNA-based techniques and a suite of multivariate statistical tools, we investigated carbonate and silicate reef sands for the effect of mineralogy, as well as season and sediment depth, on the variation in bacterial community structure. Automated ribosomal intergenic spacer analysis revealed pronounced differences in bacterial community structure depending on sand type alone, but also highlighted the importance of seasonal and vertical dynamics in determining sand-specific bacterial diversity patterns. The further combination of fingerprinting profiles with 16S sequence information confirmed these results and identified particularly *Proteobacteria* (*Alpha*-, *Gamma*-, *Delta*-) and *Actinobacteria* as prominent members of the highly diverse communities in both sand types, with specific shifts already detectable on the bacterial class level. Overall, these results suggest that mineralogy, season and sediment depth represent important drivers of spatio-temporal niche differentiation for bacteria in permeable reef sands, and emphasize the importance of carbonate and silicate reef sands as specific microbial habitats.

Abstract Category

17 Marine Microbiology

PS.17.124 ROLE OF *MARINOBACTER ADHAERENS* MOTILITY DURING INTERACTION WITH THE DIATOM *THALASSIOSIRA WEISSFLOGII*

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The sinking of marine aggregates to deeper waters and their subsequent sequestration to the sea floor majorly contribute to the ocean carbon sink. Understanding the processes that influence marine aggregate formation therefore, is of major relevance to further our understanding of the functioning of the oceanic carbon cycle. Biotic processes involved in marine aggregate formation have been reported to be mediated by transparent exopolymeric substances. These are predominantly produced by phytoplankton and bacteria, serve as biological 'glue', and both increase aggregation of particles and stabilize existing aggregates. Results of our recent *in vitro* study showed that the interaction between specific marine bacteria and the diatom *Thalassiosira weissflogii* was

instrumental for increased production of transparent exopolymeric substances and for the formation of marine aggregates. Our current research aims at understanding micro-scale phytoplankton-bacteria interactions in marine aggregate formation. Motility and chemosensing enable bacteria to interact with each other and to colonize surfaces. We hypothesize that particular adaptive strategies play key roles in mediating interactions between marine bacteria and phytoplankton. In order to address this question, we use a genetically accessible bilateral model system consisting of the heterotrophic marine *Gammaproteobacterium*, *Marinobacter adhaerens* and the diatom *T. weissflogii* to specifically investigate the roles of motility and chemotaxis. Herein, the successful generation and phenotypic characterization of two *M. adhaerens* mutants deficient in flagella and Type IV pilus formation is presented. The two mutants will be used to investigate the role of motility in *M. adhaerens* during its interaction with *T. weissflogii*.

Abstract Category

17 Marine Microbiology

PS.17.125 DIVERSITY OF PETROLEUM HYDROCARBON-DEGRADING BACTERIA IN OPEN SEA

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Petroleum hydrocarbons are ubiquitous in marine environments. The knowledge about the bacteria involving in oil-degradation in open sea is still limited, although such bacteria have been exploited intensively in coastal areas. Characterized of an oligotrophic environment with varied conditions, open sea would have unique microbes feeding on hydrocarbons and their derivatives. In the past five years, we surveyed the diversity of these bacteria in three oceans using culture-dependent and culture-independent methods. Quite diverse bacteria assimilating hydrocarbons including alkanes and polycyclic aromatic hydrocarbons (PAHs) were found. In the case of PAHs, bacteria of genus *Cycloclasticus* were found prevalent in deep sea sediments of the Pacific and Atlantic oceans as degraders, and they showed specialty in PAH substrate range and degrading mechanism (Wang et al., *Environ Microbiol* 10(8): 2138; Cui et al., *Environ Microbiol* 10(8):1948). In addition, bacteria of genus *Novosphingobium* were also found as active PAHs degraders especially in deep-sea water columns, and some novel species have been found. In the case of alkanes, bacteria of genus *Alcanivorax* were confirmed to be most ubiquitous in open sea water, both surface and deep layers (Wang et al., *Environ Microbiol*, 2010). Intriguingly, bacteria of *Alcanivorax* also frequently occurred in the PAH-degrading consortia with an uncertain role. Many other bacteria were also found for the first time as alkane or PAH degraders. These results are of interests to decipher the fate of petroleum hydrocarbons especially PAHs in deep sea environments.

Abstract Category

17 Marine Microbiology

PS.17.126 COMBINATION OF *ROSEOBACTER* CLADE AFFILIATED BACTERIA AND PHYTOPLANKTON PROMISED GREATER INHIBITION IN FISH PATHOGENIC BACTERIA

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Nannochloropsis oculata is one of the most important green microalgae and widely used for variety of applications especially for nutrition in aquaculture industry. In this study, the abundance of *Roseobacter* clade, a lineage of α -proteobacteria, is being focused, as some strains are known to produce inhibitory substances against fish pathogenic bacteria. *Roseobacter* clade abundance was being estimated by using fluorescence *in situ* hybridization (FISH). In *N. oculata* culture, *Roseobacter* clade affiliated (RCA) came to be abundant, 14 % of total bacteria compared to 4 % in coastal seawater. Out of the 14 % of RCA, 24 culturable strains were isolated from *N. oculata* culture.

Two different types of media were used to analyze the bacterial interactions: ESM medium, an inorganic medium for *N. oculata* cultivation, and *N. oculata* culture filtrate (NCF) which contained phytoplankton excreted substances. Isolated RCA was challenged against two strains of *Vibrio anguillarum*, *V. anguillarum* M93 (serotype O1, strong pathogenicity) and *V. anguillarum* ATCC 43313 (serotype O9, weak pathogenicity), respectively. In ESM, a fresh inorganic medium for phytoplankton (*N. oculata*), the growth of the both strains was suppressed by RCA strain RO3. Interestingly, in NCF medium, a filtrate of *N. oculata* culture grown in ESM, *V. anguillarum* strain M93 showed a greater suppression of the growth and disappeared completely. In the case of *V. anguillarum* ATCC 43313, the growth was also suppressed but even at the end of the experiment, some cells were still alive and remained culturable.

These results indicated *Roseobacter* clade alone come to be beneficial to larvae rearing in eliminating pathogenic bacteria. Interestingly, the combination of *Roseobacter* clade and phytoplankton reinforced the killing effect of RCA against some *V. anguillarum* strains such as M93.

Abstract Category

17 Marine Microbiology

PS.17.127 FUNCTIONAL DIVERSITY AND EVOLUTIONARY HISTORY OF MICROBIAL SECONDARY LIPID BIOSYNTHESIS

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Bacterial production of long-chain omega-3 polyunsaturated fatty acids (PUFAs), such as EPA (20:5) and DHA (22:6), is constrained to a narrow subset of marine gamma-proteobacteria. The genes responsible for de novo PUFA biosynthesis, designated pfaEABCD, encode large, multi-domain protein complexes akin to Type I iterative fatty

acid and polyketide synthases (herein referred to as "Pfa synthases"). In addition to the archetypal Pfa synthase gene products from marine bacteria, we have identified several homologous Type I FAS/PKS gene clusters in diverse microbial lineages representing 10 phyla. Phylogenomic analysis combined with hierarchical clustering of the enzymatic domain architecture of these synthase complexes reveals a high degree of functional conservation within distinct biosynthetic pathways. Incongruity between functional Pfa synthase clades and taxonomic group membership combined with the lack of orthologous gene clusters in closely related strains suggests horizontal gene transfer (HGT) has contributed to the dissemination of lipid biosynthetic activities across disparate groups. Furthermore, omega-3 PUFA production in marine Labyrinthulids protists (Eukaryota) of the genus *Schizochytrium* is known to proceed via the same FAS/PKS-related pathway providing additional evidence implicating HGT as an important contributor to chemical diversification. Based on the structural organization and functional conservation of the gene products, mutation analysis of targeted genes, and their divergence from core (primary) fatty acid biosynthetic mechanisms, we propose the term "secondary lipid biosynthesis" to describe these recently identified gene clusters and their associated reaction mechanisms - a proposition consistent with the polyketide and non-ribosomal peptide synthase-based "secondary metabolite" vernacular.

Abstract Category

17 Marine Microbiology

PS.17.128 SIP-TRACKING OF PROTEIN SYNTHESIS IN ANAEROBIC METHANOTROPHIC COMMUNITIES

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Black Sea microbial mats, capable of anaerobic oxidation of methane (AOM), were investigated to identify functional proteins, to shed light on the metabolic diversity in this consortium. We used ¹³C and ¹⁵N labelled substrates to follow their incorporation into functional proteins such as the mcrA-reductase (MCR). This enzyme is believed to be a key enzyme in anaerobic methanotrophy coupled to sulfate reduction. For this, homogenates of ANME-1 or -2 dominated mats were fed with ¹⁵N labelled ammonia, nitrate or dinitrogen. All substrates were incorporated in the bulk biomass in amounts of up to several atom percent during the incubation time of six months. Dinitrogen fixation could be observed only at lower levels. MCR subunits were the most prominent proteins in all incubations. Using FPLC followed by elemental analysis, nitrogen incorporation into selected MCR-dominated fractions reached $\delta^{15}\text{N}$ values of 1758‰ for ANME-2 dominated black mats and 9‰ for ANME-1 dominated pink mats. The more active ANME-2 mats incorporated nitrogen substrates faster than the pink mats, where almost no incorporation was observed. RNA-based molecular analyses confirmed the presence of active nitrogen cycling microorganisms. The combination of different stable isotope labelled substrates thus allows us to identify key microbial processes and players in the

complex mat systems, and to track the flow of carbon and nitrogen throughout the AOM community.

Abstract Category
17 Marine Microbiology

PS.17.129 BACTERIAL COMMUNITIES ASSOCIATED TO THE MARINE SPONGES *HYMENIACIDON HELIOPHILA* AND *POLYMASTIA JANEIRENSIS* IN RIO DE JANEIRO, BRAZIL

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In this study we performed a survey of the bacterial communities associated with the Western Atlantic demosponges *Hymeniacidon heliophila* and *Polymastia janeirensis*, based on 16S rRNA sequencing and transmission electron microscopy (TEM). We compared diversity and composition of the sponge-associated bacteria to those of environmental bacteria, represented by free-living bacterioplankton and by bacteria attached to organic particulate matter in superficial sediments. Partial bacterial 16S rRNA sequences from seawater, sediment, and sponges were retrieved by PCR, cloning, and sequencing. Sequences were subjected to rarefaction analyses, phylogenetic tree construction, and LIBSHUFF quantitative statistics to verify coverage and similarity between libraries. Community structure of the free-living bacterioplankton was phylogenetically different from that of the sponge-associated bacterial assemblages. On the other hand, some sediment-attached bacteria were also found in the sponge bacterial community, indicating that sponges may incorporate bacteria together with sediment particles. Rare and few prokaryotic morphotypes were found in TEM analyses of sponge mesohyl matrix of both species. Molecular data indicate that bacterial richness and diversity decreases from bacterioplankton, to particulate organic sediment, and to *H. heliophila* and *P. janeirensis*. Sponges from Rio de Janeiro harbor a pool of novel and exclusive sponge-associated bacterial taxa. Sponge-associated bacterial communities are composed of both taxons shared by many sponge groups and by species-specific bacteria.

Abstract Category
17 Marine Microbiology

PS.17.130 EVALUATING AND APPLYING METHODS FOR THE PRESERVATION AND EXTRACTION OF DNA AND RNA FOR ANALYSIS OF MICROBIAL COMMUNITIES IN MARINE SPONGES

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Preservation and extraction of high quality DNA and RNA from biological samples is becoming increasingly important for genomics, transcriptomics and microbial community analyses. One system of major interest, due to its ecological and biotechnological importance, is the community of microorganisms associated with marine sponges. Here, we evaluated protocols for the preservation and extraction of nucleic acids using samples of the New Zealand high-microbial-abundance sponge *Ancorina alata*. All protocols resulted in sufficiently high DNA and/or RNA (including mRNA) quantity and quality for downstream applications, as assessed via spectrophotometry and other techniques. Denaturing gradient gel electrophoresis (DGGE) analysis of community 16S rRNA gene- and 16S rRNA-derived fragments did not reveal any major changes relating to preservation or extraction method. Optimized methods were successfully tested in three other New Zealand sponges. Our results suggest that choice of preservation and extraction method has relatively little influence on the quality and composition of nucleic acids isolated from marine sponges. One of the optimized protocols for simultaneous extraction of DNA and RNA was applied to study the effect of elevated seawater temperature on the microbial community of the Great Barrier Reef sponge, *Rhopaloeides odorabile*. Sponges were exposed to four different seawater temperatures (27, 30, 31 & 32°C) for two weeks, followed by one week of recovery at 27°C. To detect changes in community diversity and activity both 16S rRNA gene- and 16S rRNA-derived fragments were used to produce DGGE profiles. Since DGGE detects only the most abundant taxa that are present in a sample, 454 tag pyrosequencing was also employed, to detect changes in rare members of the microbial community. In a time of rapidly accelerating environmental change, these results will assist in interpretation of the likely effects of elevated seawater temperature on key symbioses.

Abstract Category
17 Marine Microbiology

**PS.17.131 THE ROSEOBACTER RCA CLUSTER IN THE NORTH SEA —
POPULATION DYNAMICS, PHYSIOLOGICAL AND GENOMIC PROPERTIES**

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The RCA (Roseobacter Clade Affiliated, >98% sequence similarity of the 16S rRNA gene) cluster is the largest cluster of the Roseobacter clade. Its occurrence is restricted from temperate to polar oceans but its prominence has been shown only for the Southern and Arctic Ocean and not yet for temperate seas. Therefore, the aim of this study was to quantify the RCA occurrence in the North Sea, a typical neritic sea of the temperate zone, and to obtain an isolate for further physiological and genomic studies. Analyses by RCA-specific quantitative PCR showed that in the southern and eastern North Sea this phylogenetic lineage constituted up to 21 and 15% of total bacterial 16S rRNA genes in spring and fall, respectively. The RCA abundance was positively correlated to

phaeopigments and chlorophyll, turnover rates of dissolved free amino acids, temperature, and negatively to salinity. In most samples only one 16S rRNA phylotype and in total three phylotypes were detected. An abundant RCA strain, "Candidatus Planktomarina temperata", was isolated from the southern North Sea, applying dilution cultures. This strain has an identical 16S rRNA gene sequence to the dominant RCA phylotype detected and exhibits a growth optimum at 25°C and pH 7.5-7.8. The analysis of the closed genome of this isolate of a size of 3.2 Mb and 3042 genes showed the presence of the entire BChl a operon and of genes coding for flagellation. Comparative analyses with the GOS and other data sets revealed the presence of the RCA-specific BChl a operon in the temperate western Atlantic and the eastern coastal Pacific. Our study shows that the RCA cluster constitutes an abundant group of pelagic Alphaproteobacteria in a neritic sea of the temperate zone and indicates that it plays an important role during decaying phytoplankton blooms. The genomic analyses and comparisons to other roseobacters reveal streamlining and specific adaptations of the RCA genome to the pelagic habitat.

Abstract Category
17 Marine Microbiology

PS.17.132 EXPLORING MICROBES AND METABOLITES FROM THE MARINE SPONGE *HALICHONDRIA PANICEA*

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Marine sponges are well known sources for the discovery of secondary metabolites with potential pharmaceutical applications. The actual producers of these metabolites are often 'hidden' inside the complex sponge microcosm. Thirteen different brominated organic compounds have been found in the sponge *Halichondria panicea* of which the it is currently largely unknown whether they are synthesized by the sponge, the microbiome, or by combined activities of both fractions within the sponge-microbiota conglomerate. To initiate functional microbiomics of the sponge-associated microbiota, we have characterized the bacterial, archaeal and fungal fractions within the microbiota by 454-FLX pyrosequencing of a fragment of the 16S/18S ribosomal RNA gene and compared this to the cultivable community from the sponge. In addition, a large number of sponge-derived isolates retrieved from a broad spectrum of different media were generally screened for the presence of cytotoxic compounds and specifically for the potential to produce brominated organic compounds by using a range of PCR primer sets for the selective amplification of genes encoding the currently known halogenases.

Abstract Category
17 Marine Microbiology

PS.17.133 SPATIOTEMPORAL DYNAMICS OF NITRIFICATION AND DENITRIFICATION IN SEDIMENTS OF AN AGRICULTURALLY IMPACTED ESTUARY

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Elkhorn Slough, a small agriculturally impacted estuary, connects directly to the Monterey Bay National Marine Sanctuary. Nitrogen loading from agricultural and municipal runoff poses a threat to the health of the estuary and the bay. Long-term monitoring of nitrogen fluxes indicate that the sediments of Elkhorn Slough are a sink for dissolved inorganic nitrogen, retaining or removing approximately 50% of incoming nitrate annually. We were interested in characterizing the microbial communities involved in key nitrogen transformations in sediments, and how their activity and abundance varied seasonally. Intact sediment cores were collected monthly for one year at seven sites representing the head, the mouth, and the dominant mudflat habitats. Potential rates of nitrification and denitrification were of similar magnitude and demonstrated a distinct seasonal pattern: highest rates occurred in winter and fall with a distinct decline in late spring and summer. Bacterial ammonia monooxygenase (*amoA*) genes were orders of magnitude more abundant than those of the *Archaea*. Seasonal variations in the abundance of archaeal and bacterial *amoA* closely followed observed changes in potential nitrification rates in less impacted mudflat sites. This response pattern was also observed for *nirK* (encoding Cu-containing nitrite reductase) containing denitrifiers, suggesting a coupling between *nirK* abundance and rates of nitrification. *nirS* (encoding cytochrome-*cdl* nitrite reductase) containing denitrifiers were orders of magnitude more abundant throughout the year but were less closely associated with potential rates of nitrification and denitrification. Close agreement between changes in the abundance of bacterial and archaeal *amoA* genes and potential rates of nitrification throughout the year in the upper estuary suggests that both groups of ammonia oxidizers participate in estuarine nitrification, and respond in similar manner to governing environmental variables.

Abstract Category

17 Marine Microbiology

PS.17.134 SYNECHOCOCCUS DIVERSITY AND DISTRIBUTION IN THE TROPICAL AND SUBTROPICAL OCEANS

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The picocyanobacterium *Synechococcus* is one of the most abundant and widespread phytoplankton in the ocean and contributes significantly to primary production where it occurs. Within this genus there is a large amount of diversity, with 16 potential clades currently defined. However, it is not well understood what adaptations might differentiate the clades physiologically, and how this in situ diversity affects the amount of primary production. We aimed to understand the biogeography of *Synechococcus* in the tropical and subtropical regions of the Atlantic and Pacific oceans by mapping the abundance of

10 clades with a quantitative PCR method and comparing the distribution to physicochemical factors. We found that clade II was the most abundant and widespread of the clades, and was also found over the widest temperature range. Clades III, X, XV, and XVI were all significantly correlated to the presence of clade II; however, while clades III, XV, and XVI were not widespread or abundant, clade X was found at a number of locations in the North and South Atlantic and South Pacific at abundances up to 10^5 cells ml⁻¹. Multi-dimensional scaling (MDS) analyses comparing clade abundance with temperature, NO₃⁻, PO₄³⁻, and dissolved Fe concentrations will be discussed.

Abstract Category

17 Marine Microbiology

PS.17.135 BACTERIOPLANKTON IN THE MARINE CARBON CYCLE - RELATION OF COMMUNITY, ACTIVITY AND CARBON POOLS AT THE LONG TERM STATION HELGOLAND ROADS

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Today's ocean is the largest sink for anthropogenic CO₂ in the global carbon cycle. The influence of bacterioplankton on the biological cycling of CO₂ is manifold and includes the release of CO₂ through organic matter remineralisation as well as the stimulation of aggregate formation and export. Microbial processing of organic matter is affected by physical and biological conditions in the environment. To which extend changes in microbial activity *in situ* are related to changes in the community structure or to changes in metabolic activity itself is largely unknown. Understanding the factors that determine bacterial metabolic activity and their significance for the carbon cycle is important to put possible influence of future changes into perspective. Long term *in situ* investigations are needed to elucidate these factors. Since 1962 microbiological data have been assessed, at the long term sampling site Helgoland Roads (North Sea) representing one of the longest microbiological time series existing today. The record for other biological, hydrological and chemical data is even longer. This study is a first approach to implement investigation of the *in situ* relationship between bacterial diversity analyzed by Automated Ribosomal Intergenic Spacer Analysis, bacterial activity (respiration and extracellular enzymes) and organic matter composition in the surface water. For the transition from autumn to winter 2009, first results show a relation of carbon pools to extracellular enzyme activity that does not coincide with changes in bacterial community composition, suggesting that environmental factors rather than phylogenetic composition determine microbial carbon cycling at this time of the year. In conclusion, under certain conditions, changes in environmental factors might have the potential to change ecological and biogeochemical functions despite leaving the bacterial community rather unchanged.

Abstract Category
17 Marine Microbiology

**PS.17.136 THE PELAGIC BACTERIAL COMMUNITY IN THE GERMAN BIGHT
VARIABILITY ON DIFFERENT TIME SCALES**

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The bacterial community of the pelagic forms the largest biological active surface in the oceans. This system is influenced by a lot of physical and biological regularities. Biological processes such as phytoplankton blooms affect the bacterial community but also daily rhythms play an important role. The ecosystem of the North Sea is also influenced by the tide. The composition and structure of the bacterial community in this system remains still not fully understood. In this study the pelagic bacterial community of the German Bight was observed on different time scales from may to august 2008. Therefore samples were taken daily, within 24 hours and 1 hour to follow the variability. This was investigated via automated ribosomal intergenic spacer analysis (ARISA). The length polymorphism of the highly conserved intergenic spacer region provides a detailed insight into the bacterial community. ARISA profiles were evaluated by non metric multidimensional scaling (nMDS) and with reference to environmental parameters gradient analyses. Environmental parameters such as salinity, temperature or chlorophyll were obtained simultaneously to the sampling with a ferry box system. This study showed the highest variability in summer. Even in an interval of one hour 40 % of the entire variability occurring in a month is gained. Differences in the composition of high and low tide communities were stressed. The results concerning the influence of environmental parameters on the bacterial community revealed that the community in shorter time scales is influenced by gradients others than the community in daily samples. This signifies more variability within the community than assumed. In particular the results have shown that the tidal cycle influences the composition of the bacterial community. New insights into the composition are given which furnish more space for further analyses of their structure via metagenomic methods or clone libraries to reveal members and function.

Abstract Category
17 Marine Microbiology

PS.17.137 THE DYNAMICS AND DIVERSITY OF DIAZOTROPHS IN A COASTAL MICROBIAL MAT

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Microbial mats are usually built by the oxygenic photoautotrophic Cyanobacteria. Cyanobacteria enrich the sediment with organic matter that serves as substrate for other organisms. Many Cyanobacteria are also capable of fixing atmospheric dinitrogen. Cyanobacteria are assumed to be the main diazotrophs in microbial mats. However, the

patterns of nitrogenase activity are often complex and molecular data suggested that diazotrophs other than Cyanobacteria are responsible for the observed activities. We used a high resolution measurement of nitrogenase activity and show that different types of diazotrophs became active at different times. Although Cyanobacteria were the dominant diazotrophs in these mats, Proteobacteria and Bacteroidetes were the second most important. The filamentous non-heterocystous cyanobacterium *Lyngbya aestuarii* is structurally the dominant organism in these microbial mats and also dominated the clone libraries of *nifH*. However, expression of this specific gene was negligible and other types of Cyanobacteria appeared to more important, notably unicellular species. The transcript abundance of *nifH* varied over a day-night cycle. Cyanobacteria were dominant during the day while *nifH* transcripts of Gammaproteobacteria appeared during the night. The latter appeared to belong mainly to the anoxygenic phototrophic Ectothiorhodospira. We quantified the expression of *nifH* of a number of Cyanobacteria and of Gammaproteobacteria. The expression varied over a day-night cycle but we were unable to find a close relationship with the pattern of nitrogenase activity. We conclude that Cyanobacteria were the most important diazotrophs in these microbial mats, and that different types become active under different environmental conditions. We also concluded that the dominant active diazotrophs are not necessarily the dominant structural components of the mat. Finally, we conclude that *nifH* expression does not correspond one to one to nitrogenase activity.

Abstract Category

17 Marine Microbiology

PS.17.138 MICROBIAL-ENVIRONMENTAL NETWORKS AMONG BACTERIA AND ARCHAEA FROM THE ICOMM DATABASE

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Although their importance to global processes is clear by sheer numbers alone, uncovering interactions among marine microbes is difficult. Ocean waters and sediments host thousands of microbial types, and millions to billions of cells per liter. Microbes cannot be directly observed in the same manner as animals and plants, and their dynamic oceanic habitats pose many technical challenges to the study and experimental manipulation of microbial communities. We used a holistic approach to infer ecological

relationships among bacterial and archaeal taxa and environmental parameters in the global dataset from the International Census of Marine Microbes (ICoMM). We related both time-dependent and space-dependent changes in the microbial communities in the water column using statistical correlations which can be lagged in time. The correlations shown in the networks suggested significant ecological relationships among bacteria and archaea, ecological succession within the microbial communities, and allowed for hypotheses on ecological and biogeochemical roles for known and unknown microbes. Examination of microbial-environmental association networks from different environments allowed for the comparison of microbial community structures with implications for the fragility or robustness of specific ecosystems.

Abstract Category

17 Marine Microbiology

PS.17.139 DO INVASIVE ORGANISMS BRING INVASIVE BACTERIA? ANALYSIS OF THE BACTERIAL COMMUNITY ASSOCIATED WITH INVASIVE LIONFISH (PTEROIS VOLITANS)

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The red lionfish (*Pterois volitans*) invasion of the United States Atlantic coast and the Caribbean Sea has continued unchecked since the early 1990's, and their densities are now estimated to be approximately five times greater in the Bahamas than in portions of their native range. Studies have shown that lionfish significantly reduce recruitment of native fish on coral reefs, demonstrating their negative impact on local ecosystems. Evidence suggests that the only predators of this aggressive and venomous fish are grouper, which remain heavily overfished in the invasion range. Without discovery of a control mechanism, lionfish will continue to spread throughout the Atlantic and Caribbean Sea. To determine if this invasive species may be susceptible to a bacterial pathogen that would help control the population, we examined the bacterial communities associated with external and internal tissues of lionfish and native fish species. All fish used in this study were collected during the summer of 2009 from Lee Stocking Island, Bahamas. Total bacterial community DNA was extracted from each tissue sample and the 16S rRNA gene amplified using polymerase chain reaction. Bacterial community structure was assessed using terminal-restriction fragment length polymorphisms, and species level information analyzed through creation of clone libraries. Results showed a significantly different (analysis of similarity, $p = 0.001$) community of bacteria associated with lionfish than native fish (lane snapper, squirrel fish, and white grunt), while internal tissue was not significantly different from external tissue. Identification of a lionfish-specific pathogen may provide insight into a potential control mechanism.

Abstract Category

17 Marine Microbiology

PS.17.140 MICROBES ASSOCIATED WITH SEWAGE EFFLUENT IN DARWIN HARBOUR, AUSTRALIA

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Darwin Harbour is one of Australia's largest deep-water harbours. Every year 10,650 megalitres of sewage effluent is discharged into the harbour through Darwin's five sewage treatment plants. There is a view that because Darwin Harbour is macro-tidal, pollutants will disperse rapidly. Hydrodynamic models have shown however, that the harbour is poorly flushed, and pollutant sources such as industrial discharges including sewage have been identified in recent reports as presenting the greatest management issue for water quality in Darwin Harbour. Water and sediment samples were collected at three impacted sites and a reference site. Samples were analysed for micro pollutants, metals, nutrients and chlorophyll a. DNA was extracted directly from filtrate and sediment, and subjected to 454 pyrosequencing. Nutrients and chlorophyll levels were elevated at sewage discharge sites compared to industrial and reference sites. A number of EDCs were confirmed in effluent and sediment samples from sewage discharge sites only. Metals in water were elevated at sewage discharge sites but were similar in sediments. The microbial communities measured in the water samples were clearly different between sites, and communities measured in sediment samples were also different but not as well defined as for water. At one sewage discharge site, the microbial community was dominated by Pseudomonadales, an order that includes pathogens and antibiotic resistant microbes and is emerging as an important group in similar sewage impacted sites worldwide. We confirmed an association between sewage effluent and microbes, and now have a comprehensive microbial dataset for Darwin Harbour, which will be used to study processes in this environment and derive appropriate microbial indicators.

Abstract Category

17 Marine Microbiology

PS.17.141 ABUNDANCE AND PRODUCTION OF MAJOR PHYLOGENETIC GROUPS OF BACTERIA IN RESPONSE TO NATURAL AND MESOCOSM INDUCED DIATOM BLOOMS

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The western North Pacific (WNP) has been known as a productive region where dense patches of diatom bloom occur during the spring. The aim of this study was to examine the phylotype-specific abundance and production in response to natural and mesocosm induced diatom blooms using single-cell based method, bromodeoxyuridine immunocytochemistry-fluorescence *in situ* hybridization (BIC-FISH). Monitoring by BIC-FISH in the WNP revealed the importance of the Bacteroidetes and *Roseobacter/Rhodobacter* groups as a constantly proliferating basic population (33% and

10% of BrdU-positive cells, respectively), although their contributions were not significantly correlated with water temperature or with chlorophyll *a* or organic matter concentration. However, *Alteromonas* group, known as a numerically minor group in the open ocean, were abundant and were actively proliferating in the bloom stations (up to 18% of BrdU-positive cells). Their abundance and activity correlated strongly with chlorophyll *a* and organic matter concentrations. Mesocosm experiment induced diatom bloom in the water of WNP revealed that Bacteroidetes and *Roseobacter/Rhodobacter* groups were the major contributors to total bacterial abundance and production. Especially, the activity of Bacteroidetes group increased earlier than others just after the bloom peak, indicating that they are the major group initiating to decompose the bloom. The proliferation of *Alteromonas* group showing larger cell-size than others was observed at the peak of the bloom (up to 30% of BrdU-positive cells). Also, the drastic decrease of their abundance probably due to nanoprotozoans grazing was observed immediately after the peak of the bacterial abundance. Their large fluctuation of abundance and activity during the bloom might strongly influence total bacterial production and thus the flux of organic matters in the WNP.

Abstract Category

17 Marine Microbiology

**PS.17.142 TEMPORAL AND SPATIAL DISTRIBUTIONS OF MARINE
SYNECHOCOCCUS (CYANOBACTERIA)**

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Marine *Synechococcus* diversity has been previously described using multi-locus gene sequence phylogenies and the identification of distinct clades. *Synechococcus* from clades I, II, III, and IV and sub-clades within clades I and IV were enumerated from environmental samples by developing a hybridization assay to liquid bead-arrays (Luminex Corporation, Austin, TX). Oligonucleotide probes targeting a gene encoding a subunit of RNA polymerase (*rpoCI*) were used simultaneously in multiplexed assays to track *Synechococcus* diversity from a Pacific Ocean coastal monitoring site and a coastal to open ocean transect in the Southern California Bight. The Luminex assay demonstrated that *Synechococcus* from clades I and IV were the dominant types at the coastal site throughout the year. *Synechococcus* from clades II and III were not detected except during the late summer or early winter. Within the dominant clades I and IV, *rpoCI*-defined sub-clades of *Synechococcus* showed distinct spatial distributions from the coastal to open ocean transect coinciding with changes in the nitricline, thermocline, and chlorophyll maximum depths. In coastal waters, *Synechococcus* targeted by two sub-clade IV probes were dominant at the surface, whereas two sub-clade I probes and a third sub-clade IV probe had increased signals in deeper water near the chlorophyll maximum. In mesotrophic waters, this third sub-clade IV probe dominated deep water at the chlorophyll maximum whereas all other sub-clade probes were below detection limits. The differing distributions of sub-clades within the dominant *Synechococcus* clades

indicate that the sub-clades likely have adapted to distinct ecological niches found within the Southern California Bight.

Abstract Category
17 Marine Microbiology

PS.17.143 COMPARISON OF MICROBIAL DIVERSITIES IN SEDIMENTS OBTAINED FROM SEVERAL HYDROTHERMAL AREAS

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Microbial diversities in hydrothermal vent fluid and/or chimney used as sample have been reported. Sulfur-oxidizers, belonging to γ - and ϵ -Proteobacteria, are detected frequently in several hydrothermal vents. The reason sulfur-oxidizers are dominant is supposed that microbial source was obtained under hydrogen sulfide rich condition. Around hydrothermal vent, microbial communities and symbiosis of chemolithotrophic bacteria with hydrothermal vent animals are formed. Some members of ϵ -Proteobacteria are related to epibionts of vent animals such as bivalves. On the other hand, little is known about microbial diversity in sub-surface sediment in hydrothermal area. In this study, several submarine sediments, which were obtained from hydrothermal area around Japan, were used for investigations and comparisons of microbial diversities depending on characteristics of environments. These samplings were carried out by drilling survey in 2008. Microbial diversities were investigated using clone library analysis with 16S rRNA gene. As characteristics of sediments, concentrations of metal such as Cu, Zn, Pb, and others were analyzed. Almost clones were related to Proteobacteria, particularly γ -Proteobacteria clone were abundant in Myojin knoll and Izena caldron. This group includes many marine bacteria. The clones related to ϵ -Proteobacteria were not detected, sulfur-metabolizing bacteria seem to be minority in these sediments. In Myojin knoll sediment, other phylogenetic group such as Bacteroides, Chloroflexi, Firmicutes and others accounts for about 20% in clone library. This fact shows that carbon source might be supplied to these microbes in the microbial community of Myojin knoll sediment. In addition, some clones were not relative to known sequences. Each taxonomic group has much diversity. Analyses of other sediments obtained from different hydrothermal areas were undergoing.

Abstract Category
17 Marine Microbiology

PS.17.144 MICROALGAE NANNOCHLOROPSIS CONTROLS VIBRIO SPECIES GROWTH IN A REARING WATER MICROCOSM

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The objective of the present study was to make clear whether the addition of microalgae *Nannochloropsis* to fish-rearing water can control growth of *Vibrio* species. *Nannochloropsis* has often been used for culturing fish larvae in aquaculture industry,

which can increase their survival rates. Although the addition of the microalgae into the rearing water appears to reduce the mortality of fish larvae caused by pathogenic bacteria such as *Vibrio* species, the reason is poorly understood.

In this study, a 20-L microcosm experiment was performed to reveal the growth response of culturable and non-culturable vibrios for 15 days (Days 0 to 14). For the duplicate microcosms setup, cultured *Nannochloropsis* sp. (the inoculated abundance 1×10^8 cells L^{-1}) was added into natural seawater samples and the microcosms were enriched with $NaNO_3$ (44.1 μM) and NaH_2PO_4 (4.4 μM). Culturable *Vibrio* abundance was measured with TCBS selective medium and non-culturable *Vibrio* was analyzed by a bromodeoxyuridine magnetic beads immunocapture and PCR-DGGE (BUMP-DGGE) method.

Chlorophyll (chl) *a* concentrations of Tank 1 and 2 ranged from 8.71 to 235 $\mu g L^{-1}$ and 10.9 to 255 $\mu g L^{-1}$, respectively. Both tanks had a peak on Day 6. Culturable *Vibrio* abundances decreased dramatically in both tanks: from 1.8×10^5 colony-forming-units (CFUs) L^{-1} on Day 2 to 0.5×10^5 CFUs L^{-1} on Day 5 in Tank 1 and 1.2×10^5 CFUs L^{-1} on Day 1 to 0.2×10^5 CFUs L^{-1} on Day 6 in Tank 2. Surprisingly, after the peak of chl *a* concentration in both tanks, culturable *Vibrio* could not be detected. The relative abundance of non-culturable *Vibrio* after PCR amplification was consistent with the culturable *Vibrio*. The total *Vibrio* community was unexpectedly diverse at the early incubation day, but the structure became simple around the peak of chl *a* concentration. This study suggests the *Nannochloropsis* addition to rearing water can control *Vibrio* species growth, which is one of the key factors responsible for fish larvae survival

Abstract Category

17 Marine Microbiology

PS.17.145 DENITRIFICATION IN MARINE SEDIMENTS, WHO IS RESPONSIBLE AND WHAT IS THE EFFECT OF TEMPERATURE?

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Denitrification is an important step in the global nitrogen cycle and marine sediments are a global hotspot for denitrification. However, it is generally unknown what species of denitrifying bacteria are responsible for denitrification in marine sediments. Further, it is unknown what environmental factors are important for niche differentiation among denitrifiers. I investigated how different temperatures select for different species of denitrifiers. Therefore I incubated denitrifying microbial communities in four continuous culture bioreactors at 10, 20, 30 and 40° Celsius. The bioreactors were inoculated with marine sediment of a tidal flat south of the North Friesian island "Spiekeroog". Because the flat is exposed to the atmosphere during low tides, the temperature changes in a tidal cycle between 10 and 40 degrees Celsius. Each incubation lasted 2 weeks with a dilution rate of 4 hours (~100 generations). Nitrate, supplied in excess, was the sole electron acceptor and a defined mixture of organic substrates served as electron donor. The products of nitrate reduction were determined by mass balancing with regard to carbon and nitrogen compounds and the amount of energy that was conserved in respiration was

determined by high resolution calorimetry. The experiments were monitored by ARISA-PCR. Once a stable community was established, the species and genes involved in nitrate reduction via denitrification and dissimilatory nitrate reduction respectively, at each of the different temperatures, were determined by deep sequencing of extracted DNA and RNA.

Abstract Category
17 Marine Microbiology

**PS.17.146 METAGENOMES ALONG A STEEP OXYGEN GRADIENT;
ANAMMOX AND PLANCTOMYCETES IN THE BALTIC SEA SEDIMENTS**

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The Baltic Sea suffers from eutrophication with gradually increasing anoxic bottom areas as a consequence, resulting in a steep oxygen gradient. The large pool of inorganic nitrogen (i.e. ammonium and nitrate) at oxygen depleted depths potentially suggests the occurrence of active anammox bacteria. In order to investigate whether active anammox bacteria are present the Baltic Sea sediments we analysed a 16S cDNA clone library and T-RFLP data of total and active planctomycetes/anammox at different sediment depths, and sequenced metagenomic fosmids (454 GS-FLX). T-RFLP analyses revealed that the planctomycetes/anammox community structure was significantly different between sediment depths and that the active anammox populations were different from the dominating. Phylogenetics identified active anammox bacteria that were related, but not identical, to known sequences of the *Candidatus Scalindua* group. Out of the metagenomic fosmid sequences approximately 10% of assigned reads (MG-RAST) were affiliated to Planctomycetes. The genome fragments were further investigated using GenDB (CeBiTec) and IMG/M-ER. Furthermore, for a better understanding of the ecological role of the total microbial community along the Landsort Deep oxygen gradient we employed direct metagenomic sequencing (454 Titanium) of total DNA extracts in order to compare taxonomic and functional profiles of both water (10, 80, 400 m) and sediment (466 m). Sequencing resulted in 0.5 Gb of sequence data, which were analysed using CARMA (CeBiTec), IMG/M-ER and MG-RAST, respectively, showing putative taxa and metabolic functions specific for each oxygen depth. This study shows that active anammox bacteria are present in the Baltic Sea sediment, potentially contributing significantly to the removal of nitrogen from the sediment and water to the atmosphere. The information from the putative Planctomycetes contigs might provide additional knowledge about this groups function in the sediment.

Abstract Category
17 Marine Microbiology

**PS.17.147 ENVIRONMENTAL FACTORS AFFECTING THE
DISTRIBUTION OF AMMONIA-OXIDIZING ARCHAEA IN THE MISSISSIPPI
RIVER PLUME**

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Marine Crenarchaeota have recently been found to be capable of oxidizing ammonia via an Archaea-specific ammonia monooxygenase (*amoA*) gene. Archaeal *amoA* genes have been observed in marine environments at 10-1000 times greater abundance than the *amoA* homologue from ammonia-oxidizing Bacteria, suggesting that the ammonia-oxidizing Archaea play a major role in the marine nitrogen cycle. Pelagic ammonia-oxidizing Archaea are most abundant below 100 m depth in the water column and at higher latitudes, especially near the poles. Reasons for this distribution remain largely unknown, but previous studies have pointed to environmental factors, such as salinity, light, temperature, oxygen, and sulfide; competition with bacteria or phytoplankton have also been thought to influence this habitat partitioning between ammonia-oxidizing Archaea and Bacteria.

This study compares the distribution of ammonia-oxidizing Archaea and ammonia-oxidizing Bacteria in the Mississippi River Plume using qPCR analysis of 16S rDNA and *amoA* genes from each group combined with different environmental factors measured at the same stations. Surface and deep (200 m) water samples were also amended with ³H-leucine and ³H-D-aspartic acid for microautoradiography fluorescent *in situ* hybridization analysis to look at incorporation by single cells, in order to investigate potential heterotrophic metabolism by ammonia-oxidizing Archaea in the Mississippi River Plume.

Abstract Category

17 Marine Microbiology

PS.17.148 DYNAMICS IN BACTERIOPLANKTON AND PHYTOPLANKTON COMMUNITY STRUCTURE AT THE BERMUDA ATLANTIC TIME-SERIES STUDY SITE

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The Western Sargasso Sea is an oligotrophic gyre ecosystem that is characterized by winter convective mixing events followed by thermal stratification during the summer. These annual mixing events entrain nutrients to the surface, triggering spring phytoplankton blooms. We analysed nucleic acid samples collected from the upper 300 m of the water column at the Bermuda Atlantic Time-series Study (BATS) site between 1991 and 2004 using a community profiling approach. Four different microbial communities with distinct compositions could be discerned. One associated with the annual spring blooms of eukaryotic phytoplankton and three during seasonal stratification: a surface community in the region of lowest nutrients, a deep chlorophyll

maximum community and an upper mesopelagic community. As the water column stratified, distinct surface blooms of many microbial groups were observed, including *Cyanobacteria*, SAR86, and SAR116. These analyses also revealed different vertical and temporal distributions for three ecotypes of the SAR11 clade of Alpha-Proteobacteria. Plastid 16S rRNA genes from several eukaryotic phytoplankton clades showed bloom patterns during and following the deep mixing events, with some taxa persisting in the deep chlorophyll maximum throughout the thermally stratified summer months. Previously unseen distribution patterns emerged for photosynthetic picoeukaryotes of the clade *Prasinophyceae*, namely *Micromonas* and *Ostreococcus*. These patterns of community change suggest that highly specialized adaptations and interactions govern the success of microbial populations in the oligotrophic ocean. Long-term trends in microbial community structure were observed that are likely caused by changing environmental conditions, such as a shoaling of mixed layer depth that occurred at BATS within the time frame of this study.

Abstract Category
17 Marine Microbiology

PS.17.149 ATTACHMENT OF *VIBRIO CHOLERAE* TO AN ENVIRONMENTAL HOST — THE MARINE COPEPOD

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The copepod is a well-known reservoir and vector of *Vibrio cholerae* - the etiologic agent of cholera; and while copepods are known to be of general importance to *Vibrio* ecology, information regarding how copepod population dynamics affect the environmental prevalence of this pathogen remains largely unknown. It stands to reason that each copepod life stage (egg, nauplii, copepodite and adult copepod) may constitute a distinctly different habitat for microbial colonization. Natural plankton samples (63-200 and >200 μm size fractions), collected monthly over a one-year period, were analyzed by PCR to investigate life-stage specific associations between *V. cholerae* and the marine copepod — *Acartia tonsi*. In the 63-200 μm plankton fraction, the relative abundance of juvenile copepods (nauplii and copepodites) was directly correlated to an increased odds of detecting *V. cholerae* (odds ratio = 1.04, $p < 0.05$). Moreover, a bimodal increase in the relative abundance of juvenile copepods was accompanied by a simultaneous increase in the prevalence of *V. cholerae*. In contrast, the relative abundance of adult copepods (>200 μm size fractions) was not correlated with the prevalence of this pathogen. To further investigate potential life-stage specific associations, we are conducting in situ colonization studies using a *gfp*-tagged *V. cholerae* strain. We hypothesize that adult copepods, having attained a terminal molt and a wax epicuticle, exude less available chitin and are therefore not colonized as heavily compared to juvenile copepods, which are continuously molting.

Abstract Category
17 Marine Microbiology

PS.17.150 PHYTOPLANKTON BLOOM PROGRESSION SHAPES BACTERIAL COMMUNITY COMPOSITION ALONG THE CONTINENTAL MARGIN OF THE BAY OF BISCAY

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During three consecutive years (2006-2008), we monitored bacterial community composition (BCC) in relation to spring phytoplankton bloom development and water column stratification along the continental margin of the northern Bay of Biscay (NE Atlantic) using molecular fingerprinting and cloning techniques. Ordination and variation partitioning methods were used to assess the relationship between BCC, the physico-chemical setting and phytoplankton community composition. Due to its complex bathymetry and hydrodynamics, the area is characterized by pronounced spatial variation in bloom progression at various scales. In addition, inter-annual differences in bloom stage occurred due to short-term weather-related changes in temperature, irradiance and mixed layer depth. Despite this marked spatial and temporal variability, our results show that bacterial communities closely track the wax and wane of different phytoplankton groups (from diatoms over coccolithophores to prasino- and pelagophytes) and associated TEP production as water column stratification progressively intensifies over the shelf area. The particle-associated and free-living size fractions of the bacterial community showed overlap in their species composition but had different dominant phylotypes, affiliated to the *Alpha*- and *Gamma-Proteobacteria* and the *Flavobacterium* clade. It thus appears that although the continental margin is a hydrodynamically complex and dynamic area, the composition of pelagic microbial communities is largely predictable due to their tight relationships with the physical and chemical setting.

Abstract Category
17 Marine Microbiology

PS.17.151 QUANTITATIVE SURVEY OF BACTERIAL DMSP-DEGRADING GENE DIVERSITY AND ABUNDANCE IN MARINE SURFACE WATERS

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Primers designed on the basis of metagenomic and metatranscriptomic data are becoming an important approach for studying biogeochemically important genes in natural

environments. Here we discuss our design and analysis of environmental metagenomic-based primers for *dmdA*, a bacterial gene mediating organic sulfur degradation and incorporation into microbial biomass. Using a bioinformatic pipeline, *dmdA* primers were iteratively designed and tested against the Global Ocean Sampling (GOS) metagenomic data. To capture the tremendous *dmdA* sequence diversity observed at the nucleotide level, 9 subclade-specific primers were designed. Altogether, these yielded more than 600 gene clusters (at 90% nucleotide and 95% amino acid identity) from 454 pyrosequencing-based PCR studies using DNA from southeastern U.S. coastal communities. In the first quantitative PCR assessments of *dmdA* abundance in two different environments (North Pacific gyre and Pacific coastal bay), subclade abundance varied both by season and depth. For two subclades, termed "D/1" and "D/3", which fall into a clade that contains the *dmdAs* from SAR11 genomes, we found $\sim 10^6 - 10^8$ copies per liter of D/1 sequences and $10^3 - 10^6$ copies per liter of D/3 sequences. However, use of these primer sets in a Taqman qPCR analysis was hindered by regional-scale variations in the amplicon sequences. In several examples of spatial sequence variants of different *dmdA* subclades, we found 1) consistent mismatches to highly conserved regions between amplicons from Pacific coast vs. GOS data; and 2) subclades in the GOS data that were missing in the above environments, suggesting either that these subclades are region-specific or that their sequences have mismatches to the GOS-based primers. These results demonstrate that environment-specific sequence heterogeneity is an important consideration for assaying gene and transcript inventories in the ocean with methods requiring primers or probes.

Abstract Category
17 Marine Microbiology

PS.17.152 FUNCTIONAL ECOLOGY AND NOVEL DIVERSITY OF MYCOPLANKTON IN THE OCEANS

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Microbial community diversity and composition play critical biogeochemical roles in the functioning of marine ecosystems. Large populations of planktonic fungi exist in coastal and open-ocean waters, yet their diversity and role in ocean carbon and nutrient cycling remain largely unknown. To address this gap, we applied molecular approaches to investigate mycoplankton communities in Hawaiian coastal waters and the Western Pacific Warm Pool (WP2). Mycoplankton communities displayed distinct lateral and vertical variations in diversity and composition. Compared with the open ocean, surface (<100 m) near-shore waters had the greatest diversity and species richness of mycoplankton, whereas no differences were found among stations at depths below 150 m. Vertical diversity profiles suggested that diversity and species richness were positively correlated to phytoplankton biomass in the coastal waters. On the other hand, lateral and

vertical diversity of mycoplankton is largely associated with both physical and biological conditions (e.g., mixing and primary production) in the oceanic waters. The majority of identified fungal phylotypes belonged to the phyla Basidiomycota and Ascomycota. Particularly, the majority of the basidiomycetes are novel phylotypes showing less than 97% identity in the 18S rRNA gene with known fungal sequences GenBank. Additionally, molecular approaches in combination with flow cytometry have identified novel fungal parasites of marine phytoplankton. Our study suggests that diverse novel lineages of planktonic fungi exist in the world's oceans and may be an important functional component of marine microbial food web.

Abstract Category
17 Marine Microbiology

PS.17.153 METAGENOMIC CHARACTERIZATION OF THE MICROBES ASSOCIATED WITH ATLANTIC AND PACIFIC CORAL REEFS

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Coral reef degradation is associated with a phase-shift from a benthic community dominated by scleractinian corals and crustose coralline algae towards a community dominated by turf- and macro-algae. The mechanism whereby algae out-compete coral is not understood, however there is evidence that coral death is exacerbated via a microbial-mediated process. To investigate microbial community composition on reefs of varying condition, microbes were collected from the boundary layer overlying twenty-five reefs located in the Atlantic and Pacific Oceans. Bioinformatic analyses of five southern Line Island reef-associated microbial communities (Flint, Malden, Millennium, Starbuck, and Vostok) revealed large differences in community composition and metabolic function between near-pristine reefs and reefs associated with perturbations such as iron enrichment. Genes involved in photosynthesis were overrepresented in the metabolic profiles of microbial communities from coral dominated reefs on Flint, Starbuck, and Millennium. In contrast, the microbial community inhabiting the iron-enriched and algal covered reef on Millennium was enriched for genes involved in heterotrophy such as central carbohydrate metabolism and the degradation of amino acids and proteins. To determine whether microbial communities associated with algal-dominated reefs included more pathogenic microbes, sequence libraries were compared to the virulence factor database (www.mgc.ac.cn/VFs/). Genes for virulence factors were overrepresented on the algal-dominated reef on Millennium (n = 1068 compared to n = 18 - 341 on control reefs). The results from this study demonstrate that coral reefs undergoing phase-shifts are associated with a greater prevalence of potential pathogens, suggesting that microbes may be facilitating coral death and / or inhibiting coral recovery.

Abstract Category
17 Marine Microbiology

PS.17.154 BDELLOVIBRIOS ISOLATED FROM CORAL REEFS EXERT SIDEWAYS CONTROL OF THE POLYMICROBIAL COMMUNITIES

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The microbial communities in aquatic ecosystems such as coral reefs are subject to sideways controls from bacterial predation by *Bdellovibrios*. The predatory bacteria *Bdellovibrio* are a genus of highly motile gram negatives that have the ability to insert themselves in the periplasm of other gram negative bacteria. Once inside the host periplasm, *Bdellovibrios* can feed off the host cells without competition and replicate their genome to produce multiple progeny before lysing their host to repeat the process. Their intercellular niche, lytic progeny release, and highly motile scavenging phase indicate these organisms could play a major role in controlling bacterial communities on coral reefs. Furthermore their ability to infect a broad host range of gram negative bacteria, unlike bacteriophages which have a highly specific host, makes them particularly important for studies of polymicrobial diseases such as Black Band Disease. Coral coverage on reefs is in decline worldwide and disease is a major contributing factor. Previous studies have shown that the abundance of *Bdellovibrios* increases in nutrient enrichment treatments of experiments with adult coral fragments. We have isolated *Bdellovibrios* from coral reefs and aim to study the interactions between marine *Bdellovibrios* and the Caribbean reef coral *Montastraea cavernosa*. Gross bacterial abundance was measured by flow cytometry in interaction studies. 16s PCR primers were used to detect the presence of *Bdellovibrio* like organisms from environmental samples. Our results indicate the addition of *Bdellovibrios* produces dramatic shifts in the bacterial communities of *Montastraea cavernosa*. *Bdellovibrios* have been targeted as therapeutic agents in medical studies to control gram negative bacterial infections this study explores how marine *Bdellovibrio* control bacterial communities on corals.

Abstract Category

17 Marine Microbiology

PS.17.155 THE MICROBIOME OF MARINE COPEPODS OF THE NORTH SEA

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Marine copepods belong to the most abundant mesozooplankton in the ocean and are associated with various numbers of different bacterial species. Unfortunately, in temperate waters the knowledge on copepod associated bacteria is very much restricted and only available for few copepod genera or species. We present a comprehensive analysis of the bacterial community of four marine copepod genera *Acartia sp.*, *Temora sp.*, *Centropages sp.* and *Calanus sp.* which are key species of the North Sea. Weekly copepod samples of single specimens were analyzed using PCR-DGGE over a period of two years. DGGE fingerprints were analysed and prominent DGGE bands were identified by sequencing and phylogenetic analysis. At two dates during summer additional cloning experiments were conducted to get a more comprehensive view on the structure of the copepod associated bacterial populations. For all copepod genera no genus specific

associated bacterial population could be observed. Although the copepod associated bacterial populations varied over the year, statistical analysis using further contextual data of the sampling station (oceanographic data, nutrients, phytoplankton species) clearly prove no seasonality or correlation for any of the bacterial populations. Phylogenetic analysis of the whole microbiome revealed the presence of a number of different phylogenetic groups, in fact all four copepod genera were clearly dominated by *Alphaproteobacteria*. Within this group a distinct cluster shows up containing *Rhodobacteraceae* signatures obtained from all copepod genera. Interestingly related DNA signatures were found in a previous study with *Calanus* sp.. Besides the *Bacteroidetes* a third most prominent group were the *Actinobacteria* which are often described as capable to utilize chitin. Within the *Gammaproteobacteria* only very few DNA sequences related to the genus *Vibrio* were identified (*V. splendidus* and *V. fischeri*). Detailed results of the whole study will be presented.

Abstract Category
17 Marine Microbiology

PS.17.156 O-ANTIGEN DIVERSITY AND HABITAT DISTRIBUTION AMONG *VIBRIO SPLENDIDUS* ENVIRONMENTAL ISOLATES

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The O-antigen, a highly diverse yet strain-specific structure, covers the surface of a Gram-negative bacterium. The traditional model for the maintenance of such diversity has been from selective pressure by hosts' immune systems, in which strains expressing common O-antigens are easily detected while ones expressing rare structures evade host detection and cause disease. However, non-pathogenic bacteria also exhibit extensive O-antigen diversity, suggesting selection of this structure at levels other than the host. For instance, *Vibrio cholera* O1 and O139 are the causative agents of cholera yet >200 non-pathogenic serotypes exist. Conceivably, in nutrient-deprived water columns, the ability to associate with habitats (i.e., algae, grasses, zooplankton) offers an advantage in the acquisition of resources to marine microbes. We propose O-antigen variability among marine microbes is maintained as a direct result of habitat partitioning, in which associations with different substrates are mediated by the O-antigen. Here, we show extensive wbe (genes whose products assemble the O-antigen) genetic diversity exists among the published *V. splendidus* genomes 12B01, med222, and LGP32 which results in phenotypic O-antigen variation. These observations were extended to environmental strains isolated within various invertebrates and on suspended particles within Plum Island Estuary of coastal Massachusetts. To constrain evolution, we analyzed isolates having the same sequence type, based on three housekeeping genes, and characterized these strains by O-antigen profiling and sequence analysis of a gene closely linked to the wbe region. Results show lateral gene transfer of the wbe region and O-antigen hyper-variability among isolates. If O-antigen diversity is maintained due to habitat association

then we would predict certain serotypes to prefer particular habitats. Preliminary habitat association experiments show that different vibrios prefer one habitat over another.

Abstract Category
17 Marine Microbiology

PS.17.157 NATURAL, NON-BIOLUMINESCENT ISOLATES SHARING AN IDENTICAL *LUX* OPERON DELETION FORM A POLYPHYLETIC GROUP WITHIN THE *VIBRIO FISCHERI* CLADE

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Vibrio fischeri, a gram-negative heterotrophic marine bacterium, has been the canonical representative of bacterial bioluminescence since its characterization in the late 19th century. Over one hundred years later, non-bioluminescent (NBL) *V. fischeri* isolates have never been reported from natural populations of this bacterium, many of which are found in symbioses with fishes and squids. With this study, we describe the first NBL isolates of *V. fischeri*.

Over 60 strains of planktonic, mussel-associated, and crab-associated marine bacteria, collected from a North Atlantic estuary for another study, were identified as *V. fischeri*. The genetic and phenotypic diversity of these novel *V. fischeri* isolates was characterized using a variety of *in vitro* and *in silico* approaches. Multi-locus sequence typing was used to reconstruct the phylogeny of these isolates and other extant *V. fischeri*. This reconstruction was used to reconcile the observed intraspecific differences among *V. fischeri*, including full *lux*-operon absence in many estuary isolates, with the evolutionary history of *V. fischeri*.

From our results, we conclude that the majority of *V. fischeri* sampled from the estuary environment (62 of 65) are NBL isolates sharing an identical deletion of the *lux* operon (present in all natural isolates of *V. fischeri* studied to date). Additionally, these NBL isolates all uniformly lack *rscS*, a response-regulator linked to symbiosis specificity within the species and uniformly contain *ainS*, an autoinducer synthase protein. The hypothesis that NBL *V. fischeri* form a monophyletic clade in phylogenetic reconstructions of extant *V. fischeri* was rejected by parametric bootstrapping ($p < 0.001$). The evolutionary implications of the conserved absence of the *lux* operon in the polyphyletic NBL *V. fischeri* as well as the role(s) of various niches in selecting for/against bioluminescence in the estuary environment are discussed

Abstract Category
17 Marine Microbiology

PS.17.158 HIGH THROUGHPUT CULTIVATION OF MAJOR MARINE BACTERIAL LINEAGES FROM THE EAST SEA USING VARIOUS CULTURE CONDITIONS

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High-throughput cultivation based on dilution-to-extinction method has been successfully used to cultivate marine uncultured bacterial lineages from Oregon coast and the Sargasso Sea. In this study, we applied a modified version of high-throughput cultivation to cultivate major uncultured marine bacterial lineages in the East Sea for one year. One to 10 cells from sampled seawater were inoculated into each well of 48-well plates, which contained low-nutrient heterotrophic media prepared by adding diverse combinations of organic compounds to filtered seawater. After 4 to 8 weeks of incubation at 10-15°, microbial growth was screened by using epifluorescence microscope and flow cytometer. Positive growth was detected in 1446 wells among 7872 inoculated wells and 325 colony-forming isolates were obtained on agar plates by transferring extinction cultures. Based on phylogenetic analyses of 16S rRNA gene sequences from pure isolates, cultured strains were generally assigned to the SAR11 groups 1 and 3, Roseobacter clade and SAR92 group. The isolates belonging to the OM42, OM60, OM182, K189A clades and the phylum Lentisphaera were also recovered. Interestingly many strains were affiliated to genera of Fucophilus, Coraliomargarita and Pelagicoccus in the phylum Verrucomicrobia. This study suggests that efficient cultivation of novel members of major marine bacterial groups could be facilitated by a long-term approach using various culture conditions mimicking natural environments. [Supported by the 21C Frontier Program of Microbial Genomics and Applications from the MOST]

Abstract Category

17 Marine Microbiology

PS.17.159 AMMONIA-OXIDIZING AND DENITRIFYING COMMUNITIES ALONG A SALINITY GRADIENT FROM INTER-TIDAL SEDIMENT TO ARABLE LAND

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Microbial life in a salinity environment is energetically expensive. Large amounts of energy must be spent in adaptation of intracellular environment to the high osmotic pressures outside through various mechanisms. The adaptation cost varies with microorganisms, physiological processes and salinity levels. Certain physiological groups of microorganisms are thus absent in a high salinity environment, because they can not afford to such a high adaptation cost. Based on this theory, it is predicted that the bacterial community structure in the mudflat varies with salinity levels and that the diversity increases with the decrease of salinity levels. To test the hypothesis, a series of sediment/soil samples with different salinities were collected along a cross-shore transect from the sea-shore mudflat area of Jiangsu Province, China (Fig.1). Culturable bacteria were isolated and their 16S rRNA genes were amplified and sequenced. Bulk DNA was extracted from 0.5 g of sediment with a FastDNA SPIN Kit. Bacterial communities were fingerprinted with denaturing gradient gel electrophoresis (DGGE) based on V3 region of 16S rRNA. Clone libraries based on archaeal amoA, bacteria amoA and nosZ genes were

also constructed and analyzed. Results showed that *Bacillus* were the most abundant culturable bacteria accounting for 82% of total isolates (83), with a few isolates closely related to *Marinobacter* and *Micrococcineae*. PCR amplification for archaeal *amoA* and *nosZ* genes were successful but not for bacterial *amoA*, no matter what primers and what PCR conditions were used. DGGE fingerprint (Fig.2) showed that bacterial diversity was increased with the decrease of soil salinity, supporting the hypothesis. Library analysis revealed consistent results (Table 1). In conclusion, microbial community structure in the mudflat varies with salinity levels and that the diversity increases with the decrease of salinity levels.

Abstract Category
17 Marine Microbiology

PS.17.160 MICROBIAL DIVERSITY AND MANGANESE OXIDIZING BACTERIA IN Fe/Mn-CONCRETIONS IN THE GULF OF FINLAND, BALTIC SEA

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In the Baltic Sea large and dense (20-50 kg m⁻²) deposits of concretions (i.e. nodules) are found on silty-clayey mud bottoms. Concretions are formed from alternating Fe and Mn oxide layers and they are rich in P and organic C too. Due to their geochemical properties they may have significant effect on the nutrient balance in the Baltic Sea. Additionally, the physico-chemical characteristics of concretions indicate that the concretions themselves may serve an excellent site for microbial life. To find out whether this is true we investigated microbial diversity in the Fe/Mn-concretions and tested whether microorganisms present there are able to oxidize Mn. Microbial diversity was studied using epifluorescence microscopy and DNA-based methods such as cloning of community 16S rRNA-gene and sequencing. Mn-oxidation was studied in bottle incubation units where availability of MnSO₄, O₂ and Na-acetate as carbon source and temperature were controlled. Our results show that concretions host diverse bacteria with an average density of 6.7 x 10⁷ cells g⁻¹ DW. The sequencing studies showed that one third of the cloned sequences were related to uncultured, unclassified bacteria and that half of the cloned sequences were affiliated to Proteobacteria; gamma and alpha being the most representative classes. The closest matches to the sequences found from the concretions were obtained from ocean crust and sediments. Inoculation of microbial fraction extracted from the concretions enhanced the oxidation of Mn within a few weeks and the conditions in the experiment favored enrichment of bacteria such as *Sphingomonas*, *Pseudomonas* and *Bacillus*. Our studies demonstrate that concretions form microcosms which are colonized by diverse and mostly unknown prokaryotes and that microbes hosted by concretions have a capability to oxidize Mn.

Abstract Category
17 Marine Microbiology

PS.17.161 PROTEORHODOPSIN IS FUNCTIONING AS THE LIGHT-DRIVEN PROTON PUMP AMONG MARINE FLAVOBACTERIA

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It has been revealed that proteorhodopsin (PR) genes are widely distributed among marine prokaryotes. However, so far the function of proteorhodopsin as ion pump has only been observed heterologously in *E. coli*, but never been directly in native cells. Here, we show the confirmation of proteorhodopsin as the light-driven proton pump in all tested bacterial isolates belonging to Flavobacteria. Among 75 isolates, 38 strains were found to possess PR gene by using our newly designed primers. For the proton pump activity measurement, 8 strains were selected from genera, *Flavobacterium*, *Polaribacter*, *Tenacibaculum*, *Gilvibacter*, and *Winogradskyella*. All the cell suspensions showed marked pH drop under illumination in both low and rich nutrient conditions. Together with further data on wavelength-dependent pump activity and the effect of hydroxylamine on the spectrophotometric character, we concluded that PRs in those strains are functioning as proton pump. The results indicate that PR is widely spread among Flavobacteria and this group has evolved into light-dependent heterotrophic bacterial group highly adapted to photic-zone.

Abstract Category

17 Marine Microbiology

PS.17.162 VARIATION OF EMISSION SPECTRA OF MARINE LUMINOUS BACTERIA

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The emission spectra of marine luminous bacteria have been assumed to be common among all of them without any systematic investigation. We hypothesized that there are variations in the spectra that might reflect their phylogenetic positions. In order to verify this hypothesis, we examined the emission spectra of 777 luminous bacteria together with their phylogenetic analyses. It was revealed that the spectra are classified into 6 types according to their shapes and maximum wavelengths. The phylogenetic analysis of luciferase α -subunit gene (*luxA*) showed that except for *P. leiognathi*, each species formed individual clade and the strains sharing the same clade belong to the same spectral type. These results indicate that diversification of *luxA* lead to the diversity of light emission spectra. To our knowledge, this is the first systematic survey of light emission spectra of luminous bacteria.

Abstract Category

17 Marine Microbiology

PS.17.163 NEW METHODS FOR AUTOMATED DETERMINATION OF BIOVOLUMES AND MORPHOLOGIES OF AQUATIC BACTERIA BY IMAGE ANALYSIS.

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High-throughput analysis of aquatic bacteria by automated microscopy and image processing has become a valuable tool in microbial ecology, especially for studies at high temporal and spatial sampling resolution. While autonomous counting of multiple fluorescently stained microbes is well established, the automated determination of bacterial biovolumes and morphologies is still a major challenge. This might be the reason for these parameters being generally neglected in many studies, although they may yield ecologically highly relevant information. Therefore, we developed novel methods for the automated assessment of both, cell volume and morphology by image analysis. Both approaches are based on the contours of bacterial cells as reconstructed by elliptical Fourier descriptors. The cell volume method was validated using geometrical models of bacteria and its performance was compared to existing methods. The determination of cell morphologies is based on a nearest neighbor classifier trained on generic geometrical models; it was compared to the classification of bacterial morphologies by five scientists. In this context we also critically assessed the ability of humans to objectively judge bacterial morphologies in environmental samples. Both methods will be made freely available as stand-alone computer programs, allowing for batch processing fluorescent microscopic images from any appropriate source.

Abstract Category

17 Marine Microbiology

PS.17.164 PHYLOGENETIC DIVERSITY AND POTENTIAL FUNCTION OF MICROBIAL COMMUNITY IN SOUTH CHINA SEA MESOSCALE EDDY PERTURBATIONS

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Eddy pumping induces isopycnal displacements that lift nutrient replete waters into the euphotic zone, driving a set of biogeochemical processes. To address the potential role of such physical processes on the microbial community, the phylogenetic diversity and metaproteomics were determined in two cold-core cyclonic eddies in the South China Sea (SCS). Terminal restriction fragment length polymorphism analysis of the microbial communities through the whole water column revealed a wider intermediate transition water mass inside than outside the eddies, which contained a relatively more complex community than the euphotic and deep-water zones. A distinct partitioning of microbial diversity among the communities within the euphotic zone was revealed by clone

libraries analysis. The stratification of the prokaryotic populations between the surface and the chlorophyll maximum layer of the eddy-related sites versus the homogeneity of the communities in the euphotic zone of the reference site is most likely a reflection of isopycnal displacement induced by different water movement inside and outside eddies. Phylogenetic analysis revealed that the eddy-center sites were characterized by the nitrogen-fixing Rhodospirillaceae cluster, the deep-water group Alteromonadales-affiliated clones and the psychrophilic genus Octadecabacter cluster, while Paracoccus and Acinetobacter, as important denitrifying groups, abundantly existed at the reference site outside eddies. Metaproteomic patterns predicted to some extent the clustering relationship among the microbial communities as determined by gene sequence analysis. Taken together, phylogenetic analysis and protein identification further implied that intensifying nitrogen fixation may be occurring in the cyclonic eddy perturbations, activated by increasing iron supply from deep-water. While denitrification might greatly contribute to nitrogen removal in the SCS, causing nitrogen limitation in the unperturbed waters outside eddies.

Abstract Category
17 Marine Microbiology

**PS.17.165 MICROBIAL DIVERSITY AT TWO RECENTLY DISCOVERED
HYDROTHERMAL VENT SITES IN THE SOUTHERN OCEAN**

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Earlier this year, two sites with hydrothermal vent activity were discovered at the East Scotia Ridge in the Southern Ocean between 55-60°S. These are the first chemosynthetically driven environments to be discovered in Antarctic waters, thus providing a unique opportunity to evaluate whether they present a new distinct biogeographic province or act as stepping stones for the dispersal of chemosynthetic organisms between known hydrothermal vent sites in the Southern Pacific and Southern Atlantic Ocean. Using CTDs and the remotely operated vehicle (ROV) ISIS, a range of microbial samples was obtained. These included water samples from the buoyant plume ca. 100m above the vent site, water from within the smoke directly above the vent, high temperature vent fluid, fragments of vent chimneys, sediments and several species of vent fauna harbouring microbial symbionts. Microbial abundance in these samples was determined by DAPI (4',6'-diamino-2-phenylindole) cell counts and flow cytometry. Microbial diversity was assessed by fluorescence *in situ* hybridisation (FISH) targeting major bacterial and archaeal groups and sequencing of 16S rDNA clone libraries.

Abstract Category
17 Marine Microbiology

**PS.17.166 INFECTION OF SYNECHOCOCCUS WITH CYANOPHAGE
DRAMATICALLY INCREASES ITS PREDATION BY PHAGOTROPHIC
FLAGELLATE CAFETERIA**

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Grazing and phage infection are important factors in controlling microbial abundance and diversity in marine environments. With phage numbers of up to 10^{10} L⁻¹ seawater, a considerable fraction of all microbial cells are assumed to be infected with phage at any one time. This begs the question whether grazing is affected by this, i.e. do protozoan grazers differentiate between infected and uninfected cells? Here, we used a model system consisting of the heterotrophic nanoflagellate *Cafeteria roenbergensis*, two *Synechococcus* strains WH7803 and WH8102 and cyanophage RSM4 to investigate both ingestion and digestion of phage infected and uninfected *Synechococcus* cells. In the absence of phage infection, both WH7803 and WH8102 were shown to be ingested by *Cafeteria*. However, only WH8102 was able to support growth of grazer cells (i.e. was successfully digested). For WH8102, infection with cyanophage RSM4 had no significant effect on ingestion or digestion. In the case of WH7803, however, both ingestion and digestion increased dramatically. The effect on ingestion was most pronounced towards the end of the phage infection cycle. This distinct preference for grazing of phage infected cells has potentially important ecological implications, as it interferes with phage proliferation by removing infected cells before they burst.

Abstract Category

17 Marine Microbiology

PS.18.001 PHYSIOLOGICAL AND MOLECULAR CHARACTERIZATION OF SOIL SAMPLES AND ENRICHMENT CULTURES PRODUCING BRANCHED GDGT MEMBRANE LIPIDS

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Branched glycerol dialkyl glycerol tetraether (GDGTs) membrane lipids are ubiquitously found in soils and peat bogs and are used as paleoenvironmental proxies. These GDGTs are synthesized by as yet unidentified (anaerobic) soil bacteria, but members of the Acidobacteria phylum have been proposed as possible producers of branched GDGTs1. Aim of this study was to identify the bacteria that synthesize branched GDGTs, using an integrated cultivation- and biomolecular approach.

To this end, enrichment cultivation of branched GDGT-containing soil samples from the Têt watershed (France) and geothermally heated soils surrounding California hot springs was performed under various conditions. In addition to phylogenetic comparison of field samples by 16S rRNA gene clone library analysis, barcoded pyrosequencing analysis of PCR-amplified 16S rRNA gene fragments was used for analysis of microbiota in the Têt watershed enrichment cultures and several Californian hot springs soil samples with different content of GDGTs. Comparison of clone libraries of samples with high and low contents of branched GDGTs indicated that Acidobacteria might not be the right candidates for GDGT production.

(Traces of) branched GDGTs were detected in an enrichment culture obtained from a soil with high amounts of branched GDGTs in the Têt watershed. Presently, different strategies are followed to further enrich and isolate the GDGT-producing bacteria from this enrichment culture. Results of a more detailed physiological characterization, as well as phylogenetic comparison by pyrosequencing will be presented and discussed.

This study provides new insights into the possible identity of microbial producers of branched GDGT membrane lipids, furthering our understanding of microbial adaptation to changing environmental conditions, and allowing their exploitation as diagnostic biomarkers of such changes.

1) Weijers JWH et al. (2006) *Env Microbiol* 8:648-657

Abstract Category

18 Paleobiogeochemistry/Paleobiogeography

PS.18.002 EVOLUTION OF HIGH POTENTIAL ELECTRON TRANSFER CHAINS: CHLOROPHYLL BASED PHOTOTROPHY, AEROBIC RESPIRATION, AND DENITRIFICATION

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High redox potential metabolisms (chlorophyll based phototrophy, aerobic respiration, and canonical denitrification) play fundamental roles in structuring many modern

microbial ecosystems. Recent geochemical studies provide strong evidence that the early Earth was anoxic, suggesting that these metabolisms were not present. The high potential electron transfer chains of chlorophyll based phototrophy, aerobic respiration, and canonical denitrification share a common core architecture composed of a number of evolutionarily related proteins. Here we use genomics, coupled with data from the geological record, to understand the origin and evolution of these high potential electron transfer chains. Analysis of the quinol:electron acceptor oxidoreductases (Complex III) and oxygen reductase members of the heme-copper oxidoreductase superfamily (Complex IV) demonstrate that aerobic respiration originated within early Cyanobacteria after the evolution of oxygenic photosynthesis. All other phyla of Bacteria and Archaea received the ability to perform aerobic respiration via lateral gene transfer. In most currently known phyla early branching groups are always anaerobic, implying that at the time of the major diversification of microbial life aerobic respiration had not yet evolved. Nitric oxide reductase (NOR) members of the heme-copper oxidoreductase superfamily catalyze the reduction of nitric oxide (NO) to nitrous oxide (N₂O) in microbes capable of canonical denitrification. Analysis of NOR's demonstrate that nitric oxide reductase activity evolved many times independently from oxygen reductase members within the superfamily, suggesting that canonical denitrification evolved after aerobic respiration. Analysis of Complex III genes also demonstrate that the electron transfer chains utilized by extant anoxygenic photosynthetic clades (*Acidobacteria*, *Chlorobi*, *Chloroflexi*, *Firmicutes* and *Proteobacteria*) were assembled after the origin of aerobic respiration.

Abstract Category

18 Paleobiogeochemistry/Paleobiogeography

PS.18.003 ANAEROBIC OXIDATION OF ARSENITE BY THE PHOTOTROPH *CHLOROFLEXUS AURANTIACUS*

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On early earth, a lack of electron acceptors was a major hurdle for evolving life. Since free oxygen was absent on the young Earth and Mars, light facilitated oxidation. To demonstrate the anaerobic oxidation of arsenite under light, *Chloroflexus aurantiacus* was incubated under photoheterotrophic conditions. *C. aurantiacus* possesses genes encoding arsenite oxidase and was able to oxidise arsenite exclusively under anaerobic conditions in the light. Arsenite was also oxidised under aerobic conditions abiotically, but at significantly lower rates. Furthermore, arsenite oxidase activity was detected in cell free extracts using DCIP as the electron acceptor. Although green filamentous bacteria (e.g., *Chloroflexus*) and green sulfur bacteria both utilise the same light harvesting structure, the chlorosome, their photosystems are significantly different. *Chloroflexus* has a photosystem more reminiscent of that found in purple bacteria (e.g., Photosystem II) with Bacteriochlorophyll a (870 nm) in the reaction centre. Whether this impacts the ability to couple arsenite oxidation especially in the green sulfur bacteria needs further investigation. However, the results obtained here suggest that *Chloroflexus* is capable of

using As(III) as an electron donor in anoxygenic photosynthesis. Thus, it is quite conceivable that microbial mat communities in the Archean on Earth used As(III) as an electron donor for photosynthesis, providing a local source of As(V) prior to the advent of atmospheric oxygen. That this also occurred on early Mars is quite possible as well.

Abstract Category

18 Paleobiogeochemistry/Paleobiogeography

PS.18.004 MOLECULAR BIOMARKERS OF DIATOMS IN THE ENVIRONMENT

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Diatoms are a dominant group of phytoplankton in the present-day ocean. They also occur abundantly in freshwater and terrestrial environments. Diatoms are responsible for almost one-fifth of the world's primary productivity and are a major source of organic matter production in marine environments, especially in coastal areas. Despite their current widespread abundance, diatoms originated during the Early Jurassic and became only abundant late in the Eocene (40-35 Ma). Because of their abundance, diatoms are likely to be one of the most important sources of sterols in the marine environment. Sterols are ubiquitous components of eukaryotic membranes. These compounds are synthesized by oxidation of squalene using molecular oxygen to squalene epoxide, which is then cyclized either to lanosterol or to cycloartenol by an oxidosqualene cyclase (OSC) homologue to SHC. In this study, we investigate the sequence diversity of the gene coding the enzyme 2,3-oxidosqualene-cycloartenol cyclase (cycloartenol synthase) of the sterol biosynthetic pathway, which is thought to be the commonly used pathway within the diatoms. Degenerate primers have been designed based on the annotated cycloartenol synthase gene in the sequence genome of the diatoms *Thalassiosira pseudonana* and *Phaeodactylum tricorutum*, as well as environmental sequences. Cycloartenol synthase homologue sequences have been retrieved from a collection of diatom pure cultures showing sequence diversity at the level of species (e.g. 89% sequence identity between *Skeletonema subsalsum* and *S. costatum*). This method is currently being applied in environmental samples (water column, microbial mats) in order to estimate both diatom diversity and expression of the gene by quantitative PCR. This novel approach will increase our knowledge on the diversity and activity of a crucial gene involved in the sterol biosynthetic pathway and support the attribution of the major sources of diatom sterols in the natural environment.

Abstract Category

18 Paleobiogeochemistry/Paleobiogeography

PS.18.005 SEASONALITY OF ANAEROBIC NITRATE-DEPENDENT BIOOXIDATION OF FE(II) IN A LAKE CHEMOCLINE

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The discovery of bacteria that oxidize reduced iron with nitrate as electron acceptor at neutral pH is rather recent. In the meantime, several iron-oxidizing bacteria have been described in diverse ecosystems like freshwater and marine sediments, soils, rice fields and others. It has also been hypothesized that this metabolism might have contributed to banded iron deposit formation (BIFs) in an early ocean when nitrate became available on Earth at the end of the Archean. To date, however, nitrate dependent iron-oxidizing bacteria have not been detected in water columns. Therefore, we investigated an iron-rich meromictic lake to detect and characterize nitrate-dependant iron-oxidation. Physicochemical profiling and in situ ¹⁴C-bicarbonate incubation experiments were employed to verify the occurrence of nitrate-dependant iron-oxidation. Both summer and winter stratification of the water column were investigated to assess the seasonality of anaerobic iron-oxidation. The abundance of nitrate-dependent iron oxidizers was estimated by MPN cultivation. Several enrichments were characterized by cloning/sequencing of the 16S rDNA gene. Results indicate that nitrate-dependent chemotrophic iron-oxidation is present during summer stratification but undetectable in winter. For the first time, the activity of anaerobic microbial iron-oxidizing nitrate-dependent metabolism was detected in a modern stratified water column. Our results show that this activity can take place at the chemocline and possibly depend on the algal and cyanobacterial populations, which are present mainly in summer.

Abstract Category

18 Paleobiogeochemistry/Paleobiogeography

PS.18.006 ANAEROBIC PHOTOTROPHIC IRON-OXIDATION IN AN ANCIENT OCEAN ANALOGUE

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The Archean and early Proterozoic Ocean was anoxic, rich in iron(II), and poor in sulfide. From this ocean massive deposits of iron-oxides precipitated and formed the Banded Iron Formations (BIF). Based on stratigraphic studies, experimental data of anoxygenic phototrophic iron-oxidizing cultures, and theoretical considerations, it was proposed that some BIFs may have been formed by anoxygenic phototrophic iron-oxidizing bacteria instead of the abiotic reaction of iron(II) with oxygen produced by cyanobacteria. However, activity of such anoxygenic photoferrotrophs has not yet been demonstrated in a modern water column and the ecology of this guild is virtually unknown. We examined modern phototrophic iron-oxidation in an iron-rich meromictic lake with a water column chemistry resembling an ancient ocean. Physicochemical

profiling, ex-situ microcosm experiments under simulated environmental conditions and in-situ ^{14}C -bicarbonate incubation experiments were employed to detect and quantify phototrophic iron-oxidation. A small peak of iron(III) in the anoxic part of the chemocline, where light intensities of 0.002% PAR prevailed and where no alternative oxidants for iron oxidation were present, suggested photoferrotrophic activity. This was confirmed by radiocarbon incubation experiments where bicarbonate uptake was stimulated by iron(II) addition in the light. No stimulation was observed upon sulfide addition. Moreover, a co-culture that oxidizes iron(II) to iron(III) in presence of light and acetate was enriched and whose major components were identified as a new *Chlorobium* sp. and *Acidobacter* sp. The results are the first direct evidence of photoferrotrophic activity in an Archean Ocean analogue.

Abstract Category

18 Paleobiogeochemistry/Paleobiogeography

PS.19.001 SHIFTS IN MICROBIAL COMMUNITIES CONTRIBUTE TO FEEDBACKS BETWEEN NITROGEN MINERALIZATION AND EXOTIC ANNUAL GRASS INVASION

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Exotic species often alter ecosystem processes and create positive feedbacks that further promote invasion. During plant invasions, feedbacks occur as exotics increase the availability of limiting nutrients, especially nitrogen (N). Microorganisms mediate N cycling; however, it remains unclear if N feedbacks result from concomitant changes in microbial communities. In this study, we investigate the potential for communities to influence N cycling and the exotic annual grass invasion of *Taeniatherum caput-medusae* (Taca).

We tracked monthly shifts in communities and N mineralization and nitrification rates across an invaded shrub-steppe ecosystem (OR, USA). Specifically, we evaluate soils where: Taca dominated (exotic); Taca coexisted with the dominant native grass, *Pseudoroegneria spicata* (Pssp, front); and Pssp dominated (native) during the growing season. To verify N feedbacks, we traced inorganic ¹⁵N capture by these grasses. We measured communities using phospholipid fatty acid analyses and N cycling through *in situ* ¹⁵N isotope pool dilutions.

We found that Taca invasion dramatically influenced soil communities in exotic and front, but front communities shifted to resemble exotic or native depending on the grasses' phenologies. These differences were highlighted in the distribution of Gram+ and Gram- bacteria, and Actinobacteria. Exotic communities were associated with higher mineralization rates when Taca ¹⁵N recovery was high. Conversely, native and front communities were associated with lower mineralization rates when Pssp ¹⁵N recovery was high. Community differences did not influence nitrification or alter mineralization in early spring possibly due to high moisture and C availability.

Our results suggest that communities may alter mineralization rates that promote Taca invasion and disrupt beneficial N-limited relationships between microbes and native grasses. However, community contributions to N feedbacks are regulated by environmental conditions.

Abstract Category

19 Plant-Microbe Interactions

PS.19.002 EFFECT OF PEARL MILLET-GREEN MANURE APPLICATIONS ON POTATO YIELD, DISEASE SUPPRESSION AND RHIZOSPHERE MICROBIAL COMMUNITY

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Green manure applications are known to improve soil quality and to contribute to disease suppressiveness. A five year rotation trial of potato (*Solanum tuberosum*) and pearl millet (*Pennisetum glaucum*) was setup at two farms (AAFC and BL) near Delhi, ON, Canada. At each site there were two mirror image plots alternating millet and potato. Several methods for incorporation and cultivation of the millet were evaluated including single mowed (SGM), multi-cut (MC), multi seeded (MS) and strip spread (SS) and the effects on potato yield and disease incidence of Verticillium wilt (*Verticillium dahliae*) and common scab (*Streptomyces scabies*) were assessed. Continuous potato rotated with or without wheat served as control. At the AAFC site in 2008 and 2009 all millet treatments significantly increased yield of tubers (up to 49%) as compared to the continuous potato control. At the BL farm the millet treatments had consistently higher yields (up to 25%) but here the increases were not statistically significant. Much higher scab severity was observed in both years at the AAFC site than at the BL farm. For both years, at the AAFC farm the SS had the lowest scab disease severity and whereas at the BL farm it was the MS. The highest scab severity was found with the SGM treatment at both sites in 2008 whereas the continuous potato control had the highest incidence in 2009. In 2008, at both farms there was no significant difference in wilt incidence among treatments. In 2009, the SGM and the MS had the highest wilt incidence in the AAFC and BL farms, respectively. Interestingly, in 2008 the lowest wilt incidence was observed in SS for both farms. The effect of the millet applications on microbial composition

and functional genes is being determined by PCR-DGGE analysis of community DNA extracted from the potato rhizosphere. Overall, the mode of millet-green manure incorporation proved to be crucial as it seemed to influence potato yield and disease incidence of both scab and wilt.

Abstract Category
19 Plant-Microbe Interactions

PS.19.003 CREATION OF ARTIFICIAL ASSOCIATION BETWEEN NON-HETEROCYSTOUS CYANOBACTERIA AND CROP PLANTS

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Being a cosmopolitan organism, cyanobacteria are present in every environment. They have ability to form associations with organisms from all other domains especially with plants. Present study deals with development of artificial association between non-heterocystous cyanobacteria and plants and insight into different mechanisms involved in this relationship.

Cyanobacterial isolates from natural environment were co-incubated with ten days old plant seedlings of *Triticum aestivum*, *Vigna radiate* and *Pisum sativum* for one week. The cyanobacterial association with roots was estimated by measuring chl-a content. Roots were observed under light microscope using DIC optics and confocal laser scanning microscope to locate the presence of cyanobacteria. Indole-3-acetic acid was determined in media to view its role in colonization. Nitrogenase activity was measured by acetylene reduction assay. Nitrogen fixation of the isolates was molecularly demonstrated by amplification and sequencing nifH gene.

Cyanobacterial isolates were identified as *Chroococcidiopsis* sp., *Phormidium* sp. and *Leptolyngbya* sp. on the basis of 16S rRNA. CLSM clearly showed presence of cyanobacteria not only on rhizoplane but also invading and growing inside the root cells. Indole-3-acetic acid producing strains showed more attachment with the roots in comparison with the strains that lack this ability. As these strains have tryptophan dependent pathway for IAA synthesis, presence of IAA in culture media after co-incubation, showed IAA synthesis activity in presence of roots. *Chroococcidiopsis* sp. and *Leptolyngbya* sp. being nitrogen fixing showed much more attachment with roots in absences of nitrates in media. These strains demonstrated significantly higher nitrogenase activity in presence of plant roots. Creation of new cyanobacteria-plant associations opens new ways for cyanobacterial use in agricultural fields as biofertilizers that can also improve the plants growth and the rhizospheric conditions.

Abstract Category
19 Plant-Microbe Interactions

PS.19.004 BELOWGROUND INTERACTIONS AND ENVIRONMENTAL STRESS HAVE DIVERGENT EFFECTS IN TWO CO-OCCURRING LEGUMES

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Species do not exist in isolation and the environment does not act on them singly; yet we do not have a strong understanding of how environmental influences shape interspecific interactions. Leguminous crop species have been widely studied to improve agricultural practices, but there is large gap between agricultural knowledge about these plants and wild species growing without human intervention. We investigated the influence of soil microorganisms in general and rhizobia mutualisms specifically in two co-occurring, wild *Astragalus* species native to western North America that were subjected to selenium (Se) stress. One species, *A. bisulcatus*, has a unique adaptation to its environment and hyperaccumulates Se to levels that would be toxic to other plants and animals. The other species does not hyperaccumulate Se although it does occur in some of the same locations as *A. bisulcatus*. In a greenhouse experiment we found that in both plant species soil microorganism inoculation did not influence plant biomass or Se concentrations. Alternatively, we found divergences in the responses of the two *Astragalus* species to nodulation. The Se-hyperaccumulator concentrated 1.7 times more Se in its tissues when it was nodulated, but there was no effect of nodulation on the Se concentration in the non-accumulator. Additionally, the

hyperaccumulator concentrated 2 times more Se in the nodule than in the root, but there was no difference in belowground organ Se concentrations in the non-accumulator. These results indicate that there may be a specialized role of mutualisms in plant hyperaccumulation and that adaptation may have occurred in symbiotic rhizobia from co-occurring hosts.

Abstract Category
19 Plant-Microbe Interactions

PS.19.005 RHIZOBACTERIAL POTENTIAL TO ALTER INDOLE-3-ACETIC ACID CONTENT AND GROWTH OF VIGNA RADIATA (L.)

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Phytohormones especially indole-3-acetic acid (IAA) is considered very crucial in plant growth and development. Auxin production potential of plant associated bacteria offer good opportunity to use these microbes as biofertilizers to enhance the growth and yield and agronomically important crops. In present study, sixteen *Bacillus* strains isolated from rhizosphere, histoplane and phyllosphere of different plant species were identified by 16S rDNA gene sequencing. Potential of bacterial strains to influence the endogenous indole-3-acetic acid (IAA) content and growth of *Vigna radiata* (L.) was evaluated. Auxin production by *Bacillus* spp. in L-broth medium supplemented with 1000 µg ml⁻¹ L-tryptophan ranges from 0.60 to 3.0 µg IAA ml⁻¹ as revealed by gas chromatography and mass spectrometric (GC-MS) analysis. Plant microbe interaction experiments conducted under gnotobiotic conditions recorded 55.55%, 46.46% and 46.20% increase in shoot length with *Bacillus megaterium* MiR-4, *B. pumilus* NpR-1 and *B. subtilis* TpP-1, respectively, over control. *Bacillus* inoculations also increased shoot fresh weight with *B. megaterium* MiR-4 (60.94%) and *B. pumilus* NpR-1 (37.76%). Highly significant positive correlation between in vitro auxin production analyzed by GC-MS and shoot length ($r = 0.687^{**}$) and shoot fresh weight ($r = 0.703^{**}$) was noted under gnotobiotic conditions. Similarly, significant correlation was also found between auxin production by *Bacillus* spp. (GC-MS analysis) and different growth parameters such as shoot length ($r = 0.495^{*}$), number of pods ($r = 0.498^{*}$) and grain weight ($r = 0.537^{*}$) at full maturity under natural wire house conditions. Results showed that auxin production potential of plant associated *Bacillus* spp. can be effectively exploited to enhance the growth and yield of *V. radiata*.

Abstract Category
19 Plant-Microbe Interactions

PS.19.006 RHIZOSPHERE MICROBIAL COMMUNITY RESPONSE TO BIO-CHAR ADDITION

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Incorporation of biomass derived black carbon (bio-char) into soil has been shown to play an important role for C sequestration, increasing crop yields, reducing greenhouse gas emissions, improving biological nitrogen (N) fixation, enhancing microbial biomass and activity along with aiding nutrient retention and availability in soils. Although the suggested benefits of bio-char addition to agricultural soils are promising, it is problematic to extrapolate current knowledge to make universal statements regarding the effects of bio-char. The aim of this work is to investigate microbial community composition with bio-char addition and relate that to potential agronomic benefits.

Up to 10 % by volume additions of bio-char were added to soil at pot to field scales planted with (and without) perennial ryegrass or wheat. Treatments included bio-char, bio-char pre-conditioned in a nutrient solution and sintered glass controls. N, P and trace metals were analyzed along with soil moisture, pH and dehydrogenase activity. Microbial community development was assessed using molecular fingerprinting (T-RFLP) of bacteria and fungi with 454 sequencing to identify genus and species level peaks in T-RFLP profiles. Bulk soil and the rhizosphere were assessed. Results indicate slight elevations in pH, moisture and dry matter yields with bio-char addition but no differences in trace element uptake. Nitrate and nitrite decrease rapidly in all treatments with evidence of increased nitrogen mineralization seen at the end of 12 weeks. There was a decrease in

dehydrogenase activity without bio-char conditioning. Significant differences in microbial communities were observed between treatments with temporal shifts as well. In the system investigated, bio-char causes no obvious chemical effects but does cause significant differences in the microbial population. Whether these differences are beneficial or detrimental is yet to be established.

Abstract Category
19 Plant-Microbe Interactions

PS.19.007 MOLECULAR SCREENING OF ENDOPHYTIC ACTINOBACTERIA REVEALS A MYRIAD OF POLYKETIDE SYNTHASES

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The research of new natural products has focus efforts in study of new environments and recalcitrant organisms such as those living in association with plants: endophytic microorganisms (bacteria and fungi), and non-cultivable organisms assessing only by metagenome approaches, as important producers of new chemical entities with multiple applications. Polyketide compounds play an important role as a chemical group providing many useful compounds in medicine, veterinary and agriculture. The objectives of this work were: (i) select from a collection of endophytic Actinobacteria those with the presence of polyketides synthase genes types I, II and III; (ii) to evaluate the antimicrobial activity of the endophytic actinobacteria; (iii) to identify the selected strains by 16S rDNA analysis. The screening was carried out using primers for β -ketoacyl synthases (PKS I) and α -ketosynthase (PKS II). A third group of primers were constructed to amplify THNS (1,3,6,8-tetrahydroxynaphthalene synthase) a member of PKS III subgroup. Sequences were compared with those of GenBank, showing that 65.5-56% of the strains codified for polyketides types I and II respectively; 14% of the strains posses genes that codified for THNS, enzyme present in a new PKS III pathway, with unexplored molecules. It is interesting that 9% of the strains carry all three types of polyketide synthases, enhancing the possibilities of obtain new molecules. The antimicrobial activity assays shown high expressive antifungal and antibacterial activities. Identification studies concluded that many of the isolates are *Streptomyces* sp. The compounds were purified by HPLC/MS. This work shows the variety of possibilities to find new molecules between endophytic Actinobacteria specially those related with PKS III type.

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Abstract Category
19 Plant-Microbe Interactions

PS.19.008 DYNAMIC EXCHANGE OF EXUDATES BETWEEN BACTERIA AND PLANT ROOTS

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Plant root-microbe crosstalk is a dynamic complex process involving numerous active compounds produced by plant roots and soil bacteria. Due to high complexity in the rhizosphere the monitoring of the chemical dialog between interacting organisms is highly challenging. A new experimental design based on *Medicago truncatula*-*Sinorhizobium meliloti* interaction is presented for screening dynamics of signal exchange between plant roots and bacterial cells under laboratory condition. This setup allows studying the exudates of interacting parts during considerable period of time independently from each other. Plants of *Medicago truncatula* and *Sinorhizobium meliloti* were preincubated in the Broughton and Dilworth growth media for 6 hours. Filtrated medium from the both organisms was consequently transferred into one another. The procedure was repeated after 12, 24, 36 and 48 hours. Exudates were extracted with ethyl acetate and analyzed with mass spectrometry. Preliminary results showing changes in the metabolic profiles of the exudates in time are presented. Further examination will be done to identify novel small molecular weight compounds important in mutual recognition process between the model rhizobial symbiont *Sinorhizobium meliloti* and the model host plant *Medicago truncatula*.

Abstract Category
19 Plant-Microbe Interactions

PS.19.009 PLANT HOST INFLUENCES ON ASSOCIATED ACTINOBACTERIAL COMMUNITIES VARY WITH PLANT DIVERSITY

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Factors shaping the biogeography of microbes remain poorly understood. However, there is growing recognition of the selective influence of plant host on soil microbial communities. We explored whether host plant influences depend on a wider plant community context, and specifically whether the same host plant fosters distinct rhizosphere microbial communities when grown in plant communities of varying diversity.

We used targeted 454 pyrosequencing to characterize soil actinobacterial communities associated with four different plant hosts (*Andropogon gerardii*, *Schizachyrium scoparium*, *Lespedeza capitata*, *Lupinus perennis*) growing in 15 year-old experimental plant communities of differing diversity (1-, 4-, 8-, 16-, or 32-species plant communities).

Our sequencing effort revealed tremendous diversity among actinobacteria, with 1329 operational taxonomic groups identified and new taxa still being discovered after sampling approximately 250,000 sequences. Actinobacterial community composition and richness varied among plants and in response to plant community diversity. *A. gerardii* supported significantly lower actinobacterial richness and diversity relative to the other plant hosts, and when grown in monoculture compared to more diverse plant communities. Actinobacterial diversity associated with *L. capitata* was highest under monoculture, while diversity associated with *L. perennis* was lowest under monoculture.

Across host species, monoculture actinobacterial diversity was significantly lower than for more diverse plant community treatments. In some cases, experimental plot had a significant impact on actinobacterial community structure, indicating a biogeography independent of plant host.

Although plant-microbe interactions are critical to structuring rhizosphere microbial communities, plant-plant interactions at larger spatial scales are also important determinants of microbial community diversity and composition.

Abstract Category
19 Plant-Microbe Interactions

PS.19.010 IMPACT OF PSEUDOMONAS FLUORESCENS-MEDIATED INDUCED SYSTEMIC RESISTANCE ON RHIZOSPHERE MICROBIAL COMMUNITIES

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Selected strains of plant root colonizing fluorescent *Pseudomonas* spp. can effectively suppress plant diseases. Direct interactions with the plant pathogen can play an important role in disease suppression. However, particular strains are effective even when they are applied, and remain, spatially separated from the pathogen on the plant surface. The plant develops a state of enhanced defensive capacity and this mode of action is termed induced systemic resistance (ISR). ISR has been described for several strains of fluorescent pseudomonads in a variety of crop plants and against a wide range of pathogens. Using the model plant *Arabidopsis thaliana* progress has been made in understanding signal transduction pathways involved in induced resistance. It was established that perception of ethylene and jasmonic acid is important in ISR. The traits of *Pseudomonas* that can trigger ISR appear to be very diverse, including iron regulated metabolites, antibiotics, cyclic lipopeptides and lipopolysaccharides. Moreover there is redundancy of ISR elicitors.

Since pathogen growth is restricted on plants that are in the state of ISR, we hypothesized that the indigenous rhizosphere microflora could also be affected by ISR. Using both cultivation dependent and independent techniques, effects of plant defense signaling on the total bacterial and the *Pseudomonas* spp. microflora of *Arabidopsis* were studied. Results are discussed in the context of applications of *Pseudomonas* bacteria for biological control of diseases.

Abstract Category
19 Plant-Microbe Interactions

PS.19.011 MICROBIAL INHIBITION OF *LECANICILLIUM FUNGICOLA* IN THE MYCOSPHERE OF *AGARICUS BISPORUS*

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Lecanicillium fungicola (Preuss) Zare and Gams (synonyms: *Verticillium fungicola* (Preuss) Hassebrauk, *Verticillium malthousei* (Preuss) Ware) is the causal agent of dry bubble disease, one of the biggest problems in the commercial production of the white-button mushroom, *Agaricus bisporus*. Control of dry bubble disease relies heavily on the use of prochloraz-manganese (i.e. sporgon), but reduced sensitivity of *L. fungicola* to this fungicide has been reported (Gea et al., 2005; Grogan, 2008). Therefore, novel strategies to combat dry bubble disease are urgently needed. Biological control of dry bubble disease could prove effective and would meet the mushroom industries efforts to minimize the use of chemicals. A thorough understanding of *L. fungicola*'s life cycle and ecology is needed for sensible development of effective control of this pathogen. The most important part of the activity of *Lecanicillium* happens in the casing layer, a microbially active layer of alkalized peat soil that is needed for the formation of mushroom fruiting bodies. We studied spore germination and subsequent hyphal growth of *L. fungicola* microscopically on cyclopore membranes. In the absence of competing microorganisms spores readily germinated, but germination was reduced at high spore densities, suggesting the presence of a self-inhibitor. The microbiota present in the casing layer strongly inhibited *L. fungicola* development. This inhibition was lifted by colonization of the casing by *A. bisporus*, but also by applying a select range of simple sugars produced by *A. bisporus*. These results suggest that the fungal spores need external nutrients to germinate. However, thoroughly washed spores of *L. fungicola* germinated freely on ultra pure water, suggesting that not only nutrient availability is determinative for spore germination.

Abstract Category
19 Plant-Microbe Interactions

PS.19.012 A HIGHLY SELECTIVE QUANTITATIVE POLYMERASE CHAIN REACTION PROTOCOL FOR *BURKHOLDERIA* AND *PSEUDOMONAS* BACTERIA TESTED IN SOIL.

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The very complex soil ecosystems include an extremely high amount of bacteria, but so far detection and quantification has been very difficult. The aim of this study has been to develop two highly specific quantitative polymerase chain reaction (qPCR) assays. The assays are designed to quantify the potential opportunistic pathogenic bacteria *Burkholderia* and *Pseudomonas* in soil. The qPCR assays are functioning with both SYBR Green and Taqman probes, as well as being compatible with the high throughput 454 Pyrosequencing technique allowing species identification of the amplified sequences. To test the new methodology qPCR were used to estimate the number of the *Burkholderia* and *Pseudomonas* community in different fertilized soils. In combination with a eubacterial 16S qPCR assay the proportion of *Burkholderia* and *Pseudomonas* in soil was estimated. The qPCR estimates in soil detected bacterial values in the range of 10⁵-10⁶ *Burkholderia* and *Pseudomonas* pr g of soil, showing a larger bacterial community in the fertilized soils compared to the control soil. Pyrosequencing experiments with the developed assays showed high discriminatory powers only amplifying the correct targets (98,5% specificity), and around 200 unique *Pseudomonas* phylotypes at a 97% level. The presented material represents a new uniformed way to quantify and identify *Pseudomonas* and *Burkholderia* in different environments, which are illustrated in experiments performed on different long-term fertilized soils. The methods can easily be adapted to other environments like water or sludge, clinical use, or other bacterial subpopulations.

Abstract Category
19 Plant-Microbe Interactions

PS.19.013 PROTEOMIC ANALYSIS OF ARABIDOPSIS DEFENSE RESPONSE AFTER ENDOPHYTIC ACTINOBACTERIA INOCULATION AND CHALLENGE WITH FUNGAL AND BACTERIAL PATHOGENS

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Confronted with decline in natural resources and decrease in arable land, the discovery and utilisation of sustainable practices in agriculture are essential to keep up with demand. The use of chemical pesticides and fertilisers in agricultural crops are economically costly as well as detrimental to the environment and non-target organisms. Growing interest in the development of natural alternatives such as plant growth promoting micro-organisms that help sustain good crop production as well as defend against debilitating pathogens can help ease the use of pesticides. Endophytic actinobacteria previously isolated from wheat have been shown to enhance plant growth by suppressing wheat fungal pathogens such as *Gaeumannomyces graminis* var. *tritici*, showing potential for future use as a biocontrol agent. One such strain, *Streptomyces* sp. EN27, was used to study the effect of colonisation on the production of Arabidopsis plant defense proteins. Proteomic analysis using 2D DIGE and DeCyder software of total protein extracted from 6 week old untreated and *Str.* EN27-treated Arabidopsis plants yielded no significant differences in protein expression. However, previous analysis of transcripts using real-time polymerase chain reaction, showed that treatment of Arabidopsis with *Str.* EN27 resulted in a 19-fold induction of pathogenesis-related protein 1, a key gene expressed during plant systemic acquired resistance (SAR). The lack of correlation between proteomic and transcript analysis indicates that translation into protein product might be delayed or that the increase in protein level is not detectable. Therefore, investigation of plant protein expression after pathogen challenge of EN27 inoculated plants with *Erwinia carotovora* subsp. *carotovora* and *Fusarium oxysporum* will be compared with previous and current transcript analysis.

Abstract Category

19 Plant-Microbe Interactions

PS.19.014 CARBOHYDRATE TRANSFER MECHANISMS OF ORCHID MYCORRHIZAS FORMED BY RHIZANTHELLA GARDNERI.

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Orchids rely on their mycorrhizal fungal partners for a Carbon (C) source. The fungi forming mycorrhizas with orchids are diverse which is not surprising given there are orchid species found in almost every terrestrial ecosystem on earth. However, there is no real understanding of how different fungi are able to supply C to orchids; whether there is active nutrient transfer across plant/fungal interfaces or whether the orchid is able to 'digest' the fungus using methods akin to phagocytosis.

We used *Rhizanthella gardneri* Rogers to observe how C is transferred from fungus to orchid. *R. gardneri* is a fully subterranean (therefore obligately myco-heterotrophic) orchid that associates with a specific fungus (*Ceratobasidiales*), which in turn forms ectomycorrhizas with co-occurring autotrophic shrubs (*Melaleuca uncinata* s.l.). We grew *R. gardneri* with its specific mycorrhizal fungus and co-occurring *Melaleuca* in microcosms. We added ¹³CO₂ to *Melaleuca* foliage and traced it through the shared fungus to *R. gardneri* using isotope ratio mass spectrometry. We then used a nano scale isotope imaging method (nanoSIMS) to see if we could trace movement of added ¹³C across the orchid mycorrhizal interface.

Within the *R. gardneri* - fungus - *Melaleuca* tripartite relationship, ¹³C was being transferred across ectomycorrhizal interfaces within *Melaleuca* roots and onto the fungal portion of the orchid mycorrhizas. However, there was no evidence of any ¹³C movement across the interface formed between *R. gardneri* and its mycorrhizal fungus.

The results from this study suggest that nutrient transfer from fungus to orchid via mycorrhizas is a result of the orchid digesting the fungus - not active transfer.

Abstract Category
19 Plant-Microbe Interactions

PS.19.015 SALMONELLA ENTERICA NICHE OVERLAP IN DICKEYA DADANTII PLANT LESIONS AND HUMAN INTESTINAL EPITHELIA REVEALED BY TRANSCRIPTOME ANALYSIS

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Human enteric pathogens can exploit the plant habitat and thereby affect public health, as evidenced by the emergence of food-borne illness associated with leafy vegetables. We have investigated the fitness of *Salmonella enterica* on lettuce and cilantro leaves, and its interaction with the soft-rot plant pathogen *Dickeya dadantii* (*Erwinia chrysanthemi*). The enteric pathogen achieved large population sizes and formed complex biofilms on leaves infected with *D. dadantii*. Microarray analysis of the gene expression profile in *S. enterica* cells colonizing lettuce and cilantro soft rot lesions caused by *D. dadantii* revealed that the pathogen deploys a large part of its transcriptional machinery for anaerobic metabolism and the utilization of propanediol and ethanolamine. These pathways, which function in the human intestine, are activated in lettuce and cilantro by the presence of substrates resulting directly from the degradation of plant cells by the soft rot pathogen. PduE, EutK and CobS mutants of *S. enterica* had decreased fitness in the lesions compared to the parental strain. Genes involved in the transport of, or regulated by, quorum sensing autoinducers were induced also in soft rot lesions, indicating cross-talk between the plant and human pathogens. Additionally, *S. enterica* cells recovered from soft rot lesions were more invasive of eukaryotic cells than when grown in culture. Our observations suggest that plant pathogens contribute to creating a familiar and hospitable niche for human pathogens on plants, which may affect their infectious dose in humans.

Abstract Category
19 Plant-Microbe Interactions

PS.19.016 FACTORS CONTROLLING SOIL AND RHIZOSPHERE MICROBIAL COMMUNITY STRUCTURE IN AN AGROECOSYSTEM

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A replicated agroecological experiment was run in the field for three years in which the major factors controlling soil microbial biomass and community structure were identified. Tomato was the main crop and nine treatments were included: bare soil, black polyethylene, white polyethylene, hairy vetch cover crop, hairy vetch above-ground biomass, hairy vetch below-ground biomass, rye cover crop, rye above-ground biomass, and rye below-ground biomass. We addressed the following questions: (1) How strong are the effects of treatment and environmental variables on soil microbial community composition among the treatments? (2) Do different species of cover crops have unique root and shoot effects on soil microbial community composition? Soil factors measured included pH, texture, temperature, and moisture. Tomato rhizosphere and bulk soil were sampled and analyzed for phospholipid fatty acids (PLFA). Soil DNA was extracted from a subset of samples and analyzed by T-RFLP and pyrosequencing. Cover cropping increased the absolute amount of all microbial groups, but decreased the proportion of Gram-positive bacteria, which we believe was due to increased readily available carbon under cover-cropped treatments. Vetch shoots increased the amount and proportion of Gram-negative bacteria, fungi, and arbuscular mycorrhizal fungi in the rhizosphere of tomato plants. T-RFLP revealed differences in phylum-level responses to season, cover crop, and rhizosphere. Pyrosequencing of the 16s rRNA gene indicated positive effects of vetch on the proportion of Bacteroidetes, Chloroflexi, Nitrospira, and Planctomycetes, while negative vetch effects were observed on the proportion of Actinobacteria, Gemmatimonadetes, and Firmicutes. The lower proportion of Firmicutes and Actinobacteria under vetch was consistent with the PLFA results. The imposed treatments were much more significant than soil temperature, moisture, pH, and texture in controlling microbial biomass and community structure.

Abstract Category
19 Plant-Microbe Interactions

PS.19.017 MICROBIAL INTERFERENCE OF ZOOSPOROGENESIS IN THE OOMYCETE PLANT PATHOGEN *PYTHIUM APHANIDERMATUM*

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Pythium aphanidermatum is one of the most important causes of seed and seedling mortality of many plant species. However, in the presence of composted substrates, pathogenesis is often reduced or suppressed. Although the mechanisms of suppression are unknown, microbial activities are believed to interfere with pathogenic developmental responses to plant stimuli. An especially vulnerable developmental stage is the process in which sporangia produce vesicles that subsequently release motile zoospores under flooded conditions (a process call zoosporogenesis). Zoospores swim chemotactically to plants where they encyst, germinate and infect plants within hours after release from sporangia. Current evidence suggests that zoospore chemotaxis and encystment are suppressed by microbial consortia from vermicomposted dairy manure, yet nothing is known of the vulnerability of sporangial responses to suppression. The aim of our study was to examine zoosporogenesis development to better understand how complex microbial consortia from vermicomposts suppress *P. aphanidermatum* pathogenesis. Sporangial responses were observed under flooded conditions in the presence or absence of vermicompost substrates. Mode of germination (germ tubes versus zoospores), vesicle development, and zoospore release were monitored using time-lapse photomicroscopy. In the absence of vermicompost, vesicles formed within 1-6 hours of flooding; zoospores were released 30 minutes later. In contrast, no viable vesicles or zoospores developed in the presence of either sterile or non-sterile vermicompost and all sporangia germinated via germ tubes. Results indicate that vermicompost may disrupt zoosporogenesis of *P. aphanidermatum* and the active principle(s) may be associated with abiotic properties of the vermicompost. These results, coupled with our previous finding of zoospore chemotaxis suppression, suggest that multiple mechanisms of suppression may operate at different stages of pathogenic development

Abstract Category

19 Plant-Microbe Interactions

PS.19.018 PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF RHIZOBIAL OBTAINED NODULES OF PLANTS PEANUT

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The peanut is an important food crop and has the potential to benefit from biological nitrogen fixation through symbiosis with rhizobia. In soils of tropical regions has been occurring in populations with characteristics very different from those traditionally described for microsymbionts peanut. In order to study the diversity and phylogeny of rhizobia, 22 bacteria were isolated from nodules of peanut varieties 7 (L7, BR 1, Caipira, Jumbo, Tatu, IAC 8112 and Native), grown in soils of the Zona da Mata de Pernambuco. The isolates were characterized in culture medium (time for growth, change in pH, size, shape, edge, transparency and color of the colony and production of mucus) and tested for antibiotic resistance. We also determined the profiles of DNA after amplification (PCR) with BOX primer, which were compared with profiles of nineteen type strains. Based on molecular and phenotypic characteristics of bacteria were constructed dendrograms grouping of isolates. All isolates grew most quickly soured and the culture medium. In general, there was high resistance to the antibiotics: penicillin G, chloramphenicol and tetracycline and susceptibility to neomycin, erythromycin and rifampicin. The phenotypic characteristics (morphology of colonies and RIA) revealed little information on the phylogeny of the isolates. However, the molecular and polyphasic we observed great diversity among the rhizobia, including the possibility of new species.

Abstract Category

19 Plant-Microbe Interactions

**PS.19.019 IMPACT ASSESSMENT OF BT COTTON ON MICROBIAL COMMUNITY
STRUCTURE AND FUNCTION**

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India occupied the first position in Bt-cotton cultivation with 7.6 million hectares (ISAAA, 2008) which is the only transgenic crop under commercial cultivation. The transgenic agriculture is associated with the tangible benefits and intangible risks. Amongst the intangible risk, of special concern is the impact of transgenic crops on aboveground and belowground ecological integrity. To gauge the ecological impact of Bt cotton, a field experiment for two consecutive years was established with MRC 6301+ Bt, and its near isogenic line at IARI field, India. Mapping of microbial community structure by phospholipid fatty acids profiles in rhizosphere samples of Bt Cotton and its near isogenic lines indicated the significant difference in microbial community structure pattern through out the cropping season. Principal component analysis of the PLFA data showed 36 % of the total variation by PC1 and 24 % by PC2. Phospholipid fatty acid biomarkers such as branched (i15:0; a15:0; i17:0), straight monosaturated (18:1w9) and polyunsaturated (18:2 w6) delineated the microbial community composition of transgenic crop, from its near isogenic line at different crop stages. There was no significant difference ($p < 0.05$) in culturable community of inorganic phosphate and organic phosphate dissolving bacteria as well as nitrogen fixing bacteria. Shannon diversity index was significantly high ($p < 0.05$) in Bt cotton rhizosphere as compared to its isogenic line. Guild utilization pattern showed that microbial community in Bt cotton rhizosphere could utilize more complex compound such as phenolics, polymers and amines as compared to simple compounds by its near isogenic line. This probably may be due to the presence of Bt toxin in soil. The toxin upto the level of 0.0036 ppm was even detected in the soil after 242 days of plantation of the Bt cotton in the field. Plots planted with Bt cotton revealed more structural and functional microbial diversity.

Abstract Category

19 Plant-Microbe Interactions

**PS.19.020 DIVERSITY AND STRUCTURE OF MICROBIAL COMMUNITIES ASSOCIATED
TO THE RHIZOSPHERE OF *Rhizophora mangle* FROM A NORTHEAST BRAZIL MANGROVE**

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Mangrove biodiversity and conservation have received considerable attention in recent years since research has increased the understanding of the values, functions and attributes of these ecosystems, although studies focusing on microbial diversity are still scarce. With the advance of the molecular biology techniques, the understanding of microbial diversity has increased and also provided a powerful tool to monitoring environments. Thus, this study aimed to access the structure and diversity of microbial communities from the rhizosphere of red mangrove (*Rhizophora mangle*) in three sites along the estuary and in two periods (drought and rainy seasons) from an urban mangrove in Northeast Brazil using Denaturing Gradient Gel Electrophoresis. These data were correlated to environmental variables and soil characteristics. Results showed that soil samples are similar in number of ribotypes. In general, sites presented 36 and 42 ribotypes respectively for *Bacteria* and *Archaea*. Principal Component Analysis revealed that the distribution of the microbial communities is influenced by pH and salinity in the middle portion of the estuary, which is more exposed to tidal hydrodynamics, and by contents of organic matter and silt-clay in the upper portion of the estuary. Although the environmental variables influence the distribution of microorganisms, the sites share 61% and 50% of the total of *Bacteria* and *Achaea* ribotypes, respectively, and this can be attributed to the rhizosphere effect. The diversity indexes showed resemblances in richness, diversity and equitability among the communities. The high values of equitability (mean of 0.96) found in this work can be credited to the communities stability. In conclusion, this urban Brazilian mangrove harbor diverse and stable microbial communities and the broad spatial distribution of bacterium populations inside the red mangrove habitats seems to be controlled primarily by rhizosphere effect and secondarily by abiotic variables.

Abstract Category

19 Plant-Microbe Interactions

PS.19.021 BIOCHAR SOIL AMENDMENT: PINPOINTING MICROBIAL ELUCITATORS OF INDUCED SYSTEMIC PLANT RESISTANCE

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Biochar (charcoal) is a product of biomass pyrolysis (thermal decomposition in the absence of oxygen). When biochar is applied as a soil amendment, the carbon in biochar is permanently sequestered (half-life of 100s-1000s of years). Henceforth, biochar soil amendment has been proposed as an innovative approach to reducing global CO₂ emissions. In addition, several studies have found that biochar application to soil enhances crop production, increases nutrient and water retention, and improves soil tilth. We found that biochar soil amendment significantly suppressed (33-92%) the foliar fungal pathogens *Botrytis cinerea* (gray mold) and *Leveillula taurica* (powdery mildew) in pepper and tomato, and *Podosphaera aphanis* in strawberry. It is most likely that the biochar induced systemic resistance in the inoculated plants since the site of infection (leaves) was spatially separate from that of the amendment (roots), and soil and leaf nutrient concentrations did not differ between biochar-amended and non-amended soils. Resistance may be partially induced by chemical elicitors such as resorcinol and various fatty acids that are ubiquitous to many biochars. Nonetheless, changes in microbial profiles in the rhizosphere of biochar-amended soils indicate that microbial factors may be at least partially responsible for the observed induced plant resistance. Indeed, biochar amendments influenced abundance and diversity of culturable *Pseudomonas*, *Bacillus*, *Actinomyces*, and *Trichoderma* strains, all of which are known to potentiate plant systemic resistance. Specific char-associated strains belonging to these groups were pinpointed using molecular fingerprinting methods and isolated on selective char-amended media. These groups will be used in screening assays to pinpoint biochar-associated strains with enhanced plant growth-promoting and resistance-inducing characteristics.

Abstract Category

19 Plant-Microbe Interactions

PS.19.022 EFFECTS OF BARLEY CULTIVAR ON MICROBIAL COMMUNITY STRUCTURE: A HIERARCHICAL APPROACH

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The rhizosphere is the most active soil compartment and is strongly influenced by the activity of plant roots. Previous work has yielded conflicting results as to whether the plant is a strong or weak driver of bacterial community structure in the rhizosphere. It remains unknown whether this uncertainty relates to differences in plant biology or resolution of the methodology applied. We have applied a high throughput molecular method, terminal restriction fragment length polymorphism analysis, combined with multivariate statistics to assess the relationship between barley cultivars and bacterial populations at a range of community complexities. This allows us to assess the hypothesis that relationships between plant and microbial community are masked when assessing total bacteria which becomes clear if complexity is reduced. To achieve this we have applied a hierarchical methodology targeting bacteria both at a range of phylogenetic and functional group levels. Nitrogen cycling groups were selected for the functional group analysis using denitrifiers as a diverse and ammonium oxidizers as a restricted group. Barley lines were selected to represent a large genotypic variation and included landraces and old and new commercial cultivars with both spring and winter habits. Plants were grown in the glasshouse in three contrasting soil types and both rhizosphere and bulk soils were collected and analysed. Results show clear effects of soil type and compartment at each level of complexity and suggest that distinctive bacterial communities are generated on barley cultivars with a greater degree of separation at finer phylogenetic or functional levels, thus supporting the hypothesis. These results, in combination with differences in functional activity between barley lines, suggest a route to examine the role of the plants in driving soil community structure and function.

Abstract Category

19 Plant-Microbe Interactions

PS.19.023 ANALYSIS OF ENDOPHYTIC BACTERIAL COMMUNITIES ASSOCIATED WITH PLANTS IN A HEAVY METAL CONTAMINATED AREA

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The soil from the Park Nagib Najar (PNN), located in the city of Mogi das Cruzes (Brazil), presents high levels of iron, manganese, lead, chromium, nickel, cadmium, copper and zinc. The aim of the present study was to evaluate the endophytic bacterial communities associated with two tree species abundant in such area (*Tecoma stans* and *Blechnum brasiliensei*). A total of four samples were collected along the PNN, in transect considering different levels of soil contamination. A culture-dependent analysis showed a trend, suggesting that the contamination with heavy metals can drastically affect the density of endophytes in analyzed plants. Also, shifts were observed for distinct plant species, with log values ranging from 1.89 to 4.03 (UCF per gram of stem), in *T. stans* and *B. brasiliensei*, respectively. In addition, a clone library containing 167 sequences of the 16S rRNA gene clone libraries was constructed for the culture-independent assessment of the endophytic community of *Tecoma stans*. The sequences analysis revealed the presence of twenty seven genera constituting the endophytic community in these samples, with the finding of already known plant endophytes as the major groups; *Bacillus*, *Curtobacterium*, *Enterobacter*, *Methylobacterium*, *Rhizobium* and *Stenotrophomonas*. However, it was also remarkable the presence of sequences affiliated to the genera *Hymenobacter*, *Lysobacter*, *Massilia*, *Pandora* and *Prochlorococcus*, not described before as plant endophytes. The results have already indicated that the heavy metals contamination has a role upon the microbial community residing within plant tissues, what possibly could result in the occurrence of new groups occupation of these niches.

Abstract Category

19 Plant-Microbe Interactions

PS.19.024 ARBUSCULAR MYCORRHIZAL COMMUNITIES ASSOCIATED WITH SOIL AGGREGATE SIZE CLASSES IN THE RHIZOSPHERE OF MYCORRHIZAL INOCULATED WILLOWS (*SALIX SPP*)

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Willows are known to benefit from symbioses with arbuscular and ecto-mycorrhizal fungi, and are commercially inoculated to enhance their growth. Another advantage of mycorrhizal inoculation can be increased soil aggregation, however little is understood about how mycorrhizal inoculation affects the formation of aggregates and whether arbuscular mycorrhizal fungi (AMF) species associate with different aggregate size classes. A greenhouse study was established to determine the impact that mycorrhizal inoculation has on willow growth, percent water stable aggregates (WSA) and diversity of AMF in the rhizosphere. Cuttings of willows (*Salix viminalis* and *S. miyabeana*) were potted in 6 different soils collected from Southern Ontario and inoculated with a commercial mycorrhizal inoculant in 1 of 4 combinations: 1) *Glomus intraradices* (AM), 2) *Hebeloma cylindrosporum* (EM) 3) both (AM & EM) 4) uninoculated (control). After 8 months, willows were harvested, WSA was determined, and aggregates were separated by dry sieving (>4mm, 4-2mm, 2-0.5mm and <0.5mm). The diversity of AMF associated with each size class was assessed by nested polymerase chain reaction - denaturing gradient gel electrophoresis (PCR-DGGE) targeting the 18S rDNA gene. DGGE bands were cut, sequenced, and compared with known sequences in Genbank and a phylogenetic tree was constructed. WSA was significantly affected by mycorrhizal inoculation and soil type, the highest percent was recorded in AM > AM & EM > EM > control. The diversity of the AMF communities was significantly affected by soil type, mycorrhizal type and aggregate size class. Our results suggest that inoculation with AMF can improve water stable aggregation of soil. Furthermore, we have shown that AMF diversity differs between aggregate size classes, indicating that some populations of AMF may be associated with certain aggregate sizes. Studies are underway to determine if similar results are found under natural soil conditions

Abstract Category

19 Plant-Microbe Interactions

PS.19.025 DIVERSITY OF BACTERIA AND DIAZOTROPHS ASSOCIATED TO XEROPHYTIC PLANTS OF LIBYAN SEMI-DESERTS AND FUTURE APPLICATIONS AS BIOFERTILIZERS

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The study investigated the microflora associated to the xerophytic plants of Abu-Ghelan area, Libya. Fourteen plants were obtained and analyzed for the microbial load of total culturable bacteria and diazotrophs. Differential populations were traced outside (ecto-rhizosphere) and inside (endo-rhizosphere) roots as well as shoots (phyllosphere) of plants. Rhizobacteria were common residents to the plant roots with populations exceeded 10⁷ g⁻¹. The nitrogen-fixing bacteria (diazotrophs) constituted a significant portion of the total bacteria associated to the plant roots and shoots. More than 300 representative isolates of diazotrophs were selected and subjected to morphological, cultural and physiological studies. The taxonomic position of 33 isolated was confirmed based on API profile. Gram negative diazotrophs were represented by the genera *Citrobacter* (*C. freundii*), *Enterobacter* (*E. cloacae*, *E. agglomerans*, *E. amnigenus*), *Klebsiella* (*K. pneum. pneumoniae*, *K. pneum. ozaenae*), *Pantoea* spp., *Pseudomonas* (*P. aeruginosa*, *P. putida*). The spore-forming *Bacillus* (*B. circulans*, *B. macerans*, *B. megaterium*, *B. polymyxa*) were encountered. The high populations of diazotrophs and their wide diversity associated with tested plants is a direct clue on their very possible role played toward plant health and nutrition. Future studies should continue with the use of such diazotroph strains in the preparation of biofertilizers and application under Libyan conditions.

Abstract Category

19 Plant-Microbe Interactions

PS.19.026 ENRICHMENT AND ISOLATION OF TERRESTRIAL AND PLANT-ASSOCIATED METHYLOTROPHS DEGRADING METHYLATED SULFUR COMPOUNDS

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Methylated sulfur compounds (C1S) including dimethylsulfide (DMS) play a crucial role in the global sulfur cycle. DMS emitted from the marine environment and its oxidation products affect atmospheric chemistry and climate and are a source of sulfur input into terrestrial environments. Few microorganisms degrading C1S compounds in terrestrial habitats have been isolated so far. We investigated the culturable methylotrophic diversity in several terrestrial habitats with focus on methylotrophs able to grow on C1S compounds. Samples from two different soils, *Brassica oleracea* rhizosphere and *Brachytecium* (moss) were collected and enrichment cultures were set up with methanol, DMS and dimethylsulfoxide (DMSO). *Brachytecium* and *Brassica* leaf samples were also used to isolate methylotrophic bacteria from the phyllosphere. Diversity of the enrichments was assessed using denaturing gradient gel electrophoresis (DGGE). Soil and rhizosphere samples enriched on DMS, DMSO and methanol had similar DGGE patterns. Dominant populations in enrichment cultures were identified by sequencing as *Thiobacillus*, *Rhodococcus*, *Cytophaga*, *Flavobacterium* and bacteria of uncertain affiliation. Isolates were obtained, identified and tested for substrate range. Isolates affiliated with genera previously recognized to contain methylotrophic species (*Hyphomicrobium*, *Methylobacterium*, *Rhodococcus*, *Arthrobacter*, *Flavobacterium*, *Variovorax*) as well as novel methylotrophs were obtained, including strains of *Pedobacter*, *Rhodanobacter*, and *Stenotrophomonas*. The majority of the isolated strains from soil and rhizosphere samples were members of *Hyphomicrobium* and *Pedobacter*; however, *Flavobacteria* dominated the cultivable *Brachytecium* leaf methylotrophic community. This work demonstrates that the ability to grow on methylated sulfur compounds is more widespread in the bacterial world than previously recognized, but also shows that isolation of particular methylotrophs remains challenging.

Abstract Category

19 Plant-Microbe Interactions

PS.19.027 PREVALENCE AND COLONIZATION PERFORMANCE OF HUMAN PATHOGENIC BACTERIA IN FRESH PRODUCE

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An increase in disease outbreaks associated with the consumption of vegetables has been observed, and it has increasingly become recognized that human pathogenic bacteria can survive and multiply on and in fresh produce. Nevertheless, vegetable-linked outbreaks are not well documented from a European perspective. Thus, in four European countries field-grown vegetables and manure derived fertilizers as potential sources of pathogen infestation were screened for the prevalence of *Salmonella enterica* ssp., *Campylobacter* ssp., *Listeria monocytogenes*, *Staphylococcus aureus* and *E. coli*. Successively, the colonization behavior of these pathogens under the influence of various environmental and management-related factors was explored.

PCR- and ISO-based analyses performed on enrichment cultures revealed an unexpectedly high number of positive samples in the surveys. 38 to 70% of the manure samples from the various countries proved positive for *Campylobacter* ssp. and up to 24% for *S. aureus*, while *S. enterica* ssp. was less frequently detected. In vegetables from fields where the manure analyses had indicated a potential risk of contamination, *S. aureus* was found most prevalently in up to 20% and *S. enterica* ssp. in maximum 15% of samples. The pathogen colonization experiments using qPCR of pathogen specific marker genes evidenced a minor role of manure treatment and highlighted the significance of the type of pathogen involved. For instance, *S. enterica* ssp. seemed to be significantly promoted in growth by the presence of spinach roots in soil while *S. aureus* appeared to be unaffected. Additional analyses focus on the influence of the plant cultivar, soil management history, the pathogen genotype, and the indigenous soil microbial community on pathogen spread and persistence.

The study results shall improve our understanding of pathogen behavior in non-animal hosts and shall finally help to minimize risks of bacterial pathogen contamination in the production of fresh produce.

Abstract Category

19 Plant-Microbe Interactions

PS.19.028 SPATIAL AND TEMPORAL DISTRIBUTION OF SIDEROPHORE-PRODUCING ENDOPHYTIC BACTERIA IN RICE PLANTS

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Cultivated rice is one of the most agriculturally important grasses. In order to make rice cultivation sustainable and less dependent of agrochemicals, there is a need to know the role of the plant-associated bacteria and the strength of this association. Endophytic bacteria invade the inner tissues of the plant and spread through them without causing disease symptoms. They may confer certain benefits directly to the crop or act as a biological barrier against plant pathogens or plant growth promoting bacteria, which often are inoculated to crops to improve their yield. In this work, we report the dynamics of the endophytic bacteria community in roots and leaves of *Oryza sativa* during the crop cycle and the characterization of the dominant isolates. Triplicates from greenhouse experiments showed reliable results. The proportion of siderophore-producing bacteria in rice tissues was higher than in the surrounding soil, suggesting that iron sequestration may be relevant for the interaction with the plant. The Amplified Restriction DNA Restriction Analysis (ARDRA) of the 16S rDNA revealed that 45 from the 101 isolates obtained had a unique band profile, indicating that endophytic siderophore-producers were diverse. The genus *Pantoea* constituted the more abundant and persistent, being *Pantoea ananatis* the species more frequently isolated. BOX-PCR analysis performed for all isolates from this species showed that different strains alternated during the crop cycle. Bacteria of the genera *Sphingomonas*, *Pseudomonas* and *Burkholderia* were also sporadically

found. Among them, nitrogen-fixation and indole acetic acid producing abilities or antagonism against pathogenic fungi, were detected. This work shows that endophytic bacteria in rice plants are mainly siderophore-producers. The isolates consistently found belonged to a few genera, being *Pantoea ananatis* the species more persistent associated to grain, leaves and roots.

Abstract Category
19 Plant-Microbe Interactions

PS.19.029 GERMINATION PROCESS AFFECTING THE INTERACTION BETWEEN ENDOPHYTIC BACTERIA AND *EUCALYPTUS* SEEDS

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Endophytic bacteria are defined as all cultivable and uncultivable microorganisms that colonize the inner plant parts and do not cause apparent damage and/or visible external structures. The potential for practical applications of endophytes has led to studies addressing the bacteria's diversity and ability to control both disease and insect infestations. However, information regarding naturally occurring seed-specific endophytes and interactions is limited, mainly during the germination process. In that way, we isolated endophytic bacteria from *Eucalyptus* seeds in different germination stages. Surface disinfested seeds of the hybrid *E. grandis* x *E. grandis* were submitted to eight different times of aseptic water hydration. Additionally, were carried out analysis on Scanning (SEM) and Transmission (TEM) Electron Microscopy to study the localization of endophytic bacteria inside seeds and seedlings. The results showed that seed germination has effect on density of bacterial cells inside the seeds. We observe that late germination stages were positively correlated to higher number of bacterial cells, but the density of bacterial cell inside the seeds was least when compared to previous results obtained from leaves, stems and roots. Bacterial cells were not observed by SEM on seeds, but after seed peeling, was possible to detect bacterial cells inside seed tissues. Bacterial cells were observed mainly colonizing the space between cotyledons and primordial root. Also, the TEM analysis showed structural changes in seeds cells, but bacterial colonization was not observed. This is the first report of endophytic bacterial detection residing in *Eucalyptus* seeds during the germination process. Therefore, we suggest that seed germination stage can be positively correlated to development and colonization by endophytic bacteria.

Abstract Category
19 Plant-Microbe Interactions

PS.19.030 MOLECULAR ANALYSIS OF THE BACTERIAL DIVERSITY ASSOCIATED TO CARNIVOROUS PLANTS FROM LENTIBULARIACEAE FAMILY

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The carnivorous plants have traps able to capture several types of micro and small organisms. However, there is an indigenous microbial community living inside these traps and when preys are captured by plants these bacteria can be linked to the digestion process. The culture-dependent approaches used on bacterial community studies have showed limited efficiency, mainly due the fact that part of the microbial community present in the environment is unculturable. Studies using molecular approaches are able to minimize and to transpose these problems. In that way, we used culture-independent 16S rDNA libraries to identify and to describe the bacterial diversity associated to carnivorous plants belongs to Lentibulariaceae family. Total DNA was extracted from traps of *Utricularia hidrocarpa* and *Genlisea filiformis* and the 16S rRNA gene was partially amplified and further sequenced. A total of 291 clones from *U. hidrocarpa* and *G. filiformis* were sequenced. After sequence alignments and phylogenetic analysis, was possible to observe a high bacterial diversity.

The results showed that Proteobacteria was the most frequent group in all libraries assessed, representing about 45% of the 16S rDNA sequences. However, *Acidobacterias*, *Actinobacterias*, *Bacterioidetes*, *Chlamidia* and *Firmicutes* were also detected associated to. The results obtained in the present work provide information about the carnivorous plants associated microorganisms and show important features related to the biotechnological exploration of this interaction.

Abstract Category
19 Plant-Microbe Interactions

PS.19.031 ENHANCING LIPID AND STEARIDONIC ACID YIELDS IN FIELD GROMWELL (*BLUGOSSOIDES ARVENSIS*) THROUGH PGPR INOCULATION

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Stearidonic acid (SDA) is a n-3 polyunsaturated fatty acid (PUFA) produced in large quantity by few plant species. Dietary intake of SDA can provide human and animal health benefits similar to those associated with the consumption of fish n-3 PUFA: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The aim of this study was to develop a methodological approach to biologically enhance seed SDA yield in field gromwell (*Buglossoides arvensis*) through plant growth promoting rhizobacteria (PGPR) inoculation. Forty isolates of PGPR were screened in a series of pot experiments using non-sterilized field soil. Seed number per plant and seed weight were evaluated at plant maturity. Strain LBUM361 and LBUM677 were the only isolates which increased seed number per plant with statistical significance (26.2% and 22.4%, respectively). The highest increase in seed weight was obtained with strain LBUM395 (20.6%). Following seed lipid extraction and content analysis, an SDA yield index was calculated by multiplying the seed number per plant with the average SDA mass per seed. Five strains: LBUM288, LBUM361, LBUM395, LBUM570 and LBUM677 resulted in a statistically significant increase in SDA yield index over a range of 23-40% above the uninoculated control. Using PCR and sequencing of the 16S rDNA, *rpoB* and *gyrB* genes, the five isolates were identified as *Bacillus amyloliquefaciens*, *Stenotrophomonas rhizophila*, *Pseudomonas putida*, *Pseudomonas fluorescens* and *Pseudomonas fluorescens*, respectively. These results show the potential of using PGPR to promote the accumulation of lipids and specifically SDA in *B. arvensis*, a promising sustainable source of beneficial n-3 PUFA.

Abstract Category
19 Plant-Microbe Interactions

PS.19.032 IMPLICATION OF ANTIBIOSIS BY PSEUDOMONAS SPP. IN THE BIOCONTROL OF CLAVIBACTER MICHIGANENSIS SUBSP. MICHIGANENSIS CAUSING WILT AND CANKER OF TOMATO

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Pseudomonas spp. can use antibiosis to promote plant growth by inhibiting the attack of the root system by certain plant pathogens. 2,4-diacetylphloroglucinol (DAPG), phenazine-1-carboxylic acid (PCA) and hydrogen cyanide (HCN) have been identified as secondary metabolites produced by *Pseudomonas* spp. which show high biological activity. To date, DAPG, PCA and HCN have been mainly studied for their impact on fungal pathogens and the diseases they cause. Their impact on plant bacterial pathogens has not been thoroughly investigated. In order to address this gap in knowledge, the impact of antibiosis by *Pseudomonas* spp. on controlling *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*), causing bacterial wilt and canker of tomato, was investigated. *Pseudomonas* spp. strain LBUM223, which produces PCA, and LBUM300, which produces DAPG and HCN, were isolated from field soil in Eastern Canada. Biosynthetic genes involved in the production of DAPG, PCA and HCN were knocked-out, in the respective strains, by using the pKNOCK-Gm^r suicide plasmid technology. The impact of the mutants (LBUM223 *phzCD*⁻ (PCA mutant), LBUM300 *phID*⁻ (DAPG mutant), LBUM300 *hcnC*⁻ (HCN mutant), and LBUM300 *hcnC*/*phID*⁻ (double mutant) on the growth inhibition of *Cmm* was compared to the impact of their respective wild-type strain using in vitro confrontational assays. A significantly larger inhibition zone was observed with LBUM223, compared to LBUM223 *phzCD*⁻, as well as with LBUM300 compared to LBUM300 *phID*⁻, and LBUM300 *hcnC*/*phID*⁻. These results suggest that the production of PCA by LBUM223 and DAPG by LBUM300

can contribute to repressing the growth of *Cmm*. Studies are currently underway to validate these results in the rhizosphere of tomato plants.

Abstract Category
19 Plant-Microbe Interactions

PS.19.033 BIOGEOGRAPHY OF MICROBIAL EPIPHYTES ON TAMARIX, A SALT SECRETING DESERT TREE

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The leaf surfaces of Tamarix, a salt secreting genus of desert tree, harbor a diverse community of microbial epiphytes. This island-like ecosystem presents a unique set of biogeographic characteristics, where the environment provided by the host is extreme on one hand, and open to the surrounding environment on the other. Thus the Tamarix phyllosphere microbes are free-living, host-specific and extremophiles all at the same time.

The objective of this study was to understand the ecological gradients on which the structure of the microbial community varies by analyzing the phyllosphere microbial richness and diversity on the leaves of three Tamarix species found in Israel (*T. aphylla*, *T. nilotica* and *T. tetragina*). Leaf samples were collected from four locations in Israel and from two locations in the US. DNA was extracted from these samples and 16/18S rRNA hypervariable tag sequencing was performed. A total of over 200,000 V6 and V9 sequences were obtained, revealing a complex community, with over 400 bacterial, 80 eukaryotic and one archaeal genera. When clustered according to a 6% similarity cutoff, bacterial samples created more than 2500 clusters. Two main community types were detected. One, found on trees by the Mediterranean, is dominated by Halomonas and Halobacteria. The other, characteristic of trees around the Dead Sea, is dominated by Actinomycetales and Bacillales.

Our findings demonstrate that while the microbial community composition on Tamarix leaves remains steady on a local scale, trees of the same species that grow in different climatic regions, can host microbial communities that are almost completely unrelated.

Abstract Category
19 Plant-Microbe Interactions

PS.19.034 IAA PRODUCTION IN TWO STRAINS OF RHIZOBACTERIA ASSOCIATED TO MANGROVE AND ITS RELATION WITH N -ACYL HOMOSERINE LACTONES

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In many regions of the world, mangrove ecosystems have been damaged and reduced due to human activities. One suggested strategy for reforestation includes plantlets application with mixed inoculants of plant-growth promoting rhizobacteria. Some of these bacteria may produce the auxin indole acetic acid, improving in this way the root growth. On the other hand, *quorum sensing* is a process usually present in the complex rhizobacterial communities. Some strains may express the genes that are related with the rhizobacterial plant's beneficial action through signal molecules such as those involved in *quorum sensing*. We hypothesize that rhizobacterial auxin production would be regulated by or related to *quorum sensing* process through the production of the autoinductor acyl homoserine lactone. We quantified the production of the auxin and its relation with the acyl homoserine lactones delivered by bacterial strains isolated from mangroves' rhizosphere in Balandra, Mexico. We choose two *Vibrio* strains that produce both molecules, the autoinductor and the auxin. Then they were modified with the pDSKaiiA plasmid that codifies a lactonase enzyme. Then we quantified and compared the production of the auxin by wild and modified bacteria. A similar analysis was made with *Azospirillum brasilense* Cd used as a positive control of all

experiments. No significant differences in indole acetic acid production were detected between modified and wild *Vibrio* strains (15 to 25 $\mu\text{g}\cdot\text{mL}^{-1}$ respectively). However, *A. brasilense* did present differences between modified and wild strain (50 and 80 $\mu\cdot\text{mL}^{-1}$, respectively). Exogenous homoserine lactones were applied to cultures of one of the *Vibrio* isolates. There was a negative effect of C6 homoserine lactone in indole acetic acid production in this bacteria. Our results show evidence of a possible response of rhizobacteria auxin production to *quorum sensing* related inductor

Abstract Category
19 Plant-Microbe Interactions

PS.19.035 CONTRASTING PATTERNS OF CHANGES OF SOIL ARCHAEAL, BACTERIAL AND FUNGAL COMMUNITIES IN AN ALPINE LANDSCAPE

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With the advent of new molecular techniques in microbial ecology, understanding how regional pools of soil microbes are sorted out into communities across a range of spatial scales has gained increasing interest. However, there remains a lack of knowledge on the main drivers shaping spatial patterning of the three dominant microbial domains in terrestrial landscapes. Here, we assessed the landscape-scale spatial distribution of crenarchaeal, bacterial and fungal communities using CE-SSCP molecular profiling to characterize soil microbial diversity. The study alpine landscape (from 1900 m to 2600 m) exhibits a high turnover of plant species assemblages associated with steep environmental gradients. 33 sampling sites from eleven discrete, pre-defined habitat types that best represent the landscape heterogeneity were investigated. We used Non-Metric Multidimensional Scaling and Procrustean analyses to assess the importance of plant communities, habitat condition and geographic location on microbial community composition changes across the landscape, i.e. microbial beta-diversity. We found that fungal beta-diversity patterns were strikingly similar to those observed for plants. On the contrary, prokaryotic community changes were mainly correlated with soil pH and exhibited lower covariations with plant species assemblages. Nevertheless, fungal communities beta-diversity patterns were also related to those of prokaryotes. Our results pointed out (i) that microbial communities exhibit non-random spatial patterning in alpine landscape and (ii) that contrasting sets of drivers shape the beta-diversity of soil prokaryotes and fungi but also (iii) that fungi could, however, be involved in the prokaryotic beta-diversity patterns. This study is a milestone toward a more comprehensive picture of spatial diversification of microorganisms at the landscape scale and has implications to model biogeochemical cycling in heterogeneous environments.

Abstract Category
19 Plant-Microbe Interactions

PS.19.036 PHYLOGENETIC AND FUNCTIONAL DIVERSITY OF MICROBES ASSOCIATED WITH TROPICAL BROMELIADS; IMPLICATIONS FOR METHANE PRODUCTION AND NITROGEN FIXATION

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Plants within the family Bromeliaceae are unusual in their propensity for extreme epiphytism. "Tank" bromeliads, in particular, are those that possess foliage arranged in compact rosettes capable of retaining water. This creates an unusual freshwater environment suspended in the forest canopy; acidic and anaerobic, with decomposition of impounded leaf litter and insects. The composition and specific role of microbes within the tanks of bromeliads has not been well studied. Costa Rica is at the heart of the range inhabited by bromeliads, and thus, provided an opportunity to explore the phylogenetic and functional diversity of associated bacteria and archaea. A majority of bacteria found in association with the tanks were closely related to *Alphaproteobacteria* (*Rhodospirillales* and *Rhizobiales*), *Acidobacteria*, *Planctomycetes*, *Firmicutes*, and *Bacteroidetes*, many found in soil and peat bog environments. Bromeliads located high (~100 feet) in the canopy, as well as those with larger tanks in general, were found to possess less bacterial diversity, suggesting that both plant location and size influence the bacterial community. Many microbial

groups recovered from the tanks are known to be capable of nitrogen fixation, a process that would be beneficial to epiphytic bromeliads, which have no root system for the uptake of ammonium. Functional gene surveys confirmed the presence of nitrogenases belonging to archaea (*Methanomicrobiales*) and bacteria (*Clostridiales*, *Rhodospirillales* and *Rhizobiales*), chitinases belonging to *Firmicutes*, and archaeal methyl coenzyme m reductase genes. Archaeal communities were dominated by a unique clade of methanogens within the genus *Methanosaeta* (*Methanosarcinales*) and methane production rates of 15 ppm per ml tank fluid per day were measured in microcosm experiments. These results suggest that bromeliad-associated microbial communities may play an important role in the cycling of both carbon and nitrogen in tropical forests.

Abstract Category
19 Plant-Microbe Interactions

PS.19.037 ABUNDANCE OF COPPER AND MULTIPLE ANTIBIOTIC RESISTANT BACTERIA IN POME AND STONE FRUIT BLOSSOMS IN AUSTRALIA

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Increased prevalence of antibiotic resistant human and animal bacterial pathogens has been an issue of grave concern. Sources of antibiotic resistance may not solely derive from clinical settings. The environment provides a reservoir of diverse bacterial genotypes for transfer of heavy metal and antibiotic resistant genes. Pome and stonefruit orchards are a relatively stable ecosystem where long term use of copper fungicides exerts selection pressure for development of resistance in resident microflora. Although antibiotics are not used in Australian orchards, genetic linkage of copper and antibiotic resistance may enhance co-selection of antibiotic resistant bacteria in an orchard environment. These risks were investigated in an analysis of the resistance profile of orchard microbial populations. Pome and stonefruit blossoms are unique colonisation niches and a 'close system' until bloom occurs. An abundant and diverse bacterial community thrives in the nutrient rich habitats in the hypanthium, stigmas and pollens. Over a thousand blossom bacterial isolates collected from orchards in five eastern states of Australia were profiled. Viability of bacteria was tested against copper sulphate impregnated in culture medium, ranging from 0 to 400 ppm. Copper resistant bacteria were screened for resistance to twelve medically important antibiotics in disc diffusion assays. Copper resistant bacteria were found to be widespread in orchards in the eastern states. A high frequency (40%) of the bacteria was copper resistant, and 50-70% of copper resistant bacteria were resistant to antibiotics. Nearly 80% of these bacteria were multiple resistant to at least five to ten antibiotics. Bacteria possessing novel phenotypes including ice nucleation and inhibitory activities have been profiled. This is the first report of prevalence of both copper and antibiotic bacteria in Australian orchards.

Abstract Category
19 Plant-Microbe Interactions

PS.19.038 OCCURRENCE OF VA MYCORRHIZAL FUNGI IN THE RICE CROP FIELDS OF DEHRADUN, INDIA

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The occurrence of VA-Mycorrhizal fungi in two different rice varieties i.e. basmati and IR-36 grown in their respective climatic conditions have been studied in the present paper. Basmati rice variety was grown in crop field of Dehradun and IR-36 was grown in Deori. The results obtained from the experiments suggest that the association of mycorrhizal fungi with basmati rice was much higher than the association with IR-36. In basmati rice not only VAM fungal average spore population was higher 1236 spores/100gm of soil, average percent colonization 69% but also number of VAM species were greater in comparison to IR-36 where we observed VAM fungal average spore population 932 spores/100gm of soil, average percent root colonization 58% and lesser VAMF

species. As usual during wet/flooded condition, mycorrhizal association was reduced but at maturation / harvesting phase VAMF association was found to be increased in both the rice varieties. Interesting feature of this study was that the occurrence of ASPN (*Acaulospora spinosa*) only with IR-36 at maturation and harvesting phase whereas in basmati ADLC (*Acaulospora delicata*), ALVS (*Acaulospora leavis*) were found associated at seedling phase. ATRP (*Acaulospora trappei*), LDMR (*Glomus dimorphicum*) and LHTS (*Glomus heterosporum*) were found during maturation phase. ADLC (*Acaulospora delicata*), LABD (*Glomus albidum*), LABS (*Glomus ambisporum*), LAST (*Glomus australe*), LOCT (*Glomus occultum*) were found at harvesting phase.

Abstract Category

19 Plant-Microbe Interactions

PS.19.039 PLANT'S NECTAR AS A BACTERIAL HABITAT

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Floral nectar is regarded as the most important reward of animal-pollinated plants to attract pollinators. Despite the dominance of sugar (>90% dry weight) in nectar, non-sugar compounds such as amino acids are widespread. The rest of the nectar is made up of other chemical components such as organic acids, lipids, essential oils, polysaccharides, vitamins, antioxidants, minerals and secondary metabolites. Microorganisms are probably transferred among flowers by nectar consumers. However, it is unknown whether microorganisms in the nectar positively or negatively affect pollination. In order to better understand the role of secondary metabolites in floral nectar we studied the diversity of bacterial populations that inhabit nectar versus leaf surfaces of three plant species.

Bacterial populations that inhabit nectar and leaf surfaces of three plant species: *Nicotiana glauca*, *Amygdalus communis* and *Citrus paradise* were determined using culture dependent and DGGE.

Significant differences were found between nectar and leaf bacterial communities from all three plant species. Significant differences were also found between nectar and leaf surface bacterial communities even from the same plant species. The dominant class in the nectar was *Gammaproteobacteria* while the dominant class in leaves was *Bacilli*. About half of the isolates were novel species. The prevalence of some novel *Acinetobacter* species and a novel *Enterobacteriaceae* species were high in the floral nectar.

The findings demonstrate that the nectar is a unique microbial habitat. Every plant species had distinctive composition of nectar bacterial species which implies that bacterial presence in nectar is not accidental. Primary selection of the nectar bacteria is unclear; it seems that it is affected by the different nectar chemical composition of each plant. Further study on the effect of the microbial flora on nectar attraction and pollination is needed.

Abstract Category

19 Plant-Microbe Interactions

PS.19.040 UNRAVELING PLANT-MICROBE INTERACTIONS USING A NOVEL *IN SITU* RHIZOSPHERE SAMPLING TECHNIQUE

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The rhizosphere is the site of intense and complex interactions between soil, plants and associated microflora. Current knowledge on how root exudates affect the diversity and activity of rhizosphere organisms is hampered by the difficulties associated with sampling root exudates and rhizosphere soil without disturbing or damaging the plant root system. Therefore, this study aimed at developing and evaluating a non-destructive sampling technique to study these plant-microbe interactions *in*

situ.

Large-scale rhizotrons were used under controlled environmental conditions above and below ground. Use of an endoscope camera and sampling tool system through horizontal access portals at different depths of the rhizotrons allowed to monitor the root growth over time and to sample root exudates and rhizosphere soil *in situ* with minimal disturbance. Low molecular weight organic anions (OAs) were collected from rhizosphere and bulk soils by anion exchange membranes, after which soil samples were taken from those spots using long-armed scissors. Soil microbial structure and diversity was assessed by DGGE analysis of the general bacterial and fungal community, as well as the α - and β - proteobacteria and pseudomonads. Applying this method to the rhizospheres of two radiata pine (*Pinus radiata*) tree lines, grown in rhizotrons for ten months, revealed large spatial and temporal variations in the amount and composition of OAs secreted by the roots. However, no significant differences were detected between the tree lines. This variability resulted in considerable spatial and temporal differences in the rhizosphere microbial community structure, which showed significant but inconsistent differences between both tree lines. Rhizosphere samples significantly differed from the bulk soil, demonstrating a clear rhizosphere effect. These results showed the potential of this technique to establish relationships between root exudates and rhizosphere microbial diversity and activity.

Abstract Category

19 Plant-Microbe Interactions

PS.19.041 DOMINANCE OF *LYSOBACTER* IN NATIVE PLANT ROOTS

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Eleven wild herbaceous plants were sampled to estimate the diversity of endophytic bacteria in April and September, 2009. The bacterial diversity was analyzed by two culture independent approaches, namely denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP) fingerprinting techniques. The DGGE profiles of endophytic bacteria were simple in general, reflecting low diversity in bacterial composition. Presence of common DGGE bands was observed in all plant species, and the DNA sequence analysis of individual bands indicated that members of *Lysobacter* and *Bacillus* were the most common endophytic bacterial constituents. In particular, *Lysobacter* was predominant in all plant species. *Rhizobium* was also detected as minor constituent in many species. From the T-RFLP profiles obtained using *HaeIII* and *HhaI*, the largest T-RF peak was also derived from *Lysobacter* in both studies. In case of *HaeIII*, total areas of *Lysobacter* were measured to cover higher than 78%, and the T-RF based on *HhaI* showed that the dominant species was *Lysobacter enzymogenes*. Although no attempt to isolate *Lysobacter* strains were successful, universal and dominant presence of *Lysobacter* in plant roots suggest the potential significance of *Lysobacter* in association with plant. For further studies, isolation and cultivation of *Lysobacter* is essential, and the ongoing study focuses on the development of strategy for isolation.

Abstract Category

19 Plant-Microbe Interactions

PS.19.042 EFFECT OF POTATO GROWTH STAGE AND GENOTYPE ON FUNCTIONING AND STRUCTURE OF SOIL FUNGAL COMMUNITIES

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The structure and functioning of microbial communities in soil are strongly influenced by plants. However, it is not yet clear how much the genotype of a plant in different growth stages influences the soil fungal community. This study investigates the effect of different potato genotypes, including one genetically modified variety on soil fungal communities. We adopted a combined approach of measuring both the community structure of different fungal phyla (*Ascomycota*, *Basidiomycota* and *Glomeromycota*), the abundance of cellobiohydrolase (CBHI) genes and enzymatic measurements to gain better understanding of plant genotype mediated effects on soil fungal communities. The community structure of the three fungal phyla was assessed using phylum specific primers and TRFLP (terminal restriction fragment length polymorphism) as fingerprinting method combined with

multivariate statistics sequence analyses. Community function was studied from the same samples using CBHI gene as a marker and TRFLP and RT-qPCR were used to look at functional community structure and gene abundance. This was related to data on ligno-cellulolytic enzyme activities, fungal biomass and root colonization by AMF (arbuscular mycorrhizal fungi). Presence and growth stage of the plant was the most important factor affecting both the structure and function of fungal communities. The structure of the bulk soil communities was fairly constant throughout the season while in the rhizosphere considerable changes in the communities were observed, possibly due to changes in carbon allocation pattern of the plant which is known to be dependent on plant growth stage. Moreover, different fungal phyla responded differently to the plant growth and revealed a dynamical pattern. The effect of plant genotype was minor compared to other factors and not an important factor affecting soil fungal communities.

Abstract Category
19 Plant-Microbe Interactions

PS.19.043 MICROBIAL EUKARYOTIC 18S rRNA DIVERSITY IN AUSTRALIAN SOILS: SOIL GEOMORPHOLOGY AND LAND-USE INFLUENCE ON COMMUNITY STRUCTURE

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The sustained health of Australian soils is dependent on how land-use and management practices impact the underlying soil microbial community. We analyzed ~1,150 18S rRNA sequences for each of three samples. First, we undertook a land-use comparison in NW Victoria. Samples from 'cropped' and 'remnant' (native vegetation) were obtained from a low rainfall region with alkaline, nutrient poor soil calcarosol. A third sample was taken from SE Victoria, in a region where the soil is acidic, nutrient-rich and there is high annual rainfall. We present diversity estimates (e.g., Chao1, ACE1) and details on the degree of eukaryotic community overlap between the three sites. Sanger sequences were aligned against the SILVA reference database for 18S rRNA and BLASTed against both SILVA and NCBI sequence databases to assess taxonomic affiliations for all sequences. Sequences from the three soils were classified and grouped into different phyla and then assigned to operational taxonomic units (OTUs) as defined by 97 sequence similarity. Sequences from each site were dominated by fungal origin. Community structure was compared to results from other published soil studies to evaluate broader scales of biogeography. The results provide insight for ecosystem function and management decisions in the context of climate change and resource sustainability.

Abstract Category
19 Plant-Microbe Interactions

PS.19.044 CHARACTERIZATION OF STRAINS CONTAINED IN COMMERCIAL PRODUCTS TO IMPROVE AGRICULTURE IN AFRICA: COMPARISON WITH INFORMATION GIVEN BY PRIVATE COMPANIES

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Many commercial microbial products available on the market are claimed to significantly improve growth and yield of different crops and legumes (maize, soybean, chickpea,...). However, most of them are ineffective and no or very few scientific data are available to assess their real effect. We have been mandated by Bill and Melinda Gates Foundation to scientifically evaluate and select effective products for improving and sustaining crop yields in selected agro ecological zones in Kenya, Nigeria, Niger and Ethiopia. Before being screened under greenhouse conditions, bacterial content of the products has been assessed and compared to the information given by the private companies. Strains have been isolated in optimal conditions to allow growth of the expected microorganisms. DNA has been extracted and 16S rDNA has been amplified and sequenced. About 120 strains have been isolated from 30 products. In half of the products more strains than expected

have been isolated. That denotes an important level of contamination among the products. In the ones supposed to contain a large number of bacterial species (4 products) less strains than expected have been isolated, demonstrating the incapacity of too many bacteria to be present and active within one product. Sequencing results show that only 25% of the products contain the expected strain(s) without contamination and can be considered as "pure products". On the other hand, products supposed to contain many types of bacteria are highly contaminated and only some expected strains are isolated. These "contaminated products" represent about 25% of the products. Eventually, 10% contain none of the expected strains but only contaminations. In conclusion, the characterization of the commercial products showed that many private companies commercialize a mix of non-defined products and thus do not ensure the quality of their inoculums. This may explain their lack of impact on the plant growth under greenhouse conditions.

Abstract Category
19 Plant-Microbe Interactions

PS.19.045 THERMOREGULATED MOTILITY IN *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE* B728A AND IMPLICATIONS FOR PLANT-MICROBE INTERACTIONS.

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The effect of temperature on plant-microbe interactions has been studied extensively in diverse bacterial genera. Generally, pathogenic determinants (secondary metabolite production, T3SS, etc) are positively regulated by temperatures below the optimal growth temperature of the organism. Less studied is the effect of temperature on the motility of plant-associated bacteria. When *P. syringae* is incubated at 28-30C or greater temperatures, it is inhibited in swarming motility (a form of social, surface-associated motility). To understand the mechanism by which swarming is inhibited, we investigated the production of surfactants which are known to be required for this form of motility in other Pseudomonads. Using both an oil over-overspray assay to quantify surfactant production, as well as a transcriptional fusion between the promoter of *syfA* (the gene encoding the non-ribosomal polyketide synthase which produces syringafactin) and a GFP reporter gene, we show that syringafactin production is reduced at elevated temperatures, and that this regulation occurs at least in part or in whole at the transcriptional level. To identify thermoregulatory components, ca. 30,000 transposon mutants were screened for increased *syfA* expression at 30C. From this screen we recovered multiple, independent insertions in an ORF encoding a NUDIX hydrolase, as well as an ORF encoding an acyl-CoA dehydrogenase. Both of these mutants exhibit significantly increased expression of *syfA* at 28C. Previous work has demonstrated that flagellar motility in pathogenic *Pseudomonas spp.* is an important factor that contributes to both invasion into as well as survival on leaf surfaces. Additionally, disease of plants caused by this group of pathogens tends to be more prevalent during cool, humid conditions. We are currently performing *in planta* experiments to address the effect of temperature on the epiphytic phase as well as the pathogenic phase in this organism.

Abstract Category
19 Plant-Microbe Interactions

PS.19.046 EVIDENCE THAT PLANT-ASSOCIATED METHYLOTROPHIC BACTERIA AID IN GRASSLAND AND COASTAL SAGE SCRUB RESTORATION

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Recent evidence suggests that plant-microbe interactions can play a role in the success of biological invasions and therefore may affect restoration outcomes. Pink-pigmented facultative methylotrophic bacteria (PPFMs, *Methylobacterium*) are mutualists associated with the roots, leaves and seeds of most terrestrial plants. Previous studies have shown that PPFMs enhance plant germination, growth rates and productivity and even confer drought and pathogen resistance. Here we investigated the distribution of PPFM abundance along gradients of invasion in a California coastal sage scrub (CSS) ecosystem. We found that the abundance of PPFMs in root zones varied between plant species and that regions of mixed native/non-native species in invasion gradients harbor 33% fewer PPFMs

compared to pure CSS regions. Annual plants had 3 times more PPFMs in their root zones than perennials. Further, we found that the herbicide, glyphosate, reduces PPFM abundance. An in vitro experiment manipulating glyphosate and PPFMs showed that glyphosate-treated non-native mustard (*Hirshfeldia incana*) seeds benefited from the loss of PPFMs by germinating earlier than controls. In contrast, native shrub seedlings (*Artemisia californica*) benefited from the presence of PPFMs by reducing germination time and increasing seedling size. In a Southern California grassland restoration that had been treated multiple times with glyphosate, we found an order of magnitude fewer PPFMs in glyphosate-treated soil compared to control soil. Spraying a 20% methanol solution, a PPFM substrate, on the post-glyphosate treated soil after seeding resulted in a 30% increase in native bunchgrass (*Nassella pulchra*) germination rates and seedling size. Together, these results suggest that manipulating PPFM abundance may be a promising method for understanding plant-microbe interactions in invasions and improving restoration outcomes.

Abstract Category

19 Plant-Microbe Interactions

PS.19.047 SEED COLONIZING MICROBES ALTER ZOOSPORE CHEMOTAXIS AND ENCYSTEMENT OF THE OOMYCETE PLANT PATHOGEN *PYTHIUM APHANIDERMATUM*

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Pythium aphanidermatum causes seedling mortality of a wide range of plant species. The pathogen's life cycle includes a motile infective stage (the zoospore) that responds chemotactically to exudates released from seeds into the spermosphere. Microbially mediated suppression of *Pythium* diseases is widely documented, however the underlying mechanisms are not understood. We hypothesize that seed colonizing microbes from a disease-suppressive vermicompost alter chemotactic signals within seed exudates thus disrupting zoospore chemotaxis and subsequent disease development. Bioassays using a point source inoculation were designed to ensure that zoospore swimming was required to infect seeds. At 24 hr *P. aphanidermatum* colonization was quantified using quantitative PCR and subsets of seeds were transplanted to sterile sand to assess resulting seedling mortality. To complement bioassays, in vitro zoospore chemotaxis and encystment assays were carried out to characterize zoospore responses to exudates from seeds sown either in sand or in sand amended with 40% vermicompost. Cell-free exudates were collected from seeds germinated for 24 hr in either substrate to assess the extent of microbial modifications to chemotactic signals. No zoospore chemotaxis was observed in plant bioassays where seeds were sown in vermicompost as evidenced by a lack of seedling mortality after 8 days and seed colonization within 24 hr. However, all seedlings from sand were symptomatic with 40% mortality. High levels of seed colonization were observed within 24 hr in sand indicating that zoospores swam 2 cm through the substrate to infect seeds. In choice experiments using seed exudates from the different treatments, fewer zoospores accumulated and encysted on exudates collected from seeds sown in vermicompost than from those sown in sand. These results indicate that seed colonizing microbes may chemically alter chemotactic signals in seed exudates to disrupt zoospore homing responses.

Abstract Category

19 Plant-Microbe Interactions

PS.19.048 ASSESING POLYKETIDE SYNTHASE (PKS) BIOSYNTHETIC GENES FROM SUGAR CANE FUNGAL ISOLATES

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Polyketides are secondary metabolites with diversity in structures, functions and applications, synthesized by a polyketide synthase megaprotein. Endophytic fungi have contributed with the production of new compounds, focusing for their isolation depending on environmental characteristics of the host plant. 17 ascomycota fungi isolated from sugar cane tissues and

rhizosphere were assessed for the presence polyketide synthase genes by PCR, looking for keto-synthases (KS) and C-methyltransferase (Cmet) domains. A total of 36 sequences were similar to PKS genes according with BlastX analysis. A neighbour-joining phylogenetic analysis of the KS domain showed that 16 sequences belong to the monophyletic group of PKS evolved with synthesis of non reduced polyketides. 8 belong to the clade I responsible for the production of pigments and aflatoxins; the remaining 8 sequences belong to the clade II responsible for the synthesis of tetrahydroxynaphthalene melanin-like. Ten fit on the monophyletic group of PKS evolved with the synthesis of reduced polyketides: 4 belong to the clade II in which LNKS (lovastatin) and EquiS (Equisetin) are members; the remaining 6 fit with the clade III but are pour characterised. C-methyltransferase analysis also pointed that the ten Cmet sequences fit with groups of PKS depending on the grade of reduction of the polyketide, all of them to PKS evolved with the synthesis of reduced polyketides. Protein structural analysis pointed that these sequences are closely related with proteins from condensing enzyme family as modular PKS and fatty acid synthesis. Structural modelling showed the presence of a characteristic α helix nucleophilic elbow that bears the cystein residue responsible for the condensation activity. This work was able to prove the remarkable diversity of putative PKS genes in sugar cane endophytic fungi, perhaps with an important role on the maintenance of this tropical environment.

Abstract Category
19 Plant-Microbe Interactions

PS.19.049 GENETIC DIVERSITY OF PHYLLOSHERE PINK-PIGMENTED FACULTATIVE METHYLOTROPHS DEPENDING ON PLANT SPECIES, GEOGRAPHIC LOCATION AND LAND-USE TYPE

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Phyllosphere methylotrophic bacteria are Gram-negative organisms, which inhabit plant surfaces, mainly the leaves. They use compounds such as methanol as their sole carbon and energy source and play an important role in the methanol cycle by utilizing the methanol emitted by plants. On the other hand, they can produce plant growth promoting substances like auxins, cytokinins and vitamin B12. One objective of this project is to analyse the genetic diversity of phyllosphere methylotrophs in dependence of inhabited plant species, land-use type and geographic location of the sampling sites. Cultivation of pink-pigmented facultative methylotrophs (PPFM) was performed after isolation from leaf samples of two different plants, *Trifolium repens* and *Cerastium holosteoides*. For each plant, samples were taken from sites with different land-use types. Isolates were grouped using amplified ribosomal DNA restriction analysis (ARDRA). From representative isolates the 16S rRNA gene sequences were analysed and phylogenetic analyses were performed using the neighbour-joining method. The concentration of PPFM per gram leaf was significantly higher for *Trifolium repens* (2.0×10^7 CFU / g leave) than for *Cerastium holosteoides* (2.0×10^6 CFU / g leave). Geographic location of the sampling site, land-use type and fertilisation had no significant influence on the concentration of PPFM on leaf surfaces. Using ARDRA, 16 different groups of pink-pigmented isolates were found. The main groups of isolates could be found on leaves of both inhabited plant species, in both sampling periods, all three sampling sites and all land-use types. Analysis of the 16S rRNA gene sequences confirmed that all isolates belong to the genus *Methylobacterium* with similarities between 97.3 and 100% to described species (*M. adhaesivum*, *M. mesophilicum*, *M. organophilicum*, *M. jeotgali*).

Abstract Category
19 Plant-Microbe Interactions

PS.19.050 T-RFLP PROFILING OF SOIL MICROBIAL COMMUNITIES IN NO-TILLED UPLAND SOILS

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Agricultural practices such as tillage and crop rotation influence soil microbial community and result in changes in soil carbon and nitrogen dynamics. To investigate the soil microbial community

structure, we have developed a combined method of 16S rRNA gene-based terminal-restriction fragment length polymorphism (T-RFLP) profiling with real-time quantitative PCR (qPCR) assay. In the present study, this method was applied to monitoring of soil microbial diversity and dynamics in no-tilled upland field soils. Our experimental plots were located at Field Science Center, Ibaraki University College of Agriculture in Japan. After rye cover-cropping and fallow treatment (as control) in winter season, upland rice was grown in every summer season under a low nitrogen fertilizer input condition for 5 years. Soils were sampled in the fourth and fifth seasons and used for DNA extraction. Bacterial and archaeal 16S rRNA genes were determined by real-time qPCR with the extracted DNAs as the template. Based on the detected T-RFs profiles, the similarity of soil microbial communities was analyzed by nonmetric multidimensional scaling (MDS). The real-time qPCR assay showed that the winter rye cover-cropping resulted in increases in the amounts of bacterial and archaeal 16S rRNA genes in the soils. MDS analysis revealed that the influence of rye cover-cropping practice on the soil bacterial and archaeal community structure was small. In contrast, the soil fungal community structure was influenced more extensively by the cover-cropping practice. Further studies including clone library analysis are now in progress to identify the specific fungal group showing positive and negative responses to the cover-cropping practice.

Abstract Category

19 Plant-Microbe Interactions

PS.19.051 ZOOBOTIC PATHOGEN INTERACTION WITH THE SALAD PHYLLOPLANE

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The phylloplane is a hostile environment with many fluctuating environmental stresses, yet is host diverse microbial colonists and plant pathogens, affecting crop yields and shelf life. Recently, outbreaks of disease caused by consumption of fresh produce have turned interest to survival of zoonotic pathogens on the phylloplane. Pathogens can be derived either from the soil as the leaf emerges, particularly following application of improperly treated manures, splashing of soil onto the phylloplane during heavy rainfall, or faecal contamination from passing animals and birds. This study aims to understand the size and spatial distribution of bacterial communities persisting as biofilms on the salad phylloplane and assess their role in zoonotic pathogen attachment.

Spinach or watercress leaves were examined for biofilm and exopolymeric substance (EPS) presence using EDIC/EF microscopy. Microorganisms were confirmed and quantified using DAPI and BacLight staining. Leaves were also spiked with *Salmonella* Thompson or *E. coli* O157:H7, both carrying the *gfp* fluorescent marker, and cell-cell or cell-phyloplane interaction monitored continuously using real time fluorescence imaging.

Biofilm microcolonies and EPS slime were observed in the leaf margins, between margins and around stomata. Spiked cultures of motile *S. Thompson* showed subpopulations with different attachment strategies including directly binding to the leaf surface or to the biofilm, and also swimming directly into stomata. *E. coli* O157 attached specifically to the stomata guard cells and decorated in a characteristic pattern. The pathogens proved highly resistant to chlorine washing and became viable but non-culturable (VNC) on standard agar recovery media at 100 ppm chlorine concentrations, confirmed using an *in situ* cell elongation assay.

Attachment of pathogens to the salad phylloplane with VNC formation may account for large reported outbreaks of disease from consumption of washed salads deemed safe.

Abstract Category

19 Plant-Microbe Interactions

PS.19.052 FRANKIA AND ALNUS RUBRA CANOPY ROOTS: AN ASSESSMENT OF GENETIC DIVERSITY, PROPAGULE AVAILABILITY, AND EFFECTS ON SOIL NITROGEN

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This study investigated the *Frankia-Alnus rubra* symbiosis in forest floor and canopy roots at Olympic National Park, WA, USA. 16 *A. rubra* trees were surveyed and *Frankia* genetic diversity was assessed with sequence-based *nifH* analyses. A bioassay experiment and nitrogen analyses were also conducted to examine *Frankia* propagule availability and effects on soil nitrogen, respectively.

Frankia canopy and forest floor assemblages were similar, with both habitats containing the same genotypes. The composition of forest floor and canopy genotypes on the same tree was not always identical, suggesting dispersal was not strictly local. *Frankia* seedling colonization was similar in all soils, indicating dispersal is not a major limiting factor. Soil nitrogen was higher in canopy than forest floor soils, but the presence of *Frankia* nodules did not significantly alter soil nitrogen levels. Overall, these results indicate the *Frankia*-*A. rubra* symbiosis is ecologically similar in canopy and forest floor environments.

Abstract Category
19 Plant-Microbe Interactions

PS.19.053 CHARACTERIZATION OF AGROBACTERIUM TUMEFACIENS' TYPE IV SECRETION SYSTEM T PILIN SURFACE STRUCTURE AND ITS ROLE IN MICROBIAL ATTACHMENT

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Agrobacterium tumefaciens is a plant pathogen that transfers a segment of its own DNA into host plant cells to cause Crown Gall disease. Virulence is dependent on attachment of the bacterium to plant cells and deployment of a Type IV secretion system to deliver substrates intercellularly. This system also elaborates an extracellular filament called the T pilus which is important for establishment of bacterial/plant cell contacts. The T pilus is composed primarily of the VirB2 pilin protein along with the minor pilin protein VirB5 at its tip. T pili are sloughed from the cell and aggregate, thought to promote cell clumping and formation of biofilms. The T pilus structure, molecular basis for sloughing, and properties contributing to aggregation and biofilm formation are not yet known. Here, to gain information about the pilin structure in the T pilus, we characterized the accessibility of engineered Cys residues to the thiol-reactive reagent 3-(N-maleimidopropionyl)biocytin. We identified a surface patch marked by thiol-labeling of VirB2 Cys replacements at 64,67 & 77. This region consists of an overlap among hydrophilic and hydrophobic domains, and this surface hydrophobicity could account for aggregation and pilus-mediated biofilms. The T pilus has been visualized to extend from cell poles; however, by immunofluorescence microscopy we determined that VirB2 also accumulates around the cell surface independently of pilus formation. We propose that this cell-associated pilin also mediates contacts independently of the T pilus. Finally, diameter estimates of the T pilus, as assessed by cryo-electron microscopy suggest the pilus is too large to fit within a chamber of a recently-identified VirB/D4 channel complex that spans the *Agrobacterium* envelope. We propose a working model that the T pilus initiates assembly at the outer membrane on a platform of VirB channel subunits, and that conformational changes occurring within the channel result in pilus sloughing.

Abstract Category
19 Plant-Microbe Interactions

PS.19.054 SPECIES-SPECIFIC BACTERIAL ASSEMBLAGES ASSOCIATED WITH PERENNIAL BIOENERGY GRASSES

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Cultivation of high-yielding agricultural feedstocks for biofuel production is one attractive solution to reduce our reliance on fossil fuels and mitigate further increases in atmospheric greenhouse gases. However, sustainability of biofuel crops depends on low nitrogen fertilizer inputs and this will be best achieved by promoting beneficial associations between biofuel crops and nitrogen-fixing bacteria. Unfortunately, we currently lack a thorough understanding of the factors influencing composition and activity of nitrogen-fixing bacteria associated with non-leguminous plants. In this study, plants were collected from 19 locations across Taiwan and 8 test plots across the state of

Illinois, United States, covering a range of different soil types and plant ages. These plants included two species of native *Miscanthus* in Taiwan and the potential biofuel crops *Miscanthus x giganteus*, *Panicum virgatum* (switchgrass), and *Zea mays* (maize) in Illinois. DNA extracted from surface-sterilized root tissue and attached rhizosphere soil was used to create genetic fingerprints of the total bacterial and nitrogen-fixing bacterial communities. Endophyte and rhizosphere bacteria associated with these plants were nonrandom collections of bacteria that display consistent patterns of composition. Plant species and localization in roots or soil were the two most important factors shaping plant-associated bacterial community composition in both Taiwan and Illinois. Soil characteristics such as moisture content, percent organic matter, and calcium concentration were also shown to play a role in influencing community composition, particularly in rhizosphere soil. However, the most important environmental factors shaping bacterial communities differed among geographic regions. These results confirm that perennial bioenergy grasses select for species-specific assemblages of endophytic diazotrophs, whose colonization may be leveraged to promote sustainable growth of potential biofuel crops.

Abstract Category
19 Plant-Microbe Interactions

PS.19.055 CHARACTERIZATION OF ROOT ENDOPHYTIC ACTINOBACTERIA FROM NATIVE HERBACEOUS PLANTS

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Endophytic bacteria are defined as bacteria that reside within the internal tissue of plants. Actinobacteria may exist as endophytic bacteria within the living in plant tissues, while they do not harm the host nor develop external structures of native plants. Endophytic actinobacteria can also be a promising source of biocontrol agents, by acting directly on fungal cell walls or initiating increased plant responses against diseases.

In this study, endophytic actinobacteria were isolated from native herbaceous plants. Thirty endophytic actinobacterial strains were isolated and their 16S rDNA sequences were determined. The isolates were divided into sixteen distinctive phylogenetic clusters within the taxonomic variation encompassed by the family *Streptomycetaceae*. Fourteen clusters were assigned to *Streptomyces*, one cluster was to *Micromonospora*, and the remaining one to *Microbispora*, respectively.

The *in vitro* antagonistic activity was examined by the formation of inhibition zones against 5 plant pathogenic fungi. The proportions of the isolates exhibiting antagonistic activity were 36.7% against *Phytophthora capsici*, 43.3% against *Rhizoctonia solani*, 3.3% against *Colletotrichum gloeosporioides*, 23.3% against *Fusarium solani* and 26.7% against *Alternaria alternata*. Fifteen strains showed antagonistic activity against at least one fungal pathogen. The ongoing study also indicates that considerable numbers of strains were also positive for *in vitro* production of degradative enzymes, siderophores, and the plant growth hormone indole-1.3 acetic acid (IAA). Through this study, the endophytic actinobacteria were shown to have high potential as biocontrol agents against a range of pathogenic fungi.

Abstract Category
19 Plant-Microbe Interactions

PS.19.056 INFLUENCE OF SUCCESSIONAL VEGETATION TYPE ON MICROBIAL COMMUNITIES AND BIOGEOCHEMISTRY IN A POST-GLACIAL CHRONOSEQUENCE

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Recent research on plant-microbe interactions suggests that soil microbial community structure and function play an integral role in plant community succession through both positive and negative feedbacks; yet, plant-microbe dynamics along a successional gradient have not been well studied. Our study in the recently exposed soils of the Mendenhall Glacier forefront -- near Juneau, AK, USA -- examined the development of microbial communities in coordination with the establishment of the first plants. The Mendenhall Glacier features a perhumid climate, with moist soils throughout the year, which facilitates relatively rapid plant colonization. We sampled bulk soils (avoiding roots)

from under two different plant species (alder, *Alnus sinuata* and spruce, *Picea sitchensis*) and from unvegetated areas. All samples were gathered from within a single transect of soils which had been exposed for 6 years. For each sample site, pH, organic carbon (C), available nitrogen (N), microbial biomass carbon, and nitrogen fixation rates were determined. Significant differences were found among vegetation types for pH, organic carbon, and nitrogen fixation. Interestingly, soil under spruce plants exhibited significantly lower pH, higher organic carbon pools, and higher levels of nitrogen fixation than unvegetated soils. Our findings begin to elucidate how individual plant types may alter soil microbial community function, and, in turn, nutrient cycling. While the importance of nodule-forming alders in N-fixation in similar environments has been well-recognized, our data also support a role for non-nodulated plants in promoting N-fixation. This study begins to explore important interactions between the initial plant colonizers and the trajectory of microbial community succession, relationships that have the potential to feedback on the overall primary succession of the plant community.

Abstract Category
19 Plant-Microbe Interactions

PS.19.057 DISPERSAL OF METHYLOBACTERIUM IN THE PHYLLOSHERE

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Members of the genus *Methylobacterium* are omnipresent colonizers of plant leaves. Different factors will affect the dispersal of these bacteria on plants. In order to evaluate the importance of plant species, site and time on the *Methylobacterium* community composition in the phyllosphere, samples were collected from *Arabidopsis thaliana* and different adjacently grown plant species at five different sampling sites in two consecutive years. These samples were analyzed by cultivation-independent *Methylobacterium*-specific automated ribosomal intergenic spacer analysis (ARISA). A variation partitioning analysis based on the obtained ARISA patterns revealed that sampling site and plant species explained 50% of the variation seen in the *Methylobacterium* communities of the collected sampling material. The site at which a plant grew had a stronger impact on the *Methylobacterium* community composition than the plant species. Differences in the community composition on plants grown at different sites were mainly due to the presence/absence of distinct ARISA peaks, while the plant species specific differences seen within individual sites were a consequence of variation in the abundance of ARISA peaks that were detected on diverse plant species at that site. 16S rRNA gene sequence analysis of representative *Methylobacterium* isolates, which were obtained from the sampling material, revealed that the studied plants were colonized by 23 different genotypes, of which 10 were found at more than half of the sampling sites. Taken together, these data suggest that plant colonization is not highly host-plant specific. Plants are rather colonized by strains of those *Methylobacterium* species that are present at the respective site where the plant is growing.

Abstract Category
19 Plant-Microbe Interactions

PS.19.058 EPIBACTERIAL COLONISATION OF MARINE MACROALGAE: FROM COMMUNITIES TO COMPOUNDS

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In the marine environment macroalgae are exposed to a strong bacterial fouling pressure. Bacterial colonisation may have positive and negative effects on the host. To investigate the relationship between marine macroalgae and epiphytic bacteria we addressed whether the compositions of bacterial communities on algae were host-specific and persisted throughout the year and elucidate the underlying chemical control of bacterial colonisation at the surface of *Fucus vesiculosus*. Bacterial communities on *Fucus vesiculosus*, *Gracilaria vermiculophylla* and *Ulva intestinalis*, co-occurring macroalgae in the Baltic Sea, were repeatedly sampled in 2007 and 2008. Epibacterial communities were analysed by DGGE and 16S rDNA cloning and sequencing. A new surface

extraction method in combination with a test-system was developed to recreate the natural algal surface chemistry at an artificial substrate. Thus enables to analyse the effect of algal surface metabolites on bacterial colonisation in the marine environment.

Epibacterial community profiles differed significantly at each sampling interval among algal species and showed consistent seasonal differences on each algal species. The epibacterial composition on algal surfaces was host-specific and reproducible between different years at a bacterial phylum level but subject to strong intraspecific shifts at the bacterial species level. Several strains could be identified as host-specific. For *Fucus vesiculosus* we could demonstrate that surface abundant metabolites were able to recreate a bacterial community on an artificial substrate similar to that found on the living alga. Two repellent compounds were responsible to deter the settlement of Gammaproteobacteria and Firmicutes.

Macroalgae carry a species-specific but variable bacterial community on their surfaces. A combination of nutritive and repellent algal metabolites may lead to this selective recruitment.

Abstract Category

19 Plant-Microbe Interactions

PS.19.059 BACTERIAL COMMUNITIES IN THE BARK OF TREES ARE ASSOCIATED WITH PLANT PHYLOGENY

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Tree bark is a primary protective barrier that typically contains a suite of antimicrobial compounds such as phenolics, tannins, and terpenes. However, whether resident bacterial communities inhabit bark surfaces in spite of these compounds has not been studied. It can be hypothesized that each type of tree bark selects for its own bacterial community based on differences in the phytochemicals available for microbial growth, and the tolerances of the bacteria to specific antimicrobial chemicals. To examine this hypothesis, we evaluated 9-12 individuals of 7 tree species in a pristine Dense Ombrophilous Atlantic forest. The trees that were studied encompassed species from three orders and four families. The bacterial communities were characterized using 16S rRNA gene PCR-DGGE and clone library sequencing. Our results showed that the bacterial communities varied among tree species, but were highly similar for individuals of the same species. Moreover, the grouping pattern observed by discriminant analysis of the bacterial communities consistently reflected the phylogenetic relationships among the tree species. Approximately 65% of the bacteria on the bark were assigned to Gammaproteobacteria. The vast majority of the species are new, but included approximately 39-74% that were classified as *Pseudomonas*. Our findings reveal the high selectivity of this previously uncharacterized habitat for unique bacterial communities. Their contribution to the ecology of forest ecosystems is unknown, but can be speculated to contribute to the degradation of terpenes and phenolics that are involved in plant defense responses or chemical signaling. In addition to comprising yet another large reservoir of genetic diversity in tropical forests, the bark-inhabiting bacteria and their enzymes may have biotechnological applications. Project funded by FAPESP (São Paulo, Brazil) and CNPq (Brasília, Brazil).

Abstract Category

19 Plant-Microbe Interactions

PS.19.060 PLANT DIVERSITY IMPROVES PROTECTION AGAINST SOIL-BORNE PATHOGENS BY FOSTERING ANTAGONISTIC BACTERIAL COMMUNITIES

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Due to their inhibiting potential against plant pathogens, soil bacteria such as pseudomonads have been proposed as biocontrol agents. In order to foster bacteria-mediated plant protection factors affecting the dynamics of plant growth promoting rhizobacteria need to be understood. In particular, plants act as drivers of rhizosphere bacterial communities. In temperate grasslands, increasing plant diversity has been shown to enhance productivity, and we hypothesized that this partly relies on a positive soil feedback effect involving biocontrol bacteria. We investigated the impact of plant diversity and functional group composition on the protective function of root-associated bacteria in a long-term grassland biodiversity experiment (the Jena Experiment). The abundance of bacteria

producing the antifungal compounds 2,4-diacetylphloroglucinol (DAPG) and pyrrolnitrin (PRN) was determined by quantitative PCR. Furthermore, we investigated if plant community effects on bacterial communities are associated with changes in soil suppressiveness in an infection assay with *Rhizoctonia solani*. Plant diversity positively affected DAPG producing bacteria which in turn resulted in increased soil suppressiveness against root pathogens. PRN producing bacteria were less affected by plant diversity. Grasses increased and legumes decreased the amount of both toxin producing bacterial groups. Our results indicate that plant diversity positively affects beneficial bacterial communities, adding a novel explanation to the positive relationship between plant diversity and productivity. Polycultures of plants therefore may foster plant-protective bacterial communities and promote soil suppressiveness and the use of certain plant combinations may help establishing environmentally friendly control of plant diseases.

Abstract Category
19 Plant-Microbe Interactions

PS.19.061 DIVERSITY OF PLANT POLYSACCHARIDE DEGRADATION CAPABILITIES IN RUMEN FIBROLYTIC BACTERIA AS REVEALED BY GENOME AND METAGENOME STUDIES

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Determining the role of rumen microbes and their enzymes in plant polysaccharide breakdown is fundamental to understanding ruminant digestion and maximising ruminant productivity. To better understand the key roles microbes play in plant polysaccharide degradation we have sequenced the *Butyrivibrio proteoclasticus* B316^T genome and compared its plant degradation capabilities to other sequenced rumen bacteria and rumen microbial metagenomes.

The genome sequence of B316 was determined using a whole genome shotgun strategy and a pyrosequencing approach. Annotation and comparative genomics was performed using available public tools. A bovine rumen metagenomic study was carried out, with metagenomic DNA samples and 16S ribosomal RNA amplicons pyrosequenced and data analysed by software, including MG-RAST and RDP.

B316 devotes a large proportion of its genome to the breakdown and reassembly of complex polysaccharides and has one of the most highly developed glyco-biomes when compared to other sequenced bacteria. The secretion of a range of polysaccharide-degrading enzymes which initiate the breakdown of pectin, starch and xylan, and the occurrence of a wide range of intracellular enzymes to break down oligosaccharides, constitute the degradative capability of this organism. The number of different CAZY families represented in the B316 genome surpasses that found in two rumen cellulolytic bacteria and in a published rumen metagenome. The 16S rRNA gene sequencing revealed an abundance of sequences affiliated with the *Lachnospiraceae* family, with many assigned to the genus *Butyrivibrio*.

Analyses of rumen bacterial genomes and metagenomic data have allowed greater insight into the enzymes that are important for plant degradation in the rumen. *Butyrivibrio* strains are an important component of the microbiota and analysis of the B316 genome highlights the degradative enzyme diversity that is found within a single organism.

Abstract Category
19 Plant-Microbe Interactions

PS.19.062 PLANT DIVERSITY AS A DRIVER OF BACTERIAL DENITRIFYING COMMUNITY?

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Denitrification is the bacterial dissimilatory process that is mainly related to nitrate losses in soils and N₂O emission (greenhouse effect). Plants and bacterial communities are strongly competing for nitrate, considered as a limiting factor for plant growth. Many drivers such as soil type, nutrient availability, carbon sources, have been identified as important factors controlling denitrification. In parallel, plants can influence the activity; diversity and abundance of bacterial communities, mostly via carbon quantity and quality, either by roots exudates or litter (dead material). Thus, plant and litter diversity could constitute major factors controlling rate of denitrification. To disentangle the relations between plant diversity and denitrifying communities, a short-term pot experiment (72 days) was carried out with three European grassland plants *Mimulus guttatus*, *Lamium amplexicaule*, and *Medicago sativa*, using a factorial design in which litter amendment (none, mono and tri-specific litter) and plant association (monocultures, two- and three-species mixtures) were crossed. During plant growth, the denitrifying bacterial diversity and abundance were determined by molecular tools. In parallel, denitrifying activity was determined by measuring potential denitrification, nitrogen contents, and also carbon contents. Plants characteristics (above-ground biomass, shoot: root ratio) were measured. This multidisciplinary approach led us to better understand how plant diversity could modify the denitrifying bacterial community and the denitrification rates.

Abstract Category
19 Plant-Microbe Interactions

PS.19.063 EFFECTS OF POTATO SUBERIN ON THE MORPHOLOGY OF FOUR STREPTOMYCES SPECIES INCLUDING THE POTATO SCAB-CAUSING AGENT STREPTOMYCES SCABIEI

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Bacteria of the genus *Streptomyces* are filamentous soil-dwelling microorganisms with a saprophytic life cycle but a few species, such as *S. scabiei*, can cause common scab of potato. Their pathogenicity strongly depends on the production of a phytotoxin called thaxtomin A. Genes involved in the synthesis of this secondary metabolite are known to be induced by cellobiose. However, a previous study showed that no toxin was produced when *S. scabiei* was grown in liquid medium with cellobiose as sole source of carbon as this plant disaccharide apparently blocked the bacterium in vegetative phase. On the other hand, large amounts of thaxtomin A were recovered when cellobiose was combined to potato suberin, a complex plant polymer found on the surface of tubers. It was assumed that suberin unlocked the negative effect of cellobiose and stimulated differentiation and consequently, the onset of secondary metabolism in *S. scabiei*. In the present work, the effects of cellobiose and potato suberin regarding the morphological development of four *Streptomyces* species were tested on solid medium. The common scab-causing agents *S. scabiei* and *S. acidiscabies* and the non-pathogenic species *S. coelicolor* showed morphological patterns that strengthen the potential role of suberin in the onset of differentiation. However, suberin did not induce secondary growth phase in *S. avermitilis*. Cellular morphological changes caused by the presence of suberin were also investigated in *S. scabiei*. The presence of the plant polymer modified the fatty acids composition of the bacterial membrane which caused an increase in membrane fluidity. Suberin also induced thickening and higher density of the bacterium cell wall. These changes resulted in enhanced resistance of the *S. scabiei* to mechanical lysis. Such modifications could improve protection of the pathogenic agent from the plant defence mechanisms triggered during the infection process.

Abstract Category
19 Plant-Microbe Interactions

PS.19.064 MICROBIAL COLONIZATION OF LEGUME CROP RESIDUES DURING DECOMPOSITION

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We quantified soil microbial biomass C (MBC) and bacterial diversity (H') on decomposing residues of two pea varieties (Camry, a semi-leafless variety, and 4010, a normal-leafed silage pea variety),

faba bean grown for seed, faba bean green manure (GM) and chickling vetch GM. Nitrogen released from the residues was concurrently quantified. During 12 months of decomposition, the order of MBC on residues was faba bean GM \geq vetch GM \geq faba bean grown for seed \geq Camry pea \geq 4010 pea, and H' on Camry pea residues was less than that on the other residues. The pattern with time was a rapid increase of MBC in the first four weeks after residue placement in summer or fall, followed by a slight decrease during winter, then a gradual increase from spring to fall of the following season. The pattern for H' was similar, except that the decrease during winter was accelerated between spring and summer of the following season, and started increasing again between summer and fall. The N release pattern also followed a similar trend to that of MBC on the residues, except that N release continued to increase slowly during winter. There was positive correlation between MBC on the residues and N released from the residues even though faba bean grown for seed released the most N. H' was not related to N released, presumably because H' decreased between spring and summer when N release continued to increase. H' was negatively correlated with the residue C/N ratios. When only the first month of decomposition and N release were considered, both MBC and H' were negatively correlated with %N remaining in residues. Therefore, microbial colonization of crop residues can be a good indicator of N released from the residues.

Abstract Category
19 Plant-Microbe Interactions

PS.19.065 CHANGES IN N-TRANSFORMING ARCHAEA AND BACTERIA DURING THE ESTABLISHMENT OF BIOENERGY CROPS

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Widespread adaptation of biomass production for bioenergy may influence important biogeochemical functions in the landscape, which are mainly carried out by soil microbes. To explore the environmental impact of bioenergy crops on biological nitrogen cycling in the soil, changes in the abundance and diversity of diazotrophic bacteria, ammonia-oxidizing bacteria (AOB) and archaea (AOA), and denitrifying bacteria were monitored after planting miscanthus, switchgrass, restored prairie and corn (the only fertilized crop). Bulk soil samples were collected at monthly intervals during the growing season (June - September 2008 and 2009) from experimental plots at the Energy Farm established in spring 2008. Key N-cycling genes (nifH, bacterial amoA, archaeal amoA and nosZ genes) were analyzed using real-time PCR and bar-coded pyrosequencing methods. After planting the bioenergy crops, the quantities of these genes were relatively stable in all the crops, except corn, in which the population size of AOB doubled in less than 3 months, while denitrifiers became higher than the other crops in the second season. Deep sequencing revealed high diversity of nifH, archaeal amoA, bacterial amoA and nosZ genes with 10899, 3187, 3945 and 11242 unique nucleotide sequences observed respectively. Rarefaction analysis revealed the diversity of archaeal amoA in corn significantly decreased in 2009. Unifrac PCA analysis showed that the N-transforming microbial community structures in the soil under these crops changed following different trajectories. Thus far, our two year study has shown that specific N-transforming microbial communities develop in the soil after planting with different bioenergy crops, and that each microbial group responded in a different way. The results also suggest that application of fertilizer results in increased abundance of AOB and denitrifiers, reduced diversity of AOA, and significant changes in the structure of these N-transforming populations.

Abstract Category
19 Plant-Microbe Interactions

PS.19.066 MOLECULAR FINGERPRINTS OF MAIZE-ASSOCIATED ENDOPHYTES ISOLATED FROM BRAZILIAN SOILS.

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Because endophytes microorganisms are potentially advantageous in several aspects as promotion of plant growth and development, instead of production of metabolites of agronomical and pharmaceutical interest, the knowledge of endophytes and plants interactions became very

important. To intend the basis of those interactions, the phylogenetics of plant growth-promoting rhizobacteria (PGPR) associated with crops are now viewed as a key point to extend the biotechnological applications of this suitable resource. Focusing on maize (*Zea mays* L.) systems grown in different Brazilian soils, we decided to investigate the profile of maize-associated endophytes by means of two molecular fingerprints techniques: 1) Box-PCR supported by Box-A1R primer, and 2) isoenzymes system based on α -esterase, superoxide dismutase, alcohol dehydrogenase, and glucose-6-phosphate dehydrogenase. The twenty bacteria chosen for analysis were previously described as possessing characteristics of PGPR, phosphate solubilizing, and diazotrophic microorganisms. Box-PCR revealed two main groups including *Burkholderia* sp. and *Ralstonia* sp. in one, and *Pantoea* sp. and *Burkholderia* sp. in another, both with 67% of similarities. All isoenzymes presented a polymorphic pattern, while alcohol dehydrogenase and glucose-6-phosphate dehydrogenase were the systems that revealed the most prominent results. Bionumerics (6.0.1 - Applied Maths NV) software was applied to enclose all data from different fingerprints, and has corroborated the importance of a polyphasic analysis that is fundamental to access those bacterial diversity.

Abstract Category
19 Plant-Microbe Interactions

PS.19.067 ACCUMULATION OF THE ANTIBIOTIC PHENAZINE-1-CARBOXYLIC ACID IN THE RHIZOSPHERE OF DRYLAND WHEAT

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Natural antibiotics are thought to function in microbial defense, fitness, competitiveness, biocontrol, communication and gene regulation activity, and antibiotic-producing species are commonly found in microbial communities throughout nature. However, the frequency and amount of antibiotic production in situ are poorly understood. We addressed this issue by measuring the geographic distribution of indigenous phenazine-producing (Phz⁺) *Pseudomonas* spp. and the amounts of phenazine-1-carboxylic acid (PCA) in the rhizosphere of wheat grown in the low-precipitation zone (<400 mm) of the Columbia Plateau in the Inland Pacific Northwest, USA. In 2008-2009 we conducted a survey of commercial dryland fields situated within an area of about three million acres that is roughly bounded by 46.8° and 47.9° N and 117.5° to 119° W. Phz⁺ *Pseudomonas* spp. were detected in all sampled fields with mean population sizes ranging from log 3.2 to log 7.1 per g of fresh weight of root. PCA was detected in the rhizospheres of plants from 20 of 21 fields sampled. Linear regression analysis demonstrated a significant (P <0.0001) relationship between the population level of Phz⁺ isolates and the amount of PCA recovered from the plant rhizosphere.

Abstract Category
19 Plant-Microbe Interactions

PS.19.068 IMPACT OF IRRIGATION ON POPULATIONS OF ANTIBIOTIC-PRODUCING PSEUDOMONAS SPP. IN THE RHIZOSPHERE OF WHEAT

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This work studied the impact of irrigation on seasonal dynamics of populations of *Pseudomonas* spp. producing the antibiotics phenazine-1-carboxylic acid (Phz⁺) and 2,4-diacetylphloroglucinol (Phl⁺) in the rhizosphere of wheat grown in the low-precipitation zone (<400 mm) of the Columbia Plateau of the Inland Pacific Northwest, USA. Population sizes and plant colonization frequencies of Phz⁺ and Phl⁺ *Pseudomonas* spp. were determined in winter wheat collected during March - June, 2008 from selected commercial dryland and irrigated fields near the towns of Ritzville and Lind, WA. Only Phz⁺ bacteria were detected on dryland winter wheat, with populations ranging from log 5.2 to 7.3 CFU g root⁻¹ and rhizosphere colonization frequencies of 63 to 100%. Similar population sizes of both Phz⁺ and Phl⁺ *Pseudomonas* spp. were recovered from wheat grown under irrigation, with 88-100% plants colonized by Phl⁺ bacteria and only 13 - 38% of plants harboring Phz⁺ bacteria. These

findings were in perfect agreement with results of a second sampling in 2009 that included 16 dryland and irrigated fields in the Ritzville area.

Abstract Category
19 Plant-Microbe Interactions

PS.19.069 NAPHTHALENE DEGRADERS ACTIVE IN DIESEL RHIZOREMEDIATION WITH WILLOW

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Rhizoremediation relies on plants to biostimulate microbial degradation of pollutants, but little is known about how degradative microbial populations in a contaminated soil change in response to rhizostimulation. Outdoor pot experiments coupled with DNA-based stable isotope probing (SIP) were applied to investigate how *Salix alaxensis* (felt leaf willow) affects naphthalene degrading populations during rhizoremediation of diesel-contaminated soil. The concentration of diesel range organics (DRO) and the most probable number (MPN) of culturable diesel degrading microorganisms in the rhizosphere were monitored in planted and unplanted (control) treatments, with and without fertilizer addition, over a summer in interior Alaska. SIP incubations were performed in microcosms constructed from soils following treatment to identify and compare ¹³C-naphthalene-utilizing bacteria in the willow-planted versus unplanted (control) treatments. Terminal restriction fragment length polymorphism (T-RFLP) of ¹³C-DNA isolated by isopycnic centrifugation was conducted to examine the diversity of bacteria that derived carbon from naphthalene. In the pot study, growth of *S. alaxensis* resulted in the greatest loss of DROs, although treatments amended with fertilizer contributed to a significant increase in MPN of culturable diesel degrading microorganisms. T-RFLP profiles of ¹³C-DNA suggest that the presence of willow may have enhanced the growth of a particular bacterial taxon. Further cluster analysis showed separate clusters between control and willow samples. To identify naphthalene-utilizers, sequencing of 16S rRNA clone libraries is underway. This research suggests that *S. alaxensis* can be a useful plant for rhizoremediation of diesel-contaminated soil and that growth of this willow altered the structure and/or composition of naphthalene-utilizing populations relative to that of unplanted soil.

Abstract Category
19 Plant-Microbe Interactions

PS.19.070 MICROBIAL COMMUNITY IS AFFECTED IN FOREST SOILS HARBORING THE ECTOMYCORRHIZAL FUNGUS *TUBER MELANOSPORUM*

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Tuber melanosporum is an ectomycorrhizal fungus that produces fruiting-bodies highly appreciated because of their special taste and aroma. The worldwide demand for *T. melanosporum* has pushed researchers to understand fruiting-body formation to increase truffle production. In this framework an important result is its genome, sequenced thanks to a French-Italian Consortium. Generally associated to *T. melanosporum* presence, there is a zone around or near the host mycorrhizal tree where plant cover is very scanty. This area is described as burnt and is considered a marker of the presence of the truffle, occurring as mycelia, fruiting bodies or as mycorrhizal roots. The reasons for such a phenomenon are still unclear, likely involving phytotoxic effects of the truffle's metabolites. In the framework of a project focussed on understanding the microbial scenario of truffle-grounds, our aims were to characterize both - the fungal and bacterial populations - occurring in the rhizosphere of plants mycorrhized by *T. melanosporum*. Soil samples were collected at Cahors, France, in nine *T. melanosporum* truffle-grounds. DGGE was used to compare rRNA gene fingerprintings of fungi and bacteria inside and outside the burnt area. One truffle-ground was also assessed with ITS molecular cloning for fungal community investigation, and with a phylogenetic microarray for bacterial community investigation. DGGE led to two clusters of fungal profiles - one by the samples inside the burnt, and the second one by the

samples outside the burnt. ITS results demonstrated that percentage of ectomycorrhizal fungi was lower inside than outside. While bacterial profiles did not show presence/absence differences between inside and outside, phylogenetic microarrays revealed strong differences in taxa abundance. In conclusion, *T. melanosporum* strongly affects the microbial community inside the burnt, clearly showing a correlation with the burnt formation.

Abstract Category
19 Plant-Microbe Interactions

PS.19.071 BACTERIAL DIVERSITY OF OIL DEGRADING CONSORTIA ENRICHED FROM URBAN MANGROVE SEDIMENT AND RHIZOSPHERE AND ITS POTENTIAL FOR PHYTOREMEDIATION

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Mangroves are intertidal tropical estuarine wetlands which are disappearing worldwide. Urban mangroves receive discharges from industry and occasional oil spills. In this study, the potential use of mangrove micro-niches as reservoirs of hydrocarbonoclastic bacteria with hydrocarbon-degrading genes and broad host range plasmids was investigated. Petroleum hydrocarbon-degrading consortia (PHDC) were obtained by enrichment cultures in petroleum-amended mineral media, inoculated with bulk and *Rhizophora mangle*, *Laguncularia racemosa*, *Avicennia schaueriana* rhizosphere sediments from a polluted mangrove. Rhizosphere PHDC showed a sharp decrease in hydrocarbon levels whereas bulk PHDC was moderately lower than the control. The structural and functional diversity of PHDC were surveyed by PCR-DGGE and Southern Blot Hybridisation. Significant differences on bacterial communities, plasmids and functional genes were detected among PHDC. *Bacteria* and *Alphaproteobacteria* revealed complex DGGE profiles; lower complexity was detected for *Betaproteobacteria*, and *Pseudomonas*. Some dominant ribotypes were common to all consortia. Plasmids and *ndo* genes were enhanced by enrichment. PHDC bacteria cultures were identified by 16S rRNA gene partial sequence as *Pseudomonas*, *Comamonas*, and *Ochrobactrum* spp. IncP-1 and IncP-9 plasmids and *ndo* genes were detected by PCR in several isolates. Plants inoculated with PHDC survived well in oil-amended soil. Our results provided new insights on mangrove bacterial diversity and are relevant to improve reforestation strategies.

Abstract Category
19 Plant-Microbe Interactions

PS.19.072 COMPARATIVE PHYLOGENOMICS OF PHYLLOSHERE-ASSOCIATED ESCHERICHIA COLI ISOLATES

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The *Escherichia coli* species is mostly represented by strains that are natural commensals of the gastrointestinal tract (GIT) of vertebrates. However, a few *E. coli* strains can cause various intestinal and extraintestinal diseases, resulting in more than 2 millions deaths per year. This serious health issue has driven medical research to understand the molecular mechanisms of *E. coli* colonization and virulence. While pathogenic *E. coli* infections are commonly linked to the consumption of animal products, multiple food-related outbreaks have recently been related to the ingestion of strains associated to fresh fruits and vegetables, raising interest on the ecological links between *E. coli* and plants, and on the functions involved in this association. Indeed, little is known about how wild-type *E. coli* persist and adapt to their living environment when outside the animal gut, despite the fact that *E. coli* can be easily isolated from multiple nonhost environments such as soils, fresh waters or vegetation, where it seems to persist very well and sometimes even replicate.

In this study, *E. coli* isolates from the phyllosphere of field-grown crops were compared to strains of the widely used ECOR collection. Using Bayesian analysis, we inferred the phylogenetic relationships between the tested strains using a microarray-based comparative genomic hybridization (CGH) dataset. Additionally, we assessed the strains for various phenotypes related to life conditions on plants or in the GIT.

Our results indicate that specific plant-related metabolic abilities may be important for an increased fitness of *E. coli* on leaves. CGH did not reveal any correlation between phylogenetic relationship and sample origin (gut or plant surface). This could indicate that there is no specific adaptation to plants at the phylogenetic level, but that key functions acquired through lateral gene transfer may play an important role in the association of *E. coli* with plants.

Abstract Category
19 Plant-Microbe Interactions

PS.19.073 COMMUNITY SHIFTS IN SOYBEAN-ASSOCIATED MICROBES BY HOST NODULATION GENOTYPES AND NITROGEN APPLICATIONS

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Diverse microorganisms are living as endophytes and epiphytes of plants in nature. Legumes have developed nodulation and autoregulation systems to attain mutual symbiosis with (brady)rhizobia during their evolution. We aimed to address whether the legume symbiotic systems regulate the bacterial communities in field-grown soybeans. The diversity of microorganisms associated with roots, stems and leaves of non-nodulated (Nod-), wild-type nodulated (Nod+), and hypernodulated (Nod++) soybeans were evaluated by ribosomal intergenic transcribed spacer analysis (RISA) for roots, and clone library analysis of 16S rRNA gene using the enriched bacterial cells of soybean stem and leaf tissues. Soybeans were dressed with standard and high N fertilization to examine the nitrate effects on auto regulation. Differential RISA profiles in the roots were observed according to nodulation phenotypes. Principal component analysis revealed that bacterial communities were clustered into three groups corresponding to the nodulation genotypes. The abundance of *Alphaproteobacteria* in Nod+ soybean stems and leaves was lower than that in Nod- and Nod++ soybeans under standard N. Principal coordinate analysis showed that the bacterial communities of Nod- and Nod++ soybean stems and leaves were more similar to each other than to that of Nod+ soybeans. High N fertilization decreased the abundance of *Alphaproteobacteria*, and caused drastic shifts of the bacterial community. The clustering analyses identified a subset of operational taxonomic units (OTUs) responding to both the nodulation genotype and N levels. The relative abundances of two OTUs in *Aurantimonas* sp. and *Methylobacterium* sp. were especially sensitive to nodulation genotypes and high N fertilization. These results suggested that a subpopulation of proteobacteria in soybeans is controlled in a similar manner through both the regulation systems of plant-rhizobia symbiosis and the N signaling pathway in plants.

Abstract Category
19 Plant-Microbe Interactions

PS.19.074 IMPACT OF SNOWMELT ON THE POPULATION STRUCTURE OF THE PLANT PATHOGEN *PSEUDOMONAS SYRINGAE* IN AN ALPINE HYDROLOGICAL SYSTEM

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Recent reports reveal that the life cycle of the plant pathogen *Pseudomonas syringae* is associated with the water cycle; it inhabits snow and alpine rivers in particular. In this light, our objective is to model the transfer of populations of *P. syringae* during snowmelt towards the first overland flows in the Southern French Alps. We estimated abundance and the phenotypic diversity (ice nucleation activity, production of toxins, and pathogenicity) of *P. syringae* in the snow cover at three sites in the Southern French Alps and the associated streams. *P. syringae* was detected in the water and the snow cover at all sites during the sampling period (March to June). Abundance of *P. syringae* and population structure was stratified along the depth of the snow cover. In the 10-cm layer of snow in contact with the ground, population sizes were significantly greater and the dominant phenotypes changed during the season relative to that in snow closer to the surface where populations were less dense and more stable. Surprisingly the population structure in adjacent stream water was different from that in snow. Our field observations coupled to microcosm studies have led us to propose a preliminary conceptual model of the processes involved that modify

population structure from snowmelt to stream flow. In winter, *P. syringae* arrives in the basin with the first snow falls. Populations in this initial snow layer consist of strains arriving with the snow and those already present on the leaf litter. Populations survive, multiply and die during the winter as a function of organic matter availability, humidity, the low conductivities and the temperature (always ca. 0°C in the snow cover). Then, the meltwater infiltrates gradually into the ground; the geology of the site determines its residence time and its chemistry. The capacity of *P. syringae* subpopulations to survive transport depends on their fitness in face of the conditions determined by water chemistry and residence time.

Abstract Category

19 Plant-Microbe Interactions

PS.19.075 BIOGEOGRAPHY OF THE PLANT PATHOGEN *PSEUDOMONAS SYRINGAE* IN ALPINE HEADWATERS OF RIVERS IN THE USA, FRANCE AND NEW ZEALAND

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Non host, environmental reservoirs of pathogens play key roles in their evolutionary ecology and in particular in the evolution of pathogenicity. In light of recent reports of the plant pathogen *Pseudomonas syringae* in pristine waters outside of agricultural regions and its dissemination via the water cycle, we have examined the genetic and phenotypic diversity, population structure and biogeography of *P. syringae* from headwaters of rivers on three continents and their phylogenetic relationship to strains from crops. A collection of 236 strains from 11 sites in the USA, in France and in New Zealand was characterized for genetic diversity based on housekeeping gene sequences and for phenotypic diversity based on measures of pathogenicity and ice nucleation activity. Phylogenetic analyses revealed several new genetic clades from water. The genetic structure of *P. syringae* populations was not influenced by geographic location or water chemistry, whereas the phenotypic structure was affected by these parameters. Comparison with strains from crops revealed that the metapopulation of *P. syringae* is structured into three genetic ecotypes: a crop-specific type, a water-specific type, and an abundant ecotype found in both habitats. The abundance and diversity in water relative to crops suggest that adaptation to the freshwater habitat has played a non negligible role in the evolutionary history of *P. syringae*. In particular, it seems that adaptation to dissemination via the water cycle is linked to the epidemiological success of this plant pathogen. Our data suggest that strains that are the most active ice nuclei - and could therefore enhance their ability to re-deposit to the ground after being transported with aerosols up to clouds - are the most aggressive strains with the broadest host range and are also found among the widest diversity of non agricultural substrates associated with the water cycle.

Abstract Category

19 Plant-Microbe Interactions

PS.19.076 BIODIVERSITY AND ADAPTIVE STRATEGIES OF MYCORRHIZOSPHERE BACTERIA ASSOCIATED TO *TRISTANIOPSIS* SPECIES IN NEW CALEDONIAN ULTRAMAFIC ECOSYSTEMS

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New Caledonian ultramafic ecosystems are considered as hotspots of biodiversity, partly because of the adaptative pressure exerted by drastic edaphic conditions. These soils are composed of up to 85 % of iron oxides, are deficient in NPK, unbalanced for the Ca/Mg ratio and particularly rich in heavy metals such as Ni (2%), Cr (2%), and Mn (1%). Both plant and soil microbes play a vital role in the adaptation to harsh soil conditions mainly heavy metal uptake and tolerance. We chose an endemic species of the genus *Tristaniopsis* (*Myrtaceae*) as model plant to study the role of ectomycorrhizal fungi and associated bacteria in plant adaptation to nickel. To investigate the effect of ultramafic

soils on ectomycorrhiza and mycorrhizosphere bacterial diversities as well as on the genetic determinants of resistance/adaptation of associated mycorrhizosphere bacteria, 200 ectomycorrhizas were sampled from four different ultramafic sites (3 in Koniambo and 1 in Desmazures forest) vs two non-ultramafic ones from volcano-sedimentary soils (Arama). Molecular characterization of ectomycorrhiza (rRNA ITS) and associated mycorrhizosphere bacteria (16S rRNA) from these samples showed the presence of different dominant fungi, i.e. *Pisolithus albus*, *Russula* spp. and *Boletellus* spp., and bacteria, i.e. *Burkholderia* spp., *Bacillus* spp. and *Pseudomonas* spp., that can be found in both soil types. However, bacteria isolated from ultramafic soils could grow in the presence of Ni up to 20 mmol L⁻¹ and contained *cnrT* and *nreB* genes, known to confer heavy metal tolerance, contrarily to bacteria isolated from non-ultramafic soils. Moreover, we found a strong positive correlation between heavy metal tolerance and P-solubilizing ability. Further investigation on functional diversity of ectomycorrhiza-mycorrhizosphere bacteria associations and their role in plant adaptation to ultramafic soils would help in the understanding of plant functioning on New Caledonian mine sites.

Abstract Category
19 Plant-Microbe Interactions

PS.19.077 SELECTIVITY OF PLANT SPECIES TO RHIZOSPHERE-ASSOCIATED OOMYCETE COMMUNITIES

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Oomycetes are an important group of terrestrial and aquatic pathogens that infect hosts ranging from plants to mammals. Although we know much about specific oomycete pathogens of economically-important plants, little is known of the ecological forces that shape the assembly, distribution, and activity of oomycete communities. The aim of our study was to determine whether distinct oomycete rhizosphere communities associate with three different plant species: *Medicago sativa*, *Trifolium pretense*, and *Phleum pretense*. Rhizosphere communities were characterized using direct DNA extractions as well as baiting. Communities were then compared using a series of phylogenetic analyses and various measures of diversity and shared taxa. The α -diversity within each of the three rhizosphere communities did not differ based on calculations of θ_n . Significantly fewer total and unique taxa were detected in *Phleum* rhizospheres than in the *Medicago* or *Trifolium* rhizospheres. Estimates of β -diversity revealed that the incidence and abundance of specific taxa accounted for community differences among plant species. All rhizospheres were dominated by species of *Pythium* and *Aphanomyces*. Sequences with affinities to *Achlya caroliniana*, *Pythium arrhenomanes*, and *Aphanomyces piscicida* were most abundant in the *Phleum* rhizosphere, with few representatives in the rhizosphere of *Medicago* and *Trifolium*. Taxa recovered by baiting were also dominated by *Pythium* species; some were unique to baiting. Abundance of OTUs fluctuated across sampling dates but did not correlate with seasonal changes in temperature and precipitation. Our results demonstrate associations of distinct oomycete taxa with different plant species and further indicate that the seasonal patterns observed in many earlier studies of oomycetes may not be reflective of the changing patterns of oomycete diversity in the rhizosphere.

Abstract Category
19 Plant-Microbe Interactions

PS.19.078 STUDIES OF FUNGAL COMMUNITIES IN SOILS FROM PEA FIELDS USING NEXT GENERATION SEQUENCING

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Soil fungi are the most common causes of pea diseases. Next generation sequencing (NGS), which has recently provided new approaches to study microbial communities from environmental samples, was used in this study to characterize fungal communities in diseased and healthy pea soils. The study targeted fungal communities in pea soils using 454 pyrosequencing of the nuclear ribosomal internal transcribed spacer-1 (ITS-1). Fungal species richness, diversity, and community composition were analyzed and compared among nine different pea soils of which six were diseased and three were healthy. After filtering for quality, 68811 sequences were obtained from the nine soil

samples. These were clustered into 748 operational taxonomic units (OTUs) excluding singletons. The results showed that the fungal communities in the 9 soils were very diverse and strongly dominated by *Ascomycota* and *Basidiomycota*, *Phoma* sp. being the most dominant genus. The fungal communities were statistically different between diseased and healthy soils. Diseased and healthy soils could be classified by Linear discriminant analysis (LDA), using *Ascomycota* and *Basidiomycota*, at phylum level, and *Phoma* sp. and *Podospora* sp., at genus level, as two best variables after cross validation. The 454 pyrosequencing strategy provided detailed information on the community composition of the soils and showed that the abundance of some genera was different for diseased and healthy soils.

Abstract Category
19 Plant-Microbe Interactions

PS.19.079 HYPHAE-COLONIZING BACTERIA IN WATER-SURFACE MICROCOSMS - A NEW SOURCE OF BIOLOGICAL CONTROL AGENTS FOR *RHIZOCTONIA SOLANI* AG1-IA IN RICE

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Sheath blight disease of rice (*Oryza sativa*) caused by *Rhizoctonia solani* Kühn is of increasing concern in the Mekong Delta, Vietnam, because intensified cropping practice using semi-dwarf rice cultivars, high nitrogen input and high seeding rates leads to dense canopies with high humidity and leaf-to-leaf contact. Eco-friendly alternatives to control the disease are currently being exploited, but biological control agents (BCA) isolated directly from standard dual culture assays often show poor performance in the field. Hence, we developed a new strategy to obtain efficient BCA bacteria from rice paddy soil, based on colonization of *R. solani* hyphae floating on water surfaces. Eighty-five % of the hyphae-colonizing isolates were *Burkholderia* sp. according to 16S rRNA gene sequencing; by comparison, a majority of isolates from standard dual-culture assays were *Bacillus* sp. All isolates were tested for antagonism against *R. solani* in both the standard dual-plate assay and a microcosm assay mimicking *in situ* conditions of the irrigation water surfaces, from where sheath blight infections originates. Hyphae-colonizing isolates VL-16.5 and AG-7.3, affiliating to *Burkholderia vietnamiensis* based on *recA* gene sequencing, were found to be highly effective against *R. solani* in both assays. The hyphae-colonizing *Burkholderia* sp. isolates were further tested for *R. solani* antagonism in rice plant systems under greenhouse conditions. A strong correlation between the BCA isolates performances in microcosm and greenhouse assays was obtained. Our results indicate that isolations of BCA bacteria for sheath blight in rice are efficiently improved using the new water-surface microcosm, mimicking the ecological niche in which pathogen and BCA naturally interacts.

Abstract Category
19 Plant-Microbe Interactions

PS.19.080 MRNA STABLE ISOTOPE PROBING IN SOIL

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Stable isotope probing provides an opportunity to tie function to phylogeny in complex environments, such as soil. Currently, it is technically difficult to isolate ¹³C labeled mRNA during RNA SIP, due to its lability and low abundance relative to rRNA. We have improved the mRNA SIP process, which has allowed us to detect up to thirty times more transcripts in our SIP fractions. Here we discuss our improvements to the SIP process, as well as the limitations of this technique, in the context of ¹³CO₂ labeling of the rhizosphere.

Abstract Category
19 Plant-Microbe Interactions

PS.19.081 DIFFERENTIAL GROWTH OF *PINUS PINASTER* SEEDLINGS OBTAINED FROM SEEDS OF PLUS AND NON-PLUS TREES AFTER INOCULATION WITH SELECTED

ECTOMYCORRHIZAL FUNGI

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Seeds obtained from *Pinus pinaster* plus trees (considered phenotypically superior) have been used in forest nursery production. The effect of inoculation with ectomycorrhizal fungi on the growth of these seedlings has not been studied. We compared the growth response of *P. pinaster* seedlings obtained from seeds of plus and non-plus trees to inoculation with different selected ectomycorrhizal fungi under conventional forest nursery conditions. The growth and nutrition of *P. pinaster* seedlings obtained from seeds of plus and non-plus trees and inoculated with ectomycorrhizal fungi differed. In seedlings obtained from seeds of non-plus trees only the plants inoculated with *Suillus bovinus* + *Laccaria laccata* + *Lactarius deterrimus* had a significantly greater total plant dry weight and needles nitrogen concentration, while in seedlings obtained from seeds of plus trees this effect was seen not only in plants receiving that same ectomycorrhizal inoculation, but also in plants inoculated with *Rhizopogon vulgaris* or *Pisolithus tinctorius* + *Scleroderma citrinum*. The highest plant biomass was that of seedlings obtained from seeds of plus trees and inoculated with *R. vulgaris* or *S. bovinus* + *L. laccata* + *L. deterrimus*. The combined use of seeds obtained from plus trees and inoculation with selected ECM fungi can be an advantageous biotechnological approach to improve nursery production of *P. pinaster*. The authors wish to thank Fundação para a Ciência e a Tecnologia, POCI 2010 and FSE for the grants SFRH/BPD/23749/2005 and SFRH/BD/47722/2008.

Abstract Category

19 Plant-Microbe Interactions

PS.19.082 PLANT ROOT EXUDATES IMPACT THE DEGRADATION POTENTIAL OF A WEATHERED-HYDROCARBON CONTAMINATED SOIL

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Phytoremediation has emerged as a promising low cost technology to assist in the cleanup of contaminated sites. However, specific plants which promote degradation under one set of conditions may not under another, and knowledge limitations surrounding the mechanisms of phytoremediation hamper attempts at optimization. We addressed this issue by examining how differences in exudation patterns impact the degradation potential of microbial communities in a weathered hydrocarbon contaminated soil. Root exudates were collected from *Elymus angustus* and *Medicago sativa* grown in PAH-contaminated (400 ppm pyrene/phenanthrene) or non-contaminated sand. Mineralization assays with ¹⁴C-labeled hydrocarbons were used to determine the impact of exudates on hydrocarbon degradation in soil microcosms. Microbial taxonomic diversity in the microcosms was assessed by DGGE and functional structure was assessed by quantitative PCR of genes associated with hydrocarbon degradation (*nah*, *xyIE*). Mineralization of all hydrocarbons was initially repressed in root exudate amended microcosms. Although cumulative mineralization in many treatments reached parity by the end of the study period, the initial and variable impacts offered insights into the underlying mechanisms of plant-microbe interactions in these systems. Our results suggest that the degradation potential of this soil is directly influenced by the pattern of exudates released by a given plant. Relative concentrations of specific components within the exudates were found to be associated with either increased (acetate, alanine) or decreased (malonate) degradation potential. The success of a given phytoremediation treatment is likely linked to the specific balance of these and similar compounds within root exudates. An appropriate balance may be required such that the stimulation and transfer of catabolic competency can occur without a concomitant reduction in degradation potential due to catabolite or general metabolic inhibition.

Abstract Category

19 Plant-Microbe Interactions

PS.19.083 CATECHIN, A ROOT EXUDATE OF CENTAUREA MACULOSA (SPOTTED Knapweed) INTERFERES WITH CELL GROWTH, SPORULATION, AND GERMINATION IN

GRAM-POSITIVE BACTERIA

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Understanding the effects of root exudates on plants and soil-borne bacteria can further our understanding of what constitutes allelopathy. *Centaurea maculosa* has been an invasive species in the U.S. for over 100 years and part of its invasive success is attributed to its ability to excrete the polyphenolic catechin from its roots into the rhizosphere. Recent research has demonstrated bacteriostatic inhibition of growth by catechin on total soil bacterial communities and certain individual bacterial populations from a variety of locations. It has also been shown that pyruvate or malate restored growth to catechin-inhibited bacteria. The current study further explores specific mechanisms of catechin toxicity on Gram-positive endospore-forming bacteria. While testing Gram-positive isolates that are resistant to the bacteriostatic effects of catechin, we discovered that catechin inhibited sporulation and endospore germination of a soil *Bacillus* strain. Additional analyses demonstrated similar effects on two other endospore-forming *Bacillus* strains and a *Paenibacillus* strain. Catechin also altered cell growth patterns in these resistant strains, resulting in long chains of cells; a phenomenon also exhibited by cells grown in metal-deficient media (without catechin). The effects of catechin on cell growth, sporulation, and germination are attributed to its ability to sequester various metals necessary for these cellular processes. Catechin inhibition of sporulation was mitigated, but not completely, when media was amended with either pyruvate or malate, although bacterial growth was still filamentous under these conditions. Interestingly, spore germination was also observed with pyruvate when catechin was present, but not with malate. These findings suggest that catechin exhibits differential effects on bacterial populations in soil and may thus perturb the composition of soil bacterial communities, potentially facilitating the invasive potential of *C. maculosa*.

Abstract Category

19 Plant-Microbe Interactions

PS.19.084 BACTERIAL AUXIN PRODUCTION FACILITATES RESOURCE CONVERSION AND CARBON ACQUISITION IN THE PHYLLOSHERE

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Like many plant-associated bacteria, the phyllosphere epiphyte *Pantoea agglomerans* 299R synthesizes indole-3-acetic acid (IAA), an important plant hormone. IAA production was previously shown to confer a small but significant fitness advantage to 299R cells inoculated onto bean leaves; the mechanism by which bacterial IAA conferred this advantage was unknown, but it was hypothesized that bacterial IAA might relieve carbon limitation of bacterial growth on the leaf surface. To address this hypothesis, we used sugar-inducible bacterial biosensors to probe how IAA affects phyllosphere sugar availability. Here, we suggest that bacterial IAA promotes the conversion of sucrose, a common leaf surface disaccharide, to the monosaccharides fructose and glucose. Although 299R can use sucrose as a carbon source, it grows more efficiently *in vitro* on glucose and fructose, particularly at concentrations that would be available on the leaf surface (less than 1 mg/L). Thus, in an oligotrophic phyllosphere environment, bacterial IAA could enhance fitness by converting a less accessible form of carbon (sucrose) into more accessible forms (glucose and fructose), thereby facilitating nutrient acquisition and growth.

Abstract Category

19 Plant-Microbe Interactions

PS.19.085 SUGARCANE GROWTH PROMOTION AND CROSS-COLONIZATION BY ENDOPHYTIC BACTERIUM *PANTOEA AGGLOMERANS* 33.1

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Some micro-organisms, although found in nature as plant-pathogens, are frequently used as inoculants in agriculture. Among these micro-organisms, the endophytes have been described as a group of micro-organisms with a huge potential for application due to the fact that they are able to interact with the host acting majority as plant-growth promoters or as biocontrol agents. *Pantoea agglomerans* 33.1 strain is an endophytic bacterium isolated from *Eucalyptus grandis* and it has been described as a plant-growth promoter in this culture. Thus, the aim of this work was to explore the potential of the endophytic bacteria *P. agglomerans* 33.1 in the promotion of sugarcane growth as well as its physiological characterization. We also evaluated its capacity to cross-colonize sugarcane. 33.1 showed the potential to promote sugarcane growth and plant-protection by the increase of dried mass, and an increase in resistance-protein production of chitinases and cellulases, of inoculated plants in greenhouse assays. Biochemistry assays proved that 33.1 produces high amounts of indol-acetic-acid and different kind of phosphatases. These should be the major mechanisms involved in sugarcane growth promotion. The biological nitrogen fixing was very low. An integrative plasmid, pNKGFP, was constructed and used to transform 33.1. The marked strain was monitored during sugarcane association. The sugarcane cross-colonization by 33.1:pNKGFP was confirmed by microscopy, re-isolation and quantitative PCR (qPCR). The re-isolation and qPCR results were similar. Our results demonstrate that the sugarcane-33.1 interaction has important benefits in promoting the growth of the plant and its fitness through resistance-protein production and this bacterium is able to cross-colonize sugarcane, suggesting that 33.1 should be used in association with other crops.

Abstract Category
19 Plant-Microbe Interactions

PS.19.086 BIODIVERSITY OF ENDOPHYTES FUNGI ISOLATED FROM A MEDICINAL ENDEMIC PLANT (DENDROPANAX ARBOREUS) IN A MEXICAN FOREST RESERVE

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BACKGROUND AND AIMS: *Dendropanax arboreus* is an endemic medicine plant used for relief the fever, snake bites and stomach disorders. The leaf extract has shown cytotoxic activity. Others antimicrobial properties of this plant from an acetonic extract were antifungal and antiparasitic against dermatophytes and *Entamoeba histolytica* respectively. Nevertheless just phytochemical studies about this plant is available and there is not a report in biodiversity and if the endophytes microorganism are responsible about the therapeutic effects. Fungal endophytes are ubiquitous fungi that inhabit healthy plant tissues without causing diseases. The goal in this work was the isolation and characterization of the endophytes fungi from *Dendropanax arboreus* an important medicinal plant in endemic zones of Mexico METHODS: The samples were collected from "LA RESERVA DE LA BIOSFERA EL CIELO" Tamaulipas Mexico. The leaves, roots and stems (total of 30 samples) were obtained from 10 trees. Each part of the plant was rinsed with sterile water twice. Surface sterilization was done by immersing the samples in 96% alcohol, sodium hipoclorite 1%, and finally rinsed with sterile water. Then, the parts of the plant were cut aseptically in small fragments and placed on a PDA plates supplemented with 0.2 g.l-1 chloramphenicol and incubated to room temperature (25°C) until growth was observed. The taxonomic identification of the fungus, was made on macroscopic and microscopic features. The identification was confirmed by the molecular phylogeny with the amplification fragment ITS1-ITS4. RESULTS AND CONCLUSIONS: The preliminary fungus identification showed the genus: *Phomopsis*, *Diaphorthe*, *Fusarium*, *Alternaria*, *Encomelaconiopsis*, *Pestalotiopsis*, *Acremonium* and *Phyllosticta*. These genus have been reported commonly in other tropical plants, however, further works are necessary to elucidate whether these fungus have a relationship between the plant and their antimicrobial features.

Abstract Category
19 Plant-Microbe Interactions

PS.19.087 BACTERIAL GROWTH IN THE PLANT APOPLAST IS LIMITED BY NUTRIENT AVAILABILITY

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The presence of a functional type-III secretion system (TTSS) is known to be a requirement for pathogenic growth inside plant tissues, since both saprophytes and *hrp* (type-III secretion defective) mutants of phytopathogenic bacteria exhibit little growth in the apoplastic environment. However, we have observed high population levels of saprophytes and *hrp* mutants when they are co-infiltrated into plants with a variety of carbon sources. The increase is also dose-dependent. Metabolizable carbon-sources such as succinate and sugars can induce very high population levels of saprophytes and *hrp* mutants that are comparable to wild type *Pseudomonas syringae* pv. *syringae* B728a in compatible bean plants. This effect is not seen with non-metabolizable nutrients. Moreover, this major population increase is also evident when a *hrp* mutant of a normally incompatible pathogen such as *P. syringae* pv. *tomato* DC3000 (a pathogen of tomato and *Arabidopsis*) is infiltrated in bean leaves. Similarly high bacterial levels were found when water congestion in the apoplast was maintained after inoculation, even without nutrient addition; suggesting that inaccessible nutrients in the apoplast are made more available to bacteria by introduction of water to the intercellular spaces. This effect is transient and no population increase is seen in plants in which the introduced water is allowed to dry. Endophytic growth in leaves is thus limited by availability of apoplastic nutrients and apparently is not due to induced host defensive mechanisms. TTSS-proficient pathogens can overcome this limitation with effectors that release water and/or nutrients into the apoplast.

Abstract Category
19 Plant-Microbe Interactions

PS.19.088 13C-DNA-BASED STABLE ISOTOPE PROBING ENABLES THE IDENTIFICATION OF ACTIVE BACTERIAL SHOOT-ASSOCIATED ENDOPHYTES IN POTATOES

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Most bacterial endophytes, which colonize the intercellular space and vascular tissues of plants, are heterotrophic and thus depend on plant-assimilated photosynthetates as growth substrates to sustain their metabolism. However, as the evidence for the actual dependence of endophytes on photosynthetates and the linkage to their community structure is still lacking, we performed a ¹³CO₂ (99 atom-%, 350 ppm) incubation experiment to identify active bacterial endophytes in two cultivars of *Solanum tuberosum* L., Desire and Merkur. A specific objective was to verify if DNA-SIP is applicable to identify shoot-associated endophytes that directly utilize photosynthetates. Our results revealed that ¹³CO₂ assimilation and photosynthetic transformation into ¹³C labeled substrates by the plant was successful, and that active heterotrophic endophytes consumed these labeled metabolites as determined by DNA-SIP. Density-resolved DNA fractions obtained from SIP were further subjected to 16S rRNA gene-based T-RFLP analysis and sequence analysis of generated gene libraries. Community profiling revealed compositions dominated by plant chloroplast and mitochondrial 16S rRNA genes for the 'light' fractions of both ¹³CO₂-incubated and non-incubated potato cultivars. In the 'heavy' fractions of the ¹³CO₂-incubated endophyte DNA, especially a bacterial T-RF became abundant, which could be clearly identified as *Acinetobacter* and *Acidovorax* spp., both known as common potato endophytes with reported plant beneficial activities, in Merkur and Desire cultivars, respectively, indicating cultivar-dependent distinctions in ¹³C-label flow. The approach demonstrated the successful detection of active bacterial endophytes in potato. DNA-SIP therefore offers new opportunities in exploring the complex nature of plant-microbe interactions and plant-dependent microbial metabolisms within the endosphere.

Abstract Category
19 Plant-Microbe Interactions

PS.19.089 MOLECULAR DIVERSITY OF DIAZOTROPHIC BACTERIA ASSOCIATED WITH CHRYSOPOGON ZIZANIODES (VETIVER), AN ESSENTIAL OIL PRODUCER PLANT

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Chrysopogon zizanioides, known as vetiver, is a perennial grass (Poacea) with a great economic and ecological importance because of its ability to produce essential oil in the roots. The essential oil is used for medicinal purpose and as basic material for perfumery and cosmetics, and its production can vary in amount, composition and quality depending on the plant genotype and the bacterial community associated with vetiver roots. Some of these bacteria can also promote plant growth by fixing nitrogen. To learn more about the bacterial community, as less is known about it, the aim of the present study was to assess the molecular diversity of nitrogen-fixing bacteria associated with three vetiver genotypes. Cloning and sequencing of *nifH* PCR fragments and DGGE were used for this purpose. DGGE profiles obtained from soil, rhizosphere and root DNA amplified with *nifH* primers showed that they could be separated into three groups, and samples from rhizosphere and root were separated at 48% similarity. Ten bands, observed exclusively in root or in rhizosphere samples, were sequenced and showed high similarity with *nifH* sequences of *Bradyrhizobium* sp., *Enterobacter* sp. and *Xanthobacter* sp. Moreover, three clone libraries were generated (269 *nifH* sequences) from root DNA samples obtained from genotypes VET001, VET003 and VET004. In VET001, 24.2% (from 95 clones) were affiliated with sequences of *Mesorhizobium loti*, 18.9% with *Klebsiella pneumoniae* and 17.9% with *Bradyrhizobium* sp. In VET003, from 89 clones, 41.5% were affiliated with *Sphingomonas azotifigens*, 15.7% with *Mesorhizobium loti* and 14.6% with *Pleomorphomonas oryzae*. In VET004, from 85 clones, 36.4% were affiliated with *Klebsiella pneumoniae*, 16.4% with *Bradyrhizobium* sp. and 8.23% with *Mesorhizobium loti*. All these sequences have been previously associated with nitrogen-fixing bacterial strains isolated from different leguminous and non-leguminous plants, contributing to plant growth promotion.

Abstract Category

19 Plant-Microbe Interactions

PS.19.090 DIVERSITY ANALYSIS OF THE BACTERIAL COMMUNITY PRESENT IN THE RHIZOSPHERE AND LATEX OF *HANCONIA SPECIOSA* (MANGABEIRA)

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Hanconia speciosa, known as mangabeira, is a plant used for medicinal purpose, whose fruit is much consumed in the Northeast of Brazil. The latex extracted from all parts of the plant is used in folk medicine to treat sexually transmitted infections, wounds, warts and tuberculosis. This latex is known to present antimicrobial activity, which may be assigned to microorganisms found in the inner tissues of this plant. Given the medicinal importance of mangabeira and the lack of knowledge about the bacterial community present in the latex and rhizosphere of this plant, the aims of this study were: i) isolate bacterial strains with potential to produce antimicrobial substances against human pathogens from the latex of three mangabeira plants, ii) assess the molecular diversity of bacteria in the latex and rhizosphere by PCR-denaturing gradient gel electrophoresis (DGGE). Twelve strains were isolated from latex in Triptycase Soy Agar and purple medium. These strains were tested for the production of antimicrobial substances against *Staphylococcus aureus*, *Acinetobacter* sp., *Escherichia coli*, *Enterococcus faecalis*, *Candida albicans* and dermatophytes. However, no antimicrobial activity was observed in the tested conditions. Genotypic characterization of the isolates was performed using amplified 16S rDNA restriction analysis, and they could be separated into four groups. Moreover, to assess the molecular diversity, the DNA from latex and rhizosphere was extracted with *FastDNA Spin Kit for Soil* BIO101, amplified with 16S rRNA universal primers and submitted to DGGE. Profiles from rhizosphere showed that the bacterial community did not vary among the three mangabeira plants, while in the latex community a high number of bands were observed, suggesting a great molecular diversity. This study is relevant since it was the first time that DNA has been extracted from *Hanconia speciosa* latex and used to study the bacterial community associated with this plant

Abstract Category
19 Plant-Microbe Interactions

PS.19.091 MOLECULAR ANALYSIS OF THE BACTERIAL COMMUNITY AND THE NITROGEN-FIXING POPULATION ASSOCIATED WITH *CHRYSOPOGON ZIZANIOIDES* (VETIVER) RAROS VARIET

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Chrysopogon zizanioides, known as vetiver, is a perennial grass (Poaceae), considered a medicinal plant with aromatic properties. It is easily adaptable to climate changes, presents a rapid growth and physically resistant roots. For these reasons, vetiver has a commercial importance and can be used: (i) in the perfumery industries and also for therapeutic purpose; (ii) to avoid soil erosion; (iii) as a natural pesticide and (iv) to remediate soils contaminated with heavy metals. The composition and quality of the essential oil produced by vetiver may vary according to its associated bacterial community. These bacteria can also improve plant growth by providing nutrients, through biological nitrogen fixation. To increase our knowledge concerning the root and rhizosphere bacterial communities associated with vetiver, RAROS variety, cultivated in Brazil, the present study aimed to analyze the diversity of bacterial community and the nitrogen-fixing population after 3, 6 and 12 months post plant sowing. Molecular methods (PCR-Denaturing gradient gel electrophoresis) were used to determine any temporal or spatial (root and rhizosphere) changes that might occur. DNA extracted from root and rhizosphere samples were amplified using universal primers for 16S rRNA and *nifH* genes, and submitted to denaturing gradient gel electrophoresis (DGGE). The generated profiles showed that the total bacterial community based on 16S rRNA gene varied according to the locality (rhizosphere and roots), but only the root samples showed differences during the growth period. The *nifH* gene-based analysis showed a greater number of bands in the rhizosphere than in roots. Again, only the root samples showed differences in the DGGE profiles during the growth period. Changes in root profiles in both analyses suggest that the bacterial community and the nitrogen-fixing population are affected by the increase of oil production during plant growth.

Abstract Category
19 Plant-Microbe Interactions

PS.19.092 MUTATION OF A VFR HOMOLOGUE DECREASES SEED AND ROOT COLONIZATION BY *PSEUDOMONAS FLUORESCENS* PF-5

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We have been using a mutational approach to determine how plant-beneficial bacteria, such as *Pseudomonas fluorescens* Pf-5, colonize subterranean plant parts and suppress plant disease. Vfr, a cyclic-AMP binding protein in *Pseudomonas aeruginosa*, has been shown to be involved in regulation of traits potentially important to these plant-associated activities including: twitching motility, biofilm production, flagellar biosynthesis, and production of the stationary-phase sigma factor RpoS. Mutant Vfr-B6, a derivative of Pf-5 containing a knock-out mutation in *vfr*, was constructed to test the role of Vfr in colonization and disease suppression by *P. fluorescens*. Vfr-B6 was diminished in colonization of cucumber and pea seeds and in long-term colonization of cucumber roots in potting mix when strains were applied individually to seeds. *P. fluorescens* Vfr-B6 was essentially similar to Pf-5 in suppression of damping-off of cucumber caused by *Pythium ultimum* when applied as a seed treatment in growth chamber experiments. Vfr-B6 was also reduced in biofilm formation in microtiter dish assays and in growth on 0.5 % synthetic cucumber root exudate relative to Pf-5. Synthetic cucumber root exudate consists of a complex mixture of reduced carbon compounds supplied in the same relative proportions as detected in cucumber root exudate. These studies suggest a major role for the global regulator Vfr in colonization of seeds and roots by *P. fluorescens*.

Abstract Category
19 Plant-Microbe Interactions

PS.19.093 ACIDOVORAX SP. N35, AN AHL-PRODUCING ROOT-ASSOCIATED BACTERIUM ABLE TO PERFORM PHENOTYPIC VARIATION

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Acidovorax sp. N35 is a *Betaproteobacterium*, isolated from surface sterilized wheat roots. This bacterium undergoes phenotypic variation from the original rough colony shape on agar plates and flock formation in liquid medium to smooth colonies on agar plates without flocculation in liquid medium (N35v). A plant growth promoting effect with barley was demonstrated using both phenotypic forms in green house experiments showing more pronounced effects with the original form N35. The original type of cells showed a dominant colonization behavior on roots when coinoculated with N35v. AHL quorum sensing molecules with the structure 3-hydroxy-decanoyl homoserine lactone were detected as the major autoinducer signaling substance using a fourier transformation ion cyclotron resonance mass spectrometer (FTICR/MS). To find out genetic variations between the two forms and in order to identify the AHL synthesis gene, the whole genomes of both phenotypes were sequenced with a 454 pyrosequencer (Roche). The same sequence stretch of 555 nucleotides encoding an AHL synthase protein was identified in both phenotypic variants. Targeted mutagenesis yielded knock-out mutants of the AHL-synthase of N35, which were unable to flocculate and undergo phenotypic variation. In addition, several AHL-regulated properties, such as siderophore and lipase production were lost in these mutants. After analysis of the whole genome data, only very few differences between wild-type and variant could be detected. Interestingly, a 16 nucleotides deletion region was identified in the N35v genome compared to N35. This region shows high sequence similarity to the mismatch repair protein MutL. This protein is truncated in N35v and thus MutL might be directly or indirectly responsible for the differences in gene expression between the two variants.

Abstract Category
19 Plant-Microbe Interactions

PS.19.094 PLANT-MICROBE INTERACTIONS: SYNERGISTIC EFFECTS OF BACTERIAL ENDOPHYTES PROMOTING PLANT INVASIONS

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Invasive plants can profoundly alter ecosystem processes. Plant traits such as high growth rates and biomass, resource uptake, and novel biochemical compounds can affect ecosystem processes and have been documented for many invasive species. While these characteristics are considered plant-regulated, our work with the invasive grass *Sorghum halepense* shows microbial symbionts significantly contribute to many of these traits. Using culture and molecular approaches, we found the plant harbors several bacterial endophytes within its rhizomes including N₂-fixers, iron siderophore producers, phosphate solubilizers, and organisms capable of producing plant-growth hormones. In combination with these microbial partners, invasive *Sorghum* creates a soil environment with increased plant-available forms of nitrogen, phosphorus and potassium, in addition to altering several other soil biogeochemical cycles. Using a novel antibiotic-based approach, we manipulated the bacterial endophytes within the rhizomes and found these symbionts significantly increased plant biomass and altered resource allocation supporting more rhizomatous growth. Plants with endophytes had increased production of allelochemicals and significantly inhibited growth of *Schizachyrium scoparium*, a native prairie grass displaced by *Sorghum*. When endophyte infections were restricted, the competitive effects of *Sorghum* on *S. scoparium* were reduced or altogether inhibited. Plants with restricted endophytes experienced more insect herbivory and decreased dhurrin production, the defense compound found in leaves. These results suggest microbial interactions significantly contribute to *Sorghum* invasion by promoting invasive characteristics of the grass, including growth rate, allelochemical production and plant defense

compounds. Harboring bacterial endophytes might be a mechanism contributing to the profound alterations to ecosystem processes observed in many plant invasions.

Abstract Category
19 Plant-Microbe Interactions

PS.19.095 THE HALOPHYTE SALICORNIA BIGELOVII AND THE INNOCULATION WITH PLANT GROWTH PROMOTING BACTERIA: A MODEL OF INTERACTION OF PLANT-MICROORGANISM IN DESERTS

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The halophyte *Salicornia bigelovii* is vegetal with high potential to be improved in the deserts and sea coasts of the northwest in Mexico; however, its productivity depends of the nitrogen apportionment, and others macro and micro nutrients essentials. Nitrogen-fixation by bacteria associated to the roots of *Salicornia bigelovii* and similar halophytes is an important source of available nitrogen in salt marsh ecosystems. However, the diversity of *Salicornia*'s rhizosphere is unknown. Areas in the volcanic zone of Sonora Desert and around the Bay of La Paz in Baja California Sur, Mexico were sampled to detect nitrogen-fixing bacteria associated to this halophyte: where 38 colonies were isolated. Two isolated microorganisms showed high acetylene reduction activity. The bacterium was identified as a *Bacillus amilolyquefaciens* and *Klebsiella pneumoniae*. Those bacteria, in conjunction with *Azospirillum halopraeferens*, were tested for growth-promoting ability when inoculated on *S. bigelovii* genotypes under several saline concentration conditions. During germination and early seedling growth, *K. pneumoniae* and *Bacillus amilolyquefaciens* showed high specificity. Also, the growth and development of the same two genotypes of *S. bigelovii*, were evaluated under field conditions. *Bacillus amilolyquefaciens*, *Klebsiella pneumoniae* and *Azospirillum halopraeferens* increased some growth and development parameters, using measures of weight, length of plants, and biochemical characteristics, including total protein, ash, and total lipid content in selected plant parts. The findings suggest that yields of both genotypes of *S. bigelovii*, under field conditions, can be improved by the application of *Bacillus amilolyquefaciens*, *K. pneumoniae* or *A. halopraeferens* strains. This is the first report of *Bacillus amilolyquefaciens* as nitrogen-fixing bacterium associated to the halophyte *Salicornia bigelovii*.

Abstract Category
19 Plant-Microbe Interactions

PS.19.096 DIVERSITY OF RHIZOSPHERIC MICROBIAL COMMUNITIES OF TWO SALINE TOLERANT CHENOPODIACEAE PLANTS, FROM ARID NORTH AMERICA

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Saline environments can be found on all continents and in most countries. They consist in those that arose from seawater and those which come from nonseawater sources. The latter contain different ion ratios where the dominant anion is carbonate. Plants native to saline habitats are exposed to various levels of moisture and salinity stress during their life cycle. We analyzed both, soil and rhizospheric microbial communities, in plants from these environments. One of the genus *Suaeda* from central Mexico and other which belongs to the genus *Atriplex* from Chihuahua desert in Nuevo Mexico, USA.

In order to analyze quantitatively the microbial community rhizospheric soil and bulk soil samples were processed and analyzed by traditional surface spread plating methods. The samples were plated in at least sixteen different culture media: modified R2A; Mcasamino acids; BHAP; PDA; TYA and YCED. Each medium contained either 4% or 10% solar salt (w/v), and its pH was adjusted to

either 7.0 or 9.3. Molecular analysis was also performed. Total DNA was extracted in samples of rhizospheric soil. 16S rDNA fragments amplified by PCR, were analyzed by denaturing gradient gel electrophoresis (DGGE).

Plate count methods show a high concentration of free living bacteria, 5.9×10^8 CFU/g and fungi, 2.4×10^4 CFU/g. In bulk soil bacterial counts were 3.0×10^8 CFU/g, whereas fungal counts were only 1×10^3 . Difference was found in media formulations due to high salt concentration or high pH. Medium R2A allowed the highest cultivable microbial diversity. DNA was extracted from four selected soil samples and PCR amplified with specific 16S rDNA gene primers for both bacteria and actinomycetes. The products were analyzed under different DGGE conditions. Clear differences in microbial diversity are found in rhizosphere vs bulk soil communities, as well as in cultivable vs non cultivable communities. Implications of these results in soil management and restoration are discussed.

Abstract Category
19 Plant-Microbe Interactions

PS.19.097 THE STRUCTURE OF ACTINOBACTERIAL COMMUNITIES IS SITE SPECIFIC AND RESPONDS TO THE PRESENCE OF THE POTATO PATHOGEN STREPTOMYCES SCABIEI

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Soils known for suppressivity to plant diseases represent a natural model of proper soil functioning. The understanding of the processes involved may bring new methods of crop protection. Screening: sites differing in the incidence of common potato scab were selected in the Czech Republic. Some sites have been known for high or strongly suppressed disease level. Soil from the tuberosphere of potato plants and those of bulk soil were collected in four replicates. Actinobacterial communities were assessed by terminal restriction fragment length polymorphism analysis of the 16S rRNA gene; the pathogen *S. scabiei* was quantified by real time PCR of the virulence genes. Field experiment: two sites characterized by high and low incidence of the disease were planted with a sensitive and a resistant potato variety. Bacterial communities in tuberospheres were studied using a 16S rRNA based microarray of 1400 probes targeting taxa known for secondary metabolite production and correlated to soil and peel chemical composition. Actinobacterial communities were highly site specific and soil suppressiveness was always typical by other dominant taxa. However, the community structure was related to the presence of the pathogen i.e. incidence of the disease. Peel chemical composition corresponded with the variety resistance. Suppressiveness appears specific to a particular crop and site. Yet, producers of secondary metabolites seem to participate in soil suppression of common potato scab similarly as e.g. pseudomonads of tobacco black root rot.

Abstract Category
19 Plant-Microbe Interactions

PS.19.098 MANIPULATION OF MICROBIAL BIOMASS IN ORGANIC GROWING MEDIA TO CONTROL NUTRIENT MINERALIZATION

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The growing medium of a plant is the most important factor in a plant's life and soil microbes play vital roles in nutrient mineralization. However, due to the chemical and physical stability of organic matter, microbes can metabolize it only very slowly. It has been reported that minute quantities of glucose, vitamins, amino acids and root extracts (trigger molecules) excite the microbial biomass and improve nutrient mineralization. The overall aim of this study was to determine whether or not the microbial biomass of an organic growing media could be manipulated by the addition of 'trigger molecules' in order to optimise nutrient mineralisation. Greenhouse and laboratory experiments were carried out and the nutrient status of the medium was manipulated by adding 'trigger molecules' in the form of cellulose and amino acid. The total nitrogen (Kjeldahl Digestion method), extractable mineral nitrogen (Aquatec Auto Analyser), microbial biomass carbon (fumigation-incubation method by Jenkinson and Powlson, 1976) and microbial biomass nitrogen (Ninhydrin-

reactive method by Brookes et al, 1985) from the soil and plant were measured. Greenhouse study showed a direct correlation between the amounts of carbon as cellulose added per pot and the dry weight of plants, and also the total nitrogen uptake by a grass crop, while nitrogen addition as casein hydrolysate showed more shoot dry weight and subsequently more total nitrogen uptake by the shoot compared with the control. The laboratory experiment showed that with carbon addition, the biomass nitrogen, the ammonium nitrogen level and biomass carbon increased while biomass carbon and biomass nitrogen increased with nitrogen addition. The study concludes that the nutritional status of the medium is directly proportional to the microbial biomass in a growing medium and that manipulating the biomass in order to optimise the nutrient release for plant growth is possible.

Abstract Category
19 Plant-Microbe Interactions

PS.19.099 COMPARATIVE METAPROTEOMICS APPLIED TO PHYLLOSHERE OF TREE SPECIES FROM ATLANTIC FOREST

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The leaf surfaces of trees contain dense, complex and diverse microbial communities that are highly specific to their plant hosts. Genomic approaches have revealed the microbial species composition of communities for a few representative plants, but do not provide direct information on the functional genes that are expressed by phyllosphere microorganisms. To gain insight into this question, we performed a culture-independent analysis of the microbiota associated with leaves of four tree species from the Atlantic Forest. Different cell recovery and purification methods were tested for protein extraction and clean-up prior to 2-DE analysis. A protocol has been optimized, allowing high resolution separation of more than 500 discrete proteins. For each sample, highly reproducible proteomic maps were obtained for replicate samples generated from different individuals of the same tree species, with 86-99 % matching of protein spots. However, comparisons of microbial metaproteomes generated for different tree species showed that there was very little match of the microbial community proteomes for trees from the same genus or among different tree families. The metaproteome maps from the phyllosphere of trees from the same genus (*O. dispersa* and *O. teleiandra*) shared ~16% (154) of all detected spots; whereas metaproteomes of trees from the same order, *M. shottiana* and *O. teleiandra*, shared ~14% (103) of detected spots. All other comparisons showed less than 8% similarity among metaproteome profiles. The metaproteome comparisons are in agreement with the high endemicity of bacterial communities in which different phyllospheres shared less than 1% of bacterial species. These novel findings are now being further examined using protein iTRAQ and DIGE tools to confirm our preliminary results. The application of metaproteomics provides a valuable new tool to identify functional genes and pathways that are unique to the phyllosphere. Acknowledgements: CNPq and FAPESP

Abstract Category
19 Plant-Microbe Interactions

PS.19.100 PLANT MICROBE INTERACTIONS: A COMPARISON OF MICROBIAL RHIZOSPHERIC AND ENDOPHYTIC POPULATIONS OF POPULUS DELTOIDES IN LOWLAND AND UPLAND SOIL

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Populus roots host endophytes, mycorrhizae, and other diverse rhizosphere organisms and present ideal systems for understanding plant-microbe interactions. Here we describe the bacterial and fungal associations of the roots and rhizosphere of *P. deltoides* in lowland and upland soils in Tennessee. Bacterial community analysis targeted the 16S rRNA gene (V4), and fungal analysis the 28S rRNA (D1), using 454 pyrosequencing. Bacterial sequences were classified using the Ribosomal Database Project (RDP) and fungal sequences using the SILVA LSU database. Replicated rhizosphere samples showed much higher operational taxonomic unit (OTU) diversity than

endophyte samples, regardless of site, but endophyte samples showed greater variability from sample to sample. Rhizosphere bacterial samples were dominated by *Acidobacteria*, *Verrucomicrobia*, and *Alpharroteobacteria*, whereas endophyte samples were dominated by *Alpha-* and *Gammaproteobacteria*. Fungal rhizosphere and endophyte samples were dominated by *Ascomycetes*, rather than the *Basidiomycetes* typically more common in ectomycorrhizal systems. Variation in soil properties (%OM, pH, and Mn and Zn concentration) correlated with abundance of rarer members of the rhizosphere, such as *Chloroflexi* and *Verrucomicrobia*. Using hierarchical clustering of phyla relative abundance, some clustering by site and by tree of origin was observable in both rhizosphere and endophyte samples, however overall branch lengths indicate the extent of divergence within trees, between trees and between sites to be moderate to low. There appears to be little variation in dominant phyla within rhizosphere and within endophyte habitats between the two soils examined, indicating tree species or genotype may be a primary driver of microbial composition, rather than soil properties. However, even at the broad taxonomic levels analyzed, rhizosphere and endophyte communities are distinct, suggesting roots tissues represent a unique niche within the overall rhizosphere.

Abstract Category

19 Plant-Microbe Interactions

PS.19.101 SHIFTS IN FUNGAL AND BACTERIAL COMMUNITIES IN GRASSLAND TURFS SUGGEST THAT FUNGI CONTRIBUTE TO MICROBIAL AVAILABILITY OF SULFONATES

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Organically bound sulfur such as sulfate esters and sulfonates are the major sulfur (S) compounds in soil ecosystems where sulfate often represents less than 5% of the total S. As plants almost entirely depend on sulfate as S source, they require microbial S-supply. While some fungi are capable to desulfurize sulfate-esters the use of sulfonates as a S source seems to be limited to a defined functional bacterial guild. This study investigates whether fungi assists bacteria in sulfonate desulfurization.

Freshly sampled grassland turfs from Woburn Experimental Farm with dominance of *Agrostis sp.* plants were incubated in growth chambers and watered periodically with deionised water and 0.25 strength Hoagland solution with i) S free, ii) low molecular weight sulfonate (toluenesulfonate, TS) iii) high molecular weight sulfonate (lignosulfonate, LS) over a period of 8 weeks. The diversity of the bacterial community (16S rRNA gene), the fungal community (internal transcribed spacer, ITS) and the desulfonating bacterial community (*asfA* gene) was analyzed in *Agrostis* rhizospheres and bulk soil from the turfs, employing DNA fingerprinting methods.

Correspondence analysis of the bacterial and fungal communities and the desulfonating bacterial guild revealed a clear separation of the rhizosphere and the bulk soil samples with and without addition of sulfonates, respectively. Fungal communities were affected by the addition of sulfonates but no separation of the LS and TS treatment was possible while bacterial and desulfonating bacterial communities were affected differently by the LS and TS treatment.

The results suggest that the fungal community in *Agrostis sp.* rhizosphere is involved in the mobilization and the bio-availability of sulfonates in soil but functional bacterial guilds cleave the S group from sulfonates. Future studies will focus on how fungi support bacterial desulfonation of soil sulfonates.

Abstract Category

19 Plant-Microbe Interactions

PS.19.102 INTERACTION OF TOXIN PRODUCING CLOSTRIDIUM BOTULINUM STRAINS WITH CLOVER

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The rhizosphere of plants could act as a natural ecological niche for human pathogenic and/or toxin producing bacteria. Plants can be colonized on various surfaces epi- or endophytically by these bacteria. These microbes can be introduced into soils by e. g. agricultural practices like application of contaminated slurry or of products derived from organic waste fermentation plants and subsequently colonize the rhizosphere of crop plants. There is a high risk for systemic colonization if pathogenic bacteria exhibit the ability for endophytic colonisation of plants. In the following a transfer of pathogens via the food production chain and finally the infection of humans are possible. As an example for a toxin-producing human pathogenic bacterium we analyzed *Clostridium botulinum* concerning its colonization behaviour and efficiency on clover (*Trifolium repens*) and its plant growth promoting effect. We therefore inoculate spores of *C. botulinum* on surface sterilized clover seeds in a soil system. After growth in a phytochamber under controlled conditions we applied for *in situ* colonization studies fluorescence *in situ* hybridization with phylogenetic oligonucleotide probes (FISH) and confocal laser scanning microscopy (CLSM). Evidences of endophytic colonization were obtained which are currently extended applying targeted PCR assays for *C. botulinum* and for specific toxin-coding genes. In addition, we observed, that various *C. botulinum* strains have a plant growth beneficial potential as well. Possible mechanisms of plant growth promotion, like the production of phytohormones or volatile organic compounds (VOCs) are being studied.

Abstract Category
19 Plant-Microbe Interactions

PS.19.103 TRAITS CONTRIBUTING TO EPIPHYTIC AND ENDOPHYTIC GROWTH OF PSEUDOMONAS SYRINGAE REVEALED BY GLOBAL GENE EXPRESSION ANALYSIS

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Pseudomonas syringae is an important plant pathogen with a prominent epiphytic phase that serves as inoculum for subsequent infection. To obtain a comprehensive understanding of the traits contributing to its associations with the surface and interior of plants and their regulation, we are performing whole genome transcriptional profiling of *P. syringae* pv. *syringae* B728a, a pathogen of bean, using an ORF-based microarray. Gene expression of the wild-type strain and mutants of the global regulators *ahIR*, *aefR*, *gacA*, *salA*, *retS*, *rpoE*, *rpoN*, *rpoS*, and *hrpL* are being assessed on leaf surfaces and in the leaf apoplast as well as in culture in a basal medium and under conditions of low iron, nitrogen and water availability and high oxidative stress. Sufficient RNA could be collected from cells recovered from the surface and interior of 400 and 60 leaves, respectively, for analysis. The transcriptional responses of B728a to the leaf surface are distinct from its responses to the apoplast. For example, genes involved in flagellar motility, chemotaxis and phosphate transport were expressed more in epiphytic sites, whereas genes involved in syringomycin production, levan synthesis and sucrose catabolism, uptake of quaternary ammonium compounds, GABA degradation, tolerance to water stress and nitrogen limitation, and genes within two separate phage regions of unknown function were expressed more in endophytic sites. Conversely, genes upregulated *in vitro* under low iron conditions were not upregulated in either in planta condition indicating high iron availability. Additionally, both *ahIR* and *ahII* components of the *luxR* type quorum sensing system appear to be active in planta. Preliminary findings identify a quorum regulon *in vitro* and indicate that a smaller subset of this regulon is expressed at detectable levels both in and on leaves. The relevance of these transcriptional responses to the process of plant niche colonization is being investigated.

Abstract Category
19 Plant-Microbe Interactions

PS.19.104 GENOMIC EVIDENCE OF HOST-SPECIFIC CRYPTIC SPECIES IN TREE PATHOGEN *GROSMANNIA CLAVIGERA*: IS FUNGAL SPECIALIZATION LINKED TO TREES OR BEETLES?

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Grosmannia clavigera is an important tree pathogen and a symbiotic associate of two sister bark beetles: *Dendroctonus ponderosae* and *D. jeffreyi*. *D. ponderosae* (mountain pine beetle) is the most damaging beetle of pine forests in North America, leading to the loss of millions of trees per year. Earlier work using morphology and molecular approaches have suggested that natural populations of this fungus consist of one species. We used *G. clavigera* genome sequence and gene predictions supported by expressed sequence tags to assess whether 1) there are genetically isolated lineages within *G. clavigera* that have not been previously detected, and 2) there is evidence for host tree or beetle specialization that may have influenced the evolution of symbiosis in these lineages. First, we identified genetic variations in 76 protein-coding genes across a panel of 15 *G. clavigera* isolates. Then, we selected 16 genes that had various level of polymorphisms, and sequenced them across an additional 60 *G. clavigera* isolates from different host beetles and trees in Canada and the USA. We identified a total of 89 fixed polymorphisms distributed in 41 protein-coding genes, and infer that there are at least two independent evolutionary lineages of *G. clavigera*, which we resolved as two distinct phylogenetic species using genealogical concordance of the 16 genes. The small overall sequence differences imply that the two species diverged relatively recently. Deeper sampling of a single informative locus across 120 *G. clavigera* isolates indicates that one species is more specific to Jeffrey and Ponderosa pines isolated in the USA, while the other has a broader geographic and host distribution, occurring on different pine species in western Canada and the USA, but not on those infested by the former species. In general, host tree specialization appears more concordant with the fungal genealogy generated in this study than with its vector beetle species.

Abstract Category

19 Plant-Microbe Interactions

PS.19.105 AGRICULTURAL MANAGEMENT STRATEGIES PROMOTING SOIL MICROBIAL RESOURCES FOR IMPROVED GENERAL PLANT PATHOGEN SUPPRESSIVENESS

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Agriculture in boreal regions faces several environmental challenges. In the course of climate change the soil erosion is potentially increasing as a result of elevated winter precipitation and shorter snow cover period. Conservation tillage (stubble cultivation or zero tillage) and permanent crop cover (e.g. grass, crop rotation) practices have been reported to be advantageous in respect to erosion control in boreal areas. Over wintering crop residues however, increase fungal pathogen pressure in fields. The predicted climate change generates better growth conditions for fungal pathogens in the winter season. The aim of the project is to study agricultural management strategies employing sustainable ecosystem services. Continuous automatic measurement system and modeling were utilized to determine the soils-air interface conditions relevant to microbial populations. A collection of assays were set up to evaluate plant pathogen suppressiveness (*in vivo* plant biotests) and fungal fitness (spore germination and hyphae extension) in field soils managed with crop rotation and different tillage intensities. High fungistasis of *F. culmorum* was measured by hyphae extension and spore germination assay in clay soil. The biological nature of fungistasis was demonstrated by sterilization of the soils. PLFA and group specific molecular marker gene analysis is used to depict the microbial communities forming the bases for suppressiveness on the plots. The acquired biological data is combined with results of automatic continuous monitoring of the field site treatment plots: soil physical/chemical properties (CO₂, redox potential and electrical conductivity), soil structure and weather (soil surface temperature and relative humidity). Combining of up to date microbiological and agrotechnological analysis of agroecosystems and providing biological tools for fungal pathogen and soil erosion control the project will facilitate sustainability of future agriculture in climate change.

Abstract Category
19 Plant-Microbe Interactions

PS.19.106 COMPLEX INTERACTION OF BIOCONTROL STRAINS, *RHIZOCTONIA SOLANI* AND INDIGENOUS MICROBIAL COMMUNITY IN THE RHIZOSPHERE OF LETTUCE

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The control of the soil-borne plant pathogen *Rhizoctonia solani* is difficult because of its wide host range and its ability to survive as sclerotia under adverse environmental conditions. In the present study we aimed to explore the potentials to improve biocontrol efficiency by combining antagonists that supposedly colonize different niches in the rhizosphere and target *R. solani* via different mechanisms. *Trichoderma viride* strain GB7 and *Serratia plymuthica* strain 3Re4-18 were selected for growth chamber experiments. Different monitoring strategies were used to study the colonization patterns of the inoculants and to compare the effects of the inoculation of single and of the combined antagonists in the presence of *R. solani* AG1-IB on the composition of bacterial and fungal communities in the rhizosphere of lettuce. In addition, biocontrol efficiency was measured. At two time points during plant development lettuce plants were destructively sampled and 16S rRNA gene or ITS fragments were amplified from total community DNA and analyzed by denaturing gradient gel electrophoresis. The study showed that the applied 16S or ITS-based fingerprinting methods provided insights into the complex interaction in response to the pathogen presence and to biocontrol strain inoculation. A significantly improved biocontrol effect was observed for the combined application of *S. plymuthica* 3Re4-18 and *T. viride* strain GB7 but also a more pronounced effect on the bacterial and fungal community composition compared to the application of single inoculants.

Abstract Category
19 Plant-Microbe Interactions

PS.19.107 REVEGETATION OF BARREN LAKESIDE AREA BY PLANT GROWTH PROMOTING RHIZOBACTERIAL STRAIN OF *BACILLUS SUBTILIS*

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Bacillus subtilis RFO41 isolated from rhizosphere soil exhibits a high level of phosphate solubilizing activity and produces some phytohormones. Its promoting effect on the growth of *Xanthium italicum* Moore, a wild plant growing at lakeside barren land was evaluated in the in situ test for 19 weeks at Lake Paro, Kangwon-do, South Korea. When the strain RFO41 was applied on the soil surface at 1×10^6 cells g⁻¹ soil every week, it could promote the growth of germinated seedling of *X. italicum*, which may be a good candidate plant for revegetation of barren lakeside areas. The bacterial inoculation enhanced the dry weight of grown *X. italicum* plant by 67.7% in 19 weeks, and it also increased the shoot length of *X. italicum* plant by 21.1% compared to that of the uninoculated control. Both growth enhancements had the statistical significances. However, the bacterial inoculation did not show any effect on the root growth, which might be due to the breakage of the long and tiny roots of *X. italicum* plant. Denaturing gradient gel electrophoresis analysis showed that the inoculated bacteria were maintained in the soils, and the indigenous bacterial community did not exhibit any significant change by inoculation of *Bacillus subtilis* RFO41. This plant growth promoting capability may be utilized as an environment-friendly and low cost revegetation method, especially for the sensitive areas such as barren lakeside lands.

Abstract Category
19 Plant-Microbe Interactions

PS.19.108 GROWTH PROMOTION OF TOMATO PLANT BY TWO RHIZOBACTERIAL ISOLATES

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Plant growth may be enhanced by some bacteria which have a number of mechanisms of growth promotion such as phytohormone production, phosphate solubilization, inhibition of plant pathogen and others. Enterobacterial strains *Kluyvera intermedia* GBI and *Ewingella americana* GBL and actinomycete *Streptomyces mirabilis* strain GBQ were isolated from the rhizosphere soil collected from Mt. Gyebang, S. Korea. These bacteria could produce the plant growth hormones such as indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and gibberellin. They also inhibited growth of *Fusarium oxysporum* f. sp. *lycopersici* and *Fusarium oxysporum* f. sp. *niveum* which are important pathogenic fungi to many agricultural crops. *E. americana* strain GBL showed the highest production rates of phytohormones, including 1105.1 $\mu\text{g protein}^{-1}$ of IAA, 1313.9 $\mu\text{g protein}^{-1}$ of IBA and 799.3 mg l^{-1} of gibberellins (GA3). The lengths of shoot and root of germinated tomato seedlings in soil treated with strain GBL increased by 47.1 and 57.1%, respectively in 21 days of incubation compared to the uninoculated control. Dry weight of tomato plants grown was also higher in the inoculated soil by 60.8% compared to that in the uninoculated soil. These rhizobacterial strains may be utilized as environment-friendly biofertilizer in agriculture.

Abstract Category

19 Plant-Microbe Interactions

PS.19.109 EXPERIMENTAL MANIPULATION OF SOIL MOISTURE REGIME IMPACTS SOIL MICROBIAL COMMUNITY ABUNDANCE, DIVERSITY, AND FUNCTION IN A SEMI-ARID SAGEBRUSH STEPPE.

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Rising global temperatures are predicted to alter regional climate regimes, including the spatial and temporal distribution of precipitation. In water-limited (e.g. arid and semi-arid) ecosystems annual precipitation is low and shows a high degree of variability. In these environments, soil microbes occupy a pivotal seat coupling the effects of water availability and plant productivity to biogeochemical cycling and ecosystem function. Using a long running ecological field experiment (>15 years) sited in Idaho's sagebrush steppe, effects of experimentally manipulated soil moisture on microbial diversity, abundance, and terrestrial carbon cycling are being explored. Soils were sampled in January (Winter), April (Spring), July (Summer), and November (Fall) of 2009. Replicate cores collected at 15-20cm and 95-100cm, were taken from field plots planted with native vegetation under three different precipitation amendments; Ambient, Summer (additional 200mm in June), or Fall/Spring (additional 200mm in April or October). Bacterial and Fungal community structure was analyzed by T-RFLPs from all time points, 454 pyrosequencing was employed to assess Archaeal, Bacterial, and Fungal community diversity in Winter sampled soils. Principal Component Analysis indicates Fungal and Bacterial communities were structured by precipitation treatment and depth. Pyrosequencing analysis of soil Bacterial communities has revealed greater Bacterial community abundance and diversity across all treatments relative to Archaeal communities. Our results indicate that soil moisture is a powerful factor that can dictate soil microbial abundance and diversity. Studies are underway examining carbon resource allocation between plant-microbial communities in response to experimental soil moisture regimes. Understanding the consequences of altered soil moisture regimes will guide predictions of ecosystem function in arid and semi-arid environments, with potential feedbacks to a changing climate.

Abstract Category

19 Plant-Microbe Interactions

PS.19.110 TESTING THE GUILD-BASED DECOMPOSITION MODEL; ARABIDOPSIS THALIANA AS A MODEL SYSTEM

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Litter decay rates often correlate with the initial ratios of lignin:nitrogen (N) or lignin:cellulose in litter. Understanding the mechanisms that produce these patterns could improve current mechanistic models of litter decay. The aim of this study was to test hypothetical relationships between lignin, cellulose, and N during decomposition and use the results to test the Guild-based Decomposition Model (GDM), which models litter decay based on guilds of decomposer microbes that differ in their target C compounds and N demand.

To test lignin, cellulose, and N effects on decomposition, we used two mutants of *Arabidopsis thaliana* that were down-regulated in the biosynthesis of lignin or cellulose, and a wild type plant with low levels of tissue-N. Plants were decomposed for one year in an Alaskan boreal forest. GDM outputs were compared to the observed mass loss for each plant type.

Plants with low cellulose content and low N content decomposed slower than wild type control plants ($P = 0.0002$). For low cellulose plants, this pattern was driven by low lignin losses. Low N plants had lower total carbon and N losses compared to the wild type. GDM simulations did not match field observations of cellulose and N effects on decomposition rate, primarily due to an overestimation of labile C (non lignocellulosic) losses from litter ($P < 0.0001$). Simulations also underestimated lignin loss for low N plants and cellulose loss for all plant types.

Our results show that the cellulose and N content of litter control decay rates by acting as co-substrates for decomposer microbes that target lignin and labile C compounds, respectively. Deviations between model simulations and field data show that the GDM overestimates 1) labile C losses during decomposition and 2) the decay thresholds at which cellulose and lignin decomposition begins. Decomposition of *Arabidopsis* plants provides an opportunity to refine the GDM and increase its accuracy in predicting decomposition of natural litter.

Abstract Category
19 Plant-Microbe Interactions

PS.19.111 CAPSICUM ANNUM VAR AVICULARE WITH THE INTERACTION WITH PLANT GROWTH PROMOTING BACTERIA

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Capsicum annum var. *aviculare* known as chiltepin, is one of the hottest chilis. Although, the chiltepin plant produce a high amount of viable seed, the germination and establishment is really poor on the Tarahumara and Papago Indians area and farmers of Sonora desert, who are the main supplier of this product. The objective of the current experiment was to compare and evaluate the effects of *Azospirillum halopraeferens* (plant growth promoter microorganism) on chiltepin seed germination and seedling growth versus to traditional techniques as a control treatment. Wild chiltepin seeds were collected from the region of desert of Altar, Sonora. Five traditional processes were used as treatments control: seeds submerged in hot water at 70°C during 7, 14, 21 minutes, other was chiltepin thoroughly washed with water and detergent soap, and the fifth one was a group of seeds without any previous process. Each one of the treatments control were compared against the same, but the seeds were inoculated with *A. halopraeferens*. Seed inoculation was performed throughout a bacterial solution at 108 CFU.ml⁻¹ in an emptiness (vacuum) medium. Results on seed germination were 120 % in the treatment of washed seed plus seed inoculation with *A. halopraeferens* over its control. This treatment, also improved several plant growth parameters related to seedling survival (height plant, root length, fresh and dry weight in a 4, 40, 2 and 100%, respectively). This study adds further evidence that *A. halopraeferens* could be used to enhance seed germination and growth in nurseries.

Abstract Category
19 Plant-Microbe Interactions

PS.19.112 DEVELOPING BETTER AGRICULTURAL PRACTICES THROUGH UNDERSTANDING THE POPULATION DYNAMICS OF POTATO SCAB PATHOGENS AND THE

COMMUNITIES THEY INHABIT

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Microbial plant pathogens are influenced by the populations they inhabit. This is particularly true of pathogens occurring in the rhizosphere, where total microbial populations are particularly large, diverse and dynamic. Control of soil-inhabiting pathogens in crop plants is influenced by agricultural practices, and crop management regimes are constantly being developed and refined to achieve the best possible pathogen reduction with minimum input. For example, control of common scab disease of potato, caused by *Streptomyces* spp., is achieved largely by irrigating early in the season. In common with many disease management measures, the precise cause and effect relationship between management practice (in this case irrigation) and disease suppression or control is unknown. For example, differences in irrigation regime alone do not explain observed levels of potato scab disease. Other factors, including antagonistic micro-organisms which themselves respond differently to agricultural practices, are highly influential. We have been studying the effect of different irrigation regimes on levels of potato scab in the UK by measuring populations of pathogenic *Streptomyces* by quantitative real-time PCR as well as assessing disease levels. Our aim has been to determine the optimum time period in which populations of the pathogen can be influenced, thus achieving better targeting of control measures. These experiments have led us to define a window of control occurring for a short period following tuber initiation, and crop management recommendations based on these findings will result in considerable saving of irrigation water. Using 454 next-generation DNA sequencing of PCR amplicons from field samples under different irrigation regimes we have also identified microbial taxa which respond differentially to irrigation and which may exert a suppressive effect on the disease. We are currently investigating the disease-suppressive effects of these populations in the UK.

Abstract Category

19 Plant-Microbe Interactions

PS.19.113 INFLUENCE OF INOCULATED ARBUSCULAR MYCORRHIZAL FUNGI ON *PROSOPIS JULIFLORA* FOR THE REVEGETATION OF ACIDIC, METALLIFEROUS DESERT MINE TAILINGS

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Mine tailings are important sources of contamination in arid environments. Revegetation of tailings piles is necessary to reduce contaminants dispersion, but harsh conditions (e.g. low pH and nutrients content) in such sites prevent the occurrence of natural revegetation processes. In this study we examined the ability of a native plant, *Prosopis juliflora* (mesquite), to grow in acidic metalliferous mine tailings in association with arbuscular mycorrhizal fungi (AMF). Also, we investigated the rhizosphere microbial community structure of mesquite using denaturing gradient gel electrophoresis (DGGE) analysis of bacterial 16S rRNA and fungal 18S rRNA amplicons from community DNA extracts. The study was performed under greenhouse conditions using mine tailings from the Klondyke mill site (southwestern Arizona, US) amended with 10% compost (w/w). Mesquite seeds were inoculated with one of two commercial AMF or an AMF inoculum recovered from a mesquite rhizosphere, sown in the tailings and allowed to grow for two months. Rhizosphere samples were collected at the end of the study and DGGE profiles for bacteria and fungi were generated and analyzed *in-silico*. Results show that mesquite biomass production and root length were statistically higher (up to 76%) in AMF inoculated pots than in uninoculated pots. Further, the different AMF inocula seemed to significantly influence both the development of the rhizosphere fungal community structure and the rhizosphere bacterial community structure after two months. The changes we have observed in the rhizosphere microbial community structure may be either a direct effect of the AMF inocula, or caused by changes in plant physiology induced by AMF, or a combination of both of these mechanisms. Overall, AMF inoculation shows a good potential to support plant growth in acidic metalliferous mine tailings at reduced compost concentrations.

Abstract Category
19 Plant-Microbe Interactions

PS.19.114 CHARACTERIZATION OF A BACTERIAL CONSORTIUM WITH GROWTH PROMOTER ACTIVITY IN RICE PLANTS

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The activity of plant growth promoter bacteria has been potentiated through mixing bacterial strain with different capabilities. However, not always this approach has presented successful results. It is in part due to the lack of knowledge of the interactions bacteria-bacteria involved in the processes of plant promotion such as nitrogen fixation and auxin production. The aim of this research was to select bacterial consortia through continuous enrichment and to assess its capacity to fix nitrogen, produce indolacetic acid and promote growth of rice plants. The consortia were obtained from rhizospheric soil from 12 different rice farms. The sample was macerated and used as inoculums in a nitrogen free media and then 10 consecutive passes every 7 days were performed using same media. It was found that 58% and 50% of the consortia increased the nitrogenase activity and indolacetic acid production respectively through the passes. The F8 consortia promoted the growth of rice plants up to 72% and 57% of the dry weight of roots and shoot respectively in relation with noninoculated plants. The F8 consortium is formed by 5 different isolates. The indolacetic acid and nitrogenase activity was evaluated for each single isolate and for artificial groups of 3, 4 or whole set of bacteria, made by mixing them in similar proportions. In that context only one isolate presented nitrogenase activity (12.59 nmoles ethylene/hour), and such activity was not modified by the different evaluated groups. All single strains presented high production of indolacetic acid (52.43 - 78.33 µg/ml). However, out of 12 groups evaluated the indolacetic acid production was reduced in 11 groups to 32.15 µg/ml in average. In conclusion the continuous enrichments increase the possibility to isolate a bacterium or groups of bacteria with a higher nitrogenase activity and with the capacity to growth promotion of rice plants. This work was founded by the Agricultural Ministry of Colombia and Fedearroz.

Abstract Category
19 Plant-Microbe Interactions

PS.19.115 POSITIVE EFFECTS OF ORGANIC FARMING ON MUTUALISTIC FUNGI: LARGE SCALE COMPARISON OF MYCORRHIZAL FUNGAL COMMUNITIES IN AGRICULTURAL AND NATURAL SOILS

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Organic agricultural practices have been advocated for the maintenance and restoration of farmland biodiversity. The impact of organic farming on soil biodiversity, and in particular on arbuscular mycorrhizal fungi (AMF), is however still poorly understood. These fungi engage in symbiosis with the majority of land plants and are a key link between above- and belowground ecology. Diversity of AMF is thought to improve nutrient use efficiency, potentially negating negative effects of agriculture on the surrounding ecosystem. To assess this potential, the AMF community composition was assessed in 26 agricultural fields (13 pairs of organically and conventionally managed fields) and five semi-natural grasslands, all on sandy soil. AMF identities were assessed by T-RFLP targeting the 25S rRNA gene. The average number of AMF taxa recovered was highest in grasslands (8.8), intermediate in organically managed fields (6.4), and lowest in conventionally managed fields (3.9). Moreover, AMF richness increased significantly with the time since conversion to organic agriculture. AMF communities of organically managed fields were more similar to those of natural grasslands as compared to those under conventional management, and were less uniform, as expressed by higher β -diversity. A probable explanation is that conventional agriculture selects for a specific set of generalist AMF which outcompete AMF with other strategies. Potentially, this uniformity will reduce AMF contribution to ecosystem sustainability. An additional greenhouse experiment provided support for this conclusion. It was found that AMF from organic fields reached significantly higher densities, and that higher AMF density correlated with reduced nutrient leaching

after a mimicked heavy rain. Our results suggest that organic management in agro-ecosystems contributes to the restoration and maintenance of these important belowground mutualists.

Abstract Category
19 Plant-Microbe Interactions

PS.19.116 EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGUS (GLOMUS MOSSEAE) INOCULATION ON GROWTH OF WETLAND RICE

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Although the beneficial effects of inoculating crop plants with arbuscular mycorrhizal fungi (AMF) are well known, rice, the most important food crop in Asia, has received little attention in mycorrhiza research. This is because plants growing in marshy environments were rarely found to be mycorrhizal. *Glomus mosseae*, the most common mycorrhizal fungi ever reported to form a symbiotic relationship with wetland plant, was inoculated to observe the extent of arbuscular mycorrhizal fungi root colonization in wetland rice and to assess the effect of fungi inoculation on the growth of wetland rice. Rice was cultivated in three different soil that is sterile soil, natural field soil and *Glomus mosseae* artificial inoculated soil. Vegetative growth and reproductive characteristics were compared between three treatment rice. Result showed that *Glomus mosseae* was an indigenous fungus in native wetland soil, which could infect root and form a symbiotic relationship with rice in the natural matrix under the infection rate of $25 \pm 1.0249\%$, while it was not change the growth of rice plant obviously. Artificial inoculation enhanced the infection rate of *Glomus mosseae* up to $42 \pm 2.0036\%$ with much more spores and stronger hyphae. Mycorrhizal fungal inoculated rice seedlings grew significant better compared to non-mycorrhizal controlled seedlings, and had increased biomass (64.6%), root area (36.6%), and grain yield (45.3%) at harvesting stage. *Glomus mosseae* is available to form an association with rice plant under wetland environment, and intensified inoculation will be an effective way to increase grain yield, and reducing the agriculture pollution as chemical fertilizer using.

Abstract Category
19 Plant-Microbe Interactions

PS.19.117 REACTIONS OF PLANTS TO BACTERIAL VOLATILES: FROM LOVE TO HATE

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Increasing evidence indicates that bacteria can interact with plants through the emission of volatile compounds. Here, we report the highly variable effects of volatiles from 45 rhizosphere strains on the model plant *Arabidopsis thaliana*. Plants were grown for three weeks in the presence of bacterial strains in a compartmental Petri dish assay. Effects of bacteria ranged from over 5-fold plant growth promotion (*Pandoraea norimbergensis*) to plant killing (*Chromobacterium violaceum*). These effects were, for each strain tested, strongly dependent on culture medium and bacterial density. This led us to investigate the involvement of quorum sensing on the volatile-mediated impact of bacteria on plants. Quenching of quorum-sensing (by introducing an enzyme into a bacterial strain to degrade its signal molecules) in the plant-growth promoting strain *Burkholderia pyrrocinia* did not change its effect on *A. thaliana*. In contrast, the deleterious impact of *Chromobacterium violaceum* and *Pseudomonas aeruginosa* was significantly lower in the respective quorum sensing mutants. Further experiments testing mutants unable to produce hydrogen cyanide, or chemically applying hydrogen cyanide to growing plants, established this compound as the main candidate for the observed plant growth inhibition by bacterial volatiles. Cyanogenesis was found to be regulated by quorum sensing in *C. violaceum* and *P. aeruginosa*, suggesting that the reduced virulence of quorum sensing mutants to *A. thaliana* was caused by loss of the ability to produce hydrogen cyanide. The impact of quorum sensing on the production of other growth inhibiting volatiles, as well as on growth promoting volatiles, is currently under investigation.

Abstract Category
19 Plant-Microbe Interactions

PS.19.118 INVASIVE LEGUME, *LESPEDEZA CUNEATA*, ALTERS SOIL MICROBIAL COMMUNITIES IN HEAVILY INVADED STANDS

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Background and aims: Interactions with soil microorganisms may contribute to the success of invasive plants. The invasive legume, *Lespedeza cuneata*, has spread throughout much of the eastern United States, reducing native plant diversity. The aim of this work is characterize the impact of *L. cuneata* on soil bacterial and fungal communities at multiple scales of investigation. Methods: We sampled the root zones of *L. cuneata*, native legumes, and bulk soil from natural areas of five military installations throughout the invaded range. At each installation, collections were made in three areas: high, low, and no density of *L. cuneata*. We extracted bulk DNA and used a PCR-based, whole community "fingerprinting" analysis to characterize bacterial and fungal communities. We used multivariate data analyses and a variance partitioning scheme to characterize patterns of bacterial and fungal species turnover. Result: Bacterial and fungal communities showed similar patterns of variation in our sample set. Overall, the plant species was the greatest influence on microbial community composition, but this was largely due to unique microbial communities found in the root zones of native legumes in areas free of *L. cuneata*. Microbial communities from the root zones of *L. cuneata* were not statistically different from those of bulk soil or of co-occurring plants. However, sites with high densities of *L. cuneata* had microbial communities that were statistically distinct ($p = 0.005$) from sites with no or low densities of *L. cuneata*. This effect was consistent across the entire sampled range (i.e. independent of geographical differences). Conclusion: While *L. cuneata* has a limited influence on soil microbial communities at the scale of individual plants, it may exert a large influence on the local microbial species pool once it attains dominance at a site. This site-scale effect may feed back to plant fitness or to competitive interactions between *L. cuneata* and native legumes.

Abstract Category

19 Plant-Microbe Interactions

PS.19.119 MICROBES ASSOCIATED WITH WOODY PLANTS: ECOSYSTEM SERVICES FOR REMEDIATION OF HYDROCARBON POLLUTION

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Phytotechnologies are beginning to offer efficient tools and environmentally friendly solutions for the cleanup of contaminated sites and for the development of renewable energy sources. Microbes associated with plants influence the plant performance in remediation and may improve biomass production. Rhizoremediation of PAH- and petroleum hydrocarbon contaminated soils was carried out using birch (*Betula*) and aspen (*Populus*). Plant associated bacteria and fungi were studied with the aim of revealing microbial functions beneficial in remediation of polluted soils. The structural and functional diversity of bacteria were depicted using both traditional and molecular genetic methods including terminal restriction fragment length polymorphism (T-RFLP) of extradiol dioxygenases. Endophytic bacteria were isolated from micro environments of the plant. Degradation of PAHs was analyzed by gas-chromatography/massspectrometry. The immediate effects of petroleum pollution on aspen clones and microbial community structure and appearance of endophytic bacteria were studied. Extensive biodegradation was observed both in planted and unplanted microcosms, but with differential degradation of individual polyaromatics. Higher aromatic ring-cleavage potential was detected in both birch and aspen rhizospheres in comparison to bulk soil. The extradiol dioxygenase gene libraries revealed presence of novel degraders suggesting the presence of novel catabolic pathways in polluted soils and rhizospheres. Endophytic bacteria able to degrade benzoate, m-toluate and PAHs were isolated from hybrid aspen. *Burkholderia* sp. endophytes where PAH degraders showing promise for remediation of aromatics. Ectomycorrhizal fungal diversity of aspen included *Thelephora* sp. *Cortinarius* sp. and *Cenococcum* species. The

results facilitate understanding of plant/microbe interactions in remediation of hydrocarbon polluted soils.

Abstract Category
19 Plant-Microbe Interactions

PS.19.120 THE RESPONSE OF SOIL MICROBIAL COMMUNITIES OF LEGUME FORB AND C3 GRASS TO ELEVATED CO₂ AND N DEPOSITION

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Anthropogenic activities have increased CO₂ emissions and N-deposition into natural pools. The response of soil microbes to these environmental changes is an important, yet little-understood area. It is hypothesized that interactions between plants and microbial communities form a complex network which may modify microbial responses to elevated CO₂ (eCO₂) and N-deposition (eN). To test this, we investigated soil microbial communities at the BioCON (Biodiversity, CO₂ and Nitrogen) experimental site (Bethel, MN). In June 2009, 24 soil samples were collected from 24 plots (half with 4 species of legume forbs (LE), half with 4 species of C3 grass (C3)). Under each plant functional group, there were two treatments: CO₂ (ambient, 368 ppm vs. 560 ppm) and N (ambient vs. 4 g N m⁻²/yr). The effects of eCO₂ and eN on microbial communities were assessed by EcoPlate (Biolog, CA) and Geochip, a functional gene microarray covering various biogeochemical, ecological and environmental processes. Detrended correspondence analysis (DCA) of EcoPlate data was only able to show separation of microbial community composition along plant functional groups. DCA of Geochip data showed clear separation(s) by both plant functional groups, when pooling all data together, and CO₂ and N treatments, when analyzing LE and C3 data individually, indicating that plant functional group was the dominant factor influencing soil microbial communities at both metabolic and functional gene levels. The microbial communities had strong responses to the eCO₂ and eN at the functional gene level. Functional gene richness was higher from C3 plots than from LE plots (P = 0.007). The pattern of functional gene abundance changes under eCO₂ and eN differed between LE and C3. All these findings suggest that different plant functional groups (legume and C3), changed soil microbial communities greatly, causing different responses of microbial communities to elevated CO₂ and N deposition in this experimental field.

Abstract Category
19 Plant-Microbe Interactions

PS.20.001 BACTERIAL DIVERSITY ASSOCIATED WITH COAL IN METHANOGENIC ENRICHMENTS FROM POWDER RIVER BASIN COAL SEAMS

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The research was aimed at enhancing the fundamental understanding of the ecology and physiology of coalbed methane producing microbial communities. We conducted initial phylogenetic diversity studies using inoculated coal from methane producing wells in the Powder River Basin (southeastern Montana and Wyoming). A "bug trap" captured a coal-utilizing consortium from the bottom of a methane producing well in the Powder River Basin. Inoculum from the "bug trap" was used to create methane-generating enrichments with coal as the only energy source and compared to enrichments with acetate. Preliminary data revealed that an extremely diverse bacterial community established in coal cultures compared to enrichments without coal. The bacterial community in the coal enrichment contained at least 25 phylotypes but the acetate enrichment contained only 6 phylotypes. DNA sequences indicative of methanogens were detected in both enrichments (coal and no coal) and a majority of the archaeal sequences were closely related to a *Methanosarcina* species. The *mcrA* gene was used as an alternative to the SSU rDNA analysis to further examine the methanogenic populations. In the coal enrichment 87% of the sequences were most similar (99%) to a *Methanosarcina* species. In contrast, the acetate-only enrichment contained an almost even distribution of *mcrA* sequences most similar to a *Methanospirillum* species and a *Methanosarcina* species. These initial characterizations have identified bacterial populations that could potentially cross-feed methanogens and several that could compete with methanogens for substrates. These findings offer a compelling motive for further investigations into the biogeochemical processes that contribute to biogenic coalbed methane production.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.002 A METAGENOMIC-BASED INVESTIGATION OF METHANOGENIC COMMUNITIES IN ALBERTA COAL BEDS

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Unconventional gas sources, such as coalbed methane, or CBM, are an important source of energy that can help to offset declining conventional gas resources. CBM production in Alberta, Canada is fairly recent as the first commercial production began in 2001. Since then, 11,000 CBM wells have been drilled in Alberta and it is expected CBM to represent about 10% of future Canadian natural gas production. Geochemical and isotopic evidence as well as some microbial identification and enrichment culturing suggest that biogenic methane production occurred and is likely still occurring, albeit at very slow rates, in many coal beds in Alberta. This methanogenic activity could potentially be enhanced to increase methane yields from older or low gas content CBM wells and can be used to augment traditional CBM operations. Understanding the microbial ecology and associated methanogenesis processes in coal seams is obviously important to enhance microbial methane production to economic levels. We have started a new project involving metagenomics to gain a better insight into the microbial diversity of Alberta coal seams and to develop monitoring tools based on microbial numbers, types and activity for use in field trial applications. To date, we have collected 10 coal cuttings and 3 produced water samples from different cone zones and depths in Alberta. We have also obtained 28 core samples from different depths taken from 6 different wells. Whole community DNA has been extracted from the majority of the coal samples and the 16 S rDNA molecules sequenced by 454 pyrosequencing. We have also enriched for methanogenic consortia from the coal cuttings samples and investigated the effects of different nutrient additions to the cultures on enhancing methane production rates. This research has begun to provide an insight into the microbial diversity and ecology of coal beds and an understanding of the methanogenic processes occurring in them.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.003 METAGENOMIC ANALYSIS OF A METHANOGENIC, PARAFFIN-DEGRADING CONSORTIUM

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Very little is known about anaerobic biotransformation of paraffins. It has been hypothesized that paraffins may be activated in a similar manner as shorter alkanes. A methanogenic, octacosane (C₂₈H₅₈)-degrading enrichment culture was interrogated for its phylogenetic and metagenomic content via pyrosequencing approaches to gain insight into anaerobic paraffin degradation. Archaeal and bacterial 16S PCR products were sequenced using 454-based pyrosequencing. Sequence reads were binned into operational taxonomic units (OTUs) and analyzed via MOTHUR to determine the presence of dominant community members. Community DNA was shotgun-sequenced via 454-pyrosequencing to generate a metagenomic sequence database. Sequence reads were annotated using MG-RAST for metabolic reconstruction. More than 92% of archaeal sequences assembled into three OTUs, which classified within the families of *Methanosarcinales*, *Methanospirillaceae*, and an unclassified *Euryarchaeota* family. The three dominant bacterial OTUs accounted for 68% of bacterial sequences and belonged to two unclassified delta proteobacterial families as well as the family of *Syntrophaceae*. These results are consistent with metagenomic sequencing, which indicated that 17.7% of reads were most similar to the *Syntrophus aciditrophicus* SB genome. In addition 5.7% and 5.1% of the metagenomic reads mapped onto the genomes of *Desulfococcus oleovorans* Hxd3 and *Methanospirillum hungatei* JF-1, respectively. More than 300 out of 210,239 reads in the metagenome matched the alpha subunit of alkylsuccinate synthase (AssA), the enzyme that catalyzes the addition of alkanes to fumarate. Phylogenetic and metagenomic data are consistent with the notion that paraffin degradation proceeds via syntrophic interaction. It remains unclear how paraffins are activated anaerobically, but the high frequency of *assA*-like genes in the metagenome provides a platform for ongoing investigation.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.004 MICROBIAL DYNAMICS IN A TERRESTRIAL MUD VOLCANO OF EASTERN TAIWAN

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Understanding how microbial communities are regulated in terrestrial mud volcanoes would facilitate to unravel the carbon cycles linking deep and shallow reservoirs and to precisely assess the microbial role on the attenuation of methane emission. The objective of this study was to characterize microbial communities associated with sediments along depth in two sites of the Lei-Gong-Huo mud volcano in eastern Taiwan. These two sites distributed radially from the venting center were chosen (site A: near venting center; site B: 1 meter from venting center) in order to investigate whether the flux of ascending fluid would shape the community structures. Site comparisons revealed that *Methanosarcina* and *Methanococoides* were the major archaeal members in both sites. Methanogens were, however, not detected at the deepest interval of site B. Sequences affiliated with ANME-2 appeared to outnumber the others at shallow intervals in site A but were absent in site B. Bacterial communities were highly diverse and composed of different assemblages in both sites. *Desulfuromonas*-related sequences were prevalent at all depth intervals in both sites, whereas aerobic methanotrophs affiliated with *Methylosarcina*, and sulfide oxidizers with *Thiohalophilus* were more abundant at the deepest interval of site B. The molecular evidence combined with geochemical characteristics suggests that great fluxes of reduced, methane-rich fluid near the venting center suppress the downward percolation of atmospheric oxygen and fuel anaerobic metabolisms. The reduction of ascending, reduced fluids in the site distant from the venting center enables the enhanced oxygen intrusion from top or introduced laterally, supporting aerobic methanotrophs and sulfur oxidizers. The switch of functional expression and community structure reflects the interplay between the upwelling, hydrocarbon-rich, reduced fluids, and downward percolation of oxidative stress.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.005 MICROBIAL COMMUNITIES ASSOCIATED WITH METHANE TRANSFORMATION IN A TERRESTRIAL MUD VOLCANO, SOUTHEASTERN TAIWAN

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Terrestrial mud volcanoes are distinct geological features with fluid, sediment and hydrocarbon-enriched gas mixtures ascending from deep sedimentary environments, where microbial activities potentially influence the compositions of fluid and gas. At the Shin-Yan-Ny-Hu Mud Volcanoes in southwestern Taiwan, geochemical measurements indicated that sulfate depleted at shallow sediments while methane increased as the depth increased. The overlapping methane and sulfate profiles form a sulfate-to-methane interface (SMI, at ~10-12cm) a phenomenon commonly attributed to anaerobic sulfate-dependent methane oxidation in marine cold seeps or mud volcanoes. Phylogenetic analyses of 16S rRNA genes from erupted fluid and varying depths indicate that the dominant archaea were methanogens. Statistically cluster analyses, however, suggest that archaeal communities were structured with respect to depth. Based on the comparisons with the affiliated cultivable strains, the methanogens above the SMI preferentially used non-competitive substrates and switched to the hydrogen-utilizing pathway below the SMI. The archaeal community of the eruptive water sample appeared to be composed of diverse members, including thermophiles, marine-benthic group, and ANME. The presence of ANME group together with the most ¹³C depleted dissolved inorganic carbon observed at SMI characterized the process of anaerobic methane-oxidation. Aerobic methanotrophs affiliated with Gamma-Proteobacteria were detected at shallow depth intervals, corresponding to the decreasing methane levels and ¹³C-enriched methane. Consequently, the signature of methane detected from the surface of mud volcano reflects a mixture of deeply-sourced thermogenesis and methanogenesis overprinted by microbial alteration stratified in accordance with their respective physiological limit.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.006 CATTLE AFFECTED CHANGES IN ACTIVITY AND ABUNDANCE OF METHANOTROPHS IN UPLAND PASTURE SOIL

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Upland soils are assumed to be a sink for atmospheric methane, but impact of cattle husbandry creates conditions suitable for methanogenesis. We hypothesized that enhanced methane production in soils would facilitate the activity of methanotrophic bacteria (MOB). Thus, we investigated the relationship between the intensity of cattle impact and abundance and activity of MOB in pasture soils. Catabolized reporter deposition - fluorescence *in situ* hybridization (CARD-FISH) using probes specific to Type I and Type II methanotrophs, together with *pmoA*-targeting qPCR were employed to characterize the abundance of MOB *in situ*. Four types of soils regarding the level of cattle impact were studied: severe impacted (SI); severe in past, now regenerating (SR); medium impacted (MI); control (CO). In May 2009, the highest methane emissions both in field and in laboratory were observed in soil SI, while in October 2009, methanogenesis was insignificant. Methane oxidation rates were the highest in soil SR in May (decrease to 72% of initial concentration within 48 h) and in soil SI in October (decrease to 2%). qPCR for *pmoA* revealed significant differences among abundances of MOB in soils, being the highest in October at SI soil ($1.1 \times 10^5 \text{ g}^{-1} \text{ dw}$) and the lowest in May at BI soil ($2.2 \times 10^2 \text{ g}^{-1} \text{ dw}$). Similarly, abundances of *Methylobacter/Methylosarcina* group were the highest in October at SI soil ($3.8 \times 10^6 \text{ g}^{-1} \text{ dw}$) and the lowest in May at CO soil ($8.3 \times 10^2 \text{ g}^{-1} \text{ dw}$). On contrary, the highest relative abundance of MOB detected by CARD-FISH significantly decreased in soils under cattle impact. Type II MOB dominated over Type I in all soils and seasons. Our results indicate high potential for oxic methanotrophy in soils severely impacted by cattle (SI and SR) in comparison to CO soil. We also found discrepancy between the two methods used for molecular detection, presumably affected by the fact that FISH probes designed for 16S rDNA can target some non-methanotrophic bacteria.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.007 METHANE PRODUCTION FROM HIGH MOLECULAR WEIGHT PARAFFINS

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Geochemical and microbial cultivation studies have revealed syntrophic hydrocarbon metabolism coupled with methane production. Such activity has been shown for crude oils and for individual alkanes up to C₁₆H₃₄. However, information on the methanogenic degradation of higher chain length, waxy alkanes (> C₁₇H₃₆) is scarce. Here, we show that a highly purified methanogenic enrichment culture can biodegrade paraffins up to fifty carbons in chain length.

Paraffin-degrading cultures were enriched from hydrocarbon-contaminated marine sediments (San Diego, CA). After repeated transfers of the incubations grown with C₂₈H₅₈, C₄₀H₈₂ or C₅₀H₁₀₂, sediment-free cultures capable of long-chain alkane degradation were obtained. Alkane consumption and methanogenesis were analyzed by gas chromatography. DNA was extracted from enrichments, PCR-amplified, cloned, and sequenced to produce 16S rRNA gene libraries for archaea and bacteria.

Sediment incubations yielded methanogenic enrichments capable of degrading paraffinic substrates. The rates of methane production decreased with increasing alkane chain length. Octacosane (C₂₈H₅₈) loss was stoichiometrically coupled to methane production. Molecular analysis revealed the dominant bacterial sequence as 97% similar to an uncultured *Synthrophobacterales* clone and the dominant archaeal sequence as 100% similar to uncultured *Methanosaeta* clones, but sequences similar to *Methanoculleus* were also present.

An enrichment containing microorganisms typically associated with syntrophic alkane degradation metabolized octacosane with stoichiometric methane production. These results extend the range of hydrocarbons that be biodegraded under methanogenic conditions and may find use in microbial enhanced energy

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.008 MICROBIAL ECOLOGY OF THE RUMEN IN COWS FED DIETS OF CONTRASTING METHANOGENIC POTENTIAL

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A significant proportion of global methane emissions are accounted for by anthropogenic sources, 21 percent of which is in the form of eructation by ruminant farm animals. Reducing methane emissions from cattle would be a valuable contribution to efforts directed at minimising the scale of global temperature rise. Many studies have tested with varying success methods for reducing methane emissions from cattle, including the use of antibiotics, changes in diet and addition of food supplements. In order to understand the mechanisms underlying the reductions in methane production, it is important to study the microbial community in the rumen, and the effects that different diets and treatments have on their diversity and activity. A comprehensive study indicated that a diet of total mixed ration led to higher levels of methane production per cow than a pasture diet. Rumen liquid and solid samples for microbial analysis were collected from four fistulated cows fed either total mixed rations or pasture in a latin square design. Bacterial, archaeal, methanogenic and protozoal communities were characterised by TRFLP and ARISA of the amplified SSU rRNA or the mcrA genes. Statistical analysis of profiles generated for each community showed that bacterial communities were most affected by diet, followed by the archaea. The influence of the diets on the methanogens was not as clear as that observed for the archaea, this could be due to lower

resolution of the TRFLP analysis using the *mcrA* gene compared to the 16S rRNA gene. Protozoan communities did not appear to be significantly affected by a change in diet. These results indicate that the diet related reduction in methane production might have been due to effects on the bacterial rather than in the methanogenic community. Future characterization of the interactions of different microbial functional groups is necessary in order to successfully manipulate rumen fermentation for reduced methanogenesis in cattle.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.009 METHANOGENS IN HOT SPRING SEDIMENTS IN YELLOWSTONE NATIONAL PARK

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The goal of this project is to study the subsurface utilization of hydrogen as an energy source by methanogens in Yellowstone National Park, Wyoming, hot springs. Earlier, microorganisms and the abundance of hydrogen found in these environments have shown aerobic microbial utilization of H₂ as an energy source. Thermophilic methanogens are known to oxidize hydrogen under anaerobic conditions and here were studied in sediment environments. Two springs named Boulder and Obsidian with temperatures of 83 and 76°C were sampled and contained 3.5 and 44 nM H₂, respectively. Archaeal 16S rRNA genes were sequenced to explore community diversity. Further, the methyl coenzyme-M reductase gene, *mcrA*, was sequenced to study the presence and diversity of methanogens, and the abundance of methanogens was tested using quantitative PCR of the same gene. The archaeal 16S rRNA gene sequence libraries from both sites were dominated by Crenarchaeota. The library from Obsidian spring revealed microorganisms related to genera *Ferroglobus* and *Archeaglobus*, which include species known to utilize hydrogen as an electron donor and contain genes for methanogenesis, respectively. Sequences of the *mcrA* gene from Obsidian spring were related to sequences found in methanogenic Archaea, including the hyperthermophilic *Methanopyrus kandleri*. Further, the quantitative PCR results revealed that the *mcrA* gene was present at 10⁵ to 10⁶ copies per mL sediment at Obsidian spring, while copy numbers were under the detection limit at Boulder spring. Collectively the hot spring H₂ concentrations, the abundance of the *mcrA* gene, and the sequences found in the archaeal 16S rRNA and *mcrA* gene libraries show that methanogens are more abundant in the spring where more hydrogen is available. The findings support that methanogens may be important in the biogeochemical cycling of hydrogen in Yellowstone hot terrestrial anaerobic sediments.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.010 MICROBIAL COMMUNITY ANALYSIS, METABOLITES, AND FUMARATE ADDITION ENZYME GENE SEQUENCES IN HYDROCARBON-DEGRADING METHANOGENIC ENRICHMENTS

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Under methanogenic conditions, toluene degradation may occur via fumarate addition to form benzylsuccinate catalysed by benzylsuccinate synthase. A similar reaction was recently discovered for alkane activation, catalysed by an analogous enzyme, alkylsuccinate synthase. We investigated the microbial diversity and presence of benzylsuccinate and alkylsuccinate synthase genes in methanogenic toluene- and oil- degrading enrichment cultures that had been maintained for many transfers on the respective hydrocarbon substrates. The methanogenic cultures were assessed for (1) community composition using pyrosequencing of 16s rDNA, (2) the presence of benzylsuccinate synthase and alkylsuccinate synthase genes via PCR amplification with specific primer sets, and (3) and metabolites by GC-MS. 16s rDNA analysis of both enrichments revealed a wide diversity of bacteria that were predominated by Firmicutes, Proteobacteria and Chloroflexi. Archaeal diversity in both cultures showed a methanogenic population consisting of both acetate and hydrogen utilizing organisms. For the toluene enrichment, metabolite analysis revealed the presence of benzylsuccinate, benzoate, cresols, methylhydroquinone and hydroquinone. The presence of benzylsuccinate in this culture was consistent with the identification of benzylsuccinate synthase

genes by PCR amplification. Metabolite analysis of the oil degrading culture revealed the presence of benzoate and anaerobic naphthalene metabolites but not alkylsuccinates. Despite the lack of detection of alkylsuccinates in the culture, a PCR fragment with high sequence identity to known alkylsuccinate synthase genes was identified. These results suggest that a diverse group of microbes are involved in the degradation of hydrocarbons in methanogenic environments and that fumarate addition is an important reaction for the activation of hydrocarbons under these conditions.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.011 HIGH CO₂ CONCENTRATIONS NEGATIVELY EFFECT METHANOGENESIS AND SULFATE REDUCTION IN GAS FIELDS OF THE NORTH GERMAN PLAIN

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In consequence of their global warming potential, large-scale solutions are needed to reduce the emissions of greenhouse gases such as CO₂ or CH₄. The Carbon Capture and Storage (CCS) technique offers one option to reduce emissions. Favourable CO₂ storage sites are depleted gas and oil fields. Our study is focusing on the direct influence of high CO₂ concentrations on the autochthonous microbial population and environmental parameters at such sites. The investigated reservoir formation (Schneeren) is operated by Gaz de France Suez E&P DEUTSCHLAND GmbH. The conditions in the reservoir of two wells differ in various geochemical and microbiological parameters (Ehinger et al. 2009). Based on these results our study included cultivation and molecular-biological approaches. The two wells differed in the indigenous and inducible (with substrate addition) microbial activity. The addition of methanol to the fluids of well head A and B induced a biogenic methane production compared to the control setup. But only well B showed induced sulfide production in assays amended with hydrogen and CO₂. Results of the molecular biological analysis of the original fluids supported the activity data for both sites. The abundance of archaeal 16S rDNA and mrcA was several magnitudes higher in fluids of site A whereas site B was dominated by Bacteria. Incubations with high carbon dioxide concentrations showed a significant decrease of methane and sulfide production with increasing CO₂ levels. In a second step enrichments from the reservoir fluids and pure cultures of reference organisms were incubated under in situ pressure and temperature with high CO₂ levels. From this experiment survival rates of microorganisms together with molecular analysis of community changes were investigated. In conclusion these experiments will provide information about possible biogeochemical and microbiological changes during and storage of CO₂.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.012 ENHANCED MICROBIOLOGICAL GENERATION OF METHANE FROM COAL

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Vast reserves of coal represent a large source of energy in the United States. Research has demonstrated that microorganisms are capable of converting coal to methane under varying laboratory conditions, via a process known as methanogenesis. Stimulation of coalbed methane formation is an important process to understand because of its economic and environmental implications. Methane, the principal component of natural gas, is harnessed for use as an energy source. However, active coalbed methane wells are typically depleted within eight years and the coal is often not mined because it is not economically viable. Thus, converting uneconomical coal to natural gas could provide a means to extract valuable energy resources that would otherwise remain untapped. Additionally, if the process of coalbed methane formation is made more efficient, the dewatering step of the extraction process could be avoided, protecting surface and groundwater quality. The conversion of a complex substrate, such as coal, to methane requires a diverse microbial community that can be influenced by a wide range of environmental conditions. In order

to better understand the process of biogenic methane formation, this study aims to characterize native microbial communities responsible for anaerobic methane production from coal. Coal microcosm experiments are ongoing and methane production is being monitored by gas chromatography of headspace gas. A combination of 16S rDNA sequencing and quantitative PCR is being utilized to characterize the microbial communities and how they change throughout the incubation timeframe and are affected by different amendments. Results indicate the ability of a native microbial consortium to produce methane under anaerobic conditions, utilizing coal as a sole carbon source. Further work is planned to characterize this consortium and see how it compares to other methane-producing microbial communities.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.013 PREVALENCE OF METHANOGENIC CRUDE OIL DEGRADING CONSORTIA IN ANOXIC SEDIMENTS

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Methanogenic biodegradation of non-recoverable residual hydrocarbons to recoverable methane in heavy oil fields may be a cost-effective method of extending the operational life of petroleum reservoirs.

To determine the distribution and diversity of methanogenic crude oil-degrading consortia, anaerobic oil-amended microcosms were prepared using anoxic sediment inocula from 4 rivers (Tyne, Wear, Tees, Coquet), 3 lakes (Buttermere, Rydal Water, Priest Pot), 2 anaerobic wastewater digester sludges, and 2 aerobic wastewater sludges (anoxic incubation). The inocula encompassed both pristine and hydrocarbon contaminated environments. Conversion of crude oil to methane was monitored by gas chromatography, and compared to appropriate controls (methanogenesis inhibited, autoclaved/killed controls and microcosms without oil).

All inocula showed methanogenic activity (<50 days), however, the lag period for methanogenic oil degradation was more variable. Oil-amended River Tyne microcosms showed the highest levels of oil-induced methane production (~2 mmoles after 600 days), significantly higher than background levels of methane (0.30mmoles) detected after a 200 day lag. Oil induced methane production was much lower in microcosms inoculated with sediments from the River Wear (~600 µmoles), an anaerobic sludge (800 µmoles), and the River Tees (15 µmoles) with lag phases of 400-500 days. However, no oil induced methane production was observed in microcosms inoculated with lake sediments, river coquet sediments or the aerobic or anaerobic sludges after 600 to 800 days of incubation.

These differences may be attributable to the abundance of methanogenic hydrocarbon degrading consortia in the different inocula or their absence in some inocula suggesting that they are not ubiquitous and this may be related to current and past levels of fossil fuel contamination in the source environments.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.014 DYNAMICS AND DIVERSITY OF NON-THERMOPHILIC CRENARCHAEOTA ASSOCIATED WITH GAS HYDRATE IN THE GULF OF MEXICO

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Planktonic non-thermophilic Crenarchaeota are recently recognized a major group of metabolically active pelagic microorganisms present in the deep sea. The goal of this research is understand the distribution and abundance of Crenarchaeota in the subsurface ocean in Gulf of Mexico where hydrocarbon seeps and vents are widespread. We hypothesize that production of Crenarchaeota is

enhanced in hydrocarbon seeps which provide carbon and other essential nutrients for their growth. A gas hydrate (GC 234), a hydrocarbon vent (Brine Pool), and a control site called north brine pool (NBP-1B) which is normal marine sediment, were taken from gas hydrate mounds using a research submersible. Samples were extracted for DNA using commercially available test kits; and then amplified using the specific primers for Archaea (Arch 21F/ Arch 958R) and Crenarchaeota (Cren 344F/ Cren 517R) to detect these microorganisms in the environment. Two of these samples PCR products showed the presences of Archaea and Crenarchaeota. The third sample had negative results for both universal Archaea and Crenarchaeota perhaps due to low abundance in this sample. However, the mat sample showed strong signals for both universal Archaea and the Crenarchaeota. These results suggest Crenarchaeota may be most active at the interface between seawater and surface sediments where hydrocarbon seeping is active. Crenarchaeota are likely chemolithoautotrophs using a unique pathway for CO₂ fixation. Hydrocarbon seeps would be unique geological settings where enhanced CO₂ production from venting and hydrocarbon oxidation stimulates the growth of chemolithoautotrophic Crenarchaeota, which may play a significant role in global carbon cycling. Species diversities of the bacteria and archaea are also being determined using DGGE. The results should provide valuable information on the ecological function of microbial mats in the gas hydrate system.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.015 POSSIBLE DEPTHS FOR METHANOGENESIS IN DEEP SUBSURFACE ENVIRONMENT OF SEDIMENTARY GEOLOGICAL SETTING.

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An exciting competitive methanogenesis was found by a pseudo-in situ experiment which kept anoxic condition with adding nitrogen gas for the water taken from 500 and 140 m deep in Hokkaido, Horonobe where sedimentary horizon layered. For the water sample which biogenic methanogenesis was confirmed by stable isotope signature of $\delta^{13}\text{C}-\text{CH}_4$, incubation experiments were carried out by adding different substrates as follows; methanol, acetate, formate, H₂/CO₂, methanol + H₂/CO₂, methanol + N₂/CO₂. Though methanogenesis activity was just confirmed at around in situ temperature at 500 m of 30 to 35°C, any activity was not observed for microbial community at 140 m by neither in situ (16°C) nor 35°C temperature $\delta^{13}\text{C}-\text{CH}_4$ gas was shown to be biogenic. Methanol was the most effective substrate utilized swiftly by in situ archaeal community of 500 m. Corresponding archaea were confirmed by 16S rDNA sequencing as genus *Methanolobus*, while methylotrophic methanogen, *Methermicoccus*, were found as well as methane oxidizing bacteria for the sample taken from 140 m deep. After the consumption of added methanol in situ archaeal community exhibited methanogenesis activity from H₂/CO₂. Incubation of 500m water with an inhibitor of methanogenesis shows methanol production must be done by co-existing bacteria. This study was performed as a part of "Project for Assessment Methodology Development of Chemical Effects on Geological Disposal System" funded by Ministry of Economy, Trade and Industry, Japan.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.016 MICROBIAL METHANE PRODUCTION IN DEEP AQUIFER ASSOCIATED WITH THE ACCRETIONARY PRISM IN SOUTHWEST JAPAN

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The accretionary prism forms thick sediment that accretes onto the non-subducting tectonic plate at a convergent plate boundary. The sediment is composed mainly of non- to weakly metamorphosed sandstone and shale. The materials are derived from marine sediment; therefore, they are rich in

complex organic matter. The sediment contains layers of water-bearing permeable sandstone, and groundwater is reserved in a deep aquifer. It has been reported that methane is present in the deep aquifer. The goals of this study are to identify the origin of the methane using the stable isotopic analysis and, using microbiological methods, to explore the microbial methane production pathway in the deep aquifer. Stable carbon isotopic analysis of methane in the natural gas and bicarbonate in groundwater suggested that the methane was of methanogenic origin with small mixing of thermogenic gases. Archaeal 16S rRNA gene analysis revealed the dominance of hydrogen-using methanogens. The high potential of methane production by hydrogen-using methanogens was demonstrated on enrichments using groundwater. Bacterial 16S rRNA gene analysis showed fermentative bacteria inhabited the deep aquifer. Anaerobic cultivations using groundwater amended with organic substrates and a methanogen inhibitor suggested a high potential of H₂ and CO₂ generation by fermentative bacteria. To confirm whether or not methane is produced by a syntrophic consortium of the fermentative bacteria and hydrogen-using methanogens, anaerobic cultivations using the groundwater amended with organic substrates were performed. Consequently, H₂ accumulation and rapid methane production were observed in these enrichments. Our results strongly suggested that past and ongoing syntrophic biodegradation of organic compounds by hydrogen-producing fermentative bacteria and hydrogen-using methanogens contributes to the significant methane reserves in the deep aquifer.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.017 EFFECT OF ISO-PROPANOL ON EXPRESSION LEVEL OF ACETYL-COA SYNTHETASE GENE AND ACTIVE METHANOGENIC POPULATION IN ANAEROBIC PROCESSES

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Since acetoclastic methanogens participating in methanogenesis step play an important role in stabilizing organic pollutant and are susceptible to inhibitors, understanding the specific effects of the pollutants on acetoclastic pathway in anaerobic reactors is important. In this study, effect of iso-propanol on biogas production, microbial community structure and expression level of acetyl-CoA synthetase gene of *Methanosaeta concilii*, which is a key enzyme of methane production from acetate, were monitored. For this purpose, an anaerobic sludge with TVS of 2000 mg/L was added to the 100 ml serum bottles under anaerobic conditions. The bottles were incubated at 37 °C for 24 hours and then fed with acetate of 2000 mg/L and iso-propanol at two different concentrations of 0.1 M and 1.0 M, except the control bottle. All bottles were placed in a water bath maintained at 37 °C. When methane production reached a peak value serum bottles were given to same concentration of iso-propanol for the 2. exposure. At this stage, numbers of the gene copies per mL were found to be 1.55E+05 and 0.41E+05, for 0.1M and 1.0 M iso-propanol concentration, respectively, whereas that of was 1.66E+06 for control reactor. Due to severe inhibition, no genes can be quantified after 3. exposure which contained same concentration of the solvent. 98.5% decrease in methane production was observed for 1.0 M iso-propanol after the third exposure. The correlation between gene copies and methane production was found as 0.998. Due to FISH results, active cell percentages were 37.0%, 49.3% and 27.2% for control reactor, 0.1 M and 1.0 M iso-propanol added reactors, respectively. *Methanosaeta spp.* population in archaeal community increased to 50.0% and 43.8% for 0.1 M and 1.0 M iso-propanol concentrations whereas that was of 29.1% for the control reactor. We report that although there was no direct effect of iso-propanol on the vitality of *Methanosaeta spp.*, the decrease in methane production was dramatic.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.018 TIDAL FLAT ARCHAEA ENRICHED IN STERILIZED SEDIMENT BULKS SUPPLEMENTED WITH METHANE/METHANOGENIC SUBSTRATES

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Methane production and oxidation processes in tidal flat surface layer affect the methane flux from tidal flats to the atmosphere. Cultivation of archaea that produce or oxidize methane and are abundant in tidal flats is desirable for future studies of tidal flat's feedback to global climate change. We did qPCR to quantify archaeal and bacterial 16S rRNA, mcrA and dsrA gene abundances in the surface layer samples collected from flats located in the Yellow Sea tidal area. Bacterial 16S rRNA gene abundance was 20-80 times higher than Archaeal 16S rRNA gene. dsrA to mcrA ratio varied greatly among samples (0.14-500), and the abundance of two genes had a weak negative correlation. dsrA to bacterial 16S rDNA ratio was relatively constant among the samples, while mcrA to archaeal 16S rDNA ratio varied greatly. We hypothesized that the use of physicochemical structure of bulk sediment as medium instead of homogeneous substance would provide lower chance that rare but competitive species could dominate the community in the bottle, and be likely to represent the conditions favored by the naturally abundant species. We diluted the samples into sterilized tidal flat and added diverse substrates involved in methane biogeochemistry. After more than one month of incubation, relative abundance of archaea increased in 8 bottles: 4 of them supplemented with hydrogen, 1 with acetate, 2 with monomethylamine and 1 with methane. In 3 of 8 bottles abundance of archaea was same or greater than that of bacteria. Relative abundance of mcrA over dsrA increased greatly in 3 hydrogen-, 1 acetate- and 2 monomethylamine-supplemented bottles, suggesting that methanogens are outcompeting sulfate reducers in these bottles. Subsequently we are simplifying the community structures in bottles by diluting them into fresh sterilized sediment at high ratio. We expect that this process could lead us to the cultivation of naturally abundant microbial species important in tidal flat methane flux.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.019 COMMUNITY STRUCTURE AND ACTIVITY OF METHANOTROPHS IN ARCTIC LAKES

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Methane bubbling from newly forming Arctic lakes contributes to a large part of the high-latitude increase in atmospheric methane concentration. Biological oxidation by methane-oxidizing bacteria is significant in the methane cycle, but the sinks for arctic methane and the biological systems involved in methane consumption are poorly understood. The aim of the study was to characterize the biological processes associated with methane oxidation within water and sediment in a lake on the north slope of Alaska that is rapidly emitting methane at a substantial seep. Stable isotope probing (SIP) was applied to identify the active methane-oxidizing bacteria in the sediment from the lake with a microcosm incubated with a 10% ¹³CH₄ headspace at room temperature. Methane oxidation potential was tracked over time during the SIP incubations. Terminal restriction fragment length polymorphism (T-RFLP) analysis showed that the culturable methanotrophic community structure were very similar in water samples from different depths and sites. Initial methane oxidation potential was low in the sediment samples. However, exposure to methane increased the methane oxidation potential of the sediment samples, particularly at depths between 10-25 cm. Results from the SIP experiment indicated active methane-oxidizing bacteria were diverse in the lake sediments and community structure varied with sediment depth, and yet seemed to be a largely different consortia compared with those in the lake water. Analysis of 16S rRNA gene sequences amplified from the ¹³C-DNA also reflected the significant difference of methanotrophic bacteria between water and sediment in the lake. These findings will contribute to a better understanding the methane cycle in cold regions.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.020 THERMOPHILIC ACETATE-UTILIZING BACTERIA AND ARCHAEA IN METHANOGENIC RICE FIELD SOIL REVEALED BY STABLE ISOTOPE PROBING OF RNA AND DNA

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Acetate transiently accumulated and was subsequently consumed in methanogenic Italian rice field soil under thermophilic conditions, but the responsible microorganisms are unknown. The CO₂ produced from [2-¹³C]acetate (99% ¹³C) contained up to 27% ¹³C, indicating that acetate was not only consumed by acetoclastic methanogens, but was also oxidized. Microorganisms capable of assimilating acetate at 50°C were traced and identified by using stable isotope probing (SIP). By combining rRNA- and DNA-SIP, ¹³C-labelled bacterial and archaeal rRNA and DNA were detected after 21 and 32 days of incubation with [U-¹³C] acetate, respectively. Terminal restriction fragment length polymorphism (T-RFLP) and cloning analyses revealed species belonging to *Thermacetogenium*, *Symbiobacterium* and *Thermoanaerobacteriaceae* predominated the ¹³C-labelled bacterial rRNA. Among the *Archaea*, *Methanocellales* were dominant, indicating that CH₄ was produced via syntrophic acetate oxidation coupled to hydrogenotrophic methanogenesis. However, the rRNA of members of the acetoclastic *Methanosarcinaceae* was also labeled. Our results demonstrated that thermophilic acetate utilization was not only achieved through acetoclastic methanogens, but also through oxidation by syntrophic bacteria coupled to hydrogenotrophic *Methanocellales*.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.021 COMMUNITY ANALYSIS OF THE METHANOGENS AND STIMULATION OF METHANE PRODUCTION BY NON-COMPETITIVE SUBSTRATES IN HYPERSALINE ENVIRONMENTS

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Microbial communities in evaporation ponds at Exportadora de Sal, S. A. (ESSA) at Guerrero Negro and in natural hypersaline environments at Laguna San Ignacio, Baja California Sur, Mexico were studied. In hypersaline environments worldwide, only members of extremely halophilic methanogens of the Order Methanosarcinales and moderately halophilic methanogens as *Methanococcus* sp. are involved in methane production in these environments. However, observations of one environment are not always applicable to others because of the complexity of the different microbial and chemical processes that occur. This study elucidates the archaeal lineages responsible for this activity in two hypersaline environments and measures rates of methane production from incubated samples with competitive and non-competitive substrates. Phylogenetic trees were constructed from partial sequences of 16S rRNA and the methyl-CoM-reductase A gene obtained from environmental DNA. Measurements of methane production were done from field samples and mats incubated with different substrates: hydrogen/carbon dioxide, acetate, formate, methanol, trimethylamine. Phylogenetic analysis showed that methanogenic community in Area 1 from ESSA was dominated by members of *Methanohalophilus* spp., *Methanococoides* sp. and uncultured euryarchaeote clones. Other cluster was distantly related to *Methanolobus* sp., which was detected in samples from Laguna San Ignacio. Different rates of methane production were obtained from incubated samples from Laguna San Ignacio and ESSA. Non-competitive substrates methanol and trimethylamine stimulated methane production in samples from both sites. We conclude that the methanogen community at these sites is represented by at least by three different populations: uncultured euryarchaeote clones and members of the Order Methanosarcinales. We also found that production of methane in samples of Laguna San Ignacio and ESSA are stimulated by methanol and trimethylamine substrates.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.022 MICROBIAL COMMUNITY STRUCTURE AND ECOSYSTEM FUNCTION: LINKING METHANE PRODUCTION RATE TO METHANOGEN COMMUNITY STRUCTURE IN WETLAND SOILS

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Methane is a significant greenhouse gas, trapping up to 25 times as much infrared radiation as carbon dioxide per unit weight, and causing almost a fifth of human-caused radiative forcing. All of the methane produced by the world's biosphere is produced by methanogens, a polyphyletic group of microorganisms in the domain Archaea, in anaerobic environments. While the role of abiotic factors in controlling this process has been extensively studied, the relationship between microbial community structure and methanogenesis rates in ecosystems is not well understood. The intent of this research is to analyze the relationship between methanogenesis rates and methanogen community structure at several levels: total community composition, community functional gene transcription, and community growth profile. As it is not yet possible to culture most methanogen species, it is necessary to analyze the community structure using a culture independent, genomic approach. For all of these investigations, we are using *mcrA*, a gene encoding the active site of the terminal enzyme in the methanogenesis pathways of all known methanogens, as a molecular marker. The use of a single gene for this research unites the assays of community structure at all levels into directly comparable genomic data. DNA and RNA extracts from peat soils and lake sediments are being sequenced, and TRFLP (Terminal Restriction Fragment Length Polymorphism) community profiles will be constructed. These community data will be correlated to measured rates of acetoclastic and hydrogenotrophic methanogenesis in peat soils and anaerobic lake sediments, along with abiotic factors such as temperature, pH, and inorganic electron acceptor concentration, using non-metric multidimensional scaling (NMS). This research will be the most extensive study to date of the role of microbial community structure in the regulation of methanogenesis in terrestrial and freshwater system.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.023 MICROBIAL DIVERSITY IN A COAL-SEAM METHANE RESERVOIR IN VICTORIA, AUSTRALIA.

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There is significant interest in reducing the damage to the environment caused by coal-fired electricity generation. The use of coal-seam methane (CSM), over coal, has multiple benefits. These include notably reduced greenhouse gas emissions, significant human health benefits and relatively inexpensive power plant modifications required to convert from using coal to CSM as a fuel. CSM is generated both by geological (coalification) processes and biologically, though the latter is relatively poorly understood. The present study used a molecular approach to examine a microbial community in a brown coal seam environment in Victoria, Australia. Various Bacteroidetes, Firmicutes, Proteobacteria and Tenericutes were among the more common taxa detected. Enrichment cultures derived from this environment were able to degrade brown coal to methane. These cultures contained representatives of the bacterial phyla Firmicutes, Bacteroidetes, Spirochaetes, Tenericutes, Proteobacteria and Verrucomicrobia along with two euryarchaeal methanogens from the orders Methanobacteriales and Methanomicrobiales. The ecophysiological implications and possible pathways for coal degradation are discussed.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.024 MONITORING METHANOGEN COMMUNITIES IN ANAEROBIC DIGESTERS USING QUANTITATIVE POLYMERASE CHAIN REACTION AMPLIFICATION OF *MCR A*

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Anaerobic wastewater treatment, an economically and environmentally beneficial process, converts organic wastes into methane, a renewable energy source. However, anaerobic treatment systems depend on the biological function of microbial communities that can be stressed by digester overload or exposure to toxicants. Methanogens produce methane in digesters as the final step in the complete breakdown of organic wastes. Reduction in methane production results in digester

stress or failure. Methane production from intermediates is proportional to the number of active methanogens in the digester. Therefore, it was hypothesized that the number methanogens determined using a methanogen-specific gene and a quantitative polymerase chain reaction, would correlate with methane production rates. To test this hypothesis, the number of gene copies and mRNA transcripts of the methanogen-specific gene *mcrA* (encodes the α subunit of methyl coenzyme M reductase, the enzyme which catalyzes the final step of methanogenesis) were determined using a quantitative polymerase chain reaction on DNA and RNA extracted from 4 lab-scale anaerobic digesters maintained under different conditions and producing differing amounts of methane. The number of gene copies and mRNA transcripts were compared to rates of methane production. Both *mcrA* gene copy number and mRNA transcript numbers correlated with methane production rates ($r = 0.72-0.99$). Digesters with low rates of methane production (100.67-120.01 mL CH₄/g volatile suspended solids/hour) had fewer copies of *mcrA* per nanogram DNA and fewer transcripts of *mcrA* per nanogram RNA than digesters with higher rates of methane production (402.04-465.1 mL CH₄/g volatile suspended solids/hour). The data supported the hypothesis that quantitative polymerase chain reaction of *mcrA* genes and transcripts may be used to monitor anaerobic digesters for activity of the methanogen community.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.025 METABOLICALLY ACTIVE PROKARYOTES IN COAL BED BASIN AND THE EFFECT OF TRACE ELEMENTS ON METHANE PRODUCTION

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Biodegradation of coal organic deposits into methane is limited to anoxic conditions by the interaction of syntrophic bacteria and methanogens. In the closed system of a deep subsurface environment, trace elements are limiting factors for microbial growth and activity. In this study we estimate activity and identify metabolically-active prokaryotes involved in methane production, and determine the influences of the trace elements iron, nickel, molybdenum, and cobalt. Production water and coal were collected from various coal bed wells located in the Power River Basin, Wyoming. Identity and activity of methanogens was monitored using mRNA isolated from microcosms incubated with coals, lipid-coated slides, and with enrichment cultures. Using quantitative reverse transcription real-time PCR levels of transcripts for the methyl coenzyme M reductase genes (*mcrA*) demonstrated that groups closely related to *Methanobacterium* are active and abundant in enrichment cultures and that novel methanogens cluster in microcosms with substrates coals or lipids. Microcosms were established for different coal bed wells to test the hypothesis that trace elements may affect the microbial community composition and thus the biogenic methane production. Individual trace element incorporation into the microbial food web was quantified with ICP-MS, and correlated with total methane production. Changes in community composition over time were followed with PCR-DGGE. Increasing methane production was observed for iron, nickel, cobalt, and molybdenum amendments, respectively, and expression levels for *mcrA* provided the identity of active methanogens and their response to specific trace elements. These studies have improved our understanding of trace element effects on the activity and diversity of microorganisms involved in biogenic coal bed methane production, and will provide some insights to enhance biogenic methane production in coal bed wells as an alternative energy source.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.026 MICROBIAL CONVERSION OF BIODIESEL BY-PRODUCTS TO BIOFUEL

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Biodiesel, an alternative to fossil fuels, is derived from carbon-fixing biological sources. One of the major by-products from biodiesel production is crude glycerin. In the following study, different co- and tri-cultures of sulfate-reducing bacteria (SRB) and methanogens were grown on crude glycerin as a means to convert the waste product into a renewable energy source, methane. *Desulfovibrio*

vietnamensis and *Desulfovibrio alcoholovorans* 6133 can oxidize lactate with sulfate as the electron acceptor. Both SRB can grow syntrophically with methanogens, replacing sulfate as the terminal electron acceptor in co-culture. Cultures of *D. vietnamensis* and *D. alcoholovorans* 6133 were investigated for the ability to utilize glycerol, cleaned glycerin and crude glycerin as carbon and energy sources. The crude glycerin was a complex mixture of glycerol, salts, and methanol. Glycerol, acetate, fatty acid, and methane concentrations were measured throughout growth. *D. vietnamensis* grew faster than *D. alcoholovorans* 6133 on all 4 different carbon sources. Both *D. vietnamensis* and *D. alcoholovorans* 6133 experienced a growth lag when transferred from lactate to glycerol, cleaned glycerin, or dirty glycerin. Different co- and tri-culture combinations of *D. vietnamensis*, *D. alcoholovorans* 6133, *Methanococcus maripaludis*, and *Methanoculleus marisnigri* were tested for the ability to convert varying concentrations of glycerol, cleaned glycerin and crude glycerin into methane. The co- and tri-cultures grew fastest on pure glycerol and experienced a lag in growth on cleaned glycerin and dirty glycerin. However, methane yields and percent conversion of electron equivalents were similar on all 3 carbon sources but the most efficient methane producing co- and tri-cultures varied by carbon source. The ability to utilize glycerin as a feedstock for microbial conversions will circumvent industrial purification processes and will possibly alleviate price constraints for the biodiesel market.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.027 REVERSE METHANOGENESIS: EXPANDING THE POTENTIAL MECHANISMS WITH A LINEAGE-RESOLVED PROTEOGENOMICS APPROACH

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Microbially mediated anaerobic oxidation of methane (AOM) acts as a biological methane filter, stimulates subsurface metabolism, and supports thriving chemosynthetic communities. Molecular surveys exploring the biological component of AOM have identified microbial communities dominated by anaerobic methane-oxidizing archaea (ANMEs) and sulfate reducing bacteria. Although ANMEs bear the isotopic signature of methane-derived carbon in their cellular building blocks, the biochemical pathways underlying the conversion of methane into biomass and energy remain unresolved. Here, we describe a comparative proteogenomic approach used to constrain and validate the biochemical pathways mediating the anaerobic oxidation of methane within specific ANME lineages (1a, 1b, and 2c). A multivariate statistical analysis of oligonucleotide frequency patterns, complemented with genomic fragment clustering based on oligonucleotide frequency correlation coefficient, was used to resolve a complex pool of large insert genomic sequences derived from three distinct AOM environments (Eel River Basin, Black Sea, Hydrate Ridge). In silico pathway reconstruction of binned sequence data expanded the range of carbon, energy, sulfur, and nitrogen metabolism associated with AOM, identifying lineage-specific variation with respect to potential electron acceptors, C1 pathway components, and sulfur/nitrogen assimilation strategies. To explore these pathways, liquid chromatography (LC) tandem mass spectrometry (MS/MS) was used to identify peptides recovered directly from push core samples spanning sulfate-methane transition zones within Eel River Basin sediments. Overlapping but not identical sets of genes typically associated with methane production were expressed by different ANME lineages. These proteogenomic observations support previous hypotheses and provide an informed foundation for modeling the individual contributions of specific ANME lineages to the global carbon cycle.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.028 RHIZOSPHERIC METHANE EMISSION IN PADDY SOIL MEASURED BY LABELING WITH ¹³CO₂

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Methanogenic *Archaea* in irrigated rice fields play a key role in global methane emission. Significant carbon sources for methane formation are root exudates as well as soil organic matter and rice straw. To determine methane emission from plant secreted rhizodeposits, rice plants potted either

in pure paddy soil or in a mixture with quartz were labeled once with $^{13}\text{CO}_2$ in a closed chamber after reaching the maximum growth phase. Amounts of total and labeled CH_4 and CO_2 in the headspace were measured over a period of five days to estimate the contribution of recently assimilated carbon to the produced methane. Assimilation of carbon dioxide and emission of labeled methane occurred rapidly suggesting that root exudates were an important carbon source for the methanogenic communities. After five days, up to 1.5 % of assimilated $^{13}\text{CO}_2$ was released as $^{13}\text{CH}_4$. The amount of emitted $^{13}\text{CH}_4$ was dependent on plant age, carbon dioxide availability and light intensity. The contribution of assimilated carbon recovered in the emitted methane accounted for 4.6 - 4.8 % and 6.2 - 7.5 % in pure rice field soil and quartz mixed with paddy soil, respectively. The results illustrate that the dynamic of recently fixed carbon by plants is closely linked to microbial methane formation by methanogenic *Archaea*. The study demonstrates that single $^{13}\text{CO}_2$ feeding allows to directly estimate the contribution of currently assimilated carbon to methane emission. This approach will be useful to follow rapid shifts in carbon fluxes through the plant-soil ecosystem with changing environmental conditions in general as well as to determine rhizospheric methane emission from rice plants colonized with different microbial communities in particular.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.029 METHANOTROPHIC BACTERIA IN *SPHAGNUM* - MOVEMENT AND COMMUNITY COMPOSITION

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Peatlands are a major source of the greenhouse gas methane, CH_4 . These emissions are controlled by methanotrophic bacteria. Recently, a loose symbiotic relationship between methanotrophs and widespread peatland mosses, *Sphagnum* species, was discovered. Through CH_4 oxidation these bacteria provide CO_2 for plant photosynthesis. The CH_4 oxidation in *Sphagnum* tissues is controlled by the water table level thus existing in springtime in so far all tested *Sphagnum* species and occurs over the whole growing season in flark species.

We studied this phenomenon further to find out more about *Sphagnum* associated methanotrophic communities and their dynamics. Movement of methanotrophs was tested by transplanting *Sphagnum* lacking the CH_4 oxidation potential next to a *Sphagnum* oxidizing CH_4 . Most of the transplanted mosses showed CH_4 oxidation potential already after 3 d. Methanotrophic communities were characterized with a microarray method detecting diversity within the particulate methane mono-oxygenase functional gene. Before transplantation two out of three samples did not contain enough methanotrophic DNA to be analyzed. After 3 d all transplanted *Sphagnum* had methanotrophs and after 28 d communities had become more similar with each other. Type II methanotrophs were detected in high diversity whereas only few type I methanotrophs were present. This trial thus showed that inactive *Sphagnum* readily became active when transplanted next to active species. To further demonstrate that methanotrophs migrate through the water, another experiment was conducted: Inactive *Sphagnum* samples were watered with flark water from a peatland site with high oxidation potential. Parallel samples were watered with the same water after passing it through a 0.45 μm filter to remove methanotrophic bacteria. Only *Sphagnum* watered with unfiltered flark water became active. Through this experiment it seems clear that methanotrophs move from one *Sphagnum* plant to another in the water phase.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.030 MICROBIAL ACTIVITIES AND COMMUNITIES IN OIL SANDS TAILINGS PONDS - TOWARDS IMPROVED POND MANAGEMENT

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Vast tailings ponds store liquid and solid wastes following bitumen extraction from the oil sands deposits in Alberta, Canada, a major global source of fossil fuels. Tailings pond management concerns include biogenic gas emissions (H₂S and CH₄) and densification of solids. In order to study the roles that pond microbial communities may play to address such issues, we determined the microbial activities and communities in two tailings ponds that routinely received CaSO₄ as a densification agent. Samples were taken at the surface and at discrete depths from an active (P6) and an inactive tailings pond (P5). Sulfate and sulfide levels, pH, and rates of sulfate reduction (SRR) and methanogenesis were measured. Microbial community analysis based on the 16S rRNA gene was performed via pyrosequencing. The ponds had the highest sulfate concentrations (6-16 mM) at the surface, where no sulfide was detected. In P6, sulfide was detected in the deeper layers, where the highest SRR was measured (56 mmol sulfate/m³ tailings/d). In P5, the shallow anaerobic layers showed the highest sulfide levels and SRR (67 mmol sulfate/m³ tailings/d). Methanogenesis rates fluctuated in each pond but were generally lowest where SRR were highest (and vice versa). Community analysis showed that *Proteobacteria*, *Chloroflexi*, and *Firmicutes* were the predominant eubacterial phyla in the deep anaerobic zones of P6 but that a very different community existed at the aerobic surface. Methanogens were also detected in samples collected from beneath the pond surface. Our data reveal a broad diversity of anaerobes thriving in oil sands tailings ponds. The addition of CaSO₄ as a densification agent to tailings ponds stimulates sulfate reduction and inhibits methanogenesis which helps to lower greenhouse gas emissions. Produced sulfide appears to reoxidize in the upper oxygenated layers of the pond, offering evidence for sulfur cycling in the ponds and the prevention of H₂S release.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.031 MICROBIAL COMMUNITY ANALYSIS OF METHANOGENIC COAL BEDS IN THE POWDER RIVER BASIN

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Biogenic methane has enormous potential as a sustainable energy source and is found in a wide variety of subsurface, anaerobic, hydrocarbon bearing environments. One area of great interest for biogenic methane is the Powder River Basin in Wyoming, United States. This area has previously been shown to be an active "geobioreactor", or an area where active *in situ* methanogenesis occurs as determined by coal conversion to methane in laboratory experiments, the presence of metabolic intermediates in coalbed methane formation waters, and the ability of the consortia present in these waters to convert the metabolites to methane. Understanding the composition and metabolism of the methanogenic consortia is one of several key steps in commercializing the process of sustainable biogenic methane production. In order to identify the microbes present in these methanogenic consortia, we performed microbial community analysis using error-correcting barcode pyrosequencing analyzed with the QIIME bioinformatic pipeline. This involved hundreds of samples collected from different locations within a set geographical area over several years. We used both bacterial and archaeal specific primers to amplify these distinct populations from our DNA samples. The QIIME pipeline was used for library demultiplexing, OTU picking, alignment, taxonomic identification, and statistical analysis of community structure using Unifrac. Spotfire was used for visualization of QIIME results with metadata from the formation. Here we demonstrate the large scale sampling and community analysis of multiple coal seams within a discrete but large area of the Powder River Basin. We find distinct bacterial and archaeal populations that vary by coal seam and water chemistry, as well as over time. These community signatures may help to determine areas of greatest methanogenic potential as well as identify patterns of ground water recharge, water movement, and metabolic bottlenecks.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.032 GREENHOUSE GASES PRODUCTION/EMISSION FROM ARTIFICIAL WETLAND IN NORTHERN TAIWAN

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To investigate greenhouse gases emission from artificial wetlands, soil properties, environmental conditions, microbes and greenhouse gases emission were determined at seven decomposed ponds in the wetland of Taipei County in northern Taiwan. The concentrations of carbon dioxide and methane were between 361.6 - 428.9 and 1.6 - 3.4 ppm by Fourier-transform infrared spectroscopy measurement, and 318.56 - 414.43 and 0.45 - 2.78 ppm using point measuring method, respectively. Head space concentration of carbon dioxide and methane were 1260.24-16034.82 and 2.71-5606.37 ppm with brown bottles; and the values were 720.18-15642.56 and 3.34-5626.26 ppm with colorless bottles, respectively. The methane emission rate reached its peak 14.19 - 81.53 mg m⁻² h⁻¹ at the initial stage of waste water management at different sampling periods and then decreased gradually in the succession treating ponds for the decomposition of organic wastes. The carbon dioxide emission rate reached its peak 199.99 - 600.65 mg m⁻² h⁻¹ at the first pond and it affected by the photosynthesis obviously at the subsequently ponds. Carbon dioxide emission was very significantly negative correlation with dissolved oxygen; significantly negative with pH of water properties. Methane emission was significant positive with moisture content and total organic carbon of sediments; very significantly negative correlation with dissolved oxygen and redox potential of water properties. The experimental results suggest that the emission of methane from artificial wetland was reduced more than hundred folds during waste water management. Different stage managements have significant variation of properties and greenhouse gases emissions. Moreover, the greenhouse gases emissions may be reduced by manipulating the proper factors.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.033 METHANE PRODUCTION IN OIL SANDS MATURE FINE TAILINGS GENERATED BY DIFFERENT EXTRACTION PROCESSES

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Oil sands tailings are produced during extraction of bitumen from oil sands ores. The current inventory of tailings deposited in settling basins in northern Alberta, Canada is ~720 million m³; some basins produce biogenic methane. The oil sands operators Syncrude Canada Ltd. and Shell Albian Sands (Albian) both use hot water extraction methods but use different extraction solvents and water treatment agents. Syncrude uses naphtha (a mix of C₃-C₁₄ hydrocarbons including paraffins and monoaromatics) as an extraction solvent whereas Albian uses a C₅-C₆ paraffinic solvent and tri-sodium citrate as a water conditioner. Our laboratory studies previously showed that the source of methane in Syncrude mature fine tailings (MFT) is residual naphtha. Because only short-chain n-alkanes and BTEX compounds in the naphtha were readily metabolized by indigenous microbes in Syncrude MFT, we hypothesized that short-chain n-alkanes and possibly iso-alkanes in Albian solvent would support methanogenesis in Albian MFT. Instead, microcosm experiments suggested that citrate was the main methanogenic substrate in Albian MFT and the paraffinic solvent was recalcitrant during ~1.5 yr incubation. To assess hydrocarbon degradation potential, Albian and Syncrude MFT samples were spiked with the two solvents individually. Syncrude MFT began producing methane from Syncrude solvent after only 6 weeks but exhibited a 24-week lag before the onset of methanogenesis from Albian solvent. Conversely, Albian MFT metabolized Syncrude solvent after a 43-week lag versus a 72-week lag to begin converting Albian solvent to methane. 16S rRNA gene analyses revealed that the microbial communities present in Syncrude and Albian MFT shared some phyla but additional members were detected in Syncrude MFT. The results of this study are important in understanding methanogenic biodegradation of low molecular weight hydrocarbons as well as predicting methane production and emissions from oil sands tailings ponds.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.034 ACCELERATED METHANOGENESIS FROM ALIPHATIC AND AROMATIC HYDROCARBONS UNDER IRON AND SULFATE REDUCING CONDITIONS

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The impact of oxidants on hydrocarbon induced methanogenesis was studied. To convert hydrocarbons into methanogenic substrates, conditions to overcome the rate-limiting first hydrocarbon oxidising step are required. Thus, a high redox potential was created by addition of oxidants. Four model hydrocarbons - butane, hexadecane, ethylbenzene and naphthalene - were investigated. Hydrocarbon induced methanogenesis was observed in samples from three different locations which underscores that this pathway is a general process. Methane was released most rapidly from incubations treated with ferrihydrite and hexadecane. Ferrihydrite enhanced only alkane degradation while methanogenesis from ethylbenzene was dependent on sulfate concentration. Methane was removed in concert by anaerobic methanotrophy. The experiments presented here demonstrate the feasibility of microbially enhanced oil recovery (MEOR) by ferrihydrite or sulfate addition.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.035 DIVERSITY OF THE BACTERIAL COMMUNITY IN SHALE SAMPLES FROM A THERMOGENIC NATURAL GAS FORMATION

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The presence of indigenous microbial communities in many deep subsurface oil and natural gas reservoirs has long been recognized. However, access to these communities in such reservoirs has often been hampered by contamination introduced during various stages of operation. As a result, it is often difficult to determine whether communities identified are autochthonous or allochthonous to an oil and natural gas reservoir. Here we present a 16S rRNA gene-based pyrosequencing survey of shale samples obtained during the drilling process from seven different wells in the Barnett Shale, a thermogenic natural gas producing shale of economic relevance in north central Texas. The shale was formed thermogenically during the Mississippian age (320-360 million years old), is present at depths of 6500-6800 feet, and has a current in-situ temperature of approximately 90°C. Samples were obtained from multiple locations with gas:oil ratios and vitrinite reflectance values ranging from 1 to 10,000 mscf/bbl and 0.9 to 1.3%, respectively. A total of 396,000 sequences were obtained and analyzed. Although different shale samples were highly dissimilar at the putative species level, all samples had a core community at the class/phylum level of thermophilic Firmicutes (Bacilli, Clostridia, Thermoanaerobacter, Desulfotomaculum, and Syntrophomonadaceae), and sulfur metabolizing Epsilonproteobacteria. In addition, members of the Thermotoga, Hydrogenophilales, Thermales, Thermovibrionales, and Thermoacetogenium were identified in minor proportions in some samples but not others. Distinct correlations between the presence/absence of specific taxa and the level of gas maturation, gas:oil ratios, vitrinite reflectance and paleo-temperatures were observed. This study represents the first diversity survey of a thermogenic natural gas formation and convincingly demonstrates the repopulation of thermogenic petroleum formations upon cooling to temperatures that are habitable to microorganisms.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.036 ANAEROBIC BIODEGRADATION OF BIODIESEL AND CORROSION

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Biodiesel is composed of mono alkyl esters of long chain fatty acids derived from renewable lipid sources. It burns much cleaner than petroleum diesel, with about 60% less net carbon dioxide emissions, and it is produced domestically. While they are chemically and physically stable fuels, their susceptibility to biological attack in the absence of oxygen is underexplored.

Consequences of the storage/use of biodiesel fuels are examined by incubating five different marine and freshwater anaerobic inocula with pure biodiesel: 1) inoculum from a contaminated aquifer, 2) a defined alkane-degrading methanogenic bacterial consortium enriched from the same aquifer, 3) marine oil-degrading enrichment from the sunken remains of the USS Arizona in Pearl Harbor, HI, 4) an unenriched sample from a seawater compensated ballast tank aboard the USS Gettysburg and 5) natural Key West, FL, coastal seawater. Microbial metabolism of the biodiesel was evidenced by sulfate-reduction or methanogenesis, analyzed by ion or gas chromatography, respectively.

All inocula could consume sulfate or produce methane in ≤ 1 month in biodiesel-amended incubations. Within the same time period, biocorrosion of carbon-steel coupons was evident in the coastal seawater incubations, which indicated the direct link between biodegradation and corrosion. Gas chromatography/mass spectrometry revealed that the methyl esters of biodiesel were readily hydrolyzed by the various inocula and a complex but similar suite of fatty acids were found as metabolic intermediates.

Despite the global acceptance of biodiesel, the full impact of integrating this alternative fuel with the existing infrastructure has not been fully explored. This study showed that biodiesel can be rapidly biodegraded under anaerobic conditions by diverse microbial inocula and this metabolism facilitates the corrosion of unprotected carbon steel; the most common metal used throughout the fuel infrastructure.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.037 ANAEROBIC OXIDATION OF METHANE IN THE TERRESTRIAL SUBSURFACE ENVIRONMENT HARBORING FRESHWATER

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Anaerobic oxidation of methane (AOM) is one of the important processes involved in the global cycle of carbon. Microorganisms involved in AOM in marine sediments have been extensively studied, and various groups of methanotrophic archaea (ANME) are known to be involved in AOM in marine sediments. Although AOM activity has also been reported in several terrestrial environments, little is known about microorganisms involved in the process. Recently, '*Candidatus* Methyloirubis oxyfera' has been found to mediate AOM using nitrite (1). In addition, ANME-related 16S rRNA genes have been detected in some terrestrial environments, however, it is not still clear if ANME-archaea is active and involved in AOM especially in the terrestrial subsurface harboring freshwater. In this study, we aimed to reveal AOM driven by ANME in terrestrial subsurface environments. We obtained sediment core samples in the Kanto Plain, Japan, and conducted molecular ecological analyses (community analysis based on DNA and RNA, real-time PCR) and activity measurement using a stable isotope tracer. Potential activity measurement using ¹³C-methane revealed a continuous increase in the ¹³C content of dissolved inorganic carbon during 4 months of incubation under anaerobic condition. Methane oxidation was inhibited by the addition of nitrate, suggesting that AOM is not associated with denitrification. RNA-based community analyses using 16S rRNA gene revealed that ANME-related archaea was the dominant group in the active archaeal community. We present AOM activity possibly driven by ANME-related archaea in the terrestrial subsurface environment harboring freshwater for the first time. At least in the studied area, ANME have been widely detected. This suggests the possibility that the role of AOM in the methane flux in terrestrial subsurface has been underestimated. (1) Ettwig K.F. et al. 2010. Nature 464: 543-548.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.038 NOVEL FINGERPRINT TECHNOLOGY TO ASSESS THE DIVERSITY OF METHANOGENS

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Methanogens are important in the microbial ecology of anoxic and engineered environments. A novel high-throughput fingerprint technology was developed to determine their diversity based on the functional *mcrA* gene encoding the alpha-subunit of the methyl coenzyme M reductase, which is an enzyme involved in the terminal step of methane production by methanogens. This gene is known to have a phylogenetic marker. The technology described here, named amplicon length heterogeneity-*mcrA* (LH-*mcrA*), is based on the natural length variations in the *mcrA* gene that allows discriminating the methanogenic groups. Sequenced *mcrA* gene clones from libraries were screened to identify the LH-*mcrA* peaks from dairy and swine manures. This novel LH-*mcrA* technique revealed the diversity of several methanogenic groups: *Methanobrevibacter*, *Methanosarcinaceae*, *Methanoculleus*, *Methanogenium*, *Methanocorpusculum*, and one uncultivated group. The advantages of using LH-*mcrA* instead of T-RFLP targeting the 16S rRNA gene are that: 1) there is only one gene copy in the genome of the methanogens (unlike the 16S rRNA gene); 2) *Methanoculleus* spp. can be distinguished from *Methanosarcinaceae*; 3) The technique is less expensive and less time-consuming than T-RFLP; and 4) The main advantage is that relative abundances of the transcripts can be investigated using this novel technology by reverse transcription LH-*mcrA* (RT-LH-*mcrA*). The relative abundance of mRNA coding for the *mcrA* functional gene should more appropriately reflect the metabolic activity of specific methanogenic groups than would the abundance of 16S rRNA molecules.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.039 ABUNDANCE AND DISTRIBUTIONS OF PELAGIC METHANOTROPHS ALONG THE EASTERN PACIFIC CONTINENTAL MARGIN

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The spatio-temporal behavior of methane in the ocean is highly dynamic. Most methane released from the seabed is biologically oxidized before emission to the atmosphere, however the abundance and distribution of the planktonic microorganisms mediating methane oxidation are poorly understood. Attempts to cultivate representative species have met with limited success, necessitating the use of molecular approaches to characterize these groups. We have analyzed water column samples from a range of methane seep and non-seep sites along the California continental margin, and through oxygen minimum zones along the Costa Rica margin. Using molecular assays targeting either *pmoA*, the functional gene central to aerobic methane oxidation, or 16S ribosomal genes affiliating with gammaproteobacterial methanotrophs, we have identified putative planktonic methanotrophs (termed OPU1 and OPU3). Analysis of these groups included real-time exonuclease PCR assays (taqman assays), as well as population-profiling via amplification of the noncoding spacer between *pmoC* and *pmoA*. Above active seeps, OPU1 and OPU3 are observed in *pmoA* surveys as well as in 16S gene surveys at abundances approaching 1000 copies per milliliter seawater, suggesting that these clades represent an important pelagic methane biofilter. These phylotypes are generally widespread at significantly lower abundance along the California continental margin, and appear constrained to depths below 200 meters. Two distantly-related *pmo* clades (Groups W and X) have been recovered above methane seeps; a third distantly-related clade (Group Z) has been recovered from intermediate depths in non-seep sites. Thus, potential exists for phylogenetic diversity in biological methane cycling in the oceans. Together, these molecular methods are providing insight into the abundance and distribution of microorganisms participating in the methane cycle through many environments in the world's oceans.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.040 CHARACTERIZATION OF ARCHAEAL COMMUNITY BY 16S rRNA CLONE LIBRARIES AND CULTURE-DEPENDENT ISOLATION OF METHANOGENIC Archaea IN AN AMAZON WETLAND SOIL

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The tropical wetlands are the major natural sources of methane into the atmosphere, contributing about 60% of all natural emissions. The great wetlands areas of the Amazon basin are the largest sources of methane in this region, and it is estimated that their contribution to total emissions of flooded areas in the world is 5%. However, studies of the archaeal community composition in these environments are scarce. In this survey, four 16S rRNA archaeal clone libraries from different depths were constructed to examine the archaeal community in an Amazon wetland soil. A total of 599 clones were used to perform diversity and phylogenetic analysis. A wide archaeal community diversity was observed in the site, with diversity decreasing with an increasing of depth (*Shannon* index range: 2.40-1.94). Phylogenetic analysis revealed sequences belonging to two archaeal phylum, being classified 65% *Crenarchaeota* and 35% *Euryarchaeota*. Inside *Euryarchaeota* group, most sequence was clustered into *Methanococci* and *Methanomicrobia* classes, which comprehend organisms related to methanogenesis. In a second moment, based on abundance of methanogenic clones, culture-dependent isolation was performed to retrieve these organisms. A modified-atmosphere (H₂:CO₂, 80:20) in combination with an anoxic environment for enhancing the methanogenic *Archaea* growth was established during 18 months. Among the isolates, the genus *Methanosarcina* and *Methanobacterium* were identified, indicating its possible role on the methane production, detected throughout the anaerobic *in vitro* cultivation. In conclusion, these explorative molecular and culture-dependent approaches enhanced the understanding about archaeal community and methanogenic *Archaea* living in wetland soils from Eastern Amazon, suggesting important roles on methane production.

Support: FAPESP, CNPq

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.20.041 FIELD AND LABORATORY STUDIES ON THE BIOCONVERSION OF COAL TO METHANE

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Coal-bed methane is an important source of natural gas, but relatively little is known about the mechanisms of methane formation. In the northern San Juan basin, it has been debated whether the gas isotopic signature is: (a) solely thermogenic or (b) a mixture of early thermogenic and late-stage secondary biogenic methane. An investigation was conducted to gain insight on the processes governing the bioconversion of coal to methane. Production water was collected from coal-bed methane-producing wells for DNA extraction, enrichment studies, geochemical analyses and metabolite profiling. Community DNA was hybridized to a functional microarray (GeoChip 3.1) to survey gene diversity. Groundwater samples served as inocula for methanogenic enrichments amended with coals of differing thermal maturities. Putative metabolites were analyzed via gas chromatography-mass spectrometry. Coal enrichments demonstrated the widespread occurrence of methanogens and consortia capable of converting coal to methane, and GeoChip analysis confirmed the formation-wide presence of *mcrA* genes. Evidence for anaerobic hydrocarbon and short-chain fatty acid metabolism was relatively scarce, but some preference for low molecular weight alcohol conversion to methane was noted. Methane production rates were negatively correlated with coal

thermal maturity. The relatively high maturity range of the San Juan basin coals suggests a high level of aromatization and recalcitrance to biodegradation. However, metabolite profiling suggested that at least some aromatic compounds were biodegraded. Endogenous rates of coal methanogenesis ranged from 0.002 to 0.021 $\mu\text{mol} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$ for the Fruitland Coal at $R_o=0.87\%$. The collective integration of gas, coal, and microbial data confirm significant biogenic methane formation, resulting in mixed gases in the north. Differing coal maturities also play a role in the natural rate of methanogenesis.

Abstract Category

20 Microbe Interaction with the Production of Methane and CO₂

PS.21.001 PHENOTYPE PROFILING AND PROTEOMIC ANALYSES OF AN ENVIRONMENTALLY PERSISTENT ESCHERICHIA COLI ISOLATED FROM AN IRISH SOIL

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An understanding of the survival capacity of *Escherichia coli* in soil is critical for the evaluation of its role as an indicator of faecal pollution in the environment. Recent reports that *E. coli* can become long-term residents in Irish soils have raised the question of how the organism survives and competes for niche space in the sub-optimal cold soil environment. When compared to laboratory strains, environmental persistent isolates investigated displayed a rapid growth phenotype both at 15°C and 37°C. This work aimed to characterise one such environmental isolate using both phenotypic microarrays and proteomics. The ability of the environmental isolate to utilise 384 substrates was assessed and compared to that of a reference strain (*E. coli* K12) at both 15°C and 37°C. At 15°C, the environmental strain could utilise 139 of the 384 substrates tested with only 69 usable by the reference strain. A significantly greater rate of respiration of the environmental strain was observed for 119 substrates, while *E. coli* K12 showed a greater rate of respiration for only 6 substrates. The cold response of the two strains was evaluated using proteomics. For the environmental isolate, 13 proteins were identified as being differentially expressed at 15°C and 37°C with only one protein expressed at a higher level at 37°C, and the remaining 12 being more expressed at 15°C. For the reference strain however, 8 proteins and 6 proteins were found to be more expressed at 37°C and 15°C, respectively. Overall *E. coli* K12 reduced the expression of biosynthetic proteins to adapt to the low growth rate observed at 15°C, while the environmental isolate seemed to switch on proteins involved in stress response. The results indicate that the environmentally persistent *E. coli* is very well adapted to use a wide range of nutrient sources and to direct its protein expression to maintain a relatively fast growth rate at a low temperature.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.002 HIGH THROUGHPUT *IN SITU* CULTIVATION OF ENVIRONMENTAL MICROORGANISMS

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Although the recent advances in molecular based approaches, it is certain that cultivation is still necessary for comprehensive understanding of the physiology and ecology of environmental microorganisms. We developed a new method for cultivation of environmental microorganisms. By combined use of hollow fiber membrane chamber (HFMC), Gel Micro Droplet (GMD), and cell sorting techniques, the method enables high throughput cultivation under in situ conditions, and small enough to use for in vivo cultivation. Dual Gel HFMC, a key device, consists of a hollow fiber membrane and extensive number of (10^{6-7}) small (20 - 50 µm) agarose gel particle

(GMD) which initially contains 0 or 1 microbial cell per GMD. GMDs in HFMC are surrounded by different type gel matrix such as alginate gel. The in situ cultivation is conducted by setting the device in natural environment. Thus, they can interact with external organisms such as other microbes. After the cultivation, GMDs are applied to the flow cytometric cell sorter. Individual GMDs containing micro-colonies are selectively sorted to multi-well plate or agar plate followed by the secondary cultivation. As a result of cultivation of environmental microorganisms, such as soil microbes, the new method showed much higher cultivability and diversity compared with that in agar-plate cultivation. Interestingly, although much lower cultivability was appeared in the agar-plate cultivation than in situ, much more proportion of microbes initially grew in the in situ cultivation recovered in the conventional cultivation as a secondary cultivation. This indicated that some microbes which are originally recalcitrant to cultivation in conventional methods at first, turned into cultivable types after the in situ cultivation. Above all, the newly designed cultivation method is powerful methods for cultivation of various type environmental microorganisms and clarification of their physiological properties.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.003 EXPERIMENTAL EVIDENCE OF AN ORGANELLE-ASSOCIATED ATP SYNTHASE IN ANAMMOX BACTERIA

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Anammox (anaerobic ammonium oxidation) bacteria are important players in the global nitrogen cycle, but have also received particular attention during optimization of waste water treatment. Previous studies of key catabolic enzymes indicate that the anammox reaction takes place inside the anammoxosome, an organelle-like membranous compartment of anammox bacteria. During anammox, a proton gradient is established across the anammoxosome membrane. A lipid-based protein immobilization technique, previously used to identify key proteins of the anammox reaction, was in this study used to determine linear epitopes for antibodies specifically targeted against the identified ATP synthase. Immunocytochemical analysis followed by electron microscopy (immunogold EM) demonstrated an anammoxosome-associated ATP synthase. An ATP synthase associated with an intracellular compartment of a non-photosynthetic prokaryote has previously never been demonstrated.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.004 DISCRIMINATION OF MICROORGANISMS USING SURFACE-ENHANCED RAMAN SPECTROSCOPY

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We used surface-enhanced Raman spectroscopy to develop spectra for single, live *Escherichia coli* (DH5 α) suspended in minimal media with silver nanoparticles. The Raman signature we observed was consistent with data in the literature for aromatic amino acids and nucleic acid bases previously observed for *E. coli*, including the identification of tryptophan, guanine and adenine. The Raman signature appears to change as a function of time of exposure to Ag nanoparticles and laser power suggesting perhaps modification of the cellular membrane. We collected data for *E. coli* prepped on alternate growth media as well as for other microbial species (*Cytophaga hutchinsonii*, *Cellulomonas biazotea*, *Streptomyces griseus*) grown on the same media in order to discern whether the different species can be differentiated by Raman spectroscopy or if it is only a function of the growth medium.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.005 GLYCOGEN ALLOCATION EFFECTS REPLICATIVE LIFESPAN IN *E. COLI*

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Recent studies reported that aging - the deterioration of an organism's condition with increasing age - affects not only eukaryotes, but is also found in bacteria. This raises the question whether some of the processes underlying aging are shared between bacteria and eukaryotes, and are thus maybe common to all life forms. One process that could potentially be shared is the effect of nutrition in aging. It is known that the modulation of carbohydrate consumption and storage has serious consequences on the lifespan of many different eukaryotic organisms. We wanted to know whether nutrition also affects lifespan in bacteria. We found that changes in the carbohydrate metabolism indeed have a dramatic effect on lifespan of *E. coli*. A mutation in the carbon storage regulator gene *csrA* leads to an asymmetric glycogen accumulation at the cell poles. Cells with an old pole show a strong decrease of their reproductive rate resulting in a complete stop of cell division after short time. Fluorescent DNA staining implies that a loss of the genome is the reason for this short replicative lifespan. These results show that nutrition can also affect lifespan and aging in bacteria, but it also reveals that the molecular processes underlying this link can differ strongly between different types of organisms.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.006 GENOMIC AND PHYSIOLOGICAL INSIGHTS INTO THE CONVERSION OF PLANT LITTER TO ETHANOL BY A FOREST SOIL BACTERIUM, *CLOSTRIDIUM PHYTOFERMENTANS*

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Clostridium phytofermentans, an anaerobic bacterium isolated from forest soil near the Quabbin Reservoir in Massachusetts, U.S.A. is an emerging genetic model distinguished by its capacity to productively degrade and metabolize diverse plant polysaccharides to biofuels. Taxonomically within the Clostridia cluster XIVa, *C. phytofermentans* is closely related to uncultured bacteria in rice paddy soils, hot composting soils and growing within the gut of the composting earthworm, *Eisenia foetida*. These carbon rich environments harbor communities of metabolically diverse microbes important in microbial carbon cycling, but the details of the microbial interactions within these communities is largely unknown. Here, we investigate the unique properties of *C. phytofermentans* through analysis of its complete genome sequence and expression profile on key polysaccharides and plant litter. *C. phytofermentans*'s saccharolytic versatility is reflected in a remarkable variety of genes encoding glycoside hydrolases, with the notable absence of an extracellular protein complex (i.e. cellulosome) typical of most other cellulolytic Clostridia. Cellular uptake of diverse carbohydrates is likely mediated by numerous ATP-binding cassette (ABC)-family sugar transporters. Compared to other Clostridia, *C. phytofermentans* has a large number of transcriptional regulators for controlling switches in substrate use. The energetics responsible for the production of ethanol as the primary product appear to involve novel hydrogen signaling and metabolic complexes as well as unusual combinations of proton/sodium pumping complexes. These attributes make *C. phytofermentans* an ideal organism for studying direct conversion of plant litter to biofuels and other products. Studies involving samples isolated from the Harvard Forest's Long Term Ecological Research Site will enable the integration of the molecular and physiological data into a broader context

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

**PS.21.007 COMPARISON OF AEROBIC AND PHOTOSYNTHETIC
CHLOROFLEXUS AURANTIACUS J-10-FL PROTEOMES**

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Chloroflexus aurantiacus j-10-fl is a thermophilic green Filamentous Anoxygenic Phototrophic (FAP) bacterium that naturally forms microbial mats with cyanobacteria in hot springs. This bacterium is capable of growth as an anoxygenic photoheterotroph, a photolithoautotroph, and an aerobic chemoorganoheterotroph in the dark. Its unique photosynthetic apparatus and use of 3-hydroxypropionate pathway for CO₂ fixation are novel among phototrophic bacteria.

We quantitatively analyzed the proteomes of both oxic and anoxic, photoheterotrophic *C. aurantiacus* cell cultures, using liquid chromatography-mass spectrometry and the accurate mass and time (AMT) tag approach. Our global analysis identified approximately 13500 high

confidence peptides that matched to 1286 annotated proteins, which corresponded to 33% coverage of predicted proteins. 52 proteins were uniquely detected from the photoheterotrophically grown culture. An example of a unique protein in phototrophically grown cells is protein Caur_0133, which is a hypothetical protein for which 16 peptides were identified. A comparison of protein Z scores calculated from the estimated abundance between oxic and anoxic cultures revealed that proteins in 8 of 26 COG functional categories exhibited the largest differences between the two cultures. In particular, we identified previously characterized chlorosome proteins, proteins involved in the bacteriochlorophyll biosynthesis, 3-OHP pathway, and components of electron transport. Additionally, ~100 proteins were identified that were only observed in or increased in expression in anoxically grown cells. These proteins had not previously been reported to be involved in photosynthesis.

Our results highlighted proteins directly associated with the photosynthetic lifestyle of *C. aurantiacus*. This study also provided a list of protein candidates that may prove useful in deciphering the photosynthetic mechanism in this and other model organisms.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.008 FUNCTIONAL CHARACTERIZATION OF CRP/FNR IN *DESULFOVIBRIO VULGARIS* HILDENBOROUGH

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There are four Crp/Fnr homologues (DVU2547, DVU0379, DVU3111 and DVU2097) in the model sulfate reducing bacteria *D. vulgaris* Hildenborough. In order to understand the roles of these four predicted global regulators, knockout mutants for each of these four genes were generated. The functions of these regulators were investigated via whole genome transcriptional profiling array, physiological tests, growth under different stresses and competition of mutants. Compared to WT, the highest numbers of significantly up- or down-regulated genes were found in the DVU3111 knockout mutant, suggesting that DVU3111 may play an important role in gene regulation. Consistent with the microarray data, the involvement of these four regulators in various stress responses were supported by the more resistant growth phenotype of these four knockout mutants to increased nitrite or NaCl in the growth medium. In addition, the Cr(VI) degradation ability was studied with washed cells. The results suggested that DVU0379 and DVU2547 may play more important roles in chromium reduction. Finally, competition experiments of these four knockout mutants under salt stress (100 mM NaCl) and oxidative stress (0.5 mM H₂O₂) was conducted. Under both stress conditions, the knockout mutant of DVU3111 mutant showed the lowest population in the mixture, indicating that DVU3111 may be the most important regulator in controlling NaCl and H₂O₂ stress responses. These results

indicate that those four Crp-Fnr genes may play different roles in response to different environmental stresses.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.009 IDENTIFICATION OF METABOLIC PATHWAYS AND REGULATORY NETWORKS ASSOCIATED WITH THE UTILIZATION OF LIGNIN DEGRADATION PRODUCTS

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Identification of sensory and regulatory pathways that respond to environmental nutrients would facilitate prediction of the cellular response to the environment. For many organisms, realization of this goal is limited an incomplete knowledge of the proteins involved in sensing the environment and their connections to metabolic and regulatory networks. We evaluated an approach to remove this roadblock by characterization of transporter proteins which are an organism's primary interface with the environment. We determined the ligand-binding specificity of the genome set of ABC transporters (n = 117) for *Rhodospseudomonas palustris* CGA009 using ligand libraries consisting of common environmental and metabolic compounds and high throughput binding screens. To date, we have assigned specific binding ligands for approximately 60% of the purified and screened proteins. In most cases, the binding was observed for specific compound classes and was observed for only 1-3 compounds from the entire ligand library. Several of these transporters were demonstrated to transport aromatic compounds known to be degradation products of lignin in the soil environment. This capability for utilization of lignin degradation products was then linked to cellular metabolic pathways and regulatory networks by identification of enzymes and effector molecules for transcription factors whose activity is mediated by the environmental ligands or their metabolic derivatives. The outcome of this directed characterization strategy is the functional characterization of specific proteins and networks that mediate the utilization of lignin degradation products in *R. palustris*. The functional assignments and ability to define specific sensory and regulatory pathways will increase the predictive capability of current models and support the development of predictive systems-level models.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.010 DEEP TRENCH SINGLE CELL GENOMICS

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Deep ocean trenches are characterized by extreme hydrostatic pressure, low temperature, and reduced and altered dissolved and particulate organic carbon. Our current knowledge of psychropiezophilic (low-temperature and high-pressure adapted) microorganisms is limited to

cultivated isolates from a narrow phylogenetic grouping, despite a high diversity of bacterial phyla identified through molecular surveys. In this study, single cell genomics was employed to elucidate aspects of the genomic repertoire of four individual cells collected from a depth of 6,000 m within the Puerto Rico Trench. Fluorescence-activated cell sorting (FACS) followed by multiple displacement amplification (MDA) and 16S rRNA gene screening resulted in the identification of four cells of interest from the phyla *Planctomycetes*, *Bacteroidetes*, *Alphaproteobacteria*, and *Gammaproteobacteria*. Paired-end libraries were constructed and sequenced using 454 FLX technology. After chimeric sequence filtering and assembly, genome recovery estimates ranged from 1-11% based on both the number of predicted open reading frames (ORFs) and the average genome size of the closest sequenced relative. The total numbers of predicted ORFs are as follows: *Planctomycete* (189), *Bacteroidete* (260), *Alphaproteobacterium* (525), and *Gammaproteobacterium* (246) with an average 75.6% with functional annotations. We have successfully recovered a greater number of predicted proteins from a single microorganism than would be recovered with large-insert environmental genomic libraries, despite low estimated whole genome recovery. Analyses of gene content using COG, KEGG, and pfam databases enabled biological inferences of metabolic potential and physiochemical adaptations to the trench environment. Comparisons to environmental genomic data from other deep ocean sites, as well as surface seawater, will be presented to lend perspective into the diversity, physiology and adaptations of deep ocean microorganisms.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.011 A COMPARISON OF QUANTIFICATION AND NORMALIZATION APPROACHES TO QRT-PCR ASSAYS IN SOIL

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Of the two widely used qRT-PCR methodologies- absolute and relative, the application of latter to quantify microbial gene expression in soil has been hampered by various technical considerations. The unavailability of a suitable exogenous control(s), robust enough for a complex matrix like soil, remains the principal obstacle. Presently, we have tried to address this technical bottleneck by using an artificial gene (*MyIC*) as an exogenous spiking control and have also investigated normalization strategies on absolute quantification. In a controlled experimental setup, non-sterile field soil was inoculated with five different concentrations of *Pseudomonas* spp. LBUM300, capable of synthesizing 2,4-diacetylphloroglucinol (DAPG) and hydrogen cyanide (HCN). Total soil RNA was extracted 0, 7 and 14 days post-inoculation and, using a two-step TaqMan assay, the *phlD* (for DAPG), and *hcnC* (for HCN) gene expression was monitored. For relative quantification, *in vitro* synthesized *MyIC* RNA template was spiked during the RNA extraction procedure. In general, transcripts of both genes were detected for all five dilutions for up to 14 days; however the *phlD* transcript copy numbers detected were lower than *hcnC*, regardless of initial bacterial concentration or sampling date. Interestingly, normalization to total RNA did not significantly impact absolute quantification. The *MyIC* external spiking control was detected consistently following RNA extraction, facilitating relative quantification of both genes. Due to the inherent higher expression of *hcnC*, the sensitivity of the relative assay was lower for *phlD*, especially at lower bacterial concentrations. This suggests that

soil transcripts present in very low numbers are probably more reliably quantified with absolute quantification, whereas the relative approach is more robust and a faster alternative for highly expressed transcripts. Summarily, a generalized approach for quantifying microbial gene expression in soil is presented.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.012 EFFECTS OF SOIL CLAY CONTENT AND RNA EXTRACTION PROTOCOL ON QUANTITATIVE MICROBIAL GENE EXPRESSION IN SOIL USING QRT-PCR

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The use of quantitative real time polymerase chain reaction (qRT-PCR) to analyse microbial gene expression in soil has been increasing in interest. However, the effect of physical and chemical soil parameters, such as clay content, on microbial gene expression must be better understood to use this technique to its full potential. Numerous RNA isolation protocols have been developed for soil but no consensus has yet been reached as to which ones offer high efficiency under a large spectrum of soil conditions. This study examined the effect of clay content on RNA isolation and subsequent quantification of microbial transcripts using qRT-PCR. Three field soils with low, medium and high clay contents were inoculated with *Pseudomonas* spp. LBUM300 and RNA was extracted after 0 or 7 days. Four published protocols and two commercially available RNA isolation kits were used to isolate RNA from soil and the *hcnC* gene transcript, involved in hydrogen cyanide production, was quantified. The total amount of isolated RNA, as well as the number of *hcnC* gene transcripts detected, was significantly affected by the soil clay content, the RNA extraction protocol, and the interaction between both factors. In general, higher clay content lead to fewer quantities of RNA or fewer detected *hcnC* gene transcripts. Interestingly, the number of *hcnC* gene transcripts detected was in general not correlated to the amount of RNA extracted, suggesting that RNA purity is a more important factor than the quantity extracted. In conclusion, recommendations are made as to which protocol seems particularly well adapted to isolate microbial RNA in sufficient quantity and purity to be used in qRT-PCR amplifications in soils varying in clay content.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.013 GENOMIC ANALYSIS OF THE THERMOPHILIC AMMONIA-OXIDIZING ARCHAEON *NITROSOCALDUS YELLOWSTONII* HL72

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Ammonia-oxidizing archaea (AOA) are found in a wide range of habitats and likely play important roles in global carbon and nitrogen cycling. To date, only two AOA genomes have been completed, both of which belong to the narrow subset of mesophilic marine AOA. Based on these data, whole genome comparisons suggest that the AOA constitute a third major lineage within the *Archaea*, the *Thaumarchaeota*. Recently we described the cultivation of a deep-branching thermophilic representative of the AOA, *Nitrosocaldus yellowstonii* HL72. Here we present our analysis of the genome of HL72. The current genome draft is composed of 10 contigs comprising ~1.43 Mbp and encodes 1613 predicted genes, 23% of which are unique to HL72. The genome is 91% coding with an average GC content of 37%. We identified single copies of both the ribosomal RNA genes and the genes encoding the putative archaeal ammonia monooxygenase (*amo*). HL72 grows chemolithoautotrophically and appears to use the recently described 3-hydroxypropionate/4-butyrate pathway for carbon assimilation. There is no evidence of an identifiable homolog of the bacterial hydroxylamine oxidoreductase, supporting the proposal of an alternative pathway for ammonia oxidation in AOA. Like other AOA, HL72 appears to lack cytochrome *c* proteins; rather, it has five plastocyanin-domain proteins and two multi-copper oxidases, suggesting a copper-based electron transport chain. Genomic comparisons between the AOA and other archaea have identified 191 orthologs present in all thaumarchaea, including the archaeal *amo* genes and four plastocyanins, emphasizing the uniqueness of the respiratory chain. The AOA core genes also include five transcriptional regulators, transcription initiation factor TFIIB (TFB), and topoisomerase 1B, currently found only in eukaryotes and members of the *Thaumarchaeota*. Our study provides support for the division *Thaumarchaeota* and clarifies the genes that define this group by analyzing the most basal genome yet.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.014 QUANTIFYING TOXIGENIC AND TOXIC *MICROCYSTIS AERUGINOSA* WITH RING-FISH

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The frequency, intensity and extension of potential toxic cyanobacterial blooms is increasing due to global warming and eutrophication. As these mass events also develop in drinking water reservoirs, recreational lakes and cattle watering tanks it is of high interest to quantify toxigenic and toxic cyanobacteria and to learn more about factors which induce toxicity. We applied recognition of individual genes fluorescence in situ hybridization (RING-FISH) to quantify cells of toxigenic *Microcystis aeruginosa* strains by using a polynucleotide probe matching with the microcystinsynthetase gene D. By changing hybridization time and buffer content we could also quantify toxic cells with the gene transcript. This method in combination with flow cytometry provides the opportunity to also quantifying toxigenic and toxic cyanobacteria in a high-throughput manner. In future, it might be advantageous to use the whole cell of toxigenic or toxic cyanobacteria for applying advanced phylogenetic analysis (e.g. whole genome sequencing) after single cell sorting by fluorescence activated cell sorting (FACS). The RING-FISH method for

analyzing toxigenic and toxic cyanobacteria in combination with FACS is a rather powerful tool with several advantages compared to quantitative real-time PCR.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.015 GENOME WIDE LOCALIZATION ANALYSIS OF BPHT1 BINDING REGION ON *Rhodococcus jostii* RHA1.

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Rhodococcus jostii RHA1 was grown on a broad range of aromatic compounds and vigorously degrades polychlorinated biphenyls (PCBs). Two-component signal transduction system, BphT1T2, is necessary for activation of biphenyl degradation genes in *R. jostii* RHA1. Furthermore, transcriptome analysis of *R. jostii* RHA1 revealed that more than 1,000 genes were up-regulated in the cell grown on biphenyl compared with pyruvate grown cell. These results indicated that BphT1S1 activate many genes in addition to biphenyl degradation genes. In this study, we performed global chromatin immunoprecipitation-on-chip (ChIP-chip) analysis to identify novel BphT1 binding region in *R. jostii* RHA1. Cells were exposed on 10 mM biphenyl in 1 hour, formaldehyde was added to crosslink DNA-protein complex. BphT1 antibody was added for chromatin immunoprecipitation. Genomic DNA was used as a control for microarray hybridization. After 1 h, BphT1 activating known-promoter region, P_{bphA1}, and P_{etbA1}, were detected on ChIP-chip analysis as BphT1 binding region. Interestingly, 114 regions were detected as novel bphT1 binding regions across RHA1 genome. We also compared expression data from cells grown on biphenyl. As a result, we identified 9 ORFs as direct target of BphS1T1, which is included IclR type transcriptional regulator, possible endonuclease, and some hypothetical proteins. This result indicated that BphS1T1 is a master regulator of biphenyl degradation gene, and directly activated to transcriptional regulator and endonuclease to regulate expression level of many ORFs in RHA1. We are now characterizing direct target of BphS1T1, and also doing BphT1S1 gel mobility shift assay to demonstrate whether BphT1 bind to these regions in vitro.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.016 THE REGULATION OF THE MICROCYSTIN ENCODING GENE CLUSTER IN MICROCYSTIS

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Microcystis is a major toxic bloom-forming cyanobacterium. Toxic populations produce an array of secondary metabolites including the microcystins. These are a family of cyclic heptapeptides produced in various cyanobacteria by a complex microcystin synthetase, the mcy cluster. They

are known to inhibit protein phosphatases PP1 and PP2 in eukaryotes. They were shown to act as infochemicals, their release to the water body by lysing cells reports to the rest of the cells that the population is under stress. It has also been suggested that microcystins and other metabolites may play a role in interspecies communication. Here we show that the expression of a range of genes within the mcy cluster varies differentially in response to biotic and abiotic conditions. Our results provide better understanding of the interspecies interactions and cross-talk mechanisms within *Microcystis* populations.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.017 BIAS IN NYCODENZ EXTRACTION FROM BULK SOIL ANALYSED BY AMPLICON HIGH THROUGHPUT SEQUENCING

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Separation of bacterial cells from soil using Nycodenz density gradient centrifugation is a widely used method in microbial ecology. We hypothesized that more loosely bound bacteria will be extracted more efficiently than other bacteria. Here we use GS FLX amplicon sequencing to examine the bias in Nycodenz extraction. The study was also used to examine the bacterial diversity in the soil studied. Bacterial cells were detached from bulk soil and separated by Nycodenz density gradient centrifugation. DNA was extracted from the primary soil, from the Nycodenz extracted cells and from the soil pellet left after cell extraction. All cell and DNA extractions were carried out from three replica fields in the CRUCIAL field trial in Taastrup, Denmark. Using primers 350F and 1061R 16S ribosomal DNA was amplified from each sample and sequenced using a modified GS FLX amplicon sequencing protocol. Sequences were sorted, trimmed, clustered and classified using the Ribosomal Database Project. Each sample yielded 23,476 sequences. Comparison of clusters and rarefaction curves for the different extractions clearly showed a lower number of operational taxonomic units in sequences from Nycodenz extracted cells. After classification of the sequences an analysis of similarity at phyla level showed that the phyla composition was statistically significant ($R=0,992$, $p=0,004$) between extracted cells and primary soil. Analysis of variation showed that for some of the phyla found, the difference in the number of sequences extracted directly from the primary soil was statistically significant ($p<0,05$) from the number of sequences from the Nycodenz extracted cells. The results support our hypothesis because the majority of phyla being overrepresented in the Nycodenz extracted cells included many motile groups of bacteria. Both of the Gram-positive phyla (*Firmicutes* and *Actinobacteria*) were underrepresented in the extracted cells but no other trend was found between underrepresented phyla.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.018 CARBON AND ELECTRON OVERFLOW IN *CLOSTRIDIUM THERMOCELLUM* LQR1 GROWN UNDER CONDITIONS OF CARBON EXCESS

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Cellulosomes of cellulolytic clostridia have been the subject of considerable research, but few studies have focused on the carbon metabolic pathway. In this study, we explore the effect of nutrient excess on carbon and electron flow in cellulose-degrading, ethanol-producing *Clostridium thermocellum* LQR1, by metabolite measurements and transcriptomic analysis. When LQR1 was grown on cellobiose (29.2 mM) in DCB-1 media, fermentation of acetate, ethanol and lactate ceased immediately after entering stationary phase when cellobiose had not become limited. Extracellular accumulation of pyruvate (1.6 mM) and glucose (22.3 mM) was detected at the end of fermentation. Carbon recovery calculated from fermentation end products was 65.7% after reaching stationary phase. Accumulation of extracellular pyruvate suggests that carbon flow through pyruvate:fd oxidoreductase was a limiting step, and low carbon recovery may be due to an accumulation of other intermediate metabolites. Transcriptomic analysis revealed that expression of all genes in the glycolysis and TCA pathways were more than four-fold down-regulated during the shift from exponential to stationary phase, while expression of some genes involved in ammonia assimilation and amino acid synthesis, including ammonia permease, ABC-type urea transporter, urease, glutamine synthetase and glutamate synthase, were 7.3- to 104-fold up-regulated. LQR1 may employ a circular reaction between glutamine and glutamate to dispose of overflow carbon and reductant, and then excrete glutamate and pyruvate as overflow products into the extracellular environment, but further testing will be required to confirm this. LQR1 may have evolved in ecosystems where nutrition is limited by maximizing the production of acetate to enhance ATP generation and altering the kinetics of corresponding enzymes to facilitate metabolism at a low carbon flow. Therefore, carbon and electrons may overflow in LQR1 grown under conditions of carbon excess.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.019 SINGLE-CELL BASED GENOMIC AND STRUCTURAL ANALYSIS OF "*CAND. MAGNETOBACTERIUM BAVARICUM*"

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The giant uncultivated rod "*Cand. Magnetobacterium bavaricum*" was discovered in sediments of Lake Chiemsee (Germany). It is unusual with respect to its deep-branching affiliation with the *Nitrospira* phylum and the large number of bullet-shaped magnetosome crystals arranged in multiple chains. While magnetosome genes, and thus the mechanism of magnetosome formation, were shown to be universal among magnetotactic *Proteobacteria*, a different mechanism was postulated for the phylogenetically distinct "*Cand. M. bavaricum*". Thus, to investigate "*Cand. M. bavaricum*" magnetosome formation, we combined comprehensive ultrastructural- and

metagenomic analyses of magnetically enriched bacteria.

Using scanning electron microscopy in combination with focused ion beam milling, TEM thin sectioning and energy dispersive X-ray spectroscopy, we visualized 4-5 orbital-arranged magnetosome chains consisting of membrane-enveloped magnetite crystals. In contrast to previous reports, the presence of a magnetosome membrane points towards a conserved mechanism of magnetosome formation in "*Cand. M. bavaricum*" and other magnetotactic bacteria (MTB).

For genetic analysis of magnetosome biomineralization, we combined construction and analysis of metagenomic fosmid libraries with micromanipulation-based collection of individual cells that were subjected phi29-mediated whole genome amplification (WGA) and subsequent pyrosequencing. Fosmids corresponding to "*Cand. M. bavaricum*" were identified by mapping WGA-derived sequences onto metagenomic fosmid-derived sequences. We identified e.g. a 34kb genomic region of "*Cand. M. bavaricum*" that represents a putative magnetosome island harboring several clustered genes, which are homologous to proteobacterial magnetosome genes. Thus, structural and genetic evidence strongly suggests a universal mechanism of prokaryotic magnetosome formation, which is based on horizontal gene transfer between *Proteobacteria* and the *Nitrospira* phylum.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.020 UNDERSTANDING ECOLOGICAL FUNCTIONS OF INDIVIDUAL MEMBERS OF COMPLEX MICROBIAL COMMUNITIES

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Rapid advances in modern molecular methods such as the whole genome community sequencing approach open new ways to study microbial ecology. While application of the high-throughput sequencing could result in a blueprint of genomic content of the ecosystem of interest, generally it provides little information about ecological significance of the newly detected functions. To truly understand the role of microbes in the environment, the genomic sequences should be reconsidered in the context of physiological data. The integration of heterogeneous multi-scale physiological and genomic data in order to elucidate the functional role of yet uncultivable microbial populations involved in the cycling of C1 compounds is the major focus of our current research. We address all aspects of microbial function from single cell to community levels. (1). Flow-cytometry-based cell sorting after methane-induced activation of respiratory response, visualized by the use of a fluorescent Redox-dye, is a novel approach for characterization of microbial functions. We use this technique in combination with metagenomics and nanoSIMS analyses for a more comprehensive description of the C1-potential of the complex microbial community from Lake Washington sediment. (2). Coupling function to genomics at the single cell level. We use respiration as a core metabolic function to describe phenotypic capabilities of environmental microbes at the single-cell level. Subsequently, cells testing positive for C1-oxidation are targeted for further analyses, such as fluorescence in situ imaging (FISH) and

nanoSIMS. This single cell platform is a new technique to uncover or refine the function for uncultured members of the sediment microbial community.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.021 GENOME SEQUENCING AND COMPARATIVE GENOMICS OF PIG INTESTINAL LACTOBACILLI.

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The porcine gastrointestinal tract harbors a diverse and dense commensal microbiota. Lactobacilli are common members of this complex ecosystem and assumed to be important in supporting intestinal health and maintaining the stability of the GIT microbiota e.g. by increasing colonization resistance against intestinal pathogens. Recently, a new abundant Lactobacillus spp. was identified in the intestine of piglets that was responding to specific diet rich in fructo-oligosaccharides and found to stimulate the pig immune system (Konstantinov et al. 2004 & 2006, Roselli et al. 2007). While first classified as Lactobacillus sobrius, subsequent taxonomic studies revealed it to belong to the Lactobacillus amylovorus group that is dominant among Lactobacillus strains originating from porcine intestine (Jakava-Viljanen et al. 2008). To identify their potential functional properties, including the surface structures interacting with host cells, we have taken a comparative genome sequencing approach of a series of surface layer (S-layer) protein-carrying isolates. The genome of four L. amylovorus strains with genome size between ~1.8 to ~2 Mb were determined by pyrosequencing, further assembled, and prepared for gap closure. Primary comparisons of the four strains show a high similarity to L. acidophilus/ L. helveticus genome, the presence of several S-layer protein genes, and the clustering of CRISPR's and CRISPR associated gene cluster. Presently, the four genomes are being compared with the aim of providing a deeper insight into the genetics and metabolic capabilities of this Lactobacillus group. In addition, this and other comparative genomics studies will allow understanding the high diversity in the presently known Lactobacillus genomes. Finally, the genomic characterization of these S-layer positive L. amylovorus strains of pig origin will support to the physiological, biochemical and genetic studies that are being performed using these strains.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.022 CLONING AND CHARACTERIZATION OF A COLD-ACTIVE LIPASE FROM SALINISPHAERA SP. P7-4

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A bacterial strain that produced extracellular lipase was isolated from seawater and identified as *Salinisphaera* sp. P7-4. In the present study, the corresponding gene was cloned using shotgun method. The amino acid sequence deduced from the nucleotide sequence (951 bp) corresponded to a protein of 316 amino acid residues with a molecular weight of 34,443. The lipase contained a putative leader sequence, as well as the conserved Ser, His, and Asp residues which are known to function as the catalytic triad in other lipases. The protein P7-4 was produced in both a soluble and insoluble form when the *Escherichia coli* cells harboring the gene were cultured at 30°C. The soluble protein exhibited lipase activity in a pH-stat assay using an olive oil emulsion. The optimum pH and temperature for the enzyme activity was pH 9.0 and 10°C, respectively. The enzyme showed the highest activity toward the p-nitrophenyl ester of butyrate (C4) and metal ions such as Cd²⁺, Co²⁺, Cu²⁺, Hg²⁺ and Zn²⁺ inhibited enzymatic activity.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.023 COMPLETE GENOME ANALYSIS OF NOVEL ANAEROBIC ORGANOTROPHIC THERMOPHILIC ARCHAEA FROM UZON CALDERA, KAMCHATKA

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Kamchatka hot springs are inhabited by variety of thermophilic prokaryotes accomplishing complete hydrolysis of biopolymers. The genomes of three of these microorganisms, *Desulfurococcus kamchatkensis*, *Acidilobus saccharovorans* and *Fervidicoccus fontis*, were sequenced and annotated. Genomes were sequenced on a Roche Genome Sequencer GS FLX using standard protocols. Here we describe our genomic analysis which reflects the possible role of these microorganisms in microbial communities of terrestrial hot springs. Hyperthermophilic *D. kamchatkensis* is capable to utilize a broad range of proteinaceous substrates including keratins - extremely stable proteins. In agreement with that, *D. kamchatkensis* genome contains more than 30 peptidases including 6 secreting endopeptidases, responsible for proteins hydrolysis and a set of dipeptide/oligopeptide transporters. Moreover it is growing on dextrin, dextran and sucrose and we have found proteins responsible for degradation of these carbohydrates. *Acidophilic hyperthermophile A. saccharovorans* grows on various carbohydrates including biopolymers lichenan, starch and xylan. This correlates with the presence of genes for extracellular hydrolysis of α - and β - linked polysaccharides, transporters for monomeric sugars and oligosaccharides, as well as intracellular glycoside hydrolases, performing the final hydrolysis of the oligosaccharides. Thermophilic *F. fontis* unlike the majority of hyperthermophilic *Archaea* growing optimally at 70°C. In genome of *F. fontis* we have found several endopeptidases including the secreted ones and, surprisingly, one of them is a homologue of thermopsin, an aspartic endopeptidase, previously described only for acidophilic aerobic *Sulfolobus acidocaldarius*. These results indicate that hyperthermophilic *Archaea* possess a wide spectrum of hydrolytic enzymes and are able to degrade various biopolymers of plant or animal origin originating from the colder areas surrounding hot springs.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.024 GENOME DIVERSITY OF ACIDOVORAX TEMPERANS STRAINS ISOLATED FROM AN ACTIVATED SLUDGE ENVIRONMENT

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Members of the genus *Acidovorax* are consistently found in activated sludge wastewater treatment plants worldwide yet their functional significance in these systems is unclear. To better understand the physiological attributes that enable successful colonisation of and persistence in the activated sludge environment, we have sequenced the complete genome of *A. temperans* CB2-Hp and draft genomes of six other *A. temperans* strains.

The genome sequence of CB2-Hp was determined using a whole genome shotgun strategy combined with a pyrosequencing approach. A hybrid assembly was performed and assembly validation was confirmed by pulsed field gel electrophoresis and an optical map. Draft genome sequences of the strains CB2-Hn, CB024, CB066, CB158, CB088 and ATCC49665 were determined using pyrosequencing. Annotation, metabolic pathway reconstructions and comparative genomics were performed using available public tools.

The 4.2Mb CB2-Hp genome consists of a single chromosome and a 3Kb plasmid. Genomic analysis revealed a broad metabolic capacity which allows CB2-Hp to adapt to its activated sludge environment. Two variants of the CB2-Hp genome were identified, one of which contained an additional 17kb insert. Phenotypic characterization of the strains isolated from local activated sludge plants has revealed variability in colony morphology and differences in biofilm and floc-forming capability. Pulsed field gel electrophoresis indicates the presence of megaplasmids within this species. Comparative genomics with members of the *Acidovorax* genus isolated from other environments have identified a core *Acidovorax* genome while species and strain specific loci have been identified for *A. temperans*.

Sequencing of several *A. temperans* strains isolated from activated sludge has revealed genomic diversity within this species and has identified a number of genomic adaptations for living in this environment.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.025 EVOLUTIONARY DYNAMICS AND HISTORY OF YERSINIA PESTIS REVEALED BY MASSIVE GENOMIC SEQUENCING

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A great amount of microbe genomics studies around the world has been more and more performed. BGI is undertaking some big microbial genome projects. One of which is the *Yersinia pestis* Genome Project, *Y. pestis* is a typical natural focus-related pathogen, caused three pandemics in history. Population structure and evolutionary forces for its evolution could explain the emergence and spread of this pathogen, which will help to understand the origin of other emerging pathogens. Here we presented 118 *Y. pestis* strains were *de novo* sequenced using the next generation sequencing technology and analyzed in combination with 15 published genomes for genetic drift and natural selection forces on its evolution. The genetic drift is the main force for its evolution with predominate gene losses and a few gene acquisitions by natural selection. We constructed a neutral phylogenetic tree based on 2298 single nucleotide polymorphisms (SNPs). Instead of showing a constant molecular clock, *Y. pestis* tends to accumulate more SNPs when experiencing more transmission cycles. Major ancient trade routes connecting China and the rest of the world, like the Silk Road, seem to play a vital role in aiding the historical spreading of *Y. pestis*.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.026 SINGLE-CELL MOLECULAR DETECTION OF RAPID AND SLOW GROWING BACTERIA

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Bacteria are capable of rapid response to either positive or negative changes in their environment. Identifying immediate responders is necessary to understand individual interactions and community dynamics surrounding various environmental changes. Current methodology is very limited in distinguishing rapid versus slow responders with resolution times typically being on the order of one day. In order to rectify this lag time, we developed a protocol that couples detection of *de novo* DNA synthesis (an indirect measure of bacterial growth) using bromodeoxyuridine immunodetection (BrdU), with fluorescent-activated cell sorting via flow cytometer. We tested our protocol on seawater enrichment culture collected from the Sargasso Sea. Bacteria were separated into non-growing, slow growing, and rapid growing subpopulations. Signal intensity ranged four orders of magnitude on a logarithmic scale. Continuing research involves comparisons of the bacterial community composition within and between samples using Automated Ribosomal Intergenic Spacer Analysis (ARISA). ARISA results showed successful amplification from samples ranging between 18 and 1000 BrdU-positive cells, and currently optimizing for single cell analysis. From five samples representing fast-growing bacteria during the time course, a total of 94 ribosomal gene fragments were amplified, and ranged from 10 to 25 fragments. Sixteen of which were shared between two or more samples. No single fragment was shared across all samples, indicating the protocol was capable after capturing differential response within the community.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.027 COMPARATIVE TRANSCRIPTIONAL PROFILING OF PREVOTELLA RUMINICOLA 23 DURING GROWTH ON AMMONIA AND PEPTIDES

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The genus *Prevotella* is one of the more numerically dominant bacteria in the rumen, making significant contributions to both carbohydrate and nitrogen metabolism. Previous research has shown *P. ruminicola* 23 is able to efficiently utilize both ammonia and peptides as nitrogen sources for growth. Changes in the global gene expression profile of *P. ruminicola* 23 in response to variations in the available nitrogen source were analyzed by microarray and related to changes in enzymatic activity and the proteome. The results demonstrated changes in gene expression and enzymatic activity between the two conditions. In total, 110 genes (3.7% of the genome) were transcriptionally upregulated during growth on ammonia, while 120 genes (4.1 % of the genome) were transcriptionally upregulated during growth on peptides. Specifically, growth on ammonia induced genes whose products are predicted to be involved in amino acid biosynthesis, molecular transport and several that affect the cell envelope. Growth on peptides induced genes whose products are involved in DNA metabolism, protein fate, protein synthesis and transcription. We observed that links between carbohydrate and nitrogen metabolism were evident from glutamate biosynthesis and reverse TCA cycle upregulation when grown on ammonia. Interestingly, the GS-GOGAT (GSIII-2: 22.5, GOGAT large subunit: 26.3, and small subunit: 22.4 fold) pathways was also upregulated under these conditions. The greatest transcriptional up-regulation was observed for the ammonium transporter, *amt* (47.0 fold) and the nitrogen regulatory protein PII (46.5 fold) during growth on ammonia. Our results provide a whole genome transcriptional overview of the responses by *P. ruminicola* 23 to two different nitrogen sources and are supported by proteomic and biochemical evidence. These findings lead to an improvement in the understanding of the genetic responses and biochemical pathways used by *P. ruminicola* to obtain and utilize different nitrogen sources.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.028 IDENTIFICATION OF FRESHWATER AEROBIC PHOTOHETEROTROPHS BY SINGLE CELL SEQUENCING

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Bacterioplankton underpin ecosystem function in freshwater environments by driving most biogeochemical cycles and the flux of particulate and dissolved matter from terrestrial ecosystems to the oceans. Aerobic photoheterotrophs are a potentially significant but as of yet poorly understood functional group of freshwater bacterioplankton. They, include rhodopsin-containing prokaryotes and aerobic anoxygenic phototrophs (AAPs). These heterotrophic organisms can use light in order to produce extra ATP for their metabolic reactions.

We combined metagenomic and single cell DNA sequencing to identify the predominant photoheterotrophs in temperate freshwater lakes along a productivity gradient. First, microbial community shotgun sequencing was employed to sample the diversity of photoheterotrophy-defining genes, such as rhodopsins and bacteriochlorophylls. These sequences were used to design PCR primers targeting the discovered gene variety. Next, over 3,000 single amplified genomes (SAGs) were created from the same environmental samples by individual cell sorting and subsequent whole genome multiple displacement amplification. Each SAG was probed by PCR for SSU rRNA, rhodopsin, and genes involved in aerobic anoxygenic photosynthesis. Sequencing of the 16S rRNA gene amplicons demonstrated that SAG libraries were a good representation of freshwater microbial assemblages. They included ubiquitous groups of Actinobacteria, Betaproteobacteria (e.g. Polynucleobacter), Alphaproteobacteria (e.g. LD12 cluster), as well as novel phylogenetic groups. Sequencing of the PCR amplicons of metabolic genes from the same SAGs provided the first direct evidence for photoheterotrophy potential in multiple taxa characteristic of freshwater environments. The SAG libraries of uncultured freshwater bacterioplankton groups created here open vast opportunities for further studies by PCR screening or whole genome sequencing.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.029 DIFFERENTIAL PATTERNS OF INHIBITION OF AMMONIA OXIDATION IN NITRIFYING BACTERIA AND ARCHAEA

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In recent years it has become increasingly apparent that ammonia-oxidizing archaea (AOA) contribute significantly to global nitrogen cycling. While similarities exist between ammonia-oxidizing bacteria (AOB) and AOA (e.g., the stoichiometry of ammonia oxidation and the presence of genes encoding putative ammonia monooxygenases), there are also important differences. Genomic analyses of AOA have yet to identify archaeal homologs of key enzymes such as hydroxylamine oxidoreductase (HAO) and have suggested a copper-based electron transport chain in AOA. These analyses suggest that the ammonia-oxidation pathways of AOB and AOA may differ significantly and consequently, that their response to inhibitors of ammonia-oxidation may differ as well. Although well studied in AOB such as *Nitrosomonas europaea*, there are no published reports to date on the effect of inhibitors (e.g. allylthiourea, allylsulfide, nitrapyrin, acetylene and methane) on ammonia oxidation in AOA. The objective of this study is to compare the responses of AOB and AOA to known inhibitors of ammonia oxidation. For these studies, we have used *N. europaea* and the recently described thermophilic AOA, *Nitrosocaldus yellowstonii* HL72. Using measurements of oxygen and ammonia

consumption, nitrite production and direct cell counts, we report significant differences in the response of HL72 and *N. europaea* to known inhibitors. Complete inhibition of ammonia oxidation in *N. europaea* was observed in the presence of 10 μM of the copper-chelating inhibitor allylthiourea (ATU). In contrast, ammonia oxidation in HL72 was unaffected by this concentration and complete inhibition was only observed at concentrations of ATU above 1 mM. Our findings indicate that there are indeed significant biochemical differences between ammonia oxidation in AOB and AOA and suggest that compounds such as ATU may be important for distinguishing between the contributions of AOB and AOA to nitrification rates measured in the environment.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.030 COMPARATIVE ANALYSIS OF THE *pst* OPERON IN FIRMICUTES FROM PHOSPHATE DEPRIVED ENVIRONMENTS

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The high affinity phosphate transport system is comprised of 4 to 6 genes in a single operon that is induced by phosphate starvation. This operon has been identified in both gram positive and gram negative bacteria, but presents some variation both in structure and sequence. Due to its importance in phosphate uptake, a comparative evolutionary analysis of the *pst* operon was performed for representative species of firmicutes. Special emphasis was made on the genus *Bacillus* from marine environments and the Cuatro Ciénegas basin, which are known for their low levels of phosphates.

Maximum likelihood phylogenetic reconstructions were carried out for the whole *pst* operon and for a set of 13 genes from the core genome of 49 species of firmicutes and 3 cyanobacteria as outgroups, as well as tests for natural selection.

The structure and sequence of the *pst* operon in *Bacillus* was highly divergent and was inconsistent with the topology of the phylogenetic tree of the 13 core genes. There were two groups according to the phylogenetic reconstruction of the *pst* operon, one similar to *Listeria* that included *Bacillus subtilis* and a *Bacillus marisflavi*-like species from Cuatro Ciénegas, and another group with the other *Bacillus* that were more similar to *Staphylococcus*. A strong purifying selection was detected for most taxa, and although there were also a few cases of positive selection, these were not correlated with the low phosphate habitat.

The topological incongruity between the *pst* operon and the 13 gene phylogenies suggested a non monophyletic origin of the *pst* operon in *Bacillus*, possibly due to horizontal gene transfer. Also, there appears to be no association between the type of *pst* operon and positive natural selection with an oligotrophic habitat, thus the mechanisms used by bacteria to optimize the uptake of phosphate seem to have had different histories.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.031 DIFFERENTIAL PROTEOMICS OF *DESULFOGLAEBA ALKENEXEDENS* : NEW INSIGHTS INTO ANAEROBIC ALKANE DEGRADATION

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Desulfoglaeba alkanexedens is a sulfate-reducing bacterium that, like other hydrocarbon-degrading anaerobes, utilizes a fumarate-addition mechanism to initiate the metabolism of n-alkanes. To examine the microbial physiology of alkane degradation in this organism, we compared the metabolic pathways and proteins that were differentially expressed during growth on decane and butyrate. Protein mass spectrometry was used in combination with the draft genome sequence to achieve these goals. Proteins related to alkylsuccinate synthase were only observed during growth on decane, suggesting that this system is inducible in the presence of hydrocarbons. Specifically, the catalytic and activating subunits of alkylsuccinate synthase were detected in nine peptide fragments each, with MASCOT significance scores of 209.16 and 205.97, respectively. All proteins with significance scores of less than 20 (corresponding to $P < 0.05$) and peptide numbers less than two were removed from the analysis. Proteins necessary for sulfate reduction were also observed under both growth conditions at statistically significant levels. Other shared pathways include those involved in beta-oxidation, fatty acid metabolism, and amino acid and cofactor synthesis. Overall, 100 proteins with identifiable function were differentially expressed in the alkane condition, and 81 were differentially expressed in the butyrate condition. Other interesting findings suggest that cells grown under alkane pressure stringently control the production and utilization of the co-metabolite fumarate, and deeper interrogation of these results may lead to other pathways important to alkane biodegradation.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.032 GENOMIC INSIGHTS INTO SUBZERO GROWTH FROM A CANADIAN HIGH-ARCTIC *PLANOCOCCUS* SP.

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To date, the coldest reported temperatures for microbial growth and metabolism are -12°C and -20°C, respectively. In our investigations we have several isolates from the Canadian High-Arctic that go beyond these limits, capable of growth at -15°C and possibly colder. Among these isolates a *Planococcus* sp. originating in permafrost active layer from Eureka (Ellesmere Island, Nunavut, Canada) was chosen for genome sequencing. This is the first genome project to target a member of the Firmicutes or other Gram positive bacteria and aims to uncover novel insights into molecular characteristics required for subzero growth in contrast to the discoveries from recently published psychrophilic genomes of several Proteobacteria. Growth characteristics were determined in high salinity (10 to 20% NaCl) media over many weeks of incubation at subzero (-5, -10, -15, -18, -20°C) temperatures. Genome sequencing was performed by 454-

pyrosequencing and draft annotation was completed using automated identification of coding regions followed by protein annotation according to consensus from multiple databases (TIGRFam, Pfam, COGs, and KEGG). Manual annotation was used to verify the quality and confidence of protein annotations against the Swiss-Prot database using a basic local alignment search tool for proteins (BLASTp). The *Planococcus* sp. was characterized as a eurypsychrophile growing up to 25°C and capable of sub-zero growth down to at least -15°C as well as tolerant of high salinities (20% NaCl). Draft genome annotation is underway and thus far indicates amino acid distributions favouring increased flexibility of proteins at colder temperatures and cell envelope modifications are among the survival strategies for this High-Arctic isolate. The *Planococcus* sp. draft genome will expand on the recent insights into molecular traits required for subzero growth at the coldest temperatures described to date.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.033 ASSESING THE GENETIC ACCESIBILITY OF THE MARINE BACTERIUM RHODOPIRELLULA BALTICA SH 1^T

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The marine bacterium *Rhodopirellula baltica* is a ubiquitously distributed representative of the bacterial order *Planctomycetales* which exhibits unique cellular properties such as a cytoplasm that is composed of two compartments divided by a single-layer membrane: the peripheral, RNA-free paryphoplasm and the inner riboplasm that contains the ribosomes and the chromosomal DNA. The cell wall of Planctomycetes is a proteinaceous sacculus instead of peptidoglycan. These organisms exhibit polar cell division and crateriform structures on the cell surface. The analysis of the 7.1 Mb circular genome (1) of *R. baltica* followed by vast postgenomic studies revealed 7325 genes, 45 % of which have been assigned to a function. Functional and gene regulatory studies on this organism have been held back by the lack of genetic manipulation tools. The current study aims to render *Rhodopirellula baltica* genetically accessible and present a first set of cloning vectors that are compatible with this organism. Reduced accessibility of the chromosomal DNA due to the additional membrane and insufficient knowledge about suitable cloning vectors require testing of a variety of transformation methods and vectors. Several methods have been employed to deliver nucleic acids into *R. baltica* including electroporation, chemical transformation and liposome-mediated delivery, aided by methods to eliminate the cell wall to make cells more permeable. The response of the cells to each treatment was tracked by determining the viability and microscopic observations. The establishment of a genetic system for *R. baltica* will advance future cell biology studies as well as molecular genetic analyses to complement previous research on this organism.

1 Glockner, F. O., et al., 2003. "Complete genome sequence of the marine planctomycete *Pirellula* sp. strain 1." Proceedings of the National Acedemy of Sciences, vol. 100, no. 14. (8298-83)

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.034 CELL SURFACE DISPLAY OF THE MER—R PROTEIN IN CUPRIAVIDUS METALLIDURANS CH34

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Mercury causes severe environmental impact. Conventional treatments for contaminated water are often inadequate and alternative bioremediation approaches have been considered. Metallothioneins and synthetic phytochelatins were expressed onto the surface of bacteria to improve metal biosorption. The major problem associated with these cysteine rich peptides is their lack of specificity and low mercury affinity. Many bacteria develop resistance to mercury by inducing expression of the regulatory MerR protein, which exhibits maximal affinity for mercury. *Cupriavidus metallidurans* CH34 thrives in soils highly polluted with heavy metals, being thus a suitable candidate for application in environmental bioremediation. The present work describes the construction of a recombinant *C. metallidurans* CH34 strain displaying the MerR protein on the cell surface. The *merR* gene was PCR amplified from the *C. metallidurans* CH34 pMOL28 plasmid and inserted in the pCM2 plasmid (previously constructed in our group) between the *Neisseria gonorrhoeae* signal sequence and IgA protease β —domain. The gene fusion was placed under control of the *pan* heterologous promoter, which allows strong constitutive expression in *C. metallidurans*. The final plasmid was named pCMHg. MerR expression on the cell surface was verified upon cell fractionation by SDS—PAGE. For immunodetection, proteins were incubated with anti—Etag and secondary antibody IgG peroxidase conjugate. The successful surface display of the MerR— β —domain protein was first demonstrated in *Escherichia coli* carrying pCMHg. A protein band of 60.8 kDa corresponding to the correct size of MeR (15.8 kDa) and β —domain (45.0 kDa) was detected in the outer membrane fraction. pCMHg was then used to transform *C. metallidurans* CH34 yielding similar results. This recombinant *C. metallidurans* CH34 strain represents an attractive strategy for developing high affinity biosorbents suitable for mercury bioremediation. Support: VALE Company.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.035 GENOMIC ANALYSIS OF SINGLE TM7 CELLS FROM HUMAN ORAL SAMPLES

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Members of the uncultivated bacterial division TM7 have been detected in a wide variety of environments, including peat bogs, soils, water sources, and in the human body. TM7 is one of the most common bacterial taxa in oral cavity samples, and it is a possible risk factor for periodontal disease. Still, little is known about this phylum's phylogenetic, genomic and functional features. Here, we applied fluorescent labeling and single-cell genomic analysis to obtain insights into the genetic and functional capabilities of TM7. FISH-labeled TM7 cells from

human sub-gingival plaque were selected and sorted using a microfluidic device, followed by synthesis-based whole genome sequencing of the multiple displacement amplified DNA. The resulting genomes, estimated to have 10X(\pm 5) coverage, were annotated through gene predictions, phylogenomic and metabolic analyses, and revealed possible mechanisms by which TM7 has adapted to the oral environment. This study enhances our understanding about the evolution and function of a novel, uncultivated bacterial phylum that may play a role in human health and disease.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.036 STRUCTURE OF MUCUS-BINDING SPACBA PILI IN *LACTOBACILLUS RHAMNOSUS* GG

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Pili are proteinaceous surface appendages found in both Gram-negative and Gram-positive bacteria. The Gram-positive pilus fiber is a macromolecular assembly of protein subunits (major pilins) strung together covalently and often decorated with additional subunits (minor pilins), some of which function as adhesins and are found primarily at the pilus tip. Thus far, pili have been detected in various pathogenic Gram-positive species, such as *Corynebacterium diphtheriae*, *Actinomyces naeslundii*, *Bacillus anthracis*, *Enterococcus faecium*, and several *Streptococcus* species, and often mediate the invasive colonization of host tissues by these strains. Recently, we demonstrated that the widely used probiotic *Lactobacillus rhamnosus* GG contains cell surface-localized pili (SpaCBA) that bind to human intestinal mucus (Kankainen *et al.*, PNAS 2009). Such pili might serve as a novel colonization feature for prolonging intestinal residence time, thereby explaining the competitive advantage that this strain generally has over nonpiliated probiotics. In our present work, using immunogold electron microscopy combined with antibodies specific for each of the different SpaCBA pilin subunits, we have compared the macromolecular structure of *L. rhamnosus* GG-expressed SpaCBA pili with the same pilus fibers expressed heterologously in *Lactococcus lactis*. Herein, we report that the subunit organization of SpaCBA pili in *L. lactis* is structurally similar to the assembled pilus fibers in *L. rhamnosus* GG, suggesting that *L. lactis* is a viable host for the recombinant expression of authentic SpaCBA pilus fibers and thus useful for further characterizing the functional and structural mechanism of SpaCBA pilus biogenesis and adhesion to the intestinal mucosa by protein engineering.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.037 GENOME COMPARISON OF AN IDIOMARINA ISOLATE FROM THE GREAT SALT LAKE, UTAH, WITH THE REFERENCE STRAIN L2TR

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The reference strain for the γ -proteobacteria *Idiomarina loihiensis*, L2TR, has been originally isolated from a hydrothermal vent on a deep ocean volcano off the coast of Hawai'i, the genome sequence suggests that the organism relies primarily on amino acid catabolism for carbon and energy. We have isolated several bacteria that showed a 16S identity of >99% with L2TR from the Great Salt Lake, Utah (GSL). In an effort to better understand the microbial adaptation to extreme environments we have sequenced the genome of one of those isolates (isolate 199). The results indicated that the GSL isolate has a sequence homology to the reference strain that varies from 90 to 25%, furthermore gene annotation showed that the GSL isolate had lost some of the amino acid synthesis and degradation genes present in the reference strain as well as genes of the cell wall and capsule and potassium metabolism. To test whether positive selection is operating on individual genes the two genomes we also compared the relative abundance of synonymous and non synonymous substitutions that have occurred in the gene sequences. The results suggest that the GSL *Idiomarina* isolate is possibly relying on a different energy source than the reference strain and that it has altered the cell wall composition, and possibly, structure. Also gene sequence analysis has identified different regions, and genes, in the two genomes that are positively selected. This study may provide some insight on bacteria adaptation, genome arrangements and evolution.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.038 PREDICTING FLUXES IN MICROBIAL COMMUNITIES THROUGH PHYSIOLOGICAL PROPERTIES OF PARTICIPATING MICROBES MEASURED IN ISOLATION

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Functioning of any microbial community generally results from the interactions between various functional groups of microorganisms. We aim to establish a generalized mathematical modeling method for analytically predicting the properties of microbial communities on basis of the physiological properties of the individual member organisms. In this post genome era, metabolism of microorganisms can be modeled on basis of detailed metabolic networks generated from the whole genome data available. While, we describe the microbial metabolism using only measurable fluxes of growth, product formation, oxygen uptake, and substrate consumption etc. Such Reduced Stoichiometric Metabolic Descriptions of the member organisms are computationally less cumbersome to handle and help us determine the system - wide flux distribution of shared compounds of their environment.

A Stoichiometric Network Analysis method is established for two organisms coexisting under obligatory mutualistic relationship where one organism uses the product of the other organism and vice versa. Reduced Stoichiometric Metabolic Descriptions were used towards predicting growth rates, substrate uptake and product formation fluxes of these organisms in coexistence. These solutions were confirmed through traditional Linear Programming methods such as Flux Balance Analysis.

The virtue of being less cumbersome in modeling of such reduced but confined descriptions of the metabolism of organisms leads a new way for taking modeling to the ecological level, where one does not only model and predict the behavior of single organism in the context of its complex environment, but wants to model a whole ecosystem, consisting of up to thousands of species with variable coarse graining of the organismal metabolism.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.039 STUDY OF AROMATIC METABOLIC PATHWAYS OF THE BACTERIUM BURKHOLDERIA XENOVORANS LB400

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The development of genomics in the last decade allowed the genome sequencing of a large number of environmental bacteria. *B. xenovorans* LB400 is a model polychlorobiphenyl-degrading bacterium. The LB400 genome has been recently characterized and comprises three replicons. Genomic analysis and functional approaches of aromatic catabolic pathways were performed. For the genomic search of catabolic genes, bioinformatics tools (KEGG, Swiss-Prot/TrEMBL and BLAST) and software (Artemis and Vector NTI) were used. Metabolic reconstruction indicated that 3-hydroxybenzoate, 4-hydroxybenzoate and 3-hydroxyphenylacetate peripheral pathways were associated to the gentisate, homogentisate, catechol and protocatechuate central pathways. Catabolic genes were usually organized as clusters, and associated with transport and regulator genes. The catabolic genes of these aromatic pathways of strain LB400 showed high sequence similarity to genes of related microorganisms of the order Burkholderiales, such as *Bordetella* and *Cupriavidus*. Strain LB400 was able to degrade different aromatic compounds, such as gentisate, 3-hydroxyphenylpropionate, mandelate, 3-hydroxybenzoate, anthranilate and protocatechuate. The response of strain LB400 to specific aromatic compounds was analyzed by proteomic studies. 4-chlorobiphenyl, biphenyl and 3-hydroxyphenylacetate induced in strain LB400 stress proteins DnaK and GroEL, and other proteins involved in energy metabolism and transport. Therefore, the exposure to these aromatic compounds constitutes a stressful condition for this bacterium. The great diversity of catabolic pathways of aromatic compounds present in strain LB400 reflects a remarkable metabolic versatility that can be used in diverse biotechnological applications such as the bioremediation of polluted environments and the biosynthesis of bioactive compounds. Acknowledgments: FONDECYT 1070507 and 7090079, USM 130948 and 130826, CIT NT&SB, Milenio-P04/007-F, Conicyt fellowship (MC).

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.040 SURVIVAL OF *R. SOLANACEARUM* BIOVAR 2 (POTATO BROWN ROT) IN TEMPERATE CLIMATES

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Ralstonia solanacearum biovar 2, a devastating bacterial pathogen of potato, has recently established itself in temperate climate watercourses and its persistence is of great concern to potato production. Despite strong control measures and the absence of true hosts, the bacterium can still be found in local waterways and in bittersweet plants growing on the shores and in sediment. On the basis of isolates obtained from diseased (potato) plants, its genome is assumed to be virtually clonal, but information on environmental isolates has been lacking.

Isolates were obtained from water, bittersweet and sediment from a Dutch waterway and compared to two strains from potato using a range of molecular methods. Based on these results, we compared the genomes of novel environmental strain KZR-5 (The Netherlands) to that of potato strain 715 (Bangladesh) by suppressive subtractive hybridization. To connect genome differences to ecological behavior, we performed survival experiments, determined growth rates under different conditions and performed virulence assays in single and dual inoculation experiments.

Comparison of 42 novel isolates with two reference strains revealed they were essentially clonal. However, we identified the excision of a 17.6 Kb genomic region in strain KZR-5. The deleted region (PGI-1) shows several features of a genomic island and was, at both extremes, flanked by a composite of two insertion sequence elements. The PGI-1 region contained 13 open reading frames that putatively encoded a (p)ppGpp synthase, a transporter protein, a transcriptional regulator, a cellobiohydrolase, a site-specific integrase/recombinase, a phage-related protein and seven hypothetical proteins. Strain KZR-5 showed enhanced tolerance to 4°C, but was less competitive than strain 715 upon invasion of tomato.

We detected a recombinogenic region in the *R. solanacearum* biovar 2 genome, denoted PGI-1. The region showed features of a transposon and was flanked by identical blocks of two insertion elements. Further research is required to establish a direct link between the function of PGI-1 and ecological behavior. However, our results suggest a potential role in stress responses and/or plant interaction.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.041 METAGENOMICS AND IN SITU RCA-FISH REVEAL THAT SOME CRENOTHRIX POLYSPORA STRAINS POSSESS A CONVENTIONAL PARTICULATE METHANE MONOOXYGENASE

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Crenothrix polyspora is an uncultured filamentous methane oxidizing bacterium, which according to its 16S rRNA belongs to the gammaproteobacterial type I methanotrophs. Q-PCR data of biomass enriched in *C. polyspora* have shown that it possesses an unusual *pmoA* gene encoding a subunit of the particulate methane monooxygenase, which is more closely related to betaproteobacterial ammonia monooxygenases than to the respective enzymes of other type I methanotrophs. Interestingly, transcription of this unusual gene is induced by methane. In addition, other genes encoding methane/ammonia monooxygenase related proteins including a

canonical type I *pmoA* were present (in much lower numbers) in the enriched biomass. In the present study, more than 24 Mbp of metagenomic sequence was obtained from a new *C. polyspora* enrichment. Contigs up to 120 Kbp could be assembled and some of the large contigs with high coverage could be assigned to *C. polyspora*. The metagenome did not contain the unusual *pmoA* gene, but on contigs with low coverage two unusual *pmoC* genes were found. One of them could be linked by PCR to the unusual *pmoA* gene. Somewhat surprisingly, on a 31 Kbp contig with high coverage, indicating that it originated from *C. polyspora*, a canonical type I *pmoA* gene was found. Consistently, using in situ rolling circle amplification (RCA)-fluorescence in situ hybridization the canonical but not the unusual *pmoA* gene was detected in the *C. polyspora* filaments from the enrichment analyzed by metagenomics. Thus, different strains of *C. polyspora* were dominating the enrichments studied by and used for metagenome sequencing, respectively, and at least one strain of *C. polyspora* carries an unusual *pmoA* gene while another strain (dominating the metagenome biomass) contains a canonical type I *pmoA*.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.042 METHODOLOGICAL APPROACHES TO EXTRACT DNA FROM MARINE SEDIMENTS AND BASALT

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A contemporary research goal is to determine the microbial diversity of marine sediments and oceanic crust, since these are environments that comprise a huge but poorly understood reservoir of life on Earth. Extracting environmental DNA - the first step in diversity analyses - from both substrates is challenging due to the presence of heterogeneous mixtures of interfering molecules, such as humic acids in sediments. Humic acids that remain after a sediment DNA extraction prevent successful 16S rRNA gene amplification and whole genome amplification. Multiple DNA cleanup kits have been used to purify the DNA, but have not been successful. Our present attempts to reduce the presence of humic acids in sediment extracts include: (1) washing sediment samples with an acetate buffer (2) increasing EDTA concentrations to the cell lysis buffer and (3) a second ethanol rinse to remove contaminants from the DNA, such as phenols. Most recently we used a laser microdissection microscope to isolate single cells from a small aliquot of sediment slurry. In addition, DNA extraction techniques have also been optimized for recovering environmental DNA from oceanic crustal materials such as surficial and deep basalts. The ability to isolate single cells minimizes the presence of humic substances and allows for immediate amplification for further downstream applications, such as proteomics, metagenomics, functional gene analysis, and phylogenetic diversity analyses.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.043 COMPLETE GENOME SEQUENCE OF THE PLANT-GROWTH PROMOTING ENDOPHYTE *BURKHOLDERIA PHYTOFIRMANS* STRAIN PsJN^T

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Members of the *Burkholderia* genus are known for their adaptation to diverse environmental niches, including soil, water, human and plant ecosystems. *B. phytofirmans* strain PsJN^T is able to establish as an endophyte in a wide range of plants and is known for its plant-growth promotion capacity. The whole genome of this strain was analysed to obtain a better understanding of endophytic lifestyle and beneficial bacterial-plant interactions. Gene prediction and annotation was performed by IMG/M-ER and comparative analysis was done for selected genomes categorized into *Burkholderiaceae* and endophyte data sets. Functional analysis was based on the abundance of specific protein-encoding genes by searching for distinct domain motifs or by sequence similarity comparisons to well-studied proteins. Through initial comparative genomic analysis we could confirm the presence of the features indicated for endophytic lifestyle. Furthermore, we were able to identify numerous genes related to biodegradation functions and detoxification mechanisms indicating the potential of strain PsJN to respond and adapt to different environmental stimuli. Comparative genomics of the available endophytic genomes revealed that, although there are endophytic traits postulated, no unique gene cluster could be exclusively linked to endophytic lifestyle. Furthermore, comparative analysis with other sequenced endophytes showed a range of different strategies of endophytic life. For example, *B. phytofirmans* strain PsJN^T has a large genome (8.2 MB), harbors various functions and is thus able to cope with different environmental conditions and to establish in different habitats. On the other hand, *Gluconacetobacter diazotrophicus* PAI5 has a small genome (3.9 MB) with concentrated functional capacity, and represents an obligate endophyte known to interact with sugarcane. Various features based on genome information of sequenced endophytes will be discussed in regard to the range of endophytic lifestyles.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.044 IDENTIFYING N₂-FIXING POPULATIONS IN PHOTOSYNTHETIC MICROBIAL MATS BY COMBINING BIOGEOCHEMICAL AND MOLECULAR ANALYSIS WITH SINGLE CELL TECHNIQUES

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Photosynthetic microbial mats supply nitrogen to shallow-water marine environments, which are often characterized as nitrogen limited. The contribution of different taxa to N₂-fixation and the community response to altered environmental conditions is still poorly understood. We are characterizing these N₂-fixing microbial mat communities through a synthesis of biogeochemical

and *nifH* gene expression analysis with single cell techniques, including NanoSIMS and Element Labeling-Catalyzed Reporter Deposition Fluorescence *in situ* Hybridization (EL-FISH). Mat samples collected throughout the year from Elkhorn Slough, CA showed seasonal differences in N₂-fixation patterns. ¹⁵N₂ incubations followed by isotope ratio mass spectrometry (IRMS) as well as acetylene reduction assays indicate that the majority of N₂-fixation occurs in the upper 2 mm of the mats. NanoSIMS analysis of these samples has shown that the most abundant cyanobacteria, *Microcoleus* spp. & *Oscillatoria* spp., do not incorporate ¹⁵N. Instead, ¹⁵N incorporation was observed in filaments of *Lyngbya* spp. ((¹⁵N/¹⁴N, avg. ± std.dev.) 0.009 ± 0.001), cyanobacteria of the *Leptolyngbya*, *Plectonema*, *Phormidium* (LPP) group (0.02 ± 0.01), and in single cells (0.04 ± 0.03). Pyrotag sequencing of DNA and cDNA indicate that *Microcoleus* spp. is a dominant and active member of these communities. Based on the nitrogen fixation assays however, they do not appear to be an active participant in N₂-fixation. These data illustrate the importance of less abundant diazotrophs to this nitrogen-deprived ecosystem. The identity of these diazotrophs will be supported by a combination of EL-FISH probing and analysis of *nifH* gene expression in samples analyzed by NanoSIMS. The synthesis of biogeochemical, molecular and single cell analyses is being employed to describe the diazotrophic community structure and activity at different seasons at Elkhorn Slough

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.21.045 THE DIVERSE ARRANGEMENT AND EVOLUTION OF PHOTOSYNTHETIC SUPEROPERONS IN AEROBIC ANOXYGENIC PHOTOTROPHIC BACTERIA.

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Aerobic anoxygenic phototrophic bacteria containing bacteriochlorophyll-a represent an important functional bacterial group in marine ecosystems. The known marine AAPB species are mainly classified to three clades, *Roseobacter* clade, *Erythrobacter-Citromicrobium* clade and NOR5/OM60 clade. Ten genome sequences of aerobic anoxygenic phototrophic bacteria and two more genomes with no existence of photosynthetic genes were analyzed in this study (two of them were finished by our lab). According to the arrangement of photosynthetic genes, we discovered that the structure of photosynthetic superoperons could be grouped into four types, based on permutations and combinations of the two conserved subclusters, bchFNBHLM-LhaA-puh and crtF-bchCXYZ-puf. Homologous gene recombination occurred between the two subclusters. The genes (RPA) related to gene rearrangement were also found between the two subclusters in *Roseobacter litoralis* Och 149. In order to check the effect of the two inserted genes (RPA) on photosynthetic capacity, we measure photosynthetic ability and absorbance spectra of several strains. Interestingly, an abnormal high value of photosynthetic functional cross-section and strange absorbance spectra (very low expression of RC-LHI complex compared LHII) were obtained in *Roseobacter litoralis* Och 149. That hints the two inserted genes (RPA) suppress the expression of RC-LHI complex. Two different photosynthetic superoperons, for the first time, were found in a single strain, *Citromicrobium bathyomarinum*

JL354. But, comparison of *C. bathyomarimum* JL354 and *Citromicrobium* sp. JL1363, we thought photosynthetic genes trend to miss in environment.

Abstract Category

21 Genomic and Functional Analyses of Individual Microbial Cells

PS.22.001 INSIGHTS ON THE SULPHUR CYCLING OF MANGROVES UNDER DISTINCT HISTORIC OF CONTAMINATION IN BRAZIL

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Mangrove sediments are anaerobic and rich in organic matter, making it optimal for anaerobic microorganisms, such as sulphate-reducing bacteria. However, despite the range of methodological approaches available for assessing the major microbial players in the sulphur cycling, it is poorly described in such environment. In this study, two genes related to sulphur transformations were target (*dsrB* and *aprA*) in a survey involving three mangroves located in the coastline of the São Paulo State (Brazil): i) oil-contaminated mangrove at Bertioga, ii) non-contaminated mangrove at Bertioga, iii) non-disturbed mangrove at Ilha do Cardoso. Samples were subjected to the estimative of the diversity by denaturing gradient gel electrophoresis (DGGE), and the quantification of target genes was made by real time PCR (qPCR). Results indicated the presence of intrinsic communities of sulphur transformers microbes in each of sampled mangroves, but with a distinguishable alteration on the DGGE profiles in the area contaminated by the oil spill. These differences were consistent for both genes, indicating a shift in major players for sulphur cycling due to the oil contamination. The quantification of both genes did not reveal intense changes among mangroves in absolute numbers, ranging from 4.88 to 5.32 (log values) copies of *dsrB* and from 5.00 to 5.67 (log values) copies of *aprA* per gram of sediment. However, if a relative quantification is made (*dsrB/aprA*) a different pattern unfolds; while in non-contaminated area the *dsrB* amount is around 20% of the *aprA* in Bertioga, and 35% in Ilha do Cardoso, this value reach 90% in the area with a higher contamination with oil (oil spilled mangrove in Bertioga). These data indicate that the oil spill not only affect the structure of the microbial communities in mangroves but can also lead to changes on functional microbial populations, consequently changing the metabolic web present in this ecosystem.

Abstract Category

22 Major Biogeochemical Cycles

PS.22.002 EARTHWORMS AFFECT PRIMING EFFECT INTENSITY AND MODIFY INVOLVED MICROBIAL COMMUNITIES

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Increasing soil organic matter (SOM) contents is often considered beneficial from both agronomic (increased crop productions) and environmental (e.g., greenhouse gas mitigation) points of view. Management of organic residues is particularly important in Southern countries. Different agricultural practices have been developed in order to increase SOM contents: no-tillage, cover crops, rotation, residues management, organic amendments, etc. These practices generally positively impact physical, chemical and biological soil properties. However it has been frequently observed that a supply of fresh organic matter could result in an increase of the mineralization of (old) soil organic matter. Understand this phenomenon called "priming effect" would help to better manage organic residues in a context of carbon C sequestration. Priming effect is based on microbial processes, but all the actors involved have not been identified yet. Soil engineers such as earthworms are known to affect soil organic matter dynamics, residues decomposition and to stimulate microbial activity. Our goal is to see whether earthworms impact on the priming effect intensity, and whether this impact is linked to a stimulation/inhibition of bacterial taxa. To achieve this goal, a tropical soil from Madagascar was incubated in the laboratory, amended for 3 weeks with a 13°C highly enriched, finely ground wheat residue, in the presence or absence of a peregrine endogeic tropical earthworm, *Pontoscolex corethrurus*. Results showed that the activity of earthworms led to a stimulation of the mineralization of wheat residues, increased priming effect intensity and modified its dynamics. Moreover, the coupling between DNA-SIP (Stable Isotope Probing) and 16S pyrosequencing allowed identifying several bacterial phylogenetic groups stimulated by worms, some of taxa feeding on fresh residues and others on soil organic carbon. This work has been funded by ANR genomic (BAMBI) and INSU-Ec2Co (PrimeVers).

Abstract Category
22 Major Biogeochemical Cycles

PS.22.003 ELUCIDATING CONTROLS ON THE ACTIVE PLAYERS THAT DRIVE NITROGEN FIXATION AND DENITRIFICATION IN ESTUARINE SEDIMENTS

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Estuaries harbor groups of important microbes whose activities can influence the cycle of nutrients on global scales. Sediment nitrogen (N) cycling microbial communities are extremely diverse, and it can be difficult to identify the functional groups and elucidate controls on their activity. We have developed molecular methods to target communities that are a part of the balance of N inputs, the diazotrophs (N-fixers) and N outputs, the denitrifiers in the benthic sediments of the estuary Narragansett Bay (RI, USA). To identify potentially active groups, we are following the expression of functional genes in the denitrification pathway (*nirS*) and for N fixation (*nifH*) in sediments sampled in the Bay and in experimental mesocosm manipulations. We are using marine mesocosms to test if the amount and/or timing of organic matter deposition to the benthos controls whether the sediment microbial groups denitrify or fix N. Expression of both *nirS* and *nifH* have been detected in mesocosms with ranging flux measurements, indicating that both of these processes may be occurring simultaneously. Using the combined approach of quantitative PCR and quantitative RT-PCR, we can identify hotspots of microbial activity over time and under different conditions. *nifH* expression in the Bay sediments is limited to two phylogenetic groups of environmental sequences related, but not identical to *Pelobacter carbinolicus* and *Desulfovibrio vulgaris*, anaerobes that can reduce sulfur and sulfate compounds, respectively. We have established enrichment cultures of N fixers whose gene sequences are identical to those actively expressing *nifH* in the Bay sediments and we will use these cultures to understand controls on N fixation. By combining molecular and biogeochemical data we can better understand how microbial community composition and activity influence the nitrogen cycle and improve predictions for how estuarine ecosystems may respond to ongoing environmental change.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.004 DETERMINING THE $\delta^{18}\text{O}$ OF NITRATE PRODUCED DURING NITRIFICATION

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The isotopes of nitrate are useful tracers of microbially-mediated nitrogen transformations in the ocean. The isotope effects associated with each microbial metabolism, which alter the nitrogen and oxygen isotope ratios in nitrate, are crucial for interpreting the isotopic distributions in the water column. While much attention has been paid to the ¹⁵N isotope effects, there have been few studies that have determined the ¹⁸O isotope effects. Specifically, the $\delta^{18}\text{O}$ value of nitrification is important because it serves as the oxygen isotope source to nitrate. The $\delta^{18}\text{O}$ of nitrification is useful for interpreting new isotopic tracers such as the nitrite $\delta^{18}\text{O}$ and the "nitrate isotope anomaly", $\Delta(15,18)$, which provides insight into the role of nitrification in surface and subsurface nitrate dynamics. In previous work, we identified a large range of possible $\delta^{18}\text{O}_{\text{NO}_3}$ values produced by nitrification based on pure-culture experiments. Here we measured the oxygen isotope variations in nitrate produced in incubations of bacterial co-cultures and natural populations. In both types of incubations we manipulated the $\delta^{18}\text{O}$ of water and tracked the $\delta^{18}\text{O}$ of nitrite and nitrate produced. This allows us to determine both the $\delta^{18}\text{O}$ of newly produced nitrate, and also the amount of oxygen exchange between nitrite and water that has occurred during nitrification. When grown in co-culture, we found a low amount of exchange during ammonia oxidation by *Nitrosomonas* C-113a (0 - 8%) relative to growth in pure culture (23-25%). We found the $\delta^{18}\text{O}_{\text{NO}_3}$ of newly produced nitrate to be -0.8 to -1.5 ‰ in defined co-cultures and -0.4 ± 0.3 ‰ in surface waters of Vineyard Sound, MA. These values are near the upper end of the range predicted previously, but are within the range needed to explain low values of deep-ocean nitrate $\delta^{18}\text{O}$.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.005 UNCOVERING NEW DIVERSITY IN NITROUS OXIDE REDUCTASE (*nosZ*) GENES IN AGRICULTURAL SOILS

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The reduction of nitrous oxide to N₂, the terminal step in denitrification, is catalyzed by nitrous oxide reductase, encoded by the *nosZ* gene. Molecular ecology analyses exploring the diversity of *nosZ* in natural environments have primarily used gene sequences derived from known denitrifying bacteria. Recently, we identified a *nosZ* gene in the genomes of the non-denitrifying *Anaeromyxobacter dehalogenans* strains 2CP-C, 2CP-1 and K. These sequences were genotypically distinct from the denitrifier *nosZ* genes and are not amplified by denitrifier PCR primers, yet were shown to encode nitrous oxide respiration. Using PCR primers designed to target strain 2CP-C *nosZ* genes, 21 new *nosZ* fragments were retrieved from three agricultural soils. The nucleotide sequence identify of 12 matched closest (79-91%) to *nosZ* from strain 2CP-C, while 9 others matched closest (74-75%) to a putative *nosZ* from *Gemmatimonas aurantiaca*. In-frame translations deduced from the sequences were aligned with reference sequences and revealed at least two NosZ protein clusters distinct from denitrifiers. One cluster comprised of sequences associated with *Anaeromyxobacter* populations and the other group included NosZ deduced from phylogenetically disparate bacteria including *G. aurantiaca*, *Dyadobacter fermentans*, and *Persephonella marina*. Nucleotide alignments of the new sequences with reference denitrifiers confirmed that PCR primers commonly used to target *nosZ* in natural environments would not amplify the sequences obtained in this study. We designed new broader spectrum primers to detect and differentiate the three NosZ clusters. PCR results using these primers showed wide distribution of the denitrifier and *Anaeromyxobacter* clusters in the same agricultural soils. The results suggest that there is much higher diversity of *nosZ* in soil than previously thought and current methods must be reevaluated to investigate nitrous oxide reduction in natural environments.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.006 REAL-TIME FLUXES AND GENE COPY ASSAYS INDICATE DENITRIFICATION POTENTIAL IN SUBSOILS UNDER A GRAZED PASTURELAND

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Sub-soil denitrification is largely unexplored but is of consequence for the protection of groundwater supplies. Denitrifying microorganisms were quantified using real-time PCR assays of the nitrite reductase (*nir*) and nitrous oxide reductase (*nos*) functional genes at depths of 0-130 cm in Irish agricultural grassland. Whole-core soil incubations, consisting of the soil and overlying water in a headspace-free tube, were amended with 15N-nitrate to measure denitrification rates according to an established isotope pairing technique. Soil slurries were prepared using 1-cm depth-resolved slices of soil cores in gas-tight bags and incubated for 48 h at *in situ* soil temperatures after the addition of 15N-nitrate. The gene targets were quantified from sacrificially-sampled whole-core replicates and from temporal bag-liquor samples. Separately, soil cores from each of three depths (0-10 cm; 45-55 cm; 120-130 cm) were subjected to three alternate N- and C-source amendments (NO₃⁻; NO₃⁻ + glucose-C; NO₃⁻ + DOC) and real-time production of N₂O and N₂ was recorded using gas chromatography in a specialized He/O₂ airtight incubation vessel. During the 17-d incubation, total denitrification losses of the added N significantly decreased with soil depths and increased by the addition of either C source. The abundance of *nir* and *nos* genes decreased with depth, except in incubations amended with nitrate and DOC, where the carbon source affected gene copy numbers and fluxes of N₂O and N₂ production. The ratio of *nir*:*nos* gene copies was a useful proxy for incomplete denitrification and supported the findings of the real-time flux measurements. Ratios of

$N_2O:N_2O+N_2$ differed significantly only between soil layers, being higher in the upper layer, indicating the potential of subsoils for more complete N_2O reduction to N_2 . The data suggest that careful N management could reduce groundwater nitrate contamination and atmospheric N_2O emission, particularly through subsoil denitrification.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.007 BACTERIA INSTEAD OF ARCHAEA ARE RESPONSIVE FOR AMMONIUM INPUT IN MANGROVE MICROCOSM SEDIMENTS

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The ammonium processing is a key step in the nitrogen cycle, driving the further chemical transformation through the generation of N_2 . This process can occur by the ammonium oxidation, or by the direct conversion in N_2 , which occur in anaerobic environments, performed by the anammox bacteria. Due to the vertical stratification of the aerobic, microaerophilic and anaerobic zones, mangrove ecosystems are important areas to be used as a model in highlighting the ammonium processing in marine sediments. Here it is presented a survey based on a mangrove sediments microcosms using samples from taken two different mangroves located in the coastline of the São Paulo State (Brazil): i) oil-contaminated mangrove at Bertioaga, ii) non-disturbed mangrove at Ilha do Cardoso. Microcosms were installed to test the responsiveness of targeted communities to the ammonium input in the system. The input of ammonium was induced by the addition of artificial sheep urine (SU) amendment to the sediment cores, while control treatments were only supplemented with water. Samples were collected and analyzed in distinct times for further analysis (0, 2, 7, 14, 21, 42 and 84 days). The measures of ammonium and nitrate along the time have indicated similar ammonium consumption curves in both mangrove-based microcosms. However, low amounts of nitrate have accumulated in the microcosms, possibly indicating the direct transformation to N_2 . The 16S rRNA-based DGGE fingerprinting of Bacteria, Archaea and an anammox-related group has revealed major responsiveness to the ammonium input for bacterial communities, with higher distinctions along the period for the anammox-related group. This information combined with the low efficiency in detecting archaeal or bacterial amoA genes leads to the indication that the anammox process might have an important role in the ammonium processing in mangrove sediments.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.008 LINKING KARST AQUIFER DISSOLVED ORGANIC MATTER COMPOSITION TO CHANGES IN BACTERIAL DIVERSITY EXPLORED THROUGH PYROSEQUENCING

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Aquifer microbes can play a role in producing and cycling dissolved organic matter (DOM). However, the nature and abundance of DOM in aquifers can also influence microbial community structure. Our understanding of these biological processes is limited because, in general, knowledge of aquifer microbial diversity is lacking. Our research goal was to link changes in the fluorescence spectra of humic-like (terrigenous) and protein-like (microbially-derived) chromophoric DOM (CDOM) to the microbial diversity from the Edwards Aquifer (Central Texas). We hypothesized that the relative contributions of different CDOM would strongly reflect chemolithoautotrophic vs heterotrophic diversity and relative abundances. CDOM from filtered well and spring water was evaluated from excitation-emission matrices and calculated indices, such as the fluorescence index (FI) and humification index (HIX). Bacterial diversity from filtered water was evaluated by pyrosequencing 16S rRNA gene amplicons. Emission maxima of aquifer CDOM were distinct compared to surface water, with a more heterogeneous fluorophore collection and higher relative amounts of protein-like

CDOM. Water with the highest FI also had the highest percentage (~60%) of *Thiothrix* spp. (γ -*Proteobacteria*) and ϵ -*Proteobacteria*, groups associated with autotrophy. Water with the lowest HIX had the highest percentage (up to 40%) of *Nitrospira*, chemolithoautotrophic nitrite-oxidizers, and water with the highest HIX had higher abundances of β -*Proteobacteria* and Bacilli affiliated to heterotrophic and chemoorganotrophic metabolic groups. These results suggest that lack of humified CDOM and prevalence of protein-like CDOM, coincident with abundant autotrophic metabolic groups, may be due to either autotrophic primary productivity because terrestrial DOM does not contribute to the C reservoir, or that a terrestrial spectroscopic signature is lost through abiotic (sorption) and/or biotic processes (heterotrophy).

Abstract Category

22 Major Biogeochemical Cycles

PS.22.009 DIVERSITY OF ARSENIC REDUCING GENES IN SEDIMENT AND WATER FROM SALAR DE GORBEA, AN ACIDIC AND HIGH ALTITUDE (4,000 m) SALT FLAT

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Salar de Gorbea is an aquatic ecosystem located in the Chilean Altiplano. This salar is characterized by low pH (1-3.5), high salinity (39 g/L), aluminum and arsenic ranging from 0.5-3.5 mg/L and 1-10 mg/L respectively. In other regional saline system at neutral pH, arsenate respiratory reductase (*arrA*) was detected in a wide range of arsenic concentration and arsenate reductase (*arsC*) was only detected in samples with arsenic concentration below 10 mM (Escudero et al., 2009). The aim of that study was to investigate preliminarily the microbial population and arsenic metabolism in this particular environment through the detection of 16S rRNA, arsenate respiratory reductase (*arrA*) and arsenate reductase (*arsC*) genes.

Brine and sediments samples of Gorbea were studied. *arrA* and *arsC* genes were studied by PCR and clone libraries. Microbial assemblages were determined by DGGE of 16S rRNA gene fragments and clone libraries.

DGGE analyses revealed that bacterial community was dominated by *Gammaproteobacteria* for both brine and sediment samples. Clone libraries showed a bacterial community dominated by *Proteobacteria* and *Firmicutes* closely related to the genera, *Rhodanobacter*, *Acidisphaera* and *Desulfosporosinus*. *arsC* gene was detected in most of the samples and the sequences were similar to *arsC* of *Klebsiella pneumoniae* (90%) and *Serratia marcescens* (98%). On the other hand *arrA* was not detected in those.

Microbial communities in Gorbea exhibited a similar diversity in sediment and brine samples, but lower in comparison with other high altitude saline lakes with high arsenic concentration and neutral pH in northern Chile (Demergasso et al., 2008). The presence of *arsC* concurs with previous results obtained from other similar altiplanic systems and the absence of *arrA* is a differential trait compare to other regional environments, perhaps by acidic pH may be more important than As concentrations in determining the composition of arsenic metabolizing bacterial assemblage.

Abstract Category

22 Major Biogeochemical Cycles

PS.22.010 IMPROVED HYPOTHETICAL MODEL OF THE MICROBIAL COMMUNITY INHABITING THE LAKE OF SIMBA VOLCANO CHILEAN ALTIPLANO

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In the Chilean Altiplano, 4,000-4,500 altitude the high solar radiation and rapid changes in temperature make it a key area for studying global climate changes. The effort to improve a hypothetical model of active biogeochemical processes in a lake in the crater of Simba volcano is

described. This should permit research on the response of microbial populations facing rapid climate change. This model was built, in advance, based on the geochemistry of the system and the physiology of microbial populations inferred from phylogenetic analysis. Microbial enrichments were performed supplemented with sulfur and sulfate in aerobiosis and anaerobiosis, respectively, in the present work. The microbial diversity in amended experiments was analyzed by DGGE/sequencing.

The model considers that volcanic gases produce the acidification of the water in the crater lake and provide reduced compounds (H₂, H₂S, CO₂, CH₄). Available gases composition data from the near by Lascar volcano were incorporated in the model assuming they share the magmatic chamber. Electron donors would support microbial growth increasing the organic matter. Primary production by photosynthetic microorganisms would be increased due to UV filter activity of organic matter and volcanic ashes. Acetogenic bacteria suggest the occurrence of fermentative metabolism. The organic matter level favors the development of heterotrophic acidophilic rather than chemolithotrophic populations. Microbial growth was evidenced in both supplemented experiences. DGGE analyses of enrichments showed communities dominated by *Firmicutes* and *Alphaproteobacteria* in anaerobic and aerobic incubation, respectively. Diversity is higher in the enrichment with reduced inorganic sulfur compounds compare to the experiments for sulfate-reducing bacteria growth (Range-weight richness 113 and 16, respectively).

Growing experiences provide additional evidences of the occurrence of an active sulfur cycle and the predominant bacterial agents in Simba.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.011 SPATIO-TEMPORAL DIVERSITY OF NIRS-HARBOURING BACTERIA IN A POTATO FIELD

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The diversity of nitrite-reducing bacteria harbouring the *nirS* gene has not been extensively studied under soil conditions. To address this gap in knowledge, the diversity of *nirS*-harbouring bacteria was evaluated in a potato field over the course of a growth season using a culture-independent PCR-shotgun cloning and sequencing approach. Potato crops are of importance to the Canadian economy and this culture releases large quantities of nitrous oxide. Three different soil locations (hill, furrow, and near plant) and six sampling dates, from early spring to late fall, were targeted. For all treatments, denitrification enzyme activity (DEA), denitrification activity, nitrous oxide gas emission and nitrite were also evaluated. 182 *nirS* operational taxonomical units were identified, 8 had high homology levels to cultured microorganisms while most others had low homology levels to uncultivated organisms, suggesting the detection of novel *nirS*-harbouring bacteria. The diversity of *nirS*-denitrifiers was found to be significantly different between locations, with the highest diversity in the furrow, then the hill and finally the soil near the plant. Interestingly, the diversity was, in most cases, not significantly altered by sampling date but was found to be inversely proportional to DEA. No other significant link was found between diversity and biochemical parameters under study. In conclusion, the results suggest that the formation of hills and furrows in potato fields leads to significant differences in the diversity of *nirS*-harbouring denitrifiers and that this diversity is negatively correlated with DEA.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.012 DIFFERENCES IN GROWTH BEHAVIOR OF AMMONIA OXIDIZING ARCHAEA AND AMMONIA OXIDIZING BACTERIA

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Ammonia oxidation is the first and rate limiting step in nitrification, the process of converting nitrogen from its most reduced form (ammonium) to its most oxidized form (nitrate). Aerobic ammonia oxidation was previously thought to be carried out only by members of the *Beta*- and *Gammaproteobacteria*. Recently, ammonia oxidation was discovered among Archaea as well. We have enriched ammonia-oxidizing archaea from two freshwater lake sediments in Ohio, USA. These enrichments are 99.7% identical on the *amoA* nucleotide sequence level to clones from the freshwater area of the San Francisco Bay. One enrichment culture contains a nitrite oxidizer, while the other does not. We compared the growth response of these archaeal enrichments to those of the ammonia-oxidizing bacterial enrichment G5-7, which contains *Nitrosomonas* sp. Is79A3, under differing concentrations of ammonium, oxygen, and sodium carbonate, as well as over a range of pH. Nitrite/nitrate production was determined colorimetrically and used to calculate the growth rates. Our data reflect that the ammonia-oxidizing archaea grow faster under lower ammonium (0.015 to 0.5 mM) and are inhibited by ammonium concentrations higher than 3 mM. We did not observe growth of G5-7 under the lowest ammonium concentrations tested, however G5-7 shows increasing growth rates in ammonium concentrations of 0.25 to 5 mM. The growth of G5-7 shows a clear optimum at pH 7, while the archaeal cultures show a constant growth rate over the tested pH range from 6 to 9. When subjected to low oxygen concentrations (1%), the ammonia-oxidizing archaea displayed a faster growth rate than under normal atmospheric concentrations, whereas there was no difference in the growth rates of G5-7. Experiments with inorganic carbon are in progress. Overall, our results show that the growth of ammonia-oxidizing archaea and bacteria differs in response to culture conditions, indicating that they may occupy different niches in the environment.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.013 FUNGAL VS BACTERIAL SIGNIFICANCE FOR STREAM METABOLISM: A TEST IN EXPERIMENTAL STREAMS

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Fungi are important decomposers of leaf litter in streams with possible knock-on effects on other stream microbes and carbon cycling. To elucidate such effects, we designed an experiment in outdoor experimental flumes simulating sand-bed streams in an early successional state. We expected an overall enhanced microbial activity in the presence of fungi, accompanying a shift in microbial communities (fungi vs bacteria) on leaf litter. Fifteen experimental channels (4m x 0.12m x 0.12m) filled with sterilized sediment and a small amount of sterilized leaves were inoculated with one of four different suspensions: 1) stream water with bacterial and fungal communities, 2) stream water with bacterial communities only, 3) stream water without microorganisms, and 4) stream water with killed communities of bacteria and fungi. Water from an early-successional watershed was then circulated through the flumes for 5 weeks. We measured whole-system metabolism and sampled sediment and leaf litter at 5 occasions during that time. Whole-stream metabolism and microbial respiration associated with leaf litter were higher in experimental streams inoculated with fungi, reflecting faster fungal growth on leaves. There were no significant differences among the two control and bacteria-only treatments. Bacterial biomass on leaf litter and in sediment did not vary among treatments at any time, but sediment microbial respiration increased dramatically at the last sampling date in the streams receiving fungal inocula. Similarly, increases in net primary production and chlorophyll-*a* content were greatest in the streams inoculated with fungi. These results point to a major role of fungal communities in stream ecosystems, well beyond the direct involvement in leaf litter decomposition.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.014 DIVERSITY AND ABUNDANCE OF MEMBERS OF THE ζ -PROTEOBACTERIA IN IRON-RICH HYDROTHERMAL DEPOSITS AT MARINER AND VAI LILI VENT FIELDS (SW PACIFIC)

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Metalliferous sediments associated with hydrothermal vent systems can store much more energy for supporting microbial growth than the hydrothermal fluids. These diffuse low-temperature deposits are more extensive than previously thought and provide energy for microbes long after the hydrothermal activity has ceased. The microbial diversity in these environments is poorly studied. One group of organisms reported from diffuse sediment-hosted hydrothermal vent systems are neutrophilic Fe oxidizers like members of the ζ -*proteobacteria*. Sediment samples were collected from areas of diffuse hydrothermal venting along the Valu Fa Ridge (SW Pacific). Samples from the Mariner (22.18°S, 176.60°W) and Vai Lili (22.21°S, 176.61°W) vent fields were collected by the ROV Jason II during a research cruise on the R/V Thompson in 2009. Both classical microbiological methods and culture-independent techniques as 16S rRNA gene cloning, qPCR and amplicon analysis from 454 sequencing were applied to study the diversity and quantify the population abundance within these deposits. Phylogenetic analysis shows that the majority of the clones affiliates to *Proteobacteria* (α , β , γ , δ and ζ), but also *Chloroflexi*, *Planctomycetes* and candidate divisions were shown to be predominant members of the community. A remarkably high diversity of yet-to-be cultured microorganisms, also within the ζ -*proteobacteria* was reported. Quantification analysis shows that these chemolithotrophic organisms are particularly abundant in some of the samples (up to 10%). Moreover, various isolates were obtained. Although further characterization is needed, SEM studies reveal Fe oxide structures similar to those observed from previously known neutrophilic Fe-oxidizing bacteria. The diversity and abundance of members within the ζ -*proteobacteria* class point to the importance of these organisms within the global iron cycle and their role as pioneer colonizers of the metalliferous sediments on the seafloor.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.015 SOIL RESOURCES INFLUENCE SPATIAL PATTERNS OF DENITRIFYING COMMUNITIES AT SCALES COMPATIBLE WITH LAND MANAGEMENT

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Knowing spatial patterns of functional microbial guilds can increase our understanding of the relationships between microbial community ecology and ecosystem functions. Using geostatistical modeling to map spatial patterns, we explored the distribution of community structure, size and activity of one functional group in N-cycling, the denitrifiers, in relation to 23 soil parameters over a 44 ha farm divided into one organic and one integrated crop production system. The denitrifiers were targeted by the *nirS* and *nirK* genes that encode the two mutually exclusive types of nitrite reductases; the cd_1 -heme type and the copper reductases, respectively. The spatial pattern of the denitrification activity genes was reflected by the maps of the abundances of *nir* genes. For the community structure, which was determined by terminal restriction fragment length polymorphism of the *nirS* and *nirK* genes, it was only the maps of the *nirS* community that was related to the activity. The activity correlated to nitrate and dissolved organic nitrogen and carbon, whereas the gene pools for denitrification, in terms of size and composition, were influenced by soil structure. For the *nirS* community, pH and soil nutrients were also important in shaping the community. The only unique parameter related to the *nirK* community was soil Cu content. However, the spatial pattern of the *nirK* denitrifiers corresponded to the division of the farm in the two cropping systems. The different community patterns together with the spatial distribution of the *nirS:nirK* abundance ratio suggest habitat selection on the *nirS* and *nirK*-type denitrifiers. Our findings constitute a first step in identifying "metahabitats" for denitrifiers at scales relevant for land management.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.016 ECOLOGICAL AND EVOLUTIONARY FACTORS UNDERLYING GLOBAL AND LOCAL ASSEMBLY OF DENITRIFIER COMMUNITIES

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The conversion of nitrite to nitric oxide in the denitrification pathway is catalyzed by at least two structurally dissimilar nitrite reductases, NirS and NirK. Although they are functionally equivalent, a genome with genes encoding both reductases has yet to be found. This exclusivity raises questions about the ecological equivalency of denitrifiers with either *nirS* or *nirK*, and how different ecological and evolutionary factors influence community assembly of *nirS* and *nirK* denitrifiers. Using phylogeny-based methods for analyzing community structure, we analyzed *nirS* and *nirK* data sets compiled from sequence repositories. Global patterns of phylogenetic community structure were determined using Unifrac, whereas community assembly processes were inferred using different community relatedness metrics. Similarities between globally distributed communities for both genes corresponded to similarities in habitat salinity. The majority of communities for both genes were phylogenetically clustered; however, *nirK* marine communities were more phylogenetically overdispersed than *nirK* soil communities or *nirS* communities. A more in-depth analysis was performed using three case studies in which a comparison of *nirS* and *nirK* community relatedness within the sites could be examined along environmental gradients. From these studies we observed that *nirS* communities respond differently to environmental gradients than *nirK* communities. Although it is difficult to attribute non-random patterns of phylogenetic diversity to specific niche-based or neutral assembly processes, our results indicate that coexisting *nirS* and *nirK* denitrifier communities are not under the same community assembly rules in different environments.

Abstract Category

22 Major Biogeochemical Cycles

PS.22.017 EMISSION OF THE GREENHOUSE GAS NITROUS OXIDE BY MARINE INVERTEBRATES

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The emission of the greenhouse gas nitrous oxide (N₂O) was recently reported for earthworms and several freshwater invertebrate species. N₂O production associated with these animals is due to incomplete denitrification by ingested soil or sediment bacteria that are activated by the specific microenvironment of the invertebrate guts (i.e., low oxygen and high organic carbon and nitrate concentrations). This study investigated the N₂O emission potential of marine invertebrate species from coastal sediments of the North Sea and the Baltic Sea. The survey also included the aquacultured shrimp *Litopenaeus vannamei*, as we suspected high N₂O emission rates due to very high nitrate concentrations in the aquaculture tanks. The screening revealed that many marine invertebrate species emit N₂O. The average N₂O emission rate was 280 pmol individual⁻¹h⁻¹ for the benthic invertebrates, while the aquacultured shrimp showed an average emission rate of ~4000 pmol individual⁻¹h⁻¹. N₂O emission rates were correlated with body weight and the presence of a microbial biofilm on exoskeletal surfaces, but not with the affiliation of the animal to a certain feeding guild or taxonomic group. Incubation experiments with mussels and snails revealed a significant contribution of exoskeletal biofilms to the overall N₂O production. Considering the high abundance and high N₂O emission rate of many marine invertebrate species, a significant contribution of these animals to the overall N₂O emission from coastal marine environments and from aquaculture facilities can be expected.

Abstract Category

22 Major Biogeochemical Cycles

PS.22.018 BACTERIAL COMMUNITIES AND FUNCTIONAL GENES INVOLVED IN THE NITROGEN CYCLE ASSOCIATED TO PIONEER HEAVY-METAL TOLERANT PLANTS

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Mine tailings are crushed rock residues stored at open air, rich in heavy metals and deficient in combined nitrogen and organic matter. However, some pioneer and heavy-metal tolerant plants are growing in this environment, and the biological nitrogen fixation must be the major source of assimilable nitrogen, and fuel the nitrogen flux. In this work, the diversity of bacteria and bacterial guilds involved in the Nitrogen cycle in the rhizosphere of *Chloris virgata*, *Bahia xylophora*, *Viguiera linearis* and *Haplopappus venetus*, plants growing on mine tailings from Zacatecas, Mexico, were investigated. The diversities of total bacteria, and nitrogen fixing, ammonia oxidizing, nitrite oxidizing and denitrifying guilds were estimated by 16S rRNA, *nifH*, *amoA*, 16S rRNA of *Nitrobacter*, and *nirK-nirS-nosZ* genes libraries, respectively. *Proteobacteria*, *Acidobacteria*, *Bacteroidetes*, *Gemmatimonadetes*, *Chloroflexi*, *Firmicutes*, *Verrucomicrobia*, *Nitrospirae* and *Actinobacteria* phyla, and TM7, OP10 and OD1 divisions were recognized in the rhizospheres. Only 25% from the phylotypes were common among rhizospheres and the most abundant groups were *Acidobacteria* and *Betaproteobacteria*. In bulk soil, *Acidobacteria* and *Actinobacteria* were abundant and no common phylotypes were shared with the rhizosphere libraries. All versions of *nifH* genes of *Clostridium* spp., and *Azospirillum lipoferum* were detected. *Nitrosospira multiformis*, *Nitrosomonas communis* and *Nitrosomonas briensis amoA* genes, and *Pseudomonas* sp., *Alcaligenes xylooxidans* and *Rhizobium* sp. *nirK* genes were detected in all the rhizospheres. However, 16S rDNA of *Nitrobacter vulgaris*, and *nosZ* of *Pseudomonas fluorescens* were detected only in bulk soil, and *C. virgata* rhizosphere. Many chemolithotrophic and sulfur-oxidizing bacteria were detected, as can be expected in an environment with heavy metal-sulfur complexes, and lack in organic carbon. Apparently, in all mine tailings the nitrogen cycle is flowing in the rhizosphere of plants.

Abstract Category

22 Major Biogeochemical Cycles

PS.22.019 DIVERSITY ANALYSIS OF THE CONSTRUCTED BACTERIAL COMMUNITY OF THE AUTOTROPHIC AND THE HETEROTROPHIC *NITROBACTERIA* TREATING NH₄-N-CONTAINING SEWAGE

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6 strains of autotrophic *Nitrobacteria* and 2 strains of heterotrophic *Nitrobacteria* were screened from the bio-ceramic reactor, the nitrifying rate of the autotrophic *Nitrobacteria* ranged from 1.03mg/l.d to 1.25 mg/l.d. The removal of total nitrogen (TN) of the heterotrophic strains, which was recorded as SHY4 and SHY5, were 62.76% and 63.78%, while, the removal of nitrification of ammoniacal nitrogen (NH₄-N), were 69.73% and 80.78% after 12 days aseptic culture in ammonia oxidizing medium, and the final nitrite nitrogen (NO₂-N) concentration in the medium were 0.26mg/L, 0.45mg/l, respectively. The concentration of NO₂⁻-N decreased to 8.87mg/l after 12 days aerobic incubation in nitrous acid medium for SHY5. Nitrite accumulated in the medium and NO₂-N concentration reached 0.48mg/l. The results of the analysis through 16SrDNA sequencing, the homologies comparing and the physiology and morphology consideration showed that, SHY5 was *Pseudomonas* sp., and SHY4 was *Pseudochromobacterium* sp. The microbial consortium used for NH₄-N containing wastewater treatment in the sequential batch reactor (SBR) was constructed by equally mixing the 6 strains of autotrophic nitrobacteria and the 2 strains of heterotrophic *Nitrobacteria*. The subsequent performance for the SBR were collected, and denaturing gradient gel electrophoresis of polymerase chain reaction (PCR-DGGE) was used to analyze the species succession. The PCR-DGGE results showed that there were 19 dominant species in the reactor in day 15 and the species were similar with those in 30 (species-similarity was 85.71%). Such a result showed that the structure of the bacterial consortium tended to be steady. SHY5 was the dominant bacterium in the SBR reactor in this experiment.

Abstract Category

22 Major Biogeochemical Cycles

PS.22.020 PHYSIOLOGICAL AND ECOLOGICAL CHARACTERISTIC OF OCEANIC AMMONIA-OXIDIZING ARCHAEA DEMONSTRATED BY ENRICHMENT CULTURE

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In marine environments, archaeal dominance in ammonia oxidizing processes have been recently suggested, however, few works have been reported on characteristic of ammonia-oxidizing archaea (AOA). Here, we report nitrification activity and the diversity of ammonia monooxygenase subunit A (amoA) genes of enrichment cultures obtained from deep seawater of the Suruga Bay, Japan (maximum depth was about 2320 m) in February 2008, enriched with 50 μM $(\text{NH}_4)_2\text{SO}_4$, and then incubated at 4°C, 10°C and 20°C (n=1) in the dark for 247 days. Ammonia oxidation was observed in all enrichment cultures. Polymerase chain reaction (PCR) amplification of archaeal amoA gene was successful, whereas no amplification of bacterial amoA genes was observed. Sequencing 368 clones of amoA gene gave 175 Operational Taxonomic Units (OTUs) as grouped with more than 99% of nucleic acid sequence. All clones were fell into three clusters: "Nitrosopumilus maritimus", "water column" and "water column" clusters. Most clones retrieved from environmental samples were clustered into the "water column" cluster (94-100%) and those from the enrichment culture samples belonged to either "Nitrosopumilus maritimus" or "water column" clusters. This trend was of particular note at high temperature of the enrichment culture (26-100%). Also, one of the enrichment cultures (500 m, 20°C) contained only 1 OTU of the clones belonging to "water column" cluster. Our results suggested that "water column" cluster of AOA adapted to lower temperature and substrate concentration than "Nitrosopumilus maritimus" and "water column" clusters and dominated in ammonia oxidation in a deep layer of the Suruga Bay. We hypothesized that "water column" cluster-like AOA inhabiting deep waters could grow or maintain their life under extremely low nutrient conditions and thus could be called "super-oligotrophs".

Abstract Category
22 Major Biogeochemical Cycles

PS.22.021 IDENTIFICATION AND ISOLATION OF THE KEY N₂O-REDUCING DENITRIFIERS IN RICE PADDY SOIL

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Rice paddy soil is known to have strong denitrifying activity with N₂ as a final product. Water-dissolved N₂O is occasionally detected in surface and ground water of rice paddy fields, while little or no N₂O is emitted to the atmosphere. This indicates the presence of N₂O-reducing denitrifiers in rice paddy fields; however, these populations have not been examined well. In this study, we employed both culture-dependent and -independent approaches to identify and isolate key N₂O-reducing denitrifiers in rice paddy soil. In a soil microcosm setup, growth of N₂O-reducing denitrifiers was promoted by addition of 5% N₂O and succinate. For culture-independent experiment, ¹³C-succinate was used. DNA was extracted 24-h after incubation, and heavy and light DNA fractions were separated by density gradient ultracentrifugation. Denaturing gradient gel electrophoresis and clone library analysis targeting 16S rRNA gene and nitrous oxide reductase gene (*nosZ*) were performed. For culture-dependent analysis, N₂O-reducing denitrifiers were grown with the presence of cell-division inhibitors. The resulting elongated cells were individually captured by a micromanipulator, and transferred to a low-nutrient medium (Functional single-cell isolation method). The N₂O-reducing ability of the strains was examined by gas chromatography-mass spectrometry. Results from the stable isotope probing analysis suggested that Burkholderiales and Rhodospirillales assimilated ¹³C in the soil incubated with added N₂O and ¹³C-succinate (the soil with strong N₂O reducing activity). In contrast, Desulfuromonadales assimilated ¹³C in the soil incubated with only added ¹³C-succinate (the soil without N₂O reducing activity). Based on the functional single-cell isolation, the majority of the N₂O-reducing isolates belonged to *Herbaspirillum* (Burkholderiales) and *Azospirillum* (Rhodospirillales), similar to the stable isotope probing results. These bacteria may play a key role in N₂O reduction in rice paddy soils.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.022 EFFECT OF ANOXIA ON WATER COLUMN N CYCLING

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Anoxia in the oceans, lakes and estuaries has become a serious problem worldwide. Anoxia has the potential to both increase and reduce N removal efficiency however the mechanisms, processes and microorganisms involved remain largely unresolved. Since denitrification is an anaerobic process it usually occurs in the sediments where it is coupled to nitrification. However, during an anoxia or hypoxia event conditions within the water column may become more favourable for denitrification and other anaerobic N cycle pathways such as, dissimilatory nitrate reduction to ammonium (DNRA) and possibly even Anammox, although this has never been demonstrated. Since estuaries are generally N-limited there is a pressing need to assess and quantify changes in N cycling processes during an anoxia or hypoxia event. In the work reported in this paper we have used an integrated approach combining molecular biology (qPCR and community fingerprinting), process rates and modelling techniques to investigate the impact of seasonal anoxia events on the size and activity of N cycle functional groups within the water column. We found evidence that denitrification occurs within the anoxia bottom waters of the salt wedge during the winter. In contrast, the summer algae blooms caused a rapid depletion of oxygen and accumulation of sulphide in the bottom waters which may favour the DNRA pathway. These data are currently being analysed using multivariate statistics and Structural Equation to identify key components of the microbial community responsible for driving anaerobic N cycle pathways and initial results will be presented. The goal is to develop a complete picture of N cycling dynamics within the water column and improve our understanding of how the microorganisms involved respond to onset and relief of anoxia.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.023 FUNCTIONAL GENES RESPONSE OF NUTRIENT CYCLES TO PROLONGED NO-TILL PRACTICES AND CROP ROTATIONS IN A FARMING SYSTEM EXPERIMENT IN NORTH CAROLINA

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Two major recent modifications for sustainable agriculture are no-till practices and improved crop rotations. Both have been shown to improve soil structure and quality in supporting crop growth. Soil microbes are the mediators for biogeochemical cycles for major nutrients such as C and N. However our knowledge of microbial responses to no-till and crop rotation is limited to the physiology and structure of microbial communities at a rather broad resolution. Microarray technology targeting ~47,000 functional genes, GeoChip 3.0, was used to investigate the microbial response in detail, especially for functional constituents in nutrient cycles. A long-term experiment was designed to contrast the impact of conventional tillage (CT), no-tillage (NT) and non-managed systems called successional (SC) fields. Soil physical and chemical properties showed significant responses to both no-till (R=0.684) and crop rotations (R=0.523). The response of microbial community structure was significant to no-till (R=0.063) and crop rotations (R=0.102) as well. The percentage of functional genes detected was lowest in SC, but it was comparable between NT and CT fields. The responses in functional gene diversity (H'), richness and normalized abundance were not significant to treatments, but the responses of nitrification and N fixation functional genes in diversity and richness were significant (P<0.001). Extractable N, net mineralized N and respiration were significantly correlated with most subgroups of nutrient cycles in NT field. Multivariate correlation analysis identified similar sets of soil properties were most significantly associated with NT field functional genes. These may indicate better adaptation of microbial communities in NT soil to soil nutrients without major disturbance, such as tilling. Together the responses in nutrient cycle functional genes to no-tillage and crop rotations were apparent in the structure of microbial community, but not in diversity and abundance.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.024 DENITRIFICATION IN ANOXIC GROUNDWATER AT 140 M DEPTH IN SEDIMENTARY HORIZON

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Phylogenetically diversified prokaryotes exert various functions in deep subsurface environment. To understand microbial contribution to the geochemistry at the interface between micro-oxic and anoxic condition in subsurface environment we estimated denitrification activity and relevant players by using stable-isotope tracer technique and gene analysis in a sedimentary geological setting in Horonobe, Hokkaido, Japan. Groundwater samples were obtained from 140 m of the Ventilation Borehole (V140) and the East Borehole (E140), which is 70 m away from V140, drilled in the area of the Horonobe Underground Research Center of JAEA. Groundwater samples were added with ¹⁵N-nitrate and incubated in anoxic or micro-oxic condition. ³⁰N₂ and ⁴⁶N₂O production was measured using GC/MS over time. 16S rRNA genes for domain *Bacteria* and nitrite reductase-encoding gene fragments (*nirS*) were PCR amplified, cloned, and sequenced from the DNA extracts. Total number of cells in groundwater was 5.2×10^3 cells mL⁻¹ in V140 and 4.0×10^4 cells mL⁻¹ in E140. Nitrate concentration was below detection limit in both groundwater samples. Denitrification to N₂ gas occurred in both nitrate-added groundwater samples. Lag time in producing N₂ gas of V140 groundwater was shorter than that for E140 groundwater. It was confirmed that denitrification of groundwater were not detected in the presence of low concentration of oxygen with ca. 1 mg O₂ L⁻¹. Most of *nirS* clones did not show high similarity with known clones. Diversity of *nirS*, contrary to the measured activity, was higher in E140 than V140. Although two boreholes are located in the same formation and depth, spatial heterogeneity was observed in abundance, constituents, and potential of denitrification of prokaryotes.

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Abstract Category
22 Major Biogeochemical Cycles

PS.22.025 FUNCTIONAL COMPLEMENTARITY AMONG DENITRIFIERS IN RICE PADDY SOIL

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Rice paddy field soil shows strong activity of N₂-producing denitrification during submerged period. Denitrification is a microbial process, in which nitrate is reduced stepwise to nitrite, NO, N₂O and N₂. Although some denitrifiers can perform complete processes of the denitrification, some denitrifiers may play only part of the processes. We hypothesized that N₂ is produced in rice paddy soil as a result of complementary function of denitrifiers. In this study, we isolated bacteria capable of performing denitrification processes in rice paddy soil, and analyzed which steps of denitrification were carried out by each isolate. Soil microcosms were set up at 20°C or 30°C. Denitrifying activity was induced by addition of nitrate or nitrite, together with succinate. Single-cell isolation was performed targeting bacteria responding to the denitrification inducing conditions by using a micromanipulator. Partial sequences of the 16S rRNA gene were analyzed for the phylogenetic identification. For each isolate, abilities to reduce nitrate, nitrite, and N₂O were examined. A total of 250 strains of denitrifying bacteria were isolated. Major denitrifiers were different between soils incubated at 20°C and 30°C. Isolates capable of reducing nitrite but not nitrate or N₂O were present. They are most likely specialists for nitrite reduction. In addition, some isolates reduced only little nitrate and nitrite, but large amount of N₂O. They are probably specialist for N₂O reduction. These results indicated that denitrifiers in rice paddy soil respond differently to incubation temperature. In addition, denitrifier community might be composed of various bacteria carrying out different steps in the denitrification processes. As a total function of these denitrifiers, the rice paddy community may show N₂-producing denitrification.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.026 INFLUENCE OF THE RELATIVE SUPPLIES OF ELECTRON DONOR AND ACCEPTOR ON THE PREDOMINATING NITRATE REDUCTION PROCESS

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In the nitrogen cycle two anaerobic respiration pathways compete for nitrate: denitrification and dissimilatory nitrate reduction to ammonia (DNRA). Denitrifiers reduce nitrate mainly to nitrogen whereas dissimilatory nitrate reducers produce ammonia. The outcome of this competition has important environmental consequences, because denitrification leads to the emission of the greenhouse gas nitrous oxide to the atmosphere and a loss of fixed nitrogen, which often limits primary productivity. Thus the prevalence of one or the other pathway is also significant for the carbon cycle.

It is generally assumed that the outcome of the competition is mainly determined by the relative supply of organic carbon and nitrate. But this hypothesis has not been tested in environmental context.

To investigate the effect of the relative supplies of electron donors and acceptors on the competition between the two pathways in a natural community, sediments of a tidal flat active in nitrogen cycling were incubated in laboratory bioreactors. The electron donors/acceptors ratio was manipulated under otherwise identical conditions. The outcome of the competition was assayed by mass balancing of nitrogen and carbon compounds. The bioenergetic efficiency was measured by high resolution calorimetry (heat balancing). The competition between denitrifying and dissimilatory nitrate reducing species was monitored by metagenomics (454 and solexa sequencing of total extracted DNA).

Abstract Category
22 Major Biogeochemical Cycles

PS.22.027 CULTIVATION OF A NOVEL ACIDOPHILIC AMMONIA-OXIDIZING CRENARCHAEON FROM SOIL

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Ammonia oxidation is the first and rate-limiting step in nitrification, and vital to the global nitrogen cycle. Until the isolation of the marine crenarchaeon *Nitrosopumilus maritimus*, ammonia oxidation was thought to be carried out entirely by specific groups of bacteria. All cultivated ammonia-oxidizing organisms described to date grow under neutral or near-neutral conditions, and their growth is inhibited under acidic conditions. Approximately 30% of the world's soils are acidic (pH less than 5.5), including a wide range of natural and managed habitats. Autotrophic nitrification occurs in acidic soils and archaea, rather than bacteria, may dominate ammonia oxidizer communities in these habitats. However, the mechanism and organisms responsible for nitrification under acidic conditions are not fully understood. The aim of this study was to cultivate and characterize crenarchaea responsible for nitrification in acidic soils and to determine mechanisms for growth at low pH. Enrichment cultures were obtained by inoculation of minimal salts medium containing ammonia and streptomycin with soil from an acidic agricultural plot (pH 4.5) and incubation at 28°C. Growth was monitored by a quantitative PCR assay using 16S rRNA gene primers targeting crenarchaea. An enrichment culture was obtained containing an autotrophic crenarchaeal ammonia oxidizer. The enriched organism was representative of the main crenarchaeal phylotype present in the original soil inoculum, as revealed by denaturing gradient gel electrophoresis. This is the first report on an acidophilic ammonia-oxidizing archaeon cultivated from soil with active growth in liquid batch culture at pH 4.5. The organism falls within the crenarchaeal Group 1.1a-associated lineage previously found in acidic soils, subsurface and freshwater. Although the Group 1.1a-associated lineage is related to other ammonia-oxidizing crenarchaea, this study is the first definitive evidence of their ability to oxidize ammonia.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.028 A MICROBIAL ARSENIC CYCLE IN AN ARSENIC-RICH SHALLOW ALLUVIAL AQUIFER

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Natural arsenic in groundwater used as a source for drinking, household, and agricultural purposes represents a major health problem for humans in many places around the world. Microorganisms may play a key role in the mobilization of arsenic present in elevated concentrations within aquifers. Microcosm experiments using sediments from an arsenic-rich shallow alluvial aquifer of southwestern Taiwan showed simultaneous microbial reduction of iron and arsenate and demonstrated that the capacity for arsenic release was not limited by the availability of electron donors in the sediments. We isolated an arsenate-respiring bacterium native to aquifer sediments which showed that the direct enzymatic reduction of arsenate to the potentially more-soluble arsenite in pore water is possible in this aquifer. We also showed that a full biogeochemical arsenic cycle is operative in the shallow aquifer by microorganisms naturally coexisting in the environment at the site.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.029 CASCADING EFFECTS OF HIGH NITRATE INPUTS ON THE TRANSFORMATION OF NITROGEN BY COASTAL MICROPLANKTON

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Riverine inputs of nitrate into coastal ecosystems are increasing in most parts of the world nowadays due to increasing use of fertilizers. The assimilation of dissolved inorganic nitrogen by phytoplankton is one important pathway for the removal of nitrate from streams and coastal areas. However, mechanistic studies on the short term transformation of reactive nitrogen by microplankton are still rare. Here we report that the transformation of nitrogen critically depends on the state of the microbial food web. Incubation experiments in which a natural microplankton community (Waquoit Bay, USA) was exposed to a gradient of increasing nitrate concentrations for 6 days showed that the supply of excess nitrate immediately induced an increase in dissolved organic nitrogen uptake and eventually led to lower net nitrate removal rates. The combination of biogeochemical analytics, microbial activity measurements, stable isotope tracer techniques, and cell sorting by flow cytometry allowed new insights in the fate of reactive nitrogen within the microbial food web. Potential mechanisms for this result are discussed based on an inverse modeling approach using the experimental data set. Our finding has implications for a new understanding of nitrogen transformation in coastal areas and points out the potential for the extension of eutrophication towards the more open ocean.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.030 THE DETECTION OF ANAMMOX BACTERIA IN BRAZILIAN MANGROVES SEDIMENTS

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The process of anaerobic ammonium oxidation is based in the combination of ammonium with nitrite under anoxic conditions, resulting in the generation of dinitrogen gas. Such transformation is performed by bacteria belonging to the monophyletic group, allocated within the *Planctomyces* phylum, so-called anammox bacteria. Here it is presented a survey of anammox bacteria in three mangroves located in the coastline of the São Paulo State (Brazil): i) oil-contaminated mangrove at *Bertioga*, ii) non-contaminated mangrove at *Bertioga*, iii) non-disturbed mangrove at *Ilha do Cardoso*. The goals of this study were to estimate the amount of anammox-related bacteria in samples by quantitative real time PCR (qPCR), and also to infer about the diversity of such group by denaturing gradient gel electrophoresis (DGGE). The detection and amplification system was based on specific primers described for the anammox bacterial groups *16S rRNA* gene

Brod541F/Brod1260R. The qPCR results have shown an abundance of anammox bacteria in all mangroves; however, the samples collected closely to oil spill showed to harbor higher abundances of anammox bacteria (log values from 6.53 to 6.76) in comparison to other samples (log values from 4.66 to 5.68), possibly due to the strict anaerobiosis induced by the oil layer and the lower density of vegetation found in this area. The analyses of DGGE band patterns has indicated the presence of distinct anammox-related bacterial communities in each sampled mangrove, with a remark for samples collected near the oil spill. Such samples presented major differences in comparison to others, reinforcing that the oil contamination can directly act on the anammox bacterial community, modulating its structuring, and possibly its functioning. In summary, these results initiate a tentative in deciphering the ammonium processing in mangroves, what can add information on the diversity of functioning microbial groups involved in the nitrogen transformations

Abstract Category
22 Major Biogeochemical Cycles

PS.22.031 CHANGES IN SEDIMENT BACTERIAL COMMUNITIES ALONG NUTRIENT GRADIENTS IN NORTHEASTERN BALTIC SEA RECEIVING AGRICULTURAL DISCHARGES

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Agricultural phosphorus is the principal driver of eutrophication in estuaries. Phosphorus inputs can lead to changes in the composition of microbial communities of which other organisms are dependent on. The aim of the study was to assess whether external phosphorus loading affects bacterial community composition and how the communities are changed. To test this, we studied the relationship between bacterial communities and chemical parameters in northern Baltic Sea sediments that receive loading from an agricultural drainage area. Terminal restriction fragments of the 16S rRNA gene and chemical parameters such as different phosphorus forms were analysed by multivariate statistics. Mantel correlogram revealed that the similarity of the samples decreased along spatial distance. Moreover, canonical correlation analysis illustrated that the bacterial communities changed horizontally from the agricultural drainage area to the open sea and vertically from the surface to deeper sediment layers along the gradients of different phosphorus forms and elements linked to phosphorus cycling. Partial canonical correlation analysis excluding the spatiality showed that the bacterial communities had true relationships with phosphorus bound to aluminium oxides and with labile organic phosphorus, as well as with total and redox-sensitive iron. Deltaproteobacteria, were common throughout the study area. The presumptively sulphate-reducing bacteria were associated with labile organic phosphorus, total nitrogen and total carbon in the open-sea sediment. These bacteria may affect the release of phosphorus from sediment and therefore increase eutrophication. Our study supports our hypothesis that external nutrient loadings, especially different phosphorus forms, affect bacterial community composition. Changes in bacterial communities can dramatically alter the structure and function of aquatic ecosystems.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.032 INFLUENCE OF OXIC/ANOXIC CYCLING AND SOIL STORAGE ON TRANSFORMATIONS OF MINERAL NITROGEN APPLIED TO PEAT SOIL

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Water table fluctuations, heavy rain or flooding may have potentially significant impacts on microbial processes by influencing shifts in oxic and anoxic conditions in soil and therefore the ability of soils to convert mineral nitrogen to nitrogen gases efficiently. The impact of oxic/anoxic cycles on nitrogen transformation rates was therefore explored in the upper layer (0 - 30 cm) of partially degraded peat soil. We hypothesized that high denitrification potential would be conserved due to the high organic matter content of this soil. Mineral nitrogen was applied to approximately 1-

cm deep layers of homogenized soil in microcosms, with no external source of readily degradable carbon. Microcosms were subjected to three cycles, each consisting of an oxic phase of 8 - 11 days and an anoxic phase of 21 - 28 days. Approximately 2% of the ammonium load was lost through ammonia volatilization during oxic phases and the remainder was nitrified. Nitrification and denitrification rates during the three oxic/anoxic cycles were high, 2.9 - 3.2 kg N ha⁻¹ d⁻¹ and 1.0 - 2.3 kg N ha⁻¹ d⁻¹, respectively. However, denitrification rates decreased linearly during the three cycles, projecting cessation of denitrification activity and CO₂ production during the fifth cycle. Storage of peat soil at 4°C most probably allowed slow degradation of organic matter that was completely oxidized to CO₂ after the soil was exposed to higher temperature (28°C). Storage of soil for one year did not affect nitrification rate, but reduced denitrification rate, unless soil was amended with a readily degradable carbon source. The data suggest that, despite the high carbon content of this soil, it cannot sustain transformations of high N loads to nitrogen gases for prolonged periods without amendment with readily available carbon.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.033 DEPTH-RELATED AND AMMONIUM-RELATED DIFFERENCES IN AMMONIA-OXIDISING ARCHAEAL AND BACTERIAL COMMUNITIES IN A WETLAND SOIL

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Oxidation of ammonia, the first step in nitrification, is carried out in soil by bacterial and archaeal ammonia oxidisers and recent studies suggest possible selection for the latter in low ammonium environments. To determine depth-related and ammonia-related differences in ammonia-oxidising archaea and bacteria in a wetland soil the key functional gene for ammonia oxidation, *amoA*, which encodes ammonia monooxygenase subunit A, was targeted. Study was performed at two sites differing significantly in terms of ammonium supply but not in the groundwater level, where one site received ammonium through decomposition of organic matter, while the second, polluted site received greater supply, through constant leakage of an underground septic tank. Greater ammonium supply at the polluted site significantly increased soil nitrification potential, in comparison to the unpolluted site. Quantification of *amoA* genes demonstrated greater abundance of bacterial than archaeal *amoA* genes throughout the soil profile at the polluted, but not the unpolluted site. A strong depth-related change in community structure was observed for archaeal, but not bacterial ammonia oxidisers at both sites, supported by a corresponding increase in *amoA* gene abundance of one specific archaeal group with depth, with only a small influence of ammonium pollution on archaeal community structure. The depth-related changes in archaeal community structure in this wetland soil may reflect differences in redox potential in the examined soil profile.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.034 CRENARCHAEAL AMMONIA OXIDATION IN AN ACIDIC FOREST PEAT SOIL IS NOT INFLUENCED BY AMMONIA AMENDMENT

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Bacteria and crenarchaea contribute to ammonia oxidation in natural environments, the first step in of nitrification, which is dependent on a key functional gene, *amoA*, that encodes ammonia monooxygenase subunit A. Recent ecophysiological studies indicate that crenarchaeal ammonia oxidizers may have a selective advantage at low ammonia concentration, with potential adaptation to soils in which mineralization is the major source of ammonia. To test this hypothesis, crenarchaeal and bacterial ammonia oxidizers were investigated during nitrification in microcosms containing an organic, acidic forest peat soil, with low ammonium concentration but high potential for release during mineralization. The activity and structure of ammonia-oxidizer microbial

communities were determined by analysis of 16S rRNA and amoA genes. High rates of nitrification were observed during incubation for 20 days but rates were not influenced by addition of ammonia. Bacterial amoA genes could not be detected, presumably because of low abundance of bacterial ammonia oxidizers. Phylogenetic analysis of retrieved crenarchaeal 16S rRNA gene sequences indicated that the dominant populations belonged to Group 1.1c, 1.3 and 'deep peat' lineages, while sequences belonging to known amo-containing lineages (Group 1.1a and 1.1b) comprised only a small proportion of the total community. Growth of crenarchaeal ammonia oxidizers was indicated by increased abundance of amoA genes during nitrification, but was not affected by addition of ammonia. Similarly, denaturing gradient gel electrophoresis analysis of amoA gene transcripts demonstrated small temporal changes in crenarchaeal ammonia oxidizer community structure, but no effect of ammonia amendment. In conclusion, results suggested that crenarchaea may have the major role in ammonia oxidation arising from mineralization of organic matter rather than added inorganic nitrogen in this high organic matter soil.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.035 RELATIONSHIP BETWEEN MICROBIAL DIVERSITY AND SOIL ORGANIC MATTER TURNOVER

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Numerous human activities essential to the well-being of modern society have a severe impact on soil biodiversity. The functional significance of these losses/modifications of biodiversity, in terms of the capacity of ecosystems to maintain the functions and services on which humanity depends, is now of pivotal importance. In this context, we investigated the link between microbial diversity and the turn-over of organic matter in soil, which is a major function for soil fertility, environment quality and global changes. To simulate an erosion of soil microbial diversity, microcosms of a soil previously sterilized by gamma irradiation have been inoculated with three different dilutions of a suspension of the same but non sterile soil. By this way, three series of microcosms representing a gradient of soil diversity have been obtained, with D1>D2>D3. After microbial communities have colonized and stabilized, microcosms have been amended with ¹³C labelled wheat residues. Microcosms without wheat input were used as control. Decomposition of wheat residues and of indigenous organic matter have been assessed during 60 days by measuring ¹³CO₂ and ¹²CO₂ fluxes, respectively. Several organic families have also been followed with compound specific isotopic (¹³C) analysis: lignins, sugars, lipids including PLFA. In parallel, dynamics of microbial diversity have been characterized by both DNA-fingerprinting and pyrosequencing techniques. Results show that intensity of the soil respiration as well as of the priming-effect induced by the addition of plant residues was strongly linked to microbial diversity, with the highest values observed where diversity is greatest. The stability of microbial communities was also dependent on diversity, the least diversified communities being more greatly affected by the addition of organic matter. These results illustrate the importance of considering the microbial diversity as a predictive variable of organic carbon storage/release in soils.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.036 EFFECTS OF DIFFERENT PAIR REDOX ON ARSENATE DISSIMILATORY REDUCTION IN SEDIMENTS FROM ASCOTÁN SALT FLAT, CHILE.

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Ascotán is a hypersaline lake located at 3700 meters over the sea level in the Atacama Desert. This lake is characterized by a high content of arsenic (up to 6500 mg/Kg in sediments). In spite of arsenic toxicity, some microorganisms can respire arsenate. Previously, the biological precipitation of arsenic (as arsenic sulfide) has been demonstrated in sediments of Ascotán (Demergasso et al.,

2007). The aim of the present study was to investigate the effect of different pair redox on the reduction of arsenic and the microbial community responsible for. Sediment slurries were prepared by mixing sediments with artificial minimal medium supplemented with different electron donors (lactate, H₂ and a lactate-yeast extract) and arsenate as electron acceptor (1 mM). The DNA was extracted and *arrA*, *arsC* and 16S rRNA genes were analyzed by PCR. DGGE of 16S rRNA gene was used to analyze the bacterial community composition. Arsenic (III) was measured by HPLC. The highest arsenic reduction rate (0.152 mmol/L*day) was achieved in the presence of lactate-yeast extract and similar reduction rates were observed in the presence of hydrogen and lactate. The lowest rate (0.029 mmol/L*day) was observed when no electron donor was added to the experiment, and arsenic reduction was absent in abiotic control. Range-weight richness dominated by Firmicutes, was 20 times higher in hydrogen than in the experiment with no addition of electron donor. Similarly the cell number was 17 times higher in the experiment supplemented with lactate-yeast extract compare to the one with no addition of electron donor. In all the experiments *arsC* and *arrA* gene were detected by PCR. The microbial populations responsible for arsenic reduction are able to use organic and inorganic electron donors. Arsenic reduction rate is directly related to the energy supplied by the electron donor available. Firmicutes was evidenced as the predominant microbial agent in spite of the electron donor used.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.037 A CROSS-SYSTEMS COMPARISON OF BACTERIAL:ARCHAEAL RATIOS: THE ROLE OF NUTRIENTS AND FUNCTIONAL GROUPS IN REGULATING MICROBIAL COMMUNITY STRUCTURE

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Archaea are widely distributed in soils, sediments and aquatic environments, but little is known about their biological roles in these systems. Here, I present a survey of QPCR-based bacterial:archaeal (B:A) ratios in a variety of soil and freshwater samples from environments including tundra, northern hardwood forest and lowland tropical rainforest soils, leaf litter, and lake biofilms. B:A ratios vary dramatically between sites, ranging from less than 1 to over 600. Likewise, within-site variation in ratios is high; in some cases the magnitude of difference between B:A ratios is dramatic with no observable shift in the bacterial community composition between the same samples. Soil organic carbon (C), total nitrogen (N), C:N ratios and ammonium pools are all positively correlated with B:A ratios. B:A ratios also correlate to the relative abundance of specific bacterial groups. For example, *Gammaproteobacteria* are positively correlated with B:A ratios while *Nitrospira* are negatively correlated with B:A ratios. It is difficult to examine the relative contributions of changes in bacterial vs. archaeal relative abundance to the observed shifts in B:A ratios because of potential differences in PCR inhibitors between samples. However, the fraction of archaeal 16S rRNA genes to total soil DNA is variable across samples while bacterial relative abundance appears more stable. I present a possible mechanism to explain these observed relationships between nutrients, B:A ratios, and microbial functional groups. Specifically, I propose that when C:N ratios are high, bacterial heterotrophs (e.g., *Gammaproteobacteria*) are abundant. When C:N ratios are lower, autotrophic nitrifiers (e.g., *Nitrospira* and Archaea) are active and more abundant, consuming available ammonium.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.038 SIGNIFICANCE OF AUTOTROPHY IN SOIL CRENARCHAEA

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Mesophilic crenarchaea account for up to 6% of all prokaryotic cells and represent a major carbon reservoir of global biogeochemical significance. To understand the role of mesophilic crenarchaea in global carbon fluxes, the contributions of autotrophic and heterotrophic metabolisms within this group must be determined. Laboratory cultures belonging to three major crenarchaeal lineages

grow autotrophically, while incubation of marine waters and soil with isotopic tracers indicates representatives of the same lineages are heterotrophic. This discrepancy is supported by measurement of radiocarbon in crenarchaeal lipids extracted from ocean waters which suggests a mix of autotrophs and heterotrophs or an overall community of mixotrophs. Moreover genome analysis of the crenarchaeon *Cenarchaeum symbiosum* revealed potential for autotrophy and heterotrophy. The aim of this study was to develop PCR-based approaches to determine the significance of crenarchaeal autotrophy in the environment. The gene encoding 4-hydroxybutyryl-CoA dehydratase (4HCD) was chosen as a marker of autotrophy in mesophilic crenarchaea. 4HCD is a key enzyme of the CO₂ fixation pathway of sequenced archaeal ammonia oxidizers and, within the archaeal domain, was only reported in autotrophs. Several primer pairs specifically targeting crenarchaeal *hcd* genes were designed for clone library, fingerprinting and quantitative PCR applications. Analysis of an agricultural soil showed the existence of several clusters of sequences and an overall abundance of 10⁶ - 10⁷ gene copies per gram of soil. Growth of two *hcd* phylotypes mirrored that of two archaeal *amoA* phylotypes and growth of both *hcd* and *amoA* phylotypes was inhibited by acetylene, indicating some soil archaeal ammonia oxidizers have *hcd* genes and may be autotrophic. Stable isotope probing incubation showed most autotrophic crenarchaea in the analysed soil actively assimilate CO₂ suggesting crenarchaeal autotrophy as a major biogeochemical process.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.039 ACTINOBACTERIA ARE KEY PLAYERS IN THE BUDGET OF ATMOSPHERIC H₂

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With a typical atmospheric mixing ratio of 0.530 ppmv, molecular hydrogen (H₂) is the second most abundant reduced gas, after methane. H₂ soil uptake is responsible for 80% of the global loss of atmospheric H₂, but the exact nature of this biological process remains elusive. The activity has been attributed to hypothetical free soil hydrogenases, exhibiting an unusual high affinity for H₂ (K_{m(app)} <100 nM). This assumption has recently been challenged by the isolation of *Streptomyces* sp. PCB7, the first microorganism displaying a high affinity H₂ uptake activity. In this communication, we explore the ecological importance of H₂-oxidizing actinobacteria and present a specific functional gene marker for the microbial oxidation of atmospheric H₂. A cultivation-dependent approach was first elaborated to identify new H₂-oxidizing streptomycetes in forest and agricultural soils. These isolates, as well as natural soil samples were then screened for the presence of *hydB*-like gene encoding for the large subunit of a putative high affinity [NiFe]-hydrogenase. Six new streptomycetes isolates exhibited high to intermediate affinity for H₂ (K_{m(app)} of 39-374 nM), consumed atmospheric H₂ and possessed *hydB*-like gene. Phylogenetic analysis of *hydB*-like gene sequences revealed the occurrence of this gene in other actinobacteria. These actinobacteria, belonging to *Mycobacterium* spp., *Rhodococcus* spp. and *Saccharopolyspora* spp. also displayed the ability to consume atmospheric H₂, demonstrating that atmospheric H₂ oxidation is not limited to streptomycetes. *hydB*-like gene and transcript were detected in soils from various ecosystems, showing the ubiquity of actinobacterial high affinity hydrogenases. We postulate that H₂-oxidizing actinobacteria are responsible for the most important sink term in the budget of atmospheric H₂

Abstract Category
22 Major Biogeochemical Cycles

PS.22.040 LINKING MICROBIAL COMMUNITY STRUCTURE TO WATER QUALITY FUNCTION: INVESTIGATING NITROGEN CYCLING INFLUENCED BY HYDROLOGY IN WETLAND ECOSYSTEMS

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Changes in hydrology in wetland ecosystems can affect oxygen and nutrient availability to microorganisms. Variation in tolerance of microorganisms to the redox conditions resulting from fluctuations in soil moisture can influence the structure of microbial communities across a hydrological gradient. We investigated the response of nitrogen (N) cycling microbial communities to fluctuating water levels in wetland ecosystems. The objectives of this study were to (1) assess microbial function through measuring anaerobic denitrification and aerobic nitrification and to (2) compare microbial community composition of N cycling populations (denitrifiers and ammonia oxidizers) along a moisture gradient. Denitrifier and ammonia oxidizer groups have contrasting sensitivity to oxygen concentration, thus changes in soil moisture can result in shifts in microbial community structure and activity. Microbial structure-function relationships were compared at two natural wetlands and two restored floodplain sites. Four transects were established perpendicular to the pond/lake at each site. Four plots along each transect were placed along a moisture gradient (from dry to saturated), and soil was sampled at each site monthly from June to August. Potential denitrification and nitrification were measured to compare relative differences in microbial activity along the moisture gradient. Soil factors were also assessed. Higher ammonium and nitrate concentrations were measured in restored wetlands compared to natural wetland sites. Higher soil nitrogen levels supported higher rates of N cycling activity. Differences in soil moisture produced contrasting assemblages of denitrifiers and ammonia oxidizers (assessed by T-RFLP of functional genes). In addition, community patterns of each functional group were shown to respond to different combinations of soil factors. Niche differentiation among microbial taxa may drive ecosystem function in predictable ways.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.041 MAPPING SPATIAL PATTERNS OF DENITRIFIERS FOR BRIDGING COMMUNITY ECOLOGY AND MICROBIAL PROCESSES ALONG ENVIRONMENTAL GRADIENTS

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While there is ample evidence that microbial processes can exhibit large variations at a field scale, very little is known about the spatial distribution of the communities mediating these processes. The objective of this study was to explore spatial patterns of size and activity of the denitrifying community, a functional guild involved in N-cycling, in a grassland field subjected to different cattle grazing regimes. We used geostatistical modeling to map the distribution of size and activity of the denitrifier community in the pasture. Size of the denitrifier community was estimated by PCR quantification of the denitrification gene copy numbers while its activity was estimated by measuring potential denitrification activity and potential N₂O emissions. We found non-random distribution patterns of the size and of the activity of the denitrifier community were observed with a field-scale spatial dependence. The soil properties, which were strongly affected by presence of cattle, imposed significant control on potential denitrification activity, potential N₂O production but not on the size of the denitrifier community. The relative abundance of bacteria possessing the *nosZ* gene encoding the N₂O reductase within the total bacterial community was a strong predictor of the N₂O/N₂ ratio. Overall, our results indicated that patterns of distribution of denitrifiers can be modelled at the field scale. Characterization of such pattern at a field-scale constitutes the first step in modelling distribution of functional bacterial communities at a scale compatible with land management strategies. The abundance of most denitrification genes was not correlated with potential denitrification activity or potential N₂O production. However, the relative abundance of bacteria possessing the *nosZ* gene in the total bacterial community was a strong predictor of the N₂O/(N₂+N₂O) ratio, providing evidence for a relationship between ecosystem processes and bacterial community composition.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.042 POLLEN DEGRADATION STRONGLY IMPACTS LAKE NUTRIENT CYCLES

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Pollen from conifers can represent a major influx of terrestrial carbon into limnetic systems. However, in lakes pollen has been greatly ignored as a nutrient and organic matter source as well as a microbial habitat. Therefore, in our study we aimed to elucidating the release of pollen-derived organic matter and nutrients into the ambient water. Furthermore, we phylogenetically characterized bacteria and fungi to targeting the key players of pollen mineralization processes. We incubated pollen of *Pinus sylvestris* in prefiltered water from 3 different lakes for 16 days and measured a variety of biological and chemical parameters, e.g. bacterial growth as well as fungal abundances. Both, bacterial community structure and composition of aquatic fungi were studied by DGGE analysis of rRNA marker genes.

Pollen grains were rapidly colonized by heterotrophic bacteria and aquatic fungi in parallel to a strong decrease in particulate organic carbon (POC) between days 2 and 3. Both, chemical leaching and microbial decomposition of pollen were highest within 2 weeks of incubation leading to a marked release of inorganic nutrients into the ambient water, e.g. soluble reactive phosphorus (SRP) and total dissolved nitrogen (TDN).

Our results demonstrate that pollen provides high amounts of organic substrates which are easily accessible for aquatic microorganisms. Pollen serves as important microbial habitat and carbon/nutrient source for heterotrophic bacteria and fungi. Hence, we conclude that the ecological consequences of pollen input into lakes, its subsequent chemical leaching, and microbial degradation are by far underrated. In particular, at times when nutrient concentrations are low, i.e. at the end of the phytoplankton spring bloom, coniferous pollen fuels the microbial loop in temperate lakes.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.043 SEDIMENT DARK CARBON FIXATION AS AN IMPORTANT PROCESS IN LAKE SYSTEMS

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Chemoautotrophic fixation of CO₂ represents an autochthonous source of highly labile organic matter. Little is still known about chemoautotrophy in sediments, yet it may constitute a significant pathway close to redox boundaries. We here present comparisons between dark carbon fixation, bacterial production, and oxygen consumption in sediments from four Swedish boreal lakes and seven tropical Brazil lakes. Sediment oxygen consumption was measured with a start-end sediment-water interface incubation and after that, the sediment cores were sliced and the uppermost centimetre was used for slurry incubations of bacterial production and dark carbon fixation via leucine and ¹⁴C labelled inorganic carbon incorporation, respectively. Sediment oxygen consumption average rates varied from 14.50 mmol m⁻² d⁻¹ to 97.27 mmol m⁻² d⁻¹ in studied lakes. Two tropical environments presented the lowest (1.19 mmol m⁻² d⁻¹) and the highest (48.60 mmol m⁻² d⁻¹) rates of sediment bacterial production. Dark Carbon Fixation rates varied from 0.01 mmol m⁻² d⁻¹ to 3.98 mmol m⁻² d⁻¹, being generally higher in temperate environments. Dark carbon fixation contributed between 8.41 % and 37.4 % of the total heterotrophic bacterial production in the studied temperate sediments and between 0.40 % and 80.4 % in the tropical ones. This study present estimates of the quantitative importance of chemoautotrophic activity in lake sediments, indicating that dark carbon fixation can represent a substantial contribution to bacterial biomass, especially in sediments with low organic matter content. Considering the previous suggestions of a tighter trophic linkage between sediment bacteria and macrobenthos in lakes, dark carbon fixation accounted for up to 80 % of the total heterotrophic bacterial production, drawing attention to this important energy way in limnic sediment food webs.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.044 ENRICHMENT AND CHARACTERIZATION OF AMMONIA-OXIDIZING ARCHAEA FROM THE OPEN OCEAN: PHYLOGENY AND STABLE ISOTOPE FRACTIONATION

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Archaeal genes associated with ammonia oxidation are widespread in the marine environment, but direct physiological evidence for ammonia oxidation in marine archaea is limited. We report the enrichment and characterization of three strains of pelagic ammonia-oxidizing archaea (AOA) from the north Pacific Ocean from depths of 25, 75, and 150 m. Phylogenetic analyses based on 16S rRNA genes and ammonia monooxygenase subunit a (*amoA*) genes indicate that all three enrichments contain a single strain of *amoA*-containing archaea and no *amoA*-containing bacteria. The three AOA strains possess genes highly similar (98-99% identity) to those recovered in environmental clone libraries from the open ocean, and 92-98% identical to the 16S rRNA gene and 83% identical to the *amoA* gene from *Nitrosopumilus maritimus*, the only isolated mesophilic marine archaeon. The strains exhibit growth in natural seawater-based liquid medium while stoichiometrically converting ammonium (NH₄⁺) to nitrite (NO₂⁻) with bicarbonate as the sole carbon source. In contrast to cultivated strains of ammonia-oxidizing bacteria, the enriched archaeal strains showed minimal inhibition of NH₄⁺ oxidation upon the addition of allylthiourea to the culture medium. Nitrogen stable isotope discrimination (ε_{AMO}) during NH₃/NH₄⁺ oxidation ranged from 13 - 41 ‰, within the range of that previously reported for ammonia-oxidizing bacteria. Thus, despite relatively low amino acid identity between the archaeal and bacterial AMO proteins, their functional diversity as captured by ε_{AMO} is relatively similar.

Abstract Category

22 Major Biogeochemical Cycles

PS.22.045 AQUARIUM NITRIFICATION REVISITED: ARCHAEA ARE THE DOMINANT AMMONIA OXIDIZERS IN FRESHWATER AQUARIUM BIOFILTERS

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The recent discovery of ammonia-oxidizing archaea and investigations into their ecology have implicated these organisms as numerically dominant over ammonia-oxidizing bacteria in many terrestrial and aquatic environments. Although nitrification is the primary function of aquarium biofilters, very few studies have investigated the microorganisms responsible for this process in aquaria. This study used quantitative real-time PCR to quantify the ammonia monooxygenase (*amoA*) and 16S rRNA genes of Bacteria and Archaea in freshwater aquarium biofilters, in addition to assessing the diversity and phylogeny of archaeal *amoA* genes by denaturing gradient gel electrophoresis (DGGE) and clone library analysis, respectively. The results demonstrate that ammonia-oxidizing archaea dominated 14 of 19 freshwater biofilters, and in seven of these biofilters, ammonia-oxidizing archaea contributed all detectable *amoA* genes. In contrast, the saltwater aquaria and commercial aquarium supplements included for comparison were dominated by bacterial *amoA* genes. In freshwater aquaria, the proportion of *amoA* genes from Archaea relative to Bacteria was inversely correlated with ammonium concentration of the aquarium water. Sequencing of freshwater archaeal *amoA* genes revealed clades of gene sequences that clustered with environmental clones derived from a variety of freshwater sources. The crenarchaeal *amoA* genes and DGGE fingerprint derived from a saltwater aquarium clustered in a distinct clade with marine *amoA* genes and the *amoA* sequence from *Nitrosopumilus maritimus* strain SCM1, an isolate from a marine aquarium substrate. These results reveal surprising new insight into commonplace residential biofilters and have important implications for the management of water quality in the commercial aquarium industry, aquaculture and wastewater treatment.

Abstract Category

22 Major Biogeochemical Cycles

PS.22.046 SINGLE CELL ISOLATION AND CHARACTERIZATION OF DENITRIFYING BACTERIA FROM RICE PADDY FIELD SOILS WITH DIFFERENT SOIL TYPES AND CROPPING SYSTEMS

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Rice is cultivated on 80% of Japanese arable land area, which include continuous rice cropping fields and rotational cultivation fields of paddy rice and upland crops. It has been shown that the emission of nitrous oxide (N₂O) in the field is extremely low under paddy condition, while it increased when the field is converted to upland condition. Little N₂O emission from rice paddy is likely due to strong denitrification activity where nitrate is completely reduced to N₂. Our previous PCR-DGGE study revealed that the key denitrifiers were similar among paddy soils with same soil type regardless of the cropping system (continuous or rotation). The purposes of this study are (1) to isolate the denitrifying bacteria, (2) to examine the activity and end-product of denitrification of each isolate, and (3) to consider the reason for little N₂O emission from paddy field soil.

Microcosms were established using six rice paddy soils of three different soil types (Andosol, Gley soil, and Gray lowland soil) and two different cropping systems. Single-cell isolation was carried out targeting denitrifying bacteria in the soils by using micromanipulator. The 16S rRNA gene sequences were analyzed to identify phylogenetic affiliation of the isolates. In addition, denitrifying activity and the end product of denitrification (N₂ and/or N₂O) were examined by GC.

A total of 111 strains were isolated, and 85% of the strains were identified as the members of the genera, *Pseudogulbenkiania* and *Bradyrhizobium*, in agreement with the previous PCR-DGGE analysis. The diverse denitrifying activity was observed among the isolates. Both N₂-producing and N₂O-producing denitrifiers were present in the soils regardless of the soil type or cropping system. Low N₂O emission from rice paddy field could be attributed to the presence of N₂O-reducing denitrifiers. These denitrifiers may be able to reduce floodwater-dissolved N₂O, produced by other denitrifiers, to N₂.

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Abstract Category
22 Major Biogeochemical Cycles

PS.22.047 SEASONAL CHANGES IN ACTIVITY AND DIVERSITY OF METHANE-OXIDIZING BACTERIA IN A GRASSLAND SOIL

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We assessed the diversity, structure, and seasonal dynamics of methanotrophic communities in a grassland soil near Giessen, Germany. Seasonal samples were taken from the surface layer (0-10 cm) of three different sites (I, II, III) in August 2007, November 2007, February 2008, and May 2008. The grassland soil always acted as a sink for atmospheric methane as revealed by methane flux measurements. The moisture content ranged from 30-70 % with highest moisture content in site III throughout all seasons. The methanotroph cell numbers were determined by real-time PCR of *pmoA* and ranged between 1.9×10^5 - 9.3×10^5 cells/gram of dry soil. T-RFLP fingerprinting and pyrosequencing of *pmoA* showed that the methanotrophic communities in all three sampling sites were dominated by members of the "Upland Soil Cluster alpha" (USCa). *Methylocystis* spp. were detected as a minor component. The sequence data revealed six species level clusters of USCa. These as-yet-uncultured bacteria are assumed to be specialized on the consumption of atmospheric methane, which agrees well with the *in situ* methane concentrations (<1.8 ppmv) in our sampling sites. Seasonal dynamics of the methanotrophic communities were most pronounced in site III, less pronounced in site II, and almost not detectable in site I. Throughout all seasons, methanotroph cell numbers and potential methane oxidation rates were significantly higher ($P < 0.05$) in site III than in sites I and II. Among the different environmental factors (pH, C, N, and moisture) tested, the moisture content was found to be the major factor ($P < 0.01$) in structuring the methanotrophic communities.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.048 IMPACT OF CRUDE OIL POLLUTION ON NITROGEN CYCLING AND DEHYDROGENASE ACTIVITY IN SOIL

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Soil is a unique natural environment characterized by enormous abundance of microorganisms and complex interactions among microbial processes, soil properties and plant roots. Microbial abundance, diversity and activity are considered to be important indicators of soil health. Since the soil microorganisms respond to changes in their environment very fast, they are also considered as very useful indicators of unfavourable changes in soil caused by different pollutants. All phases of crude oil exploitation are accompanied by interventions and procedures hazardous to the environment. The main aim of the present study was to evaluate the microbial response to changes in soil caused by addition of crude oil and drilling fluids as well. The impact of these pollutants on abundance of microorganisms involved in nitrogen cycle as well as on dehydrogenase activity was studied. The field trial was set up on luvisol contaminated with crude oil and drilling fluids. Oilseed rape, winter wheat, winter barley were used as test plants. The results revealed marked variability in abundance of ammonifiers although pronounced regularity was less expressed. Low values of the most probable number of nitrifiers were determined in all trial treatments. Besides, the results clearly showed that nitrifiers were more susceptible to soil pollution than other groups. Among particular pollutants clear differences were found. Significantly lowest dehydrogenase activity was determined in the soil treated with the highest amounts of drilling fluids. The results revealed that microorganisms involved in nitrogen cycling strongly differ in their overall susceptibility to pollutants as well as in their particular response to crude oil and drilling fluid pollution. Detrimental effect of soil pollution on dehydrogenase activity was less expressed in comparison to particular groups of microorganisms involved in nitrogen cycle.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.049 MICROARRAY ANALYSIS OF MICROBIAL GENE EXPRESSION ASSOCIATED WITH NITROGEN CYCLING IN THE COLUMBIA RIVER COASTAL MARGIN

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The Pacific Northwest coastal margin is a highly biologically productive system, and the Columbia River system makes an important contribution to this productivity. As the second-largest river in the United States, the Columbia River has profound influence on biogeochemical processes occurring in the coastal ocean through the delivery of nutrients in a massive plume. Physical and chemical gradients are established in the coastal margin due to varying seasonal discharge volumes, upwelling events, tidal mixing and formation of estuarine turbidity maxima zones. These gradients influence microbial community composition and metabolic activities. We investigated metabolic properties of microbial populations involved in nitrogen cycling (nitrogen fixation, assimilatory nitrate reduction, dissimilatory nitrate reduction to ammonium, denitrification, and nitrification) using microarray analysis of gene expression. Thus, over 80 coastal margin water samples were collected during spring pre-freshet and peak freshet, summer, and late fall in 2007 and 2008; for each, physical, chemical and biological parameters were analyzed. Total RNA was isolated from each sample, reverse-transcribed into cDNA and hybridized to custom-synthesized DNA oligonucleotide microarrays. Microarray probes were designed for 5000 genes involved in carbon and nitrogen metabolisms in environmental bacteria and archaea. Both empirical and bioinformatics approaches were used to determine specificity of the probe set. Hybridization to pooled water samples was done to select a final set of 2226 probes for microarray synthesis, 1648 of which were genes involved in nitrogen metabolism. The microarrays were then hybridized with individual water samples to assess differential microbial gene expression across environmental gradients. Expression profiles of genes involved in nitrogen cycling provide the basis for testable hypotheses about related biogeochemical activities in the Columbia River coastal margin.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.050 ANAEROBIC AMMONIUM OXIDATION (ANAMMOX) IN NITROGEN CONTAMINATED GROUNDWATER

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Dissolved inorganic nitrogen is often found in groundwater due to anthropogenic inputs from fertilizer, sewage, and feedlots. The presence of nitrate/nitrite is of particular concern because nitrite causes blue baby syndrome (methemoglobinemia). Previously, denitrification was considered to be the main nitrogen removal process in groundwater. Anaerobic ammonium oxidation (anammox) recently discovered in wastewater treatment plants and marine environments may be another important N removal pathway in aquifers. Thus, we conducted molecular and stable isotope analyses to detect and measure anammox in a nitrogen contaminated aquifer on Cape Cod, Massachusetts. The study site, located near the Massachusetts Military Reservation, has a linear plume of nitrogen contaminated groundwater as a result of continuous discharge of treated wastewater over 60 years. Groundwater was collected from multiport sampling devices installed at two sites, near the waste-water disposal location (A) and more than 3 km down gradient (B) along the contamination plume. Biomass was collected from water samples for DNA extraction and ¹⁵N tracer incubation experiments. PCR of 16S rRNA and hydrazine oxidase genes detected the presence of anammox bacteria at both sites. Phylogenetic analysis of 16S rRNA genes revealed that the anammox community at site A was most associated with *Kuenenia* spp. while site B had a community more closely related to *Brocadia* spp. ¹⁵N tracer incubation experiments revealed that the anammox rate was higher than the denitrification rate at site A and accounted for up to 62% of total N₂ production. The denitrification rate was higher at site B, although anammox contributed 40% of total N₂ production. This study clearly demonstrates the potential importance of anammox in the nitrogen removal from groundwater and suggests that detailed characterization of the process under in situ subsurface conditions could provide new information regarding the ecology of these microbes.

Abstract Category

22 Major Biogeochemical Cycles

PS.22.051 IMPORTANCE OF ANAEROBIC AMMONIUM OXIDATION (ANAMMOX) IN AGRICULTURAL SOIL

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Anaerobic ammonium oxidation (ANAMMOX) produces N₂ by coupling ammonium oxidation and nitrite reduction under anoxic conditions. Anammox bacteria have been detected in wastewater treatment plants and marine environments. Until recently, there was limited evidence for the existence of anammox bacteria in terrestrial environments. The diversity and activity of anammox bacteria in soil has yet to be examined. Therefore, we investigated various soil communities to detect anammox bacteria and to measure anammox rates and its contribution to total N₂ production. Soil core samples were collected from agricultural fields in North Carolina, Kansas, Indiana, and Iowa. Each core sample was divided into surface and deep or surface, mid, and deep layers based upon the depth of the water table. By targeting hydrazine oxidase genes (*hzoA* and *hzoB*) as a genetic marker, anammox bacteria were detected from 16 out of 54 samples. Anammox bacteria were mostly found above 30 cm in surface or mid layers. Based on phylogenetic analysis of the detected *hzoA/hzoB* genes, soil anammox bacteria were closely associated with either *Jettenia* or *Anammoxoglobus* species. The highest diversity was observed in the soil communities in North Carolina samples. Soil slurry incubation experiments with ¹⁵N tracer were conducted with the samples that were positive for *hzoA/hzoB* gene detection. The rates of anammox and denitrification were simultaneously measured using isotope ratio mass spectrometry. Preliminary data suggests that anammox contributes substantially to total N₂ production in agricultural soils. More than 50% of N₂ production was mediated by anammox in North Carolina soil samples. We found that anammox bacteria are not ubiquitous in soil ecosystems. However, they may play an important role

in removing nitrogen fertilizers in agricultural fields in which they are present. Therefore, anammox should be considered an integral pathway in the soil nitrogen cycle.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.052 PHYLOGENETIC DIVERSITY OF PLANCTOMYCETES IN LOW TEMPERATURE IRON-HYDROXIDE DEPOSITS AT THE JAN MAYEN HYDROTHERMAL FIELD

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Planctomycetes is a Bacterial phylum frequently reported in aquatic environments. Recent studies from hydrothermal areas including iron-hydroxides and newly formed seafloor lavas have reported *Planctomycetes* sp. to be highly abundant and diverse in these systems. This phylum remains one those underrepresented in microbial culture collections and few studies have been specifically aiming to investigate *Planctomycetes* diversity at these sites. At 600 meters depth along the Mohns Ridge in the Norwegian Arctic Sea, large deposits of iron-hydroxides are associated with diffuse hydrothermal venting of low-temperature fluids. To investigate the diversity of *Planctomycetes* in these iron-hydroxides, clone libraries were made using primers specifically targeting *Planctomycetes* 16S rDNA sequences. In addition enrichment cultures selecting for the growth of *Planctomycetes* were set up to complement the molecular data. The sequences obtained were diverse, and affiliated with the order *Planctomycetales*, with the anammox Candidatus order "*Brocadiales*" and with the putative clade WPS-1. Two new strains from the order *Planctomycetales* were isolated. One strain highly similar to the previously isolated *Planctomyces maris*, and one strain, Pr1d, affiliated with the *Pirellula-Rhodopirellula-Blastopirellula* clade (PRB-clade). This novel strain (Pr1d) revealed a 16S rDNA sequence identity of only 96% to the closest related isolated but uncharacterised strain (strain 678 Schlesner), and 86% identity to the nearest characterised strain (*Blastopirellula marina*). Strain Pr1d appears to be a unique strain within the PRB-clade, and is to our knowledge the first *Planctomycete* to be isolated from low temperature, marine iron-hydroxides. No 16S rDNA sequence from either of the two strains was detected in the clone libraries, giving further indications of a substantial diversity of *Planctomycetes* sp. in this enviro

Abstract Category
22 Major Biogeochemical Cycles

PS.22.053 SOIL TYPE AFFECTS N₂O EMISSIONS AND POPULATIONS OF NITRIFYING AND DENITRIFYING BACTERIA IN MYCORRHIZAL-INOCULATED WILLOWS

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Nitrous oxide (N₂O) is a potent greenhouse gas emitted from soil ecosystems by nitrifying and denitrifying bacteria. It is well established that N₂O emissions are influenced by the soil physical environment, but attempts to correlate emission rates to soil microbial communities have been inconclusive. The soil physical characteristics, along with nitrogen availability, may be altered by the presence of mycorrhizal fungi. We hypothesized that by establishing a more efficient nutrient cycle in the rhizosphere, mycorrhizae may alter emission rates of N₂O from soils. A greenhouse experiment was designed to test the effects of different soil types and mycorrhizal inoculation on N₂O emission rates and populations of N₂O emitting bacteria from potted soils containing willow (*Salix* spp.) trees. Willow cuttings were potted in 6 Southern Ontario soils representing a range of soil textures. Mycorrhizal fungal inoculant was added in 1 of 4 treatments: 1) Arbuscular Mycorrhizae (AM) (*Glomus intraradices*), 2) Ectomycorrhizae (EM) (*Hebeloma cylindrosporium*), 3) AM and EM, or 4) uninoculated. Four month old potted trees were placed in flow-through chambers for 72 hours to determine N₂O fluxes using tunable diode laser technology. N₂O emissions and concentrations of nitrate (NO₃⁻) and ammonium (NH₄⁺) were significantly influenced by soil type, plant presence and inoculation type. Soil DNA was extracted and quantitative polymerase chain reaction (qPCR) was used to quantify genes coding for bacterial nitrification and denitrification. Sandy soils exhibited the lowest N₂O emission rates and lowest denitrifying gene copy numbers. Our data indicates that mycorrhizal inoculation does affect N cycling in soil. This may be due to the

interactive effect that we see between microbial populations, soil type and N₂O emissions. However, this did not result in significantly lower N₂O emissions from soils containing the inoculated willows.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.054 DIFFERENCES IN CONTRIBUTIONS OF AMMONIA OXIDIZING ARCHAEA AND AMMONIA OXIDIZING BACTERIA TO THE NITRIFICATION POTENTIALS OF OREGON SOILS

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An assay was developed to determine the contributions of ammonia (NH₃) oxidizing archaea and NH₃ oxidizing bacteria to the nitrification potential of soils. Slurries were prepared from Oregon soils sampled under forest, pasture, cropped and fallowed land and exposed to acetylene to irreversibly inactivate ammonia monooxygenase. Recovery of nitrite (NO₂⁻) and nitrate (NO₃⁻) production was followed in the presence of protein synthesis inhibitory antibiotics after removal of acetylene. In pasture, fallowed, and cropped soils nitrification recovered following lags that ranged from 6 to 18 h post acetylene removal, with rates that represented 56, 56 and 42%, respectively, of the non-acetylene treated control. Nitrification failed to recover in forest soil despite nitrification potentials being greater than either pasture or fallow soils. Recovery of NO₂⁻ and NO₃⁻ production in cropped soils under N fertilized wheat was completely prevented by the bacterial protein synthesis inhibitor kanamycin (800 µg/ml), suggesting that NH₃ oxidizing bacteria were the major contributors to the nitrification potential of these soils. In contrast, recovery of NO₂⁻ and NO₃⁻ production of grazed, permanent pasture soils was completely insensitive to kanamycin or gentamycin (800 µg/ml), and was also insensitive to fungal inhibitors. In addition, recovery of nitrification in pasture soil was insensitive to 500 µM allyl thiourea, and occurred at 40°C. This combination of properties suggests that NH₃ oxidizing bacteria did not contribute to nitrification in the pasture soil, and raises the possibility that NH₃ oxidizing archaea were responsible for the recovery of nitrification. Recovery of NO₂⁻ and NO₃⁻ production in fallowed soils was insensitive to fungal inhibitors, and a combination of kanamycin and gentamycin only prevented 63% of nitrification recovery, suggesting that both archaeal and bacterial NH₃ oxidizers were responsible for recovery of the nitrification potential in this soil.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.055 CULTURED AND UNCULTURED MICROBIAL COMMUNITIES ASSOCIATED WITH NITROGEN CYCLING FROM THE RHIZOSPHERE OF TWO SOIL MANagements OF SOLANUM PHUREJA CROPS

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Nitrogen transformations in soil are carried out by diverse microorganisms involved in the availability of this element for the plant. The aim of the present study is to provide a comprehensive analysis of the role of the microbial communities related with the nitrogen cycle in agricultural soils sown with the native potato *Solanum phureja* managed under two contrasting agricultural practices: traditional (where chemical inputs are used) and organic (where no chemicals are used). Rhizospheric soil samples were taken from two crops of each contrasting soils. Total soil DNA was extracted and the V5/V6 region of the 16S rDNA was amplified by PCR and sequenced using the 454 FLX Titanium pyrosequencing system. Besides, microbial populations related to nitrification, denitrification and nitrogen fixation were estimated by culture-dependent techniques and physical-chemical properties and the nitrogenase activity from each soil sample were measured. 16S rDNA sequence from the diazotrophic bacteria was obtained from the culturable fraction and taxonomic

comparisons were made with the sequences obtained by 454 pyrosequencing. Microbial abundances were compared using the Z-test proportion analysis and with the different tested variables a hierarchical clustering analysis was done. Contrary to culturable counts, the physical-chemical analysis clustered the soils by the agricultural-management system. When pyrosequencing and culture-dependent approaches were compared, we found that there is not a good correspondence between the potential microbial populations related to each nitrogen-cycle step. However, measuring soil nitrogenase activity gives us a better correlation with the potential diazotrophic population obtained by pyrosequencing. In conclusion, the study of the microbial population related to the nitrogen cycle is complemented when using culture-dependent and culture-independent techniques as well as soil enzymatic activities and physical-chemical properties.

Abstract Category
22 Major Biogeochemical Cycles

PS.22.056 AGRO-ECOLOGICAL COMPARISON OF MICROBIAL FUNCTIONAL GROUPS FROM FOUR CONTRASTING RICE PRODUCTION AREAS FROM COLOMBIA

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Microbial communities associated to rice crops have great influence over nutrient uptake for plants. However, it is not well known the effect of intensive management of such soils on the microbial activity, the microbial composition and the role of them on crop productivity. The aim of this work was to compare the microbial activity of different microbial functional groups associated to the nitrogen, phosphorus and sulphur cycling from four rice producers regions from Colombia. These regions differ in terms of soil origin (Oxisols and Alfisols), and crop management system (flooded and aerobic). In this context 16 rice crops (4 for each region), were sampled to make a comprehensive analysis of 21 physical-chemical variables, plus the enzymatic activity of five soil enzymes: nitrogenase, proteases, arylsulphatase and acid and alkaline phosphatase. Also total counts on agar plates were done to estimate culturable bacteria, actinomycetes, fungi, phosphate solubilizing microorganisms and diazotrophic bacteria. At the end of each productive cycle, the rice yield (tones/hectare), was determined. Sampled zones were differentiated by the physical-chemistry characteristics of the soil. Nitrogenase, proteases, phosphatases activities and plate counts of bacteria and actinomycetes allowed the separation of flooded crops from aerobic soils at the Meta region (Oxisols). Such variables were influenced by the percentage of humidity, pH and nutrient availability. For the Tolima region (Alfisols), the production zones were differentiated by the distribution of cations and cation exchange capacity. It was not found relationships between production, enzymatic activity and microbial plate counts. It was interesting that productivity was better correlated with micronutrients availability. This work was founded by the Agricultural and Rural Development Ministry of Colombia and Fedearroz

Abstract Category
22 Major Biogeochemical Cycles

PS.22.057 ACTINOBACTERIAL COMMUNITIES ALONG A GRADIENT OF HEAVY METAL CONTAMINATION AND THEIR POTENTIAL INVOLVEMENT IN ORGANIC MATTER TRANSFORMATION

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Some actinobacteria - like fungi - produce extracellular enzymes involved in the degradation of polysaccharides or phenolic compounds contained in dead plant biomass. The highly active secondary metabolism, mycelial growth and spore production represent selective advantages for their survival in heavy metal-contaminated soils where some species (esp. *Streptomyces* spp.) may dominate. The aims of this work were (1) to characterize actinobacterial communities along two gradients of heavy metal contamination - one in a grassland soil and the other in a forest soil and (2) to characterize the abilities of isolated strains to degrade polysaccharides, lignin and other small-molecular-mass carbon compounds. The study site was situated in the vicinity of a metal smelter near Příbram, Czech Republic. T-RLFP and 454-pyrosequencing were used for the

characterization of communities while diagnostic media, enzyme activity measurements and Biolog™ plates were used for strain characterization. Significant differences in community composition were found between sites with the highest and lowest heavy metal content. Isolated strains showed significant variation in their ability to produce cellulose- and hemicelluloses-degrading enzymes and only a few strains were able to oxidize phenolic substrates. It is likely that the decomposition of biopolymers (cellulose, hemicelluloses or lignin) is limited to a small group of strains of the total community, while the range of simple carbon-containing compounds serving as sources for actinobacterial growth is relatively wide. The community changes induced by heavy metals may cause changes in organic matter transformation rates in contaminated soils.

Abstract Category

22 Major Biogeochemical Cycles

PS.22.058 DIVERSITY OF AMMONIA OXIDIZING BACTERIA: TRFLP GONE BAD

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Ammonia oxidizing bacteria play a critical role in nitrogen cycling in estuarine and marine sediments where they link the mineralization of nitrogen and its removal by feeding nitrite to the diverse denitrifying pathways. We have used clone libraries and the terminal restriction fragment length polymorphism (tRFLP) of the *amoA* gene to study their diversity in the sediment of the Gulf of Finland, Baltic Sea. By using *in silico* tRFLP, we have identified that the restriction enzyme Sau3AI was ideally suited to resolve the diversity found within the clone library. However, the actual tRFLP experiments revealed that some of the expected restriction fragments were lost to an unidentified effect of the enzyme. Experiments with different isoschizomers of Sau3AI have confirmed the creation of very short terminal fragments. Tests with several commonly used restriction enzymes demonstrated that the Sau3AI tRFLP underestimated the abundance and sometimes failed to detect the presence of Nitrosomonas-type sequence while over-representing Nitrosospira-like fragments. Altogether, this study shows that although the tRFLP has been long praised for its robustness, its use with DNA fragments populations with expected low diversity can be troublesome and its results misleading. The use of techniques that do not require a treatment of the PCR products such as Single Strand Conformation Polymorphism or Denaturing Gradient Gel Electrophoresis are thus highly recommendable over tRFLP for such DNA fragments.

Abstract Category

22 Major Biogeochemical Cycles

PS.22.059 EXPLORING MICROBIAL IRON OXIDATION IN WETLAND SOILS

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The release of oxygen by the roots of wetland plants creates suboxic conditions that may favour the growth of iron-oxidizing bacteria (FeOB). Given their importance in iron cycling, little is known about the diversity or distribution of these bacteria. This is largely due to the lack of efficient methods to study them and the difficulty in isolating them in pure culture.

Our study is focussing at the ecology of FeOB by developing molecular and microbiological methods to investigate them *in situ*. Firstly, gradient tubes were used to enrich and isolate iron-oxidizing bacteria. A novel iron-oxidizing strain related to *Gallionella* spp. was isolated from an irregularly flooded grassland soil. Secondly, a specific primer set 122f/998r targeting the 16S rRNA gene was developed on the basis of the isolate. The primer set was applied to community DNA obtained from three contrasting wetland environments, followed by DGGE analysis. The PCR products were also used to construct a 16S rRNA gene library. The cloned sequences all represented novel iron oxidizers most closely related to *Gallionella* spp., and were comparable to the PCR-DGGE result. Thirdly, a SYBR Green qPCR assay was developed. Together with the previously mentioned nested PCR-DGGE method, the qPCR assay was used to quantify the distribution of FeOB in the tidal freshwater marsh. The effects of plant niches, soil depth, elevation gradient and season were investigated. The results showed that the composition of the FeOB community varied with sediment

depth, elevation gradient and season, while no influence was observed from plant species. A strong correlation between relative abundance and Fe(III) concentration indicated that the bacterial distribution might be influenced by the iron dynamics. Moreover, oxygen availability and moisture content could serve other steering factors.

Abstract Category

22 Major Biogeochemical Cycles

PS.22.060 EFFECTS OF LONG-TERM FERTILIZATION AND LIMING ON MICROBIAL FUNCTIONS AND DIVERSITY IN THE GRASSLAND SITE AT ROTHAMSTED RESEARCH, UK

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The effects of long-term N fertilization and liming on microbial functions in grassland soil at the Park Grass Experiments site in Rothamsted, UK were evaluated. Soils were collected from 4 subplots (a-d) of 3 plots NA (nil), AS (ammonia sulfate amendment), and SN (sodium nitrate amendment). Subplots a-c were limed to adjust the soil pH to 7, 6, and 5, respectively, and subplot d was a non-limed control. Soils were incubated for carbon mineralization with addition of ¹⁴C-labelled ryegrass, for nitrification with enrichment of ammonia, for denitrification with enrichment of K₁₅NO₃, and for methane oxidation with addition of methane gas. The carbon mineralization potential of subplots a & b (pH 7 and 6) of all three plots (13.5-19.9 μg g⁻¹soil d⁻¹) were significantly higher than those of the subplots c & d (pH 5, non-limed; 9.7-15.9). The carbon mineralization rate of plots NA, AS, and SN were 11.9, 14.4, and 17.1, respectively. Carbon mineralization rate was correlated to pH when all data were considered (r=0.58, p=0.05). Almost no nitrate accumulated in any of the NA subplots, but ammonia was rapidly depleted in subplots a & b, while remaining high in subplots c & d. Nitrate accumulated quickly in subplots a & b of plot AS and at a high concentration (97.4 and 103.3 μg g⁻¹ soil), but not in subplots c & d. Nitrate accumulation was observed in all subplots of plot SN with higher concentrations in subplots a & b. Nitrification potential was significantly correlated to pH based on ammonia depletion, but only marginally based on nitrate accumulation. Preliminary results showed that decreasing pH positively affected N₂O production. N fertilization had significant effect on methane oxidation, but no significant correlation to pH was found. These results indicated that N fertilization and liming affected the soil microbial functions. The effects of fertilization and liming on the microbial community structure and composition are being investigated using Geochip.

Abstract Category

22 Major Biogeochemical Cycles

PS.22.061 EXPRESSIONS OF DENITRIFICATION FUNCTIONAL GENES IN RICE PADDY SOIL

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Denitrification is a microbial respiratory process and has an important role in N-cycling. Rice paddy soil is known to have strong denitrifying activity. Previous DNA-based clone library analysis targeting functional genes for denitrification (*nirK* and *nirS*, *nosZ*) suggested the presence of diverse denitrifiers in rice paddy soil. However, it is unclear if they really performed denitrification. In this study, we tried to identify active denitrifiers in paddy soil by using RNA directly extracted from soil. In a soil microcosm experiment, rice paddy soil was anaerobically incubated with nitrate and succinate additions at 30°C. DNA and RNA were extracted from soil 0-, 6-, 12-, 16-, 20-, and 24-h after incubation, and RNA was reverse-transcribed to synthesize cDNA. Real-time PCR was performed targeting 16S rRNA, *nirK*, *nirS*, and *nosZ* to quantify abundance of each gene and gene product in soils. In addition, clone library analysis was performed targeting 16S rRNA and *nirS* from soils 0- and 20-h after incubation to study the diversity of denitrifiers.

In the microcosm setup used, strong denitrifying activity was observed with its maximum at 20-h after incubation. Results from our quantitative PCR showed the gradual increase in the copy numbers of each gene. The abundance of the gene products increased and decreased in response to

the denitrification activity. Although clone library analyses targeting DNA suggested the presence of diverse denitrifiers, those targeting RNA indicated only some of them performed denitrification. In particular, clones closely related to Burkholderiales, Rhodocyclales, and Neisseriales were dominant in the sample with strong denitrifying activity.

These results indicate that the expressions of the functional genes change drastically within 24-h of incubation, and the levels of expression are compatible with denitrifying activity. Diverse denitrifiers are present in the rice paddy soil, but only some of them might be responsible for denitrification.

Abstract Category

22 Major Biogeochemical Cycles

PS.22.062 DISTRIBUTION, DIVERSITY AND ACTIVITY OF ANAMMOX BACTERIA ALONG A SOIL TRANSECT IN THE FRENCH JURA

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Anaerobic ammonium oxidation (anammox) has been recognized as important process in some marine and lacustrine ecosystems yet little is currently known about its role in the terrestrial realm. We studied, therefore, the ecology of anammox bacteria along a soil transect ranging from a fertilized pasture to nitrophilic grassland, a minerotrophic fen and further to an ombrotrophic bog. This transect encompassed thus two major opposing ecological gradients: soil nitrogen and soil water content. The abundance, diversity and activity of anammox bacteria were assessed by qPCR, cloning/sequencing, and 15N incubations. Physico-chemical properties of soil interstitial water (e.g. dissolved oxygen, nitrate, nitrite, ammonium) and the level of the groundwater table was followed during an annual cycle using simple and multilevel piezometers. Results show that anammox bacteria were detectable by PCR in the nitrophilic grassland and the minerotrophic fen but not in the two other soils. Retrieved 16S rRNA sequences from the nitrophilic grassland were most closely related to *Candidatus Anammoxoglobus*. In the fen soil sequences related to *Candidatus Brocadia*, *Candidatus Jettenia* and a yet undescribed cluster were detected, whereby the composition of the anammox guild changed along the soil profile. Anammox activity was detectable by incubation experiments with 15N labeled compounds, but its contribution to dinitrogen formation did not exceed a few percent. The distribution of anammox bacteria seems to be controlled by the presence of free interstitial water, anoxic conditions and the availability of oxidized nitrogen compounds.

Abstract Category

22 Major Biogeochemical Cycles

PS.23.001 SPATIAL DISTRIBUTION OF PHOTOSYNTHETIC EFFICIENCY IN MICROBIAL MAT ECOSYSTEMS

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The use of microsensors has revolutionized our understanding of the function of microbial communities and how microbial activity is regulated by biotic and abiotic factors. Although microsensors allow measurements on a μm scale, they represent a limited amount of point measurements that describe complex and inherently heterogeneous microbial communities from a one-dimensional point of view. Thus, it is rather difficult to extrapolate towards quantitative relationships in three dimensions at larger scales [1, 2]. Here, we applied variable chlorophyll fluorescence and hyperspectral imaging for a detailed analysis of the spatial heterogeneity in oxygenic photosynthesis and pigment composition of microbial mats. Additionally, Automated Ribosomal Intergenic Spacer Analysis (ARISA) and statistical methods were used to assess changes in bacterial community structure upon changes in functional and contextual parameters. The combined imaging approach enabled us to co-localize variations in the activity (PSII quantum yield) with the corresponding pigment content for each pixel in the image. Furthermore, statistical analysis quantified activity variations within the same mat sample as well as within samples collected from the same and different geographic regions. Variations in microbial community composition in samples of different geographical origin were explained mainly by quantum yield, $E_{1/2}$ (the irradiance where half of the maximal quantum yield was observed) and the time between sample collection and measurements. Furthermore, we found a significant variation between samples from the same region. Biogeographical variations between mats collected from different geographical locations, were best described by microbial composition, light adaptations of the microbial mat ecosystems, and the time of sampling.

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Abstract Category

23 Spatial Heterogeneity - Small Volumes with Big Impacts

PS.23.002 SMALL SCALE SPATIAL DISTRIBUTION OF MCPA MINERALISATION CHANGES WITH DEPTH IN A DANISH AGRICULTURAL SOIL PROFILE

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Background: Spatial distribution of mineralisation potentials of xenobiotics in soil is typically studied using homogenised soil samples, while little is known about small-scale spatial distribution of specific degradation potentials. Our aim was to study small-scale spatial distributions of phenoxyalkanoic acid (MCPA) mineralisation potentials in a Danish agricultural soil profile.

Method: Arrays of 96 small soil cores (300 μl soil) were sampled in 7 x 10.5 cm grids at five

different depths (8, 28, 48, 85 and 115 cm). Subsamples of 0.20 g from the cores were transferred to deep-well micro plates (96 wells) according to their original sample position. Samples were spiked with ^{14}C -labelled MCPA (10 mg l⁻¹, 2500 dpm per well) and the plates were sealed with real-time PCR sealing tapes. Each sealing tape was fitted with an array of 96 Ca(OH)₂ impregnated filters to trap the evolved $^{14}\text{CO}_2$, and the trapped radioactivity was quantified by digital autoradiography. Real Time qPCR was used to estimate the total microbial populations (16S rDNA genes) and numbers of MCPA degraders (*tfdA* genes) at the five depths. Results: 16S rDNA copy number decreased from 4.3x10⁸ in the top soil to 9.4x10⁷ at a depth of 115cm. The *tfdA* gene copy numbers decreased from 1.2x10⁴ to < 1x10³ MCPA was mineralised in all wells in the top soil (8cm), whereas MCPA was only mineralised in 60% of the samples at 28cm, 4% of the samples at 48cm, 10% of the samples at 85cm and 0% of the samples at 115cm. Geostatistics was applied on data to analyse spatial variation. Conclusions: The mineralisation of MCPA at each depth was unevenly distributed within the sampling grid with clearly defined hotspots and cold spots. Our study demonstrated an uneven distribution of degrader organism activity and that spatial heterogeneity is most profound in the intermediate soil layers (depth 28-85cm), whereas the top (8cm) and sub soil (115cm) are more homogeneous in respect to MCPA mineralis

Abstract Category

23 Spatial Heterogeneity - Small Volumes with Big Impacts

PS.23.003 IMPACT OF PROTOZOA ON 2-METHYL-4-CHLOROPHENOXYACETIC ACID (MCPA) MINERALISATION IN SMALL-VOLUME SOIL SLURRIES

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Mineralisation of xenobiotics is often studied in slurries of soil and water, where the soil structure is broken up and grazing protozoa may have easy access to soil bacteria. We have previously experienced that mineralisation of the phenoxyacetic acid herbicide MCPA was considerably lower in soil slurries compared to intact soil, probably due to protozoan grazing. The aim of the present study was to investigate the impact of protozoa on the mineralisation of MCPA in soil slurry samples.

Small-volume soil slurries (200 µl) and sieved soil samples (200mg) of agricultural top soil (8cm depth) and underlying subsoil (48cm depth) were compared. The impact of protozoan grazing in soil slurry was studied using the protozoan inhibitor cycloheximide (400 mg l⁻¹). Mineralisation was studied by use of ^{14}C -labelled MCPA, and the numbers of culturable protozoa and MCPA degraders were determined by most probable number methods.

High densities of protozoa (20.000 g⁻¹) and MCPA degraders (2000 g⁻¹) were found in the top soil, whereas the subsoil contained only low densities of both protozoa (2400 g⁻¹) and MCPA degraders (15 g⁻¹). Addition of cycloheximide inhibited protozoan activity considerably in both top- and subsoil slurries, but the inhibition led to increased MCPA mineralisation only in the

subsoil. A 100-fold dilution of the top soil, resulting in densities of protozoa and MCPA degraders similar to the subsoil, showed MCPA mineralisation only when the protozoa were inhibited by cycloheximide.

Protozoan grazing caused a severe inhibition of MCPA mineralisation in soil slurry. The inhibition was most pronounced when the density of degrader bacteria was low such as in the subsoil slurry and the 100-fold diluted top soil. Therefore, soil slurry samples should be used with caution in mineralisation studies, especially for soils with low densities of degrader bacteria.

Abstract Category

23 Spatial Heterogeneity - Small Volumes with Big Impacts

PS.23.004 BIOLOGICAL ACTIVITIES IN INDIVIDUAL SOIL AGGREGATES

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Soil is a three-dimensional system made up physically of aggregates of mineral and organic material. The pore spaces within these aggregates serve as potential sites of microbial establishment. However, the small (sub-millimeter) scale of these aggregates makes them difficult to study, even though they may be discrete habitats for microbial communities. We measured the distribution of biomass (measured as ATP) and enzyme activity (β -glucosidase) in 90-member populations of individual aggregates, from each of three size classes (250-425 μm , 425-841 μm , and 841-1000 μm diameter). Aggregate weight, enzyme activity and ATP content were measured sequentially on each individual aggregate within each size population. Additional experiments measuring β -N-acetylglucosaminidase, leucine aminopeptidase, and lipase activity on individual aggregates were also performed. Total activity (per aggregate) was greater for largest size class. However, when the activities were expressed as a function of the aggregate volume, the smallest aggregates revealed the most intense enzyme activities. There was a weak, but significant, relationship between total ATP (microbial biomass) and β -glucosidase activities; R^2 values were 0.22, 0.51, and 0.43 for the small, medium, and large aggregate categories, respectively ($P < 0.001$).

Abstract Category

23 Spatial Heterogeneity - Small Volumes with Big Impacts

PS.23.005 FUNCTIONAL SIGNIFICANCE OF NITRIFIER AND DENITRIFIER SPATIAL VARIABILITY IN THREE ARCTIC ECOSYSTEMS

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Permafrost soil ecosystems dominate about one-fifth of the world's and the majority (40%) of the Canadian landscape. Permafrost regions are likely to undergo the greatest degree of climate change and sequestered soil organic matter may be released to the atmosphere in the form of greenhouse gases. Soil microbial transformations contribute 60 to 90% of the world's total nitrous oxide (a potent greenhouse gas) emissions. Owing to the characteristic variability of soil physical and chemical properties nitrifier and denitrifier abundance and their activities may vary across multiple spatial scales. A thorough understanding of this spatial variability is important to assess the relationships between nitrifier and denitrifier communities and their functions. The objective of this study was to investigate the spatial association between nitrifier and denitrifier abundance and nitrification and denitrification potential in three Arctic ecosystems. Following a variable lag-distance design along three transects 93 soil samples were collected at each site. For each soil sample, nitrification and denitrification potential was measured and microbial abundance was estimated by real time PCR. Spatial variability and correlation was assessed by using geostatistical techniques. Ammonia oxidizing bacterial (10^5 - 10^7) and archaeal (10^6 - 10^8) and denitrifying bacterial population size (10^5 - 10^9) is similar to other ecosystems. The potential nitrification and denitrification in Arctic soils is in the similar range as reported in agricultural soils. Nitrifier and denitrifier population in Arctic soils is spatially well structured and they have a significant spatial correlation with nitrification and denitrification potential. This study highlights that nitrifier and denitrifier spatial heterogeneity can play a key role in regulating nitrification and denitrification in Arctic soils.

Abstract Category

23 Spatial Heterogeneity - Small Volumes with Big Impacts

PS.23.006 BACTERIAL COMMUNITY STRUCTURE IN SOIL MICROAGGREGATES AND ON PARTICULATE ORGANIC MATTER FRACTIONS LOCATED OUTSIDE OR INSIDE SOIL MACROAGGREGATES

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Soil aggregates and particulate organic matter (POM) are thought to represent distinct, specific soil habitats for microbial communities. Soil macroaggregates (> 200 µm), microaggregates and smaller soil fractions (< 200 µm) have been also described as potentially, relatively stable, habitats for microorganisms. This study investigated whether POM and organo-mineral soil fractions represent distinct microbial habitats and whether the location of these habitats outside or inside macroaggregates affects features such as the amount and quality of organic matter and the structure of bacterial communities. Organic carbon (OC) content and quality expressed as the C:N ratio, and bacterial communities structure (DGGE profiles) were determined for three organo-mineral soil fraction sizes (0-20 µm, 20-50 µm, 50-200 µm) and two POM sizes (> 200 µm: coarse POM and < 200 µm: fine POM) located outside or inside water-stable macroaggregates of a well-aggregated cultivated soil. DGGE profiles mainly revealed different bacterial communities on POM fractions and to a lesser extent in organo-mineral soil fractions in comparison to unfractionated soil. Bacterial community structures were strongly correlated to C content (P=0.001, $\rho=0.64$) and only slightly to C:N ratios (P=0.005, $\rho=0.28$). There was little

difference in C content, C:N ratios or DGGE profiles in organo-mineral fractions. POM fractions showed different DGGE profiles than organo-mineral fractions. Only coarse POM showed slightly different C:N ratios but clearly different DGGE profiles according to its location. This study showed that POM provides distinct microhabitats, harboring specific bacterial communities in comparison to organo-mineral soil fractions. This may be due to high soil aggregate turnovers in the soil studied or to the C resources in different soil fractions being too similar to reduce the differentiation of the bacterial community structures.

Abstract Category

23 Spatial Heterogeneity - Small Volumes with Big Impacts

PS.23.007 CONNECTIVITY IN INTERMITTENT STREAM SEDIMENTS SUPPORT DYNAMIC BACTERIAL COMMUNITIES

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Watersheds comprise hundreds of small, headwater streams within their boundaries and their biodiversity remains largely underestimated. We assessed the influence of water connectivity on bacterial community composition in an intermittent headwater stream. In situ sediment bacterial communities were described across consecutive seasons in 2007 and 2008 (i.e., dry and wet years, respectively). Although the stream did not hold surface water at several times during the study, sediments remained saturated, especially deeper into the hyporheic zone. Significant variations in bacterial community composition were attributed to spatial and seasonal changes in environmental factors. Prior to the onset of flooding, bacteria were isolated in pools or at certain depths in the hyporheic zone. Communities were observed to change from being rich in operational taxonomic units (OTUs) in isolated pockets at the surface, to a few OTUs overall, including an overall decline in both common and rare bacterial forms. We examined the plausibility of the hyporheic refuge hypothesis for bacteria, which predicts a migration of biota into hyporheic sediments following periods of drought. Only 15% of all OTUs existed in both surface and sediment samples, suggesting that only a few phylotypes may use hyporheic sediments as refuge and were likely transported through connected pore spaces, or isolation at the streambed surface. Changes to connectivity impacted bacterial diversity on rapid timescales (i.e., within days), below-ground and across the sediment-water interface (i.e., the hyporheic zone). Due to the coupling of intermittent streams and their sediments to the surrounding watershed, we stress the importance of understanding connectivity at the pore-scale, consequences to biodiversity and over longer time periods.

Abstract Category

23 Spatial Heterogeneity - Small Volumes with Big Impacts

PS.23.008 MULTI-TEMPORAL SCALE VARIATION OF ENZYME ACTIVITY AND MICROBIAL COMMUNITY COMPOSITION

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Marine microbial communities in the Southern California Bight are subjected to various influences throughout the year. Upwelling and terrestrial runoff deliver nutrients, seasonal variation affects water temperature, and tidal changes can affect dissolved oxygen and nutrient concentrations. Changes in these influences can result in variations in microbial community composition and enzymatic activity. Similar to spatial biogeographic patterns, is community composition more similar when sampled over shorter time intervals, and does enzyme activity vary at the same time scales as community composition? To address these questions, water samples were collected at time intervals ranging from thirty minutes to months over a one-year period. The water was filtered for enzyme, nutrient, and genetic analysis of microbial communities. Enzyme activity was measured using a fluorometric assay, and communities were analyzed by sequencing the 16S rDNA region. Enzyme activity showed seasonal variation. Of the four enzymes measured most had sharp increases of activity in the winter and gradual increases over spring with declining activity over the summer. Bacterial communities also varied seasonally. The winter was dominated by alphaproteobacteria and cyanobacteria. Bacteroidetes was the dominant taxa during the spring, and the summer was dominated by bacteroidetes and cyanobacteria. Based on the enzyme analysis and the community analysis, both factors follow patterns of seasonal variation which seem to correspond to increased rain-fall in the winter and plankton bloom conditions in the spring. However, further analysis is being completed using similarity matrices. Changes in community composition over various temporal scales will be determined using a Mantel test. Future work will investigate links between community variation and changes in enzyme activity. This work will attempt to understand how changes in community composition influence ecosystem function.

Abstract Category

23 Spatial Heterogeneity - Small Volumes with Big Impacts

PS.23.009 AGGREGATES PROVIDE IMPORTANT MICROHABITATS FOR MICROBIAL COMMUNITIES IN NO-TILL AND CONVENTIONALLY-TILLED AGRICULTURAL SOILS

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Aggregates provide important physical microhabitats for the growth and activity of microorganisms in soil. The three-dimensional structure of soil influences the spatial distribution of water, nutrients and organisms themselves. Tillage management can affect soil microorganisms through changes to the broader soil structure, redistribution of crop residues and by physical disruption of aggregates themselves. We studied microbial biomass and community structure in a long-term tillage experiment comparing no-till and conventional tillage practices at Swift Current, Saskatchewan, Canada. Sampling strategy included the collection of 0-15 cm

cores, segmented into 5 cm depth increments, as well as 0-10 cm samples dry-sieved into five aggregate size fractions. Phospholipid fatty acid analysis was conducted on both bulk soils and aggregates to assess microbial biomass and community structure. Community fingerprinting of 16S and 18S rDNA using denaturing gradient gel electrophoresis was also used to assess bacterial and fungal community structure in the bulk soils. Total microbial biomass and biomass of different functional groups was greater in no-till than conventional-till bulk soils and aggregates. Conversely, no significant tillage-induced shift in microbial community structure was detected in the bulk soils. However, a significant shift was observed between communities in no-till vs. conventional-till aggregates, indicating that tillage management was in fact an important determinant of community structure. Our results highlight the importance of aggregates as microbial habitats under different tillage management practices. Further investigation is needed to elucidate how tillage management affects microbially-driven processes that occur within aggregates in this semi-arid prairie agroecosystem.

Abstract Category

23 Spatial Heterogeneity - Small Volumes with Big Impacts

PS.23.010 IMPROVED *IN SITU* METHODS FOR THE CO-LOCALISATION OF MICROBES IN SOIL

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The physical structure of soil is highly complex and it is this complexity that permits the coexistence of air and water in soil that is essential for supporting terrestrial life. That same structure provides a habitat for plant roots and soil microbial community that represent the most diverse component of the terrestrial biosphere, and is responsible for processing the soil carbon store. Recently, X-ray CT imaging has captured the details of soil pore geometry from which information on water, gas and nutrient delivery can be predicted and related to function using 3D modelling. However, a key limitation of this approach lies in locating the position of microorganisms within the soil. This could be overcome by labelling soil microorganisms with a biomarker able to attenuate X-rays, such as, heavy metals. It may be then possible to co-locate microorganisms that have accumulated these heavy metals using the CT system. To this end, we explored the possibility of using Alexa Fluor 594 Nanogold probe to co-locate micro-organisms within the soil. We initially developed an *in situ* hybridisation (ISH) protocol for nanogold probing on pure culture (*Pseudomonas*) using combined Fluorescence and Electron microscopy. The combined ISH and Electron Microscopy approach makes it possible to distinguish microorganisms from soil particles and thereby overcoming the problems of autofluorescence normally associated with conventional Fluorescent *in situ* hybridisation (FISH). The protocol was developed further to locate microorganisms within soil plugs (soil embedded in agarose) and root plugs. This ensured that protocol and sample preparation was optimised before attempting to co-localise microorganisms using CT approaches.

Abstract Category

23 Spatial Heterogeneity - Small Volumes with Big Impacts

PS.23.011 BIOGEOGRAPHIC PATTERNS IN SOIL BACTERIAL AND FUNGAL COMMUNITIES, WITHIN AND AMONG SIX TERRESTRIAL BIOMES

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The relevant spatial scales and biogeochemical factors influencing soil microbial community structure remain unclear. We examined the influence of plant cover, edaphic factors, and distance on variability of fungal and bacterial communities at the hectare scale within six terrestrial biomes (pine, sweetgum, aspen, desert, scrub oak/palmetto, estuary). We also compared compositional similarity between the regionally separated biomes.

Highly replicated rDNA bacterial and fungal community sequence surveys (n=12-18 per site) were compared within and across biomes using multidimensional visualization tools and resampling tests. At two sites, the influence of soil depth was also assessed.

Within each site, we identified edaphic factors that contributed to spatial heterogeneity. At the desert site where plants and soil crusts are patchily distributed, the presence of plants or crusts had a major effect on underlying soil community structure. In the sweetgum, scrub oak, and aspen sites, communities were highly structured by soil depth. Although the trees within the sweetgum, aspen and pine sites were of identical age and spacing, the bacterial community in each replicate sample diverged. Despite the within-site variability, bacterial and fungal community composition was more similar within biomes than between biomes. Each biome contained a distinctive fungal community. In contrast, some overlap occurred with the bacterial communities across the forest sites. Conclusions. Spatial heterogeneity occurs at different scales in these biomes, and is strongly influenced by both plant and soil conditions. Despite local heterogeneity, there appears to be bacterial and fungal meta-communities specific for each biome.

Abstract Category

23 Spatial Heterogeneity - Small Volumes with Big Impacts

PS.23.012 EXTREME HETEROGENEITY AMONG DRY VALLEY SOIL BACTERIAL COMMUNITIES—ANTARCTIC MICROBIOLOGY REVAMPED

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Although recent reports on the microbial ecology of McMurdo Dry Valley soils have rejected the notion that Antarctic Dry Valley soils contain limited microbiota, little is known regarding how these microbial communities are influenced by harsh physicochemical conditions that preclude the existence of vascular plants and macrofauna. Comprising of only 0.03% of Antarctica, the

Dry Valley soil ecosystem is exceptionally simple and provides a highly tractable framework for elucidating interactions between abiotic factors and soil microbial communities. Reported dominance of aeolian transport in biota distribution and extremely low estimated turnover rates contribute to an assumption that Dry Valley soil microbial communities are relatively homogeneous despite clear physicochemical heterogeneity within the region. Here we present evidence that challenges this belief and reveals remarkably localized phylogenetic diversity in Dry Valley soil microbiota. Soil samples collected in four Dry Valleys were analyzed using biogeochemical analyses and DNA-based molecular tools, including pyrosequencing of bacterial 16S rRNA gene PCR amplicons. The results show that the four communities are structurally and phylogenetically distinct, and possess significantly different levels of diversity. Geochemical analyses reveal correlation between physicochemical parameters and compositions of bacterial and cyanobacterial communities. Our results indicate that physical and geochemical factors play major roles in shaping microbiology of ice-free areas of Antarctica, and the astonishingly localized diversities of Dry Valley soil microbial communities indicate extraordinary levels of spatial heterogeneity in an area widely believed to harbor a homogeneous microbial population. These observations underscore the need to re-evaluate current ideas of microbial biogeography and raise issues regarding potential loss of biodiversity induced by changes in macroclimatic conditions in Antarctica.

Abstract Category

23 Spatial Heterogeneity - Small Volumes with Big Impacts

PS.23.013 EFFECTS OF LONG-TERM FERTILIZATION ON SPATIAL DISTRIBUTION PATTERNS OF MICROBIAL FUNCTIONAL GENES FROM THE ROTHAMSTED EXPERIMENTAL SITE

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Understanding the diversity and spatial patterns of microbial communities and the mechanisms shaping biotic communities is a central goal in microbial community ecology. The tendency that species richness increases with area (taxa-area relationships, TARs) is a well-known law in microbial ecology. Here, to test the TARs of soil microbial communities and the effects of long term fertilization and liming, a nested sampling scheme was designed at Rothamsted Park Grass field plots with a more than 150-year fertilization history. Soil cores (0-10 cm) were taken at the four corners of the 5 nested squares at increased areas (10 cm², 25 cm², 1 m², 2.5 m², and 5 m²). Plant species in the same area at the same scale were taken to relate the above-ground botanical diversity to the microbial functional diversity. The same sampling scheme was applied on two plots, plot 11/2C with ammonium sulphate and triple superphosphate as fertilizer, and plot 12D with no fertilizer and organic manure. Lime was applied to plot 11/2C since 1863 onto adjust the pH to the same level as that of plot 12D. GeoChip 3.0, covering >37,700 gene sequences from 290 gene families involved in N, C, S, P cycling and other important biogeochemical processes, was used to determine the gene-area relationship (GAR) for both functional and phylogenetic genes. The functional gene numbers were significantly different between the two plots by t-test (P = 0.004). Detrended corresponding analysis (DCA) also indicated that long term fertilization

profoundly changed the microbial functional pattern that the microbial communities were separated by fertilization and non-fertilization. Soil microbial communities exhibited higher z value with long term fertilization, $z_{11/2C} = 0.0864$, than non-fertilization, $z_{11D} = 0.0742$ ($P < 0.05$), which was in accord with the tendency of plant diversity turnover, $z_{11/2C} = 0.419$ and $z_{11D} = 0.225$.

Abstract Category

23 Spatial Heterogeneity - Small Volumes with Big Impacts

PS.23.014 AQUIFERS - HOMOGENEOUS SATURATED SYSTEMS OR MICROBIAL HABITATS WITH SURPRISING FINE-SCALE SPATIAL AND TEMPORAL HETEROGENEITY?

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Aquifers deliver a number of important ecosystem services to our society, amongst the most important of which are the sustaining of water resources and the attenuation of anthropogenic contamination. In dichotomy to this relevance, the scientific understanding of the ecological principles controlling aquifer functioning, especially as habitats for a diverse intrinsic microbiota, is still in its infancy. Classically, aquifers are considered as systems low in spatial or temporal heterogeneity. Here, we challenge this perception based on fine-scale qualitative and quantitative microbial community data obtained by depth- and time-resolved sampling of sediments at a BTEX-contaminated aquifer. By T-RFLP fingerprinting, qPCR and 454 Pyrosequencing of ribosomal and functional marker gene fragments we show, that pronounced cm-scale distinctions actually govern the structure and distribution of aquifer microbiota. Especially in different compartments of the BTEX-plume, as well as at biogeochemical redox gradients, characteristic microbial community patterns were established. When functionally dissected for dominating respiratory and catabolic potentials, these were surprisingly well correlated to the fine-scale localization of respective processes. Thus, plume zones characterized by highest anaerobic BTEX degradation activity were coupled to the dominance of distinct anoxic degrader populations, as well as to high catabolic (*bssA*) gene abundances. At the oxic/anoxic capillary fringe, cm-scale distinctions in the sequential detectability of typical aerobic degrader lineages, thiotrophic chemolithoautotrophs, as well as anaerobic respiratory guilds unveiled unparalleled fine-scale heterogeneities in aquifer microbial community distribution. We discuss how these findings can help to understand the controls of natural attenuation at contaminated sites, and whether similar heterogeneity may be found also in non-contaminated groundwater environments.

Abstract Category

23 Spatial Heterogeneity - Small Volumes with Big Impacts

PS.23.015 CENTIMETER SCALE ANOXIC SITES AND N₂O DYNAMICS IN SOIL

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Periodic depletions of O₂ due to rainfall or application of animal slurry is an essential factor in the control of N₂O production in the soil environment. In this study distribution of O₂ and N₂O in the soil was monitored with microsensors during a four week period following injection of animal slurry in tracks five cm below the surface. Emission rates of N₂O from the soil surface were measured by the closed chamber method. A combination of high O₂ consumption and a low diffusivity ($8 \times 10^{-4} \text{ m}^2 \text{ s}^{-1}$) created an anoxic 3-cm thick cylindrical soil volume which persisted for 7 days. Two N₂O emission peaks from the soil surface were detected with the emission chamber; first a high transient peak 1 day after slurry injection and secondly a longer lasting peak after 6-17 days. However, with the microsensors N₂O was only detected during the first two days and only within the anoxic cylinder. The anoxic cylinder functioned as a hotspot for denitrification of the NO₃⁻ originally in the soil, and denitrification was responsible for the N₂O production causing the first emission peak. Transport limitation in the saturated cylinder resulted in accumulation of N₂O within the anoxic volume and delayed the N₂O emission peak as compared to the net N₂O production. Depletion of the NO₃⁻ in the cylinder two days after injection caused a shift from net N₂O production to net consumption and thereby resulted in a rapid exhaustion of the accumulated N₂O and a consequently cease of the N₂O emission. It was calculated that 20-30% of the NO₃⁻ initially present in the anoxic volume was emitted as N₂O. The second hot-moment of net N₂O production and emission was ascribed to increasing nitrification in the aerated soil and coupled nitrification-denitrification in the periphery of the anoxic cylinder. This study illustrates directly how the N₂O emission from the soil is controlled by temporal and spatial variations in diffusivity as well as the availability of O₂, NO₃⁻, NH₄⁺ and organic carbon

Abstract Category

23 Spatial Heterogeneity - Small Volumes with Big Impacts

PS.23.016 SPATIAL VARIABILITY OF NITROUS-OXIDE PRODUCTION, DENITRIFICATION GENES AND DENITRIFICATION ENZYME ACTIVITY IN A TABLE GRAPE VINEYARD

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Nitrous-oxide (N₂O), a potent greenhouse gas contributes to ozone layer depletion and global climate change. Denitrifying bacteria are major controllers of N₂O, through their ability to perform either complete or incomplete denitrification based on their production of either dinitrogen or N₂O as an end product, respectively. Previous studies, in a California table grape vineyard had determined large N₂O fluxes in the berm compared to the row. The goal of this study was to determine if the ratio of denitrification genes (*nir:nos*) would correlate with N₂O fluxes. Denitrification enzyme activity in the berm ranged from 10.3-11.5 ng N₂O-N min⁻¹ g dry soil⁻¹ and from 1.1-1.8 ng N₂O- N min⁻¹ g dry soil⁻¹ in rows. This corresponds to a 7.5 fold increase in denitrification activity in the berm. Denitrification genes were quantified using quantitative polymerase chain reaction and reported as a percent of 16S rRNA. There were 7-10% *nirS* genes in the row and 20-50% in the berm; 0.6-0.7% *nirK* genes in the row and 1-1% in the Berm; 0.4-0.5% *nosZ* genes in the row and 0.3% in the Berm. N₂O reductase (*nosZ*) was

enriched 1.7 times in the row compared to the berm whereas nitrite reductase, *nirS* and *nirK*, were enriched 3.0 and 1.6 times in the berm compared to the row, respectively. The ratio of *nirS*:*nirK* indicated that there was a preference for *nirS* in the berm. The ratio of *nir*:*nos* was 17-21 in the row and 80-244 in the berm which correlates with the N₂O fluxes measured in the field and the denitrification enzyme activity data. Whereas *nosZ* gene abundances were inversely correlated with denitrification enzyme activity and N₂O fluxes recorded in the field. The abundance of denitrification genes can be used to predict overall denitrification rates and incomplete denitrification, N₂O production.

Abstract Category

23 Spatial Heterogeneity - Small Volumes with Big Impacts

PS.23.017 ARE MEASURED NITRIFICATION RATES A GOOD INDICATOR OF SOIL HEALTH?

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The concept of soil health encompasses the complex interactions of plants, hydrology, and nutrient cycling. Microbial indicators have been highlighted as superior in determining the healthy functioning of the soil microhabitat, due to their rapid response to environmental change. Modern molecular methods allow for rigorous assessment of soil health through their ability to measure both biodiversity and functional processes in the soil environment. Microbial health restoration and maintenance in new soils created from Brownfield land remediation, is important for flexible and safe end uses such as recreation and residential housing. Currently soil health guidelines do not exist for reclamation of contaminated land. This study aimed to define rapid high throughput indicators for use in contaminated land assessment and remediation that directly link functional metabolic processes to community diversity. A range of soils have been analysed to evaluate the effects of pollutants on basal soil health characteristics. Soil health has been monitored through the measurement of three main soil functions: respiration, nitrification and denitrification rates. Key populations related to soil health have been assessed using culture-independent community analysis (Polymerase Chain Reaction, real time PCR and Denaturing Gel Gradient Electrophoresis) to target 16S rRNA and functional genes (*amoA*, *nirS/K*). In this study, Potential Nitrification Rates (PNR) did not indicate significant effects of contamination upon function despite measurable changes in community diversity. PNR measurements of highly contaminated soils show rates, often equating to and exceeding uncontaminated soils. Functional gene profiling of Ammonia oxidizing Bacteria and Archaea identified strong reductions in diversity with contamination. This indicates that PNR measurements may be insensitive to impacts on soil health in polluted environments.

Abstract Category

23 Spatial Heterogeneity - Small Volumes with Big Impacts

PS.23.018 COMMUNITY DIVERSITY IN INDIVIDUAL SOIL AGGREGATES

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Soil aggregates are one example of a discrete habitat experienced by soil microbial communities. These aggregates are the building blocks of soils, and highly stable individual microaggregates bind together to form more dynamic, larger aggregates (up to 2 mm diameter and larger). These individual and compound aggregates thus potentially form different habitat types, and may feature different microbial community structures and functions. We therefore examined the microbial community structures in samples of individual aggregates, organized by size (250-425 μm , 425-841 μm , and 841-1000 μm). We developed an approach to extract DNA from individual soil aggregates, weighing between 0.03 and 0.5 mg. The 16S rDNA from 16 individual aggregates, as well as pools of aggregates, was amplified and sequenced using barcoded universal primers (27F and 338R). Over 314,000 sequences were obtained, assigned to Operational Taxonomic Units (OTUs), and used to examine the community diversity among and between individual aggregates. There was substantial variability in the number and identity of OTUs identified in individual aggregates, indicating considerable beta-diversity (differences in community composition between sites) at the scale of soil aggregates.

Abstract Category

23 Spatial Heterogeneity - Small Volumes with Big Impacts

PS.23.019 DISTURBANCE FREQUENCY AFFECTS MORPHOLOGY AND COMMUNITY COMPOSITION OF A BIOFILM

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Biofilms are spatially heterogeneous at the scale of micrometers in terms of microbial composition, physiological state, and biofilm morphology. It is often neglected that maturing biofilms in a flow field also develop a heterogeneous spatial structure on the order of millimetres and centimetres. At this larger scale, the heterogeneity of biofilms is likely an important parameter affecting substrate diffusion into the biofilm and consequently influences the availability of substrate to the microbial community. How disturbance frequency, in our case the interval of monochloramine addition, influences the evolution of biofilm morphology and bacterial community structure on a larger scale is the objective of our study.

Multispecies biofilms are grown on defined synthetic substrate in three 5-liters bubble column reactors. While one reactor serves as control without biocide addition, two reactors receive a biocide pulses daily and every other day. In each reactor, over a period of 2 months, biofilm is sampled on 133 removable coupons for the acquisition of images with a size of nearly 4 cm² and for DNA extractions. DNA and images allow us to perform community fingerprinting using single strand conformation polymorphisms and automated image analysis of biofilm morphology.

We showed in initial experiments that a minimum image size is necessary to capture heterogeneities in biofilm morphology relevant for mass transfer and thus substrate availability. This size is larger than typical dimensions of images acquired with e.g. confocal microscopes.

We introduce a novel strategy for large-scale biofilm imaging to relate biofilm inhabitants to macroscopic heterogeneities on the order of millimetres and centimetres. In three parallel reactors, we relate microbial diversity to disturbance frequency and biofilm morphology.

Abstract Category

23 Spatial Heterogeneity - Small Volumes with Big Impacts

PS.23.020 LINKING BACTERIAL ABUNDANCE TO HUMAN DENSITY AT A CONTINENTAL SCALE

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Assessing the global importance of bacteria and their major regulating factors require knowledge of their abundance at large spatial scales, only by which general patterns could emerge. Published comparative analysis at large spatial scales were performed using local environmental factors as independent variables, but no attempt was performed using a broader, landscape approach, that also includes the human impacts on aquatic ecosystems. We demonstrated that there is a strong-positive relation between bacterial and human densities when analyzing a unique large-continental data set. Bacterioplankton samples (N = 1,160) were collected in large rivers, lakes and reservoirs of the 12 largest Brazilian hydrographical regions (from Brazil das Águas project, www.brasildasaguas.com.br), which encompass the overall Brazilian territory and approximately 50% of South American continent. Bacteria were counted by flow-cytometry, while human density in each region was obtained by the last Brazilian census. We observed a positive-linear relation between aquatic bacterial abundances and human densities in hydrographic regions. Overall, almost 50% of variation of aquatic bacterial abundance may be explained by this sole regional factor, which is surprising since several local factors may independently, or combined, regulate natural bacterial abundances. This whole idea seems to be trivial or well-documented, however the direct relationship between these smallest organisms of from aquatic ecosystems and human occupation in drainage areas is unlikely and was never shown, since several local factors acting on lower scales usually regulate bacterial abundances and processes

Abstract Category

23 Spatial Heterogeneity - Small Volumes with Big Impacts

PS.23.021 NEOTROPICAL ISLAND BIOGEOGRAPHY FOR SULFATE-REDUCING BACTERIA ACROSS MANGROVES

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Global phylogeography across disturbed sediments has revealed a patchy distribution for sulfate-reducing bacteria (SRB). However, a distinctive cluster was formed by several Neotropical mangrove samples. Mangroves swamps represent a major habitat for SRB while prevailing in the tropical and subtropical shorelines. Our objective is to examine the heterogeneity of SRB in mangroves along the coast of Puerto Rico to assess their island biogeography. Sulfidogenic community composition was characterized with terminal restriction fragment length polymorphisms of the dissimilatory sulfite reductase gene (*dsr*-TRFLP) amplified from soil/sediment samples. A total of 2640 phylotypes (TRF) were detected (representing 373 different TRF). The sulfidogenic community ranged from 8 (Vieques) to 103 (Loiza). No TRF was common to all samples analyzed so far. In contrast, 14% were detected only in one sample (potential endemic taxa). Few major peaks and minor peaks dominated the overall communities. Similarity analysis, based on the Sorensen's index, illustrated clustering together with minimal geographical proximity. Additional samples to process and application of multivariate analyses will provide a more complete description of the island biogeography for SRB in Neotropical mangroves. Sulfidogenic communities sustain a patchy distribution within the continue contour of the island likely due to biogeochemical conditions, geomorphological characteristics, and hydrological regimes of each sites. The application of *dsr*-TRFLP has provided a description of the functional diversity for SRB in one of the most relevant habitats in Earth. The genetic pool being disclosed will assist in understanding the prevailing taxa and resilience of the sites to environmental disturbances and changes.

Abstract Category

23 Spatial Heterogeneity - Small Volumes with Big Impacts

PS.23.022 DIVERSITY AND DISTRIBUTION OF SULFATE-REDUCING BACTERIA AT THE JOBOS BAY NATIONAL ESTUARINE RESERVE (PUERTO RICO, USA)

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The Jobos Bay National Estuarine Research Reserve has suffered anthropogenic and natural disturbances that affect environmental quality compromising its sustainability. Government agencies monitor its water quality and sediments chemistry, and benthic habitats. The chemical character of water and sediments is greatly influenced by microbial activities. Sulfate-reducing bacteria (SRB) predominate in marine sediments and play key roles in biogeochemical cycles, including transformations of pollutants. In order to understand the contributions of SRB to the environmental quality of the sediments, we examine the diversity and distribution of SRB along transect of sediments monitored. SRB were described using terminal restriction fragment length polymorphism analysis of dissimilatory sulfite reductase genes (*dsr*-TRFLP). A total of 1388 phylotypes (TRF) were detected (representing 273 different TRF). The sulfidogenic community ranged from 10 (most polluted area) to 98 (most pristine area) TRF. No TRF was common to all samples analyzed so far. In contrast, 22.7% were detected only in one sample (potential endemic taxa). Few major peaks and many minor peaks dominated the overall communities. Similarity analysis, based on the Sorensen's index, illustrated clustering together with minimal geographical proximity. Additional samples to process, clonal sequencing, and application of multivariate

analyses coupled to chemical data will provide a more complete description of the benthic biogeography for SRB in the estuary. Sulfidogenic communities sustain a patchy distribution within the sediments examined likely due to biogeochemical conditions influenced by anthropogenic influences. This study will help to understand SRB's roles in natural attenuation of contaminants and dynamics within the marine sediments of the Jobos Bay, specifically the impact of non-point source pollution in SRB diversity and distribution.

Abstract Category

23 Spatial Heterogeneity - Small Volumes with Big Impacts

PS.23.023 EFFECTS OF DISTURBANCE AND RECOLONIZATION ON BENTHIC MICROALGAL COMMUNITY STRUCTURE AND SPATIAL PATTERNS

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Benthic microalgae (BMA) are important primary producers in intertidal and shallow subtidal sediments, serving as a vital food resource for heterotrophs. BMA also release extracellular polymeric secretions that stabilize sediments. Primary objectives of the research reported here were to assess the effects of biotic and abiotic disturbances on microalgal community structure in sediments, and to determine mechanisms by which BMA recover from such disturbances.

Using field comparative studies, we characterized the effects on BMA of two prominent types of disturbance: macroinvertebrate ingestion and tidal resuspension. BMA biomass and composition in fecal materials from the enteropneust, *Balanoglossus aurantiacus*, and sediments were followed through time using fluorometry, microscopy and molecular techniques (DGGE and sequencing). We also determined experimentally the mechanisms and rates of recolonization of disturbed sediment patches, comparing regrowth, recruitment and immigration. Last, we examined effects of disturbance on spatial patterns of BMA biomass over the larger sedimentary landscape using correlative studies.

Deposit-feeder ingestion was found to significantly reduce BMA biomass and alter BMA composition, although qualitative changes were comparatively less remarkable. BMA biomass recovery was significant in < 3h, with migration dominating. A significant difference was also found between average biomass before and after tidal immersion, a physical disturbance. Spatial autocorrelation revealed heterogeneity in BMA distribution during low tide, and that this distribution correlated with *B. aurantiacus* fecal coils. Samples taken after tidal immersion showed no patchiness, nor was there a correlation between BMA biomass and pre-immersion fecal cover. At least in intertidal habitats, the qualitative and quantitative impacts of deposit feeding, and the resulting landscape-scale patchiness, appear to be short-lived due to frequent tidal resuspension.

Abstract Category

23 Spatial Heterogeneity - Small Volumes with Big Impacts

PS.23.024 BIOGEOGRAPHICAL PATTERNS OF SOIL MICROBIAL COMMUNITIES AT THE SCALE OF FRENCH METROPOLITAN TERRITORY

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Ecologists studying plant and animals have long recognized that the modifications of diversity across a landscape are central for understanding the environmental factors driving the magnitude and the variability of community assembly. Despite the statement that this conceptual vision is also relevant for microorganisms, since it can offer valuable insights into the relative influence of dispersal limitations, environmental heterogeneity, and environmental and evolutionary changes in shaping the structure of ecological communities, studies integrating wide spatial scale have been poorly investigated and the environmental filters structuring microbial biodiversity remain largely unknown. In this context, we have investigated the characterisation of indigenous microbial communities from soils sampled on a broad scale. This characterisation relied on analyses of density, diversity and genetic structure of microbial communities by using molecular tools directly on DNA extracted from the soil library of RMQS ("Rseau de Mesures de la Qualit des Sols" = French soil quality monitoring network) which cross-rules all the French territory with about 2,200 soils sampled. Geo-positioning, physico-chemical characteristics, climatic factors, floristic composition and land use of these soils were also recorded. The geostatistical interpolation of soil microbial density and diversity revealed a heterogeneous distribution of microbial communities on a wide extent which is spatially structured in bio geographical patterns. Microbial density and diversity seems to be more influenced by local filters such as soil properties and land use but is relatively independent of global filters such as climatic factors or the presence of natural barriers. Our study confirms that the biogeography of microorganisms differs fundamentally from the biogeography of "macro-organisms" and that soil management can have significant large-scale effects.

Abstract Category

23 Spatial Heterogeneity - Small Volumes with Big Impacts

PS.23.025 THE ROLE OF SPATIAL HETEROGENEITY ON PRODUCTIVITY/DIVERSITY RELATION IN A MICROBIAL BIOFILM

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A strong correlation between ecosystem's productivity and diversity has been proposed in many studies. However, there is a lack of agreement concerning the pattern of this relation (unimodal, positive linear, negative linear), as well as the processes behind these patterns. Changes on the levels of heterogeneity on resource availability have been proposed to explain, at least, the decrease on diversity levels in an unimodal ("hump-shaped") relation. Nevertheless, due to ecosystem complexity, heterogeneity has not been characterized and therefore its role in shaping these patterns has not been determined. Bacterial systems provide an excellent model where populations and communities can be propagated in controlled environments in order to reduce

ecosystem complexity. In this work we varied the degree of heterogeneity in the limiting resource (oxygen) in tubes with LB broth, by two means: 1) shake the tubes or leave them static 2) using different diameter tubes, changing the area of the air-broth interface. We inoculated a *Pseudomonas* sp. in the different tubes and after four days it diversifies into different morphs each one with a particular niche. After plating a sample of each tube, we measured both, productivity and diversity. Heterogeneity was calculated as the variance in oxygen concentration (measured with a microelectrode) inside the tubes. Results show that there is no relation between productivity and diversity in homogeneous environments. In contrast, in heterogeneous environments there is a high influence of resource availability on both: diversity and productivity. We conclude that there is an important effect of spatial heterogeneity in shaping the relation between productivity and diversity.

Abstract Category

23 Spatial Heterogeneity - Small Volumes with Big Impacts

PS.23.026 IMPACT OF DISTURBANCE ON SOIL MICROBIAL ACTIVITY IN THE NORTHERN CHIHUAHUAN DESERT

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Cryptobiotic soil crusts in arid regions contribute to ecosystem stability through increased water infiltration, soil aggregate stability, and nutrient cycling between the soil community and vascular plants. These crusts are particularly sensitive to compaction/fracturing disturbances such as livestock grazing, off-road vehicle use, trampling by humans, and drilling and mining activities. Loss of soil crusts is believed to increase the rate of desertification, and recent findings indicate that crusts are extremely slow to recover, on the order of hundreds of years. However, post-disturbance recovery rates for soil bacterial and fungal populations is vastly understudied, and it is suggested that loss of soil crusts leads to decreased abundance and diversity of these non-crust soil biota. Soil microbial activity within and around two natural gas well pads embedded within a livestock grazing area were investigated. During the natural gas extraction process, the shallow surface soil is stockpiled near the well pad. Surface and subsurface soil samples were taken from the heavily compacted well pad, the surface soil stockpile (fallow 12yr), and the grazed area outside of the well pad. Microbial activity was measured using the MicroResp™ system, which measures respiration of microbes within whole soil samples supplemented with various carbon sources (simple and polymeric sugars, amino acids, carboxylic acids, and fatty acids). Preliminary results indicate slightly reduced activity in heavily disturbed well pad areas compared to grazed areas, although overall activity for all samples was extremely low, and not significantly different from controls. Activity was marginally higher in surface soils (top 5cm) compared to subsurface soils (5-30cm). Greatest microbial activity was measured in the soil stockpile at a depth of 5-30cm. Further testing is underway to compare microbial activity in these disturbed areas to samples from nearby areas with intact crusts.

Abstract Category

23 Spatial Heterogeneity - Small Volumes with Big Impacts

PS.23.027 KARST POOLS IN SUBSURFACE ENVIRONMENTS: COLLECTORS OF MICROBIAL DIVERSITY OR TEMPORARY RESIDENCE BETWEEN HABITAT TYPES

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Groundwater is a major source of drinking water for a significant proportion of the world's population. Subterranean karst aquifers are structurally diverse and they may play an important role in groundwater formation. So far little is known about the microorganisms inhabiting karst-derived subsurface aquatic habitats and about the factors influencing microbial assemblages in such environment. We studied bacterial diversity and community composition in three shallow pools of a Swiss karst cave system with contrasting hydrological and hydrochemical properties. A comparative analysis of 16S rRNA genes showed remarkable differences between the microbial assemblages in the pools. Only one operational taxonomic unit (OTU, 97% similarity) was shared between the three of them (total OTU number in all pools: 150). Unexpectedly high microbial phylotype richness was found even in the two pools without groundwater contact and with low concentrations of organic carbon and total cell numbers ($< 10^4 \text{ ml}^{-1}$). One of these seepage water fed systems harbored 15 distinct OTUs from several deeply branching lineages of the candidate phylum OP3, whereas representatives of OP3 were not found in the other two pools. A tentative phylogeographic analysis of available OP3-related sequences in the context of our data set revealed that there was generally little agreement between the habitats of origin of closely related sequence types. Two bacterial clades affiliated with the obligate methylamine utilizer *Methylothermobacter mobilis* were only found in the pool that was exposed to repeated flooding events. Direct microscopic analyses by fluorescence in situ hybridization revealed that these bacteria formed relatively stable populations of up to 6% of total cell counts over periods of several months irrespective of inundation by groundwater. This suggests that karst water may provide a means of transport for these bacteria from terrestrial to freshwater habitats.

Abstract Category

23 Spatial Heterogeneity - Small Volumes with Big Impacts

PS.23.028 AN INDIVIDUAL-BASED APPROACH TO SURFACE COLONIZATION BY BACTERIA

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Most microbial habitats are characterized by physical, chemical, and biological conditions that differ significantly along micrometer distances. This environmental heterogeneity at the scale of individual microbes may account for much of the nonrandom distribution of microorganisms observed in natural settings. Here we report on an individual-based approach that combines spatially explicit modeling and bioreporter-driven experimentation to improve our basic understanding of the impact of small-scale heterogeneity on the bacterial colonization of

surfaces. We used bioreporters of *Erwinia herbicola* 299R tagged with the green fluorescent protein (GFP) in two ways. One type of bioreporter constitutively expressed GFP which allowed for easy visualization of the location of bacteria in relation to one another. In the second type of bioreporter, preformed GFP was diluted from cells as they divided, rendering GFP content of daughter cells an inverse function of the reproductive success of their mothers. These bioreporter cells were inoculated on a defined agarose surface and analyzed by fluorescence microscopy to measure bacterial growth and colony formation during incubation. *E. herbicola* cells all grew at the same rate on the surface of the gel medium, regardless of the bacterial spatial density. This suggests that under these conditions, growth of bacteria was not influenced by the presence of others nearby. Fluorescence intensity and surface area measurements of the reproductive success were in good correlation. The pattern simulation matched the experimental data when it introduced initial variations in the cell cycle of colonizers. This approach of validating bioreporter output in simple environments with defined complexity are essential for our ability to explain their performance in more complex environments, such as the natural habitat of *E. herbicola* 299R, i.e. the plant leaf surface.

Abstract Category

23 Spatial Heterogeneity - Small Volumes with Big Impacts

PS.23.029 BIODIVERSITY OF THE WORLD'S MOST ALKALINE ENVIRONMENT: SOIL AND GROUNDWATER COMPARISON

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The microorganisms in the groundwater of the Lake Calumet wetlands, Chicago, IL, have been described as the world's most extreme alkaliphiles. Over the 100 years, the wetlands have been infilled with steel slag. The slags were deposited from local blast furnace mills and were comprised primarily of high-temperature calcium silicate minerals and could contain as much as 50% metallic iron, manganese, and other steel additives (Cr, Mo, V) which can vary in composition within in meters of each other.. The weathering of the calcium silicates within the slag subsequently formed an aquifer with groundwater pH up to 12.8. Previous research on the microbial community has been performed on the groundwater but nothing has been done to examine this community in the soils in contact with this high pH groundwater. The objective of this research project is to determine the structure of the microbial communities at different locations with the Calumet aquifer systems and to determine if the microbial communities are similar in the groundwater and the soils. Preliminary screening of three soils and two groundwater samples with utilizing sole source carbon utilization plates (Biolog® Ecoplates) revealed low microbial diversity. It also determined that the microbial community in the soil was significantly different than that found in the soil especially in highest pH setting. In addition, the spatial distribution of the microbial community in relationship to the mineralogy was determined using scanning electron microscopy. It was determine that the different chemical composition of the aquifer materials impacted the biofilm that formed on the soil associated with the extreme alkaphile conditions. The microbial community will also be examined using different molecular

techniques. The findings of this study will reveal novel insights into the extreme alkaliphilic microbiology.

Abstract Category

23 Spatial Heterogeneity - Small Volumes with Big Impacts

PS.23.030 A LANDSCAPE PERSPECTIVE ON ANTIBIOTIC RESISTANCE IN SOIL BACTERIA

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The extent, pattern, and causes of antibiotic resistant bacteria in soil are not well understood. Antibiotic resistant in the environment may arise from the use of antibiotics in agricultural practices, from indirect selection for resistance to heavy metals, and from natural interactions among microbes. We performed a novel study of the large-scale distribution of antibiotic resistance in soil bacteria. We determined the relationship of resistance in *Enterobacter aerogenes* to land use and concentration of metals during July of 2007 and 2009 in Lancaster County, PA, USA. Sites were randomly chosen within each land use. Microbial analyses involved isolation and replica plating of 48 isolates per site. Metal analyses were performed with a MS-ICP. From soils collected from 84 sites in 2007, we found mean proportions of isolates resistant to ampicillin (0.50), chloramphenicol (0.49), kanamycin (0.05), and tetracycline (0.03). From soils collected from 73 sites in 2009, we found mean proportions of isolates resistant to ampicillin (0.64), chloramphenicol (0.12), trimethoprim-sulfamethoxazole (0.04), kanamycin (0.002), and tetracycline (0.02). Levels of resistance were typically lower in forest versus other land uses. In both years, bacteria from forests had significantly less resistance to ampicillin (2007: $F_{3,80} = 6.7$, $p = 0.001$; 2009: $F_{3,69} = 5.6$, $p = 0.002$) than those in residential, crop, or pasture sites. In 2007, bacteria from forests and residential sites had significantly less resistance to kanamycin ($F_{1,79} = 11.88$, $p = 0.001$) than from agricultural sites. We did not detect an effect of soil metal concentration on antibiotic resistance. We document microbial resistance in a human-dominated landscape and provide an assessment of variables affecting that resistance. A greater effort should be made to understand the relationship between land use, soil characteristics, and resistance and the implications of these relationships on public health.

Abstract Category

23 Spatial Heterogeneity - Small Volumes with Big Impacts

PS.23.031 INFLUENCE OF SOIL STRUCTURE ON MICROBIAL INTERACTIONS AND DIVERSITY

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Soil habits are extremely heterogeneous and are inhabited by a tremendous number and diversity of microorganisms. Although recent studies have provided insight into some primary drivers of soil-borne microbial community structure, little is known about how the physical structure of soil

influences the establishment and maintenance of microbial diversity. Soil structure and stability determines the connectivity (flow of chemical compounds such as nutrients, antibiotics, and signaling compounds) of soil-borne microhabitats, thereby influencing bacterial interactions such as resource- and interference competition. In a series of microcosm experiments, we therefore sought to gain insight into the influence of soil structure and soil particle connectivity on microbial diversity. We hypothesized that small-scale spatial isolation fosters greater biodiversity by allowing for the co-existence of otherwise competing species. To test this hypothesis, a series of artificial soil communities were constructed, using different numbers of soil isolates introduced into microcosms of different pore-size-distributions with different levels of mixing. Resulting communities were followed in time, and population densities tracked by specific plate counting and quantitative real-time PCR. These communities were also examined by fluorescent in situ hybridization (FISH) to assess the physical distribution of the different populations in the developing microcosms. Experiments involved inoculation with two different hierarchies of postulated competitive interactions: perfectly intransitive competition (i.e. strain A beats B, B beats C and C beats A) and perfectly hierarchical competition (A beats B, B beats C, etc.). Results show that spatial isolation at the micro-scale can greatly affect competitive relationships, thereby stabilizing microbial community composition and diversity.

Abstract Category

23 Spatial Heterogeneity - Small Volumes with Big Impacts

PS.24.001 VIRAL LIFECYCLES IN THE OXYGEN MINIMUM ZONE OFF IQUIQUE, CHILE

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Oxygen minimum zones (OMZ) are defined as coastal areas in the ocean containing < 20 µM of dissolved oxygen. Throughout the Earth's history, these areas have expanded and contracted during times of warmer and colder geological climates, respectively. Biologically, OMZs are dominated by microbes, which may play important roles in carbon and nitrogen cycling. Metagenomic techniques allow us to combine the DNA and RNA signatures of all microorganisms within a sample and characterize their metabolic potential and taxonomic makeup. Analysis of microbial and viral fractions at different depths in an oxygen gradient from the OMZ off Iquique, Chile have shown low viral to microbe ratios at specific depths. These low numbers indicate that the viruses may be adopting a lysogenic rather than lytic lifestyle in the low oxygen regions of the OMZ. To explore the hypothesis that OMZ phages are lysogenic rather than lytic cycle, induction experiments were conducted. Water samples at depths containing 0.1 µM dissolved oxygen were treated with mitomycin C to induce the prophages. Metagenomes were constructed from the induced viral community. Viral DNA from treated metagenomes were compared to non-induced viral DNA metagenomes to identify changes in gene content. We expect to see more prophage genes from the induced samples such as serine or tyrosine integrases, CI-type repressors, and transposases, compared to the non-induced sequences. Both non-induced and induced sequences should include phage genes such as capsid and tail genes. The occurrence of anoxic metabolic genes involved in marine microbial survival, including sulfur, nitrogen, and carbon metabolism, will be identified from all samples. Since phages act as mobile genetic elements for microbes, they may be shuttling anoxic metabolic genes throughout OMZ microbes via a lysogenic lifestyle. This research will shed light upon the role of microbes and viruses in nutrient cycling of low oxygen marine systems.

Abstract Category
24 Marine Oxygen Minimum Zones

PS.24.002 COMMUNITIES OF NOSZ-TYPE DENITRIFIERS IN THE OXYGEN MINIMUM ZONE IN THE EASTERN SOUTH PACIFIC

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The oxygen minimum zones (OMZ) such as in the Eastern South Pacific (ESP) off the Chilean Coast are considered as important sites of N₂O and N₂ efflux into the ocean. Therefore, samples which differed with respect to biogeochemical parameters (e.g. O₂, NO₃⁻, NO₂⁻, N₂O, and NH₄) were collected from several depths in the water column of three areas within the Chilean and the Peruvian OMZ over two consecutive years. To understand the structure of denitrifying microbial communities presumably related to N₂O reduction in this area, these communities were explored by terminal restriction fragment length polymorphism (T-RFLP) analysis of nosZ genes. The nosZ gene which encodes nitrous oxide reductase has been frequently used to target denitrifying bacteria capable of the reduction of nitrous oxide to dinitrogen. T-RFLP showed generally a low diversity of nosZ genes within the water column especially at depths of limited oxygen availability. Denitrifier community composition was very different between distant geographical locations and differed with time which presumably reflected shifts in the environmental conditions. Canonical correspondence analysis indicated that nitrous oxide, ammonium and oxygen were important environmental factors governing these communities along the gradient in the water column at different localities. However, despite differences in the physical-chemical conditions along the water column within the Chilean OMZ, identical T-RFs occurred. Interestingly, within the OMZ the water depth showing the highest NO₃⁻ and N₂O concentrations harbored the most distinct and least diverse denitrifier community suggesting that this community is limited in its ability to denitrify and especially to fully reduce N₂O to dinitrogen.

Abstract Category
24 Marine Oxygen Minimum Zones

PS.24.003 ENHANCED ANAPLEROTIC CARBON FIXATION IN HETEROTROPHIC BACTERIA IN HYPOXIC WATERS

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Carbon fixation in heterotrophic bacteria in marine environments is often attributed to respiration processes in which the organism consumes O₂ produced by autotrophs while fixing dissolved organic carbon (DOC) through glycolysis and the citric acid cycle. Some heterotrophic bacteria may have a more dynamic role in the marine carbon cycle than previously believed. Our project utilizes stable isotope probing with ¹³C-NaHCO₃ to determine which microbial taxa use inorganic carbon in hypoxic waters taken from 10km off the coast of Newport, Oregon. Results implicated several known heterotrophic bacterial taxa active in the assimilation of dissolved inorganic carbon (DIC) as well as DOC. Anaplerotic reactions are well known in many heterotrophic organisms, where pyruvate carboxylase and phosphoenolpyruvate carboxylase (PEPC) facilitate CO₂ fixation in the presence of HCO₃⁻ resulting in the anaplerotic generation of oxaloacetate, a TCA cycle intermediate required for amino acid and biomass production. However, little is known about the importance and function of anaplerotic carbon fixation in heterotrophic organisms in the environment. The data suggests hypoxic conditions influence bacterially mediated carbon fixation as the number of heterotrophs fixing CO₂ increases and the diversity of active chemolithoautotrophs decreases. The heterotrophic assimilation of CO₂ may reflect an adaptive response to a depletion of complex organic carbon sources following a hypoxic event as heterotrophic organisms continue to consume O₂ in an already starved system consuming DOC contributed by bloom decay. The bacterial PEPC gene was detected in all environmental samples suggesting presence of the anaplerotic carbon fixation pathway. To further our understanding of heterotrophic anaplerotic processes we are focusing on *Polaribacter irgensii* 23-P as a model for heterotrophic DIC assimilation in hypoxic environments.

Abstract Category
24 Marine Oxygen Minimum Zones

PS.24.004 THRIVING IN AN OXYGEN MINIMUM ZONE: PROTEOMIC ANALYSIS OF SUP05 ENERGY METABOLISM

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Oxygen minimum zones (OMZs) are regions of hypoxia which support a microbiota whose metabolic activity impacts nutrient and trace gas cycling within the oceans. The OMZ microbial community plays an important role in the nitrogen cycle as sinks for nitrogen and production of nitrous oxide. Currently, the contributions of denitrification, anammox and dissimilatory nitrate reduction to ammonia (DNRA) to nitrogen loss are under debate. Recent work has demonstrated the presence of two closely related and co-occurring lineages, ARCTIC96BD-19 and SUP05, in hypoxic systems globally. While SUP05 populations have been implicated in chemolithotrophic sulfide (H₂S) oxidation coupled to nitrate (NO₃⁻) reduction, both ARCTIC96BD-19 and SUP05 are found in sulfidic and non-sulfidic OMZs. Metagenomic analysis of SUP05 revealed a versatile energy metabolism with multiple and integrated pathways for sulfur oxidation and denitrification. Here we explore environmental and genetic factors regulating SUP05 energy metabolism by proteogenomic analysis of SUP05 within the stratified water column of Saanich Inlet, a seasonally anoxic fjord on the coast of Vancouver Island British Columbia. Using the SUP05 genome as a recruitment platform for peptide identification we identified differential gene expression in hypoxic and anoxic regions of the water column specifically in the integration of sulfur-oxidation and nitrate reduction pathways. Additionally, we detected a c-type polyheme cytochrome related to hydroxylamine oxidoreductase with the potential to mediate

DNRA in anoxic basin waters. Collectively, these observations validate proposed models for SUP05 energy metabolism while uncovering previously unrecognized modes of electron transfer and provide a functional basis for constraining the ecological role of SUP05 in relation to sulfur detoxification and biological nitrogen loss in sulfidic and non-sulfidic OMZs.

Abstract Category
24 Marine Oxygen Minimum Zones

PS.24.005 PROGRESSIVE OXYGEN DEPLETION AT THE BOTTOM OF LAKE BIWA AND CHANGES IN DISTRIBUTION OF SULFATE-OXIDIZING BACTERIA *THIOPLOCA* SP.

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The bottom of Lake Biwa (the largest monomictic lake in Japan: 104m in depth at the deepest point; mean depth 41m) has been exposed to low oxygen conditions during the season before vertical mixing every year since the late 1960s as well as progressing water eutrophication. Furthermore, recent conditions due to global warming and climate change have often extended lake stratified periods, which induce a less than 2 mg•L⁻¹ dissolved oxygen concentration at the bottom layer of water, where it is difficult for most aerobic creatures to survive. Dense mats of *Thioploca* spp., filamentous, gliding, sulfur-oxidizing bacteria that use oxygen and/or nitrate were first found on the sediment in the deep water area (water depth 90m) of Lake Biwa in 1991. It was considered that the increased microaerophiles was a noteworthy phenomenon during the decreasing process of oxygen. Monthly monitoring and distribution research at the 90m area of the lake was carried out in the 1997-2000 period and during the winter of 1997, respectively. The biomass was about 10⁶ μm•cm³, and the distribution was relatively homogenous in the area deeper than about 40m in depth. We also investigated the monthly biomass change at the same points in 2007 and distribution in 2009; however, a significant decrease at the surface layers of the sediment core of the 90-m area to about 10⁵ μm•cm³ was observed in 2007, and the distribution pattern had become heterogeneous in 2009. Oxygen concentration levels have fluctuated, but cumulate depletion has become increasingly severe over the past 10 years, which may suggest that the oxygen level has dropped below the suitable level for microaerophiles such as *Thioploca* sp.

Abstract Category
24 Marine Oxygen Minimum Zones

PS.24.006 GENE EXPRESSION ANALYSES IN PELAGIC SUBOXIC ZONES OF THE CENTRAL BALTIC SEA ARE STRONGLY INFLUENCED BY THE SAMPLING PROCEDURE

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Metatranscriptomics, as an important tool for the analysis of microbial activities, has become a major interest in aquatic microbiology. Unfortunately, short half life times of some prokaryotic mRNAs and the possibility of metabolic shifts under changing abiotic conditions during and after sampling are factors that potentially alter the original expression profile of a habitat. In consequence, the study of the actual in situ gene expression is a demanding task and requires efficient sampling and preservation procedures. We investigated mRNA profiles from suboxic areas of Baltic pelagic redoxclines, located around 100 m depth. Our aim was to compare the mRNA profiles of these habitats gained through both more traditional and a newly developed sampling procedure. As traditional sampling 5-liter Free-Flow bottle (Hydrobios) and a Pump-CTD were used (duration approximately 20 min), followed by filtration and sample storage on board. For comparison, the Automatic Fixation Injection Sampler (AFIS) was developed. AFIS enabled the fixation of water samples exactly at the time of sampling directly in the original habitat. Samples were then filtered and stored on board. In the laboratory, RNA was extracted from the filters and gene expression profiles for all different sampling procedures were investigated by 454-pyrosequencing. Moreover, archaeal amoA transcripts which are specific for Baltic suboxic zones were quantified using Real-time PCR. Both methods demonstrated that (1) the metatranscriptomic profile was specific for the sampling technique, and (2) archaeal amoA transcripts were only

preserved in higher numbers when immediately fixed with the AFIS system. Our results demonstrate that routine sampling techniques introduce qualitative and quantitative biases in gene expression profiles which can be overcome by in situ fixation procedures. The magnitude of this bias probably depends on the particular habitat conditions and on the time needed to process the samples.

Abstract Category
24 Marine Oxygen Minimum Zones

PS.24.007 COMMUNITY FEATURES OF NOVEL VIRUSES FROM THE OXYGEN MINIMUM ZONE

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Oxygen minimum zones are the result of reduced ocean ventilation combined with degradation of organic matter. They affect ocean biogeochemistry as well as commercially important fisheries. Due to the low oxygen concentrations, these zones are microbe dominated and their respiration contributes to the flux of carbon dioxide and nitrous oxide into the atmosphere. In an effort to understand the role that marine viruses play in the microbial ecology of these extreme ecosystems, six metagenomic samples were obtained representing three depths in the oxygen profile within two locations of a longitudinal transect. MG-RAST analysis using the SEED database revealed the novelty of the dataset as less than 3% of the sequences matched known genomes. PHACCS analysis shows this ecosystem has lower viral diversity than other marine viromes. Dinucleotide odd ratio analysis confirms the marine-hypersaline signature of the metagenomes demonstrating that although novel, these viruses are oceanic. Reciprocal best blast among metagenomes identified overlapping sequences among all metagenomes, including a low percentage (2-3%) between oxygen deficient waters (<1 ml l⁻¹) and highly oxygenated (5 ml l⁻¹) ones. Further computational analysis expose the presence of diverse unknown contigs whose abundances vary with depth. Functional metagenomic studies show the presence of the malate dehydrogenase gene of the glyoxylate cycle, a process mostly used by Archaea, suggesting the presence of viruses that infect this taxon. Enumeration of microbes and viral like particles indicates that viral microbial ratios varied greatly (8.5 - 0.5) along a depth profile (10-500 m), suggesting distinct microbial dynamics within the oxygen minimum zone. Given the importance of viruses in controlling microbial (Bacteria and Archaea) populations in most aquatic environments, our research focuses on the role that these viruses play in contributing to the establishment and/or maintenance of these near dead zones

Abstract Category
24 Marine Oxygen Minimum Zones

PS.24.008 KINETICS OF OXYGEN RESPIRATION BY SEAWATER MICROBIAL COMMUNITIES

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There has always been oxygen deficiency in some marine areas, but during the most recent decades, anthropogenic eutrophication has resulted in a massive growth in oxygen deficient marine environments. One of the basic parameters for understanding and modeling oxygen deficiency is the respiration rate in the water. Very sensitive isotope methods are available for determination of N-dependent respiration rates and sulfate reduction rates in water samples, whereas the low expected rates of oxygen respiration are difficult to measure and therefore rarely quantified. We here present a new method for measuring oxygen respiration in water samples where respiration rates of less than 1 nM h⁻¹ (i.e., < 9 μM year⁻¹) can be determined. The method is based on the measurement of the decrease in O₂ concentration during a 24 h incubation of a 1-2 L water sample using a novel O₂

sensor that can quantify O₂ concentrations down to 1-5 nM. To measure the usually very low rates of decrease in O₂ concentration, the initial O₂ concentration should be low, and the highest resolution is obtained with water containing < 1 μM O₂. Water with high O₂ concentrations should thus be partially deoxygenated by bubbling with an N₂/CO₂ mixture before the assay. We have determined rates of oxygen respiration and studied how oxygen respiration is regulated in various waters bodies from the Danish seas and from the OMZ region in the Pacific Ocean off Northern Chile. In the Pacific, the O₂ respiration rate in the water just above the oxic-anoxic boundary was about 12 nM h⁻¹, which was >10 times higher than the N-dependent respiration (denitrification plus anammox) in the anoxic water layers just below. A linear decrease in O₂ down to very low concentrations suggests half-saturation O₂ concentrations for this microbial community of less than 40 nM. Our ability to directly monitor microbial metabolism in natural seawater opens for new integrated studies of metabolic activity and the biological community

Abstract Category

24 Marine Oxygen Minimum Zones

PS.24.009 INSIGHTS INTO THE MICROBIAL ECOLOGY OF MARINE HYPOXIA

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Marine hypoxia is increasing throughout the world, influencing biogeochemical cycles and the distribution of marine life. Natural hypoxia occurs in oxygen minimum zones (OMZs) and in the bottom water of silled basins and semi-enclosed seas, where weak circulation and high remineralization rates maintain low oxygen levels. Currently, the number of hypoxic "dead zones" in coastal oceans is increasing due to anthropogenic fertilization. Likewise, global warming is intensifying OMZ hypoxia through reduced oxygen solubility and enhanced stratification. Ecosystem models demonstrate that increasing hypoxia leads to a diversion of energy from higher trophic levels into microbial pathways. Moreover, the extent of hypoxia regulates the microbial-mediated loss of fixed N from the oceans and controls the oceanic production of N₂O, a potent greenhouse gas. Given the ecological impact of marine hypoxia, and the contribution of microbial processes to oxygen-sensitive biogeochemical cycles, it is important to further understand the microbial ecology of hypoxic systems. To this end, we report on a four-year time-series study of a seasonally hypoxic coastal basin (Saanich Inlet) in British Columbia. Conditions typically range from mild hypoxia to anoxia, an ideal dynamic for investigating and modeling microbial responses to variable oxygen levels. Molecular and genomic profiling identified spatiotemporal interactions amongst microbial taxa, including several implicated in nitrogen cycling (e.g. Marine Group I Crenarchaea, Nitrospina, SUP05 and Scalindua). In addition, we identified co-occurrence patterns of genes involved in carbon fixation, denitrification, nitrification and sulfur oxidation, implicating novel microbes in C, N and S cycling under hypoxia. Currently, we are using the Saanich inlet time-series data, including the molecular and genomic data, to model the physical and biogeochemical processes that result in hypoxia and predict microbial community response.

Abstract Category

24 Marine Oxygen Minimum Zones

PS.25.001 DIVERSE PHAGE IN THE SWINE INTESTINE

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The complex effects of antibiotics on indigenous microbial ecosystems are poorly understood despite the widespread use of antibiotics in agricultural animals. An increase in resistant bacteria is one obvious effect of antibiotics; additional activities of some antibiotics at subinhibitory concentrations include the modulation of gene expression and the stimulation of gene transfer. Previous work from our lab showed that the veterinary antibiotic carbadox induced a phage-like gene transfer agent in the swine intestinal pathogen *Brachyspira hyodysenteriae*. We therefore hypothesize that one collateral effect of antibiotic treatment in animals is an increase in phage lysis in the gastrointestinal microbiota. The current study aims to characterize shifts in the swine intestinal phage population following different antibiotic dosages. Feces were collected at one day, three days, and 14 days post-dosage from 18 piglets that were reared on antibiotic-free feed, or feed containing subtherapeutic or therapeutic levels of carbadox. We developed a method to isolate phage from 10 grams of feces that included blending, centrifugation, cesium chloride gradient separation, and syringe filtration. Phage particles were then visualized by electron microscopy prior to DNA isolation for pyrosequencing. Electron microscopy showed many different viral morphologies in both control and treated animals, including enveloped virions and morphotypes of the myoviridae, siphoviridae, and podoviridae phage families. The results show a great diversity of viruses in the swine intestinal microbiome. Sequencing the viral assemblage followed by comparative metagenomic analyses will demonstrate the effect of antibiotics on the phage population in the gastrointestinal tract. Learning the effect of antibiotics on intestinal phage is critical to understanding all of the ecosystem-level effects of agricultural antibiotics, which in turn is essential to animal health and food safety.

Abstract Category

25 The Gut Microbiome

PS.25.002 METAGENOMIC AND 16S rRNA ANALYSES OF FECAL BACTERIA FROM SWINE GIVEN DIETS CONTAINING ANTIBIOTICS

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Antibiotics are commonly given to livestock to treat disease and promote growth. For the most part, effects of these dietary additives on intestinal microbes and their ecology are unknown. The goal of this study was to evaluate the influence of antibiotics on swine intestinal microbial communities. ASP250 (chlortetracycline, sulfamethazine, and penicillin) is added to swine diets for both therapeutic and performance enhancing applications. Three pigs received ASP250 feed and three received feed without antibiotics. Feces were collected from the animals before treatment (zero time) and after 14 days of continuous treatment. Metagenomic and 16S rRNA gene (V3 region) sequence analysis was used to characterize the microbial communities for each time and treatment. According to the V3 analysis, the majority of the reads grouped with the

Bacteroidetes (53%) and *Firmicutes* (30%) phyla. After 14 days, the group given antibiotics showed a two-fold increase in *Proteobacteria* (9% to 18%) compared to the community of the same pigs at zero time. The largest shift within *Proteobacteria* was with *Escherichia*, which increased from 1% to 11% with antibiotic treatment. Comparisons between the antibiotic-fed and control groups at 14 days reflected the same shift with the use of antibiotics. Metagenomic sequence analyses of the pooled DNA from each timepoint and treatment confirmed the 16S results. To assess the antibiotic resistance potential of the treated and untreated metagenomes, the antibiotic resistance gene database was used to identify resistance genes in the four metagenomes. At a cutoff of 35% amino acid identity, 190 antibiotic resistance genes were identified across all metagenomes, most of which were present regardless of antibiotic treatment. This indicates swine can be a reservoir for resistance genes even in the absence of antibiotic use. The increase in *Escherichia* levels with the use of antibiotics suggests implications for food safety and animal disease.

Abstract Category

25 The Gut Microbiome

PS.25.003 THE GUT MICROBES AND FORAGING BEHAVIOR OF WILD, BLACK HOWLER MONKEYS (*A. PIGRA*) IN PALENQUE NATIONAL PARK, MEXICO

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Within primate species, energy and nutrient needs vary among age and sex classes due to body mass, growth, and reproduction. However, few studies have demonstrated age and sex differences in energy and nutrient consumption. Studies of mice and humans suggest that differences in gut microbial community composition allow individuals to extract different amounts of energy and nutrients from the same diet. Non-human primates of different age, sex and reproductive status may use differences in gut microbial community composition to meet distinct nutritional demands. This study combines community fingerprinting (ARISA), pyrosequencing and fermentation end-product assays of fecal samples with field observations of behavior to compare age- and sex-based variation in foraging behavior and gut microbial community activity and composition in wild, black howler monkeys (*Alouatta pigra*). Data were collected over an 8-week period from 14 howlers in two groups in Palenque National Park, Mexico. Few differences were detected in foraging behavior between age and sex classes. Fecal pH was similar among individuals, but adults exhibited higher fecal ammonia, acetate, and isovalerate concentrations than juveniles. Adult females exhibited higher concentrations of butyrate than males. Community fingerprinting revealed weekly turnover of microbial communities within individuals and differences in gut microbial community composition between social groups and between age and sex classes in one social group. Pyrosequencing analyses of weekly fecal samples from all 14 individuals are being used to validate correlations between metabolic efficiency and the relative abundance of specific taxa such as *Bacteroidetes* and *Firmicutes* that have been observed in other species. However, it appears that, in this wild primate population, differences in gut microbial community activity and composition play a greater role than behavior in compensating for individual differences in nutritional demands.

Abstract Category
25 The Gut Microbiome

PS.25.004 DIVERSITY OF INTESTINAL VIRAL COMMUNITY FROM HUMAN FECES

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The human gut is a nutrient-rich environment, where a huge amount of the microbes reside, including viruses. The symbiotic contribution of the microbes in human gut to normal physiology and predisposition to disease has been well-studied. Viruses are most abundant biological entities on the earth and can influence on microbial community, but little characterized so far. Viruses in human gut, recognized mostly as bacteria-infecting viruses (bacteriophage), have not been well-established, especially for single-stranded DNA viruses. In this study, we established uncultured DNA viral community archived from each 10 g of feces from five individuals. Virus-like particles were collected by CsCl gradient ultracentrifugation after serial-filtration. Viral DNAs were extracted and amplified with phi29 polymerase in multiple displacement amplification method, and then sequenced by high-throughput next generation sequencing, pyrosequencing. We believe that metagenomic approaches in large scale provide deep knowledge to understand uncharacterized viruses in human gut. The results from this study are described below.

Abstract Category
25 The Gut Microbiome

PS.25.005 MOLECULAR DIVERSITY OF BACTERIA AND ARCHAEA IN THE RUMEN OF GOATS FROM THE SEMI-ARID REGION OF BRAZIL

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The rumen is a complex ecosystem harboring a rich microbial community. Ruminants have a powerful system for fiber fermentation, mainly due to the presence of anaerobic organisms. The aim of this study was to assess the diversity of the microbial ecosystem associated with the rumen of goats from the semi arid region of Brazil using a molecular approach. Total DNA was extracted from the solid and liquid fractions of the rumen; PCR was used to amplify 16S rDNA using primers for the domains Bacteria and Archaea. Approximately 200 non-chimeric sequences with lengths greater than 400 bp were obtained for each Bacteria library and 80 sequences for each library of Archaea. The rarefaction curves revealed that at the distance level of 3%, the sampling effort was sufficient to cover the diversity existing in all libraries. Analysis of 16S rDNA sequences from the Bacteria domain showed the presence of 5 phyla: *Bacteroidetes*, *Firmicutes*, *TM7*, *Verrucomicrobia*, *Actinobacteria* as well as unclassified bacteria representing 27.5% in the solid fraction and 15% in the liquid fraction. The dominant classes: *Clostridia* and *Bacteroidetes*, with 34% and 23% in the solid fraction and 52% and 30% respectively in the liquid fraction, are known to play an important role in the degradation of

fibers in other ruminants. Within the domain Archaea, only the phylum *Euryarcheota* was obtained, especially sequences belonging to the methanogenic archaea *Methanobrevibacter* and *Methanosphaera*. In our study we have identified a group of Archaea sequences not previously reported to be associated with the rumen of animals. Despite the fact that the rumen has been studied for years with traditional techniques, the relatively high number of sequences belonging to Bacteria not yet classified and a new group of Archaea found in this study demonstrate that the microbiota of the rumen of goats is not well known.

Abstract Category
25 The Gut Microbiome

PS.25.006 GUT MICROBIAL COMMUNITY STRUCTURE IS ASSOCIATED WITH DIETARY INTAKE OF FIBER AND STARCH IN HEALTHY PREMENOPAUSAL WOMEN

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Dietary intake is known to influence digestive health and disease. While the role of the gut microbial community (GMC) in this relationship has been acknowledged, the extent to which diet influences whole GMC structure is not well characterized. In this study we aimed to evaluate relationships between intake of 15 nutrients and food groups, and GMC structure in healthy premenopausal women. 115 women provided fecal samples and completed 3-day food records. Bacterial community analysis was performed using terminal restriction fragment length polymorphism (TRFLP) analysis, and diet-GMC associations were evaluated using linear regression models and multi-response permutation procedure (MRPP). We also evaluated dietary trends in major groups of GMC organisms using quantitative PCR specific to these groups at the phylum and family levels. Two independent analyses (linear regression and MRPP) returned overlapping nutrients significantly associated with differences in GMC structure. Univariate linear regression showed fiber, starch, vegetable and refined grain intake were associated with GMC ($P < 0.05$); fiber ($P < 0.001$) and starch ($P < 0.003$) were most predictive in multiple linear regression models. MRPP indicated that GMC differed significantly between low- and high-level consumers of refined grains, starch and soluble fiber ($P < 0.05$). GMC structure was not associated with differences in protein, fat or iron intake, or body mass index in this population. In addition, bacterial taxonomy was inferred for each of seven TRFLP peaks found to be significant indicators of high fiber or low starch intake. Variability in fiber and starch intake among this group of healthy women was consistently reflected in GMC structure. While diet is one of many factors influencing the GMC, we report that its influence is detectable in the context of an observational study.

Abstract Category
25 The Gut Microbiome

PS.25.007 METAGENOMIC ASSESSMENT OF MICROBIAL COMMUNITY REESTABLISHMENT FOLLOWING GASTROINTESTINAL TRACT RESECTION

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The microbial community of the gastrointestinal tract plays a vital role in the homeostasis of the healthy gut, whereas disruptions in community structure and function may underlie disease states, such as ulcerative colitis. Our longitudinal study tracked the luminal and mucus-associated microbial communities in patients that had undergone total colectomy with the construction of an ileal pouch anal anastomosis for the treatment of ulcerative colitis. Over 20 Gb of shotgun metagenomic data generated using the 454 GS-FLX and Illumina sequencing platforms was analyzed with the MetaGenome Analysis Server (MG-RAST) to organize the taxonomic composition and functional gene content of the metagenomes. Taxonomic distributions inferred from protein encoding genes revealed a dominance of Bacteroidetes within luminal contents reflecting the abundance of di-, and oligo-saccharide utilization associated genes (subsystems) detected within these populations relative to mucus-associated communities. Furthermore, subsystems representing cellulosome formation (multi-enzymatic structures involved in lignocellulose degradation) were detected in higher abundance within the luminal aspirates versus mucus-derived samples. A comparison of functional gene content across a series of longitudinal mucus-associated samples within a single patient (one at an initial timepoint prior to ileostomy takedown and two timepoints following takedown) revealed an increased abundance and representation of carbohydrate utilization subsystems at time points following ileostomy takedown. The cellulosome subsystem increased ten-fold between the pre-takedown sample and the final post-takedown sample. These results indicate a trajectory of microbial community colonization and re-establishment in a newly formed pouch that has adopted more characteristics of a normal lower intestine (i.e. ability to degrade more complex carbohydrates) following the ileostomy takedown.

Abstract Category

25 The Gut Microbiome

PS.25.008 REGULATORY T CELLS AND GUT MICROBIOME IN COW'S MILK ALLERGIC INFANTS DURING ACQUISITION OF ORAL TOLERANCE

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Cow's milk allergy (CMA) is the most common food allergy in infants, affecting 2 % of children under 2 years of age and is considered as a breakdown of oral tolerance. The clinical spectrum of CMA ranges from IgE-mediated immediate-type hypersensitivity reactions (ITH), to CD4⁺ T cell-mediated delayed-type hypersensitivity (DTH) reactions. DTH reactivity to cow's milk can cause significant morbidity in rapidly growing infants and young children. Unlike other food allergies, CMA in infancy is associated with spontaneous remission before the age of 3 years. Several lines of evidence suggest that regulatory T cells (Treg) play a decisive role in the acquisition of oral tolerance to dietary and gut microbial antigens. Another important issue relates to the contribution of gut commensal flora in the protection against allergies. The gut microbiota, which develops shortly after birth, participates in the development of the immune system and acquisition of oral tolerance. Whether structural diversity of gut microbiota is involved in Treg development and function in the context of food allergy and acquisition of oral tolerance in infants remains to be understood. In this clinical study, the frequency and suppressive function of CD4⁺ Treg as well as ribosomal intergenic spacer analysis and phylogenetic microarray of the gut microbiota extracted from feces of ITH and DTH infants (age < 5 months) were compared to healthy controls over a 9 month period. We observed in both groups of patients a selective quantitative defect in a discrete subset of activated Foxp3⁺ICOS⁺ Treg, which normalized with time only in the DTH group together with acquisition of tolerance to cow's milk. In addition, the gut microbial community appeared more diverse in both ITH and DTH patients as compared to the controls. The evolution of the gut microbiota was clearly different in ITH and DTH groups, suggesting two different relationships between microbial diversity and the type of CMA.

Abstract Category

25 The Gut Microbiome

PS.25.009 DECOLORIZATION OF SUDAN DYES (1-AMINO-2-NAPHTHOL-BASED AZO DYES) BY HUMAN INTESTINAL BACTERIA

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Background and aims: Sudan dyes are red dyes. Although illegal to use, they have been found as contaminants in food products such as chili powder and shown to be potentially genotoxic and carcinogenic in experimental animals. Since ingestion of food products contaminated with Sudan I, II, III, IV, and Para Red could lead to exposure in the human gastrointestinal tract, we screened human intestinal microbiota to evaluate their capacity to degrade Sudan dyes and analyze their potential to form toxic metabolites. Methods: Fecal samples of healthy adults and 37 bacterial species representative of the human gastrointestinal microbiota were grown anaerobically at 37°C by using BHI or MRS broth supplemented with various dyes. Metabolism of Sudan dyes were determined by HPLC. Metabolites were isolated and identified by HPLC and LC/ESI-MS analyses and compared with authentic standards. Results: All Sudan dyes were reduced at different rates by the intestinal microbiota. Among the tested bacterial strains, 23, 13, 35, 32, and

29 out of 37 species were able to reduce Sudan I, II, III, IV, and Para Red, respectively. *Bifidobacterium infantis*, *Clostridium indolis*, *Enterococcus faecalis*, *Lactobacillus rhamnosus*, and *Ruminococcus obeum* were able to reduce completely all tested Sudan dyes. *Escherichia coli* and *Peptostreptococcus magnus* were the only two strains that were not able to reduce any of the tested Sudan dyes. Metabolites of the degradation of the dyes were isolated and identified as aniline, 2,4-dimethylaniline, o-toluidine (2-methylaniline), and 4-nitroaniline. Conclusions: Most of the intestinal bacterial species screened in this study were able to metabolize the tested Sudan dyes except Sudan II. The human intestinal microbiota were able to reduce Sudan dyes via azoreduction to form potentially carcinogenic aromatic amines. These results suggest that human exposure to ingested food residues contaminated by Sudan dyes could be a potential risk for human health.

Abstract Category
25 The Gut Microbiome

PS.25.010 PATTERNS OF IMMUNOGLOBULIN BINDING TO MICROBIAL POPULATIONS DURING COLONISATION OF THE GASTROINTESTINAL TRACT.

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Human gut microbial communities show stable inter-individual differences, contributing significantly to human diversity. This individuality is thought to arise from the stochastic nature of early microbial colonisation processes and postnatal development of the host ability to influence community structure by selectively stimulating or preventing colonisation. Previous studies have observed shifts in immunoglobulin A (IgA) levels corresponding to important colonisation phases and changes to gut microbial diversity and stability. Our study aims to identify the potential role of IgA as a host-mediated regulator influencing persistence/ transience of specific microbial populations within the gut community structure. Using pig models, we previously observed a sudden change in community dynamics at approximately 3 weeks of age, and a corresponding drop in levels of free IgA. Our current strategy employs flow cytometry (FCM) to quantify IgA-bound cells and non-bound cells followed by fluorescence-activated cell sorting (FACS) to separate these fractions for community profiling. We predict that binding of IgA to specific bacterial populations at 3 weeks of age in pigs relates to persistence within the community. Preliminary results indicate that this method permits quantification and observation of differential binding of IgA to microbial populations in the pig gut. FCM showed that the fraction of IgA-bound cells increases from weeks 2 to 4. Fractions from FACS were successfully amplified for community profiling by denaturing gel gradient electrophoresis. IgA-bound populations at week 3 were found to be present in the stable community profile observed after 4 weeks of age in piglets indicating a possible role of IgA binding in marking specific bacterial populations for anergy. We expect that further refinement of this method will aid understanding of the mechanism by which IgA excludes/selects for certain microbial populations within the gut for persistence/transience.

Abstract Category
25 The Gut Microbiome

PS.25.011 A COMPREHENSIVE SURVEY OF MICROBIOTA DIVERSITY IN THE GUT OF PIG SUPPLEMENTED WITH FERMENTED BROCCOLI IN THE DIET USING PHYLOGENETIC MICROARRAY

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Broccoli is known to have an antibacterial activity against *Helicobacter pylori*, anti-oxidative and anti-inflammatory activities. We analyzed the effect of the Broccoli on the intestinal microflora of pig supplemented with fermented organic broccoli enriched with lactic acid bacteria in the diet using phylogenetic microarray. We developed a microbiota microarray with COMBIMATRIX CustomArray 4x2K platform, it contains 747 probes as well as nine negative controls. Microarray experiments were performed four times (n=4) using DNA extracts of total microbiota isolated from the feces in different groups (control, 0.2% antibiotics, 0.2% broccoli powder, 0.2% fermented broccoli, and 0.2% mixed lactic acid bacteria). Microarray analysis identified 450 phylospecies in controls, 514 in antibiotics supplements, 517 in broccoli powder, 533 in fermented broccoli, and 561 in lactic acid-mixed bacteria. *Firmicutes* is most abundant (176-216 phylospecies), and *Proteobacteria* (phylospecies 122-161) were identified in the control and treatment groups at the phylum level. *Firmicutes* (41~50% of the total signal) and *Proteobacteria* (21~23% of the total signal) are predominant in the distal gut microbiota. *Firmicutes* was decreased from 50.07% to 41.44-45.26%, whereas Bacteroidetes was increased from 5.7% to 6.55-8.3%. The major classes in microbiota composition are *Bacilli* (91-108 species) and *Clostridia* (77-94 species). In all treatment groups, *Bacilli* is the most abundant species. Compared to the control (33.24%), *Clostridia* is decreased to 28.51-31.55% in all treatment groups. *Gammaproteobacteria*, contains lots of virulent microorganisms, was also reduced to 10.91-12.63% in all treatment groups compared to the control (14.08%). These results suggest that the treatments with antibiotics, broccoli, fermented broccoli, and the mixed lactic acid bacteria increase the microbiota diversity whereas exert negative effect on the colonization of *Clostridia* and *Gammaproteobacteria*.

Abstract Category

25 The Gut Microbiome

PS.25.012 ANALYSIS OF THE GUT MICROBIOMES CONTAINED IN THE APC MIN MOUSE, MODEL OF COLON CANCER, AS COMPARED TO THAT OF HUMANS

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Studies have shown that host-associated microbial communities play an important role in colonic health. To better understand gastrointestinal disease, researchers often use mouse models that

mimic the genetic background of human disease genotypes. However, not much is known about the resemblance between the mouse and the human GI microbiome. In order to assess the appropriateness of using a mouse as a model system to examine the potential link between colon associated microbes and the development of colon cancer, we compared the microbial flora from mouse colon to that of the human colon. Microbial 16S rRNA gene sequences from the human gut were obtained from a previous study (Dethlefsen et.al, 2008), while the microbial 16S rRNA gene sequences from APC min mouse which is used as a model for colon cancer due to the mutated adenomatous polyposis coli (Apc), a tumor suppressor gene, was isolated in our lab using 454-pyrosequencing. Data were cleaned using a bioinformatic pipeline, resulting in 10,082 mouse and 125,615 human high quality sequences. These sequences were subsequently classified using the Ribosomal Database Project and a distance matrix was generated in order to assess the extent of sequence coverage and diversity within each sample. The relative abundance of each classified sequence was then compared to identify differences and similarities between the 16S rRNA gene sequences isolated from the mouse and the human samples. It was found that there is less than 1% overlap between microbial community of the mice and the human samples. Although, further sequencing is required to get better coverage for the mice microbial community, it appears that there is not much overlap between the mouse and human gut microbiome.

Abstract Category

25 The Gut Microbiome

PS.25.013 DESCRIPTIVE GENE ANALYSIS OF INTESTINAL MICROBIOTA ON NEWBORNS WITH INTESTINAL SURGERY

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Initial colonization occurs during birth and shortly afterwards until a dense, complex microbiota develops with a gradient from stomach to colon. Establishment of normal microbiota is important for physiologic, nutritional, and immune development. Perturbations, especially during early development, may have consequences that extend well beyond the neonatal period. The aim of this study was to take advantage of newborn intestinal surgery to analyze the microbiota all along the intestinal track. Neonates (0-30 days old) were recruited to the neonatal intensive care unit at Nantes (FRANCE) following the local Ethics Committee. Sampling: During surgery, preoperative samplings were obtained. Analysis: After bacterial DNA isolation, V6 to V8 region of the 16S rDNA amplification, samples were analyzed using a fingerprinting method, the denaturing high-performance liquid chromatography (dHPLC). 53 samples from 42 babies have been analyzed. Bacteria genes per babies: 1 baby gave flat dHPLC curve, 30 babies were found with only one bacteria gene (Staphylococcus), 9 with 2 (Staphylococcus and Enterobacteriaceae), 2 with 4 (Staphylococcus, Enterobacteriaceae, Pseudomonaceae, Peptostreptococcaceae). Origin of the bacterial 16S rDNA: All together 139 peaks of dHPLC profiles have been eluted and sequenced: 27 sequences from 14 newborns, belonging to the

bacterial family Pseudomonaceae, shared in 2 groups (1 major from in doors environment, 1 close to *P aeruginosa*) ; 45 sequences from 16 newborns, belonging to the bacterial family Enterobacteriaceae: *Escherichia*, *Morganella* ; 39 sequences isolated from 16 newborns, belonging to the bacterial family *Staphylococcus*, shared in 2 groups (the human skin microbiome and soil and environment contaminant); 28 sequences, isolated from 6 newborns, belonging to the bacterial order Clostridiales shared in 5 groups. This snapshot description of intestinal microbiota implantation may constitute a marker of pathological status.

Abstract Category

25 The Gut Microbiome

PS.25.014 EFFECT OF EARTHWORM FEEDING GUILDS ON SELECTIVE ACTIVATION OF INGESTED DISSIMILATORY NITRATE REDUCERS AND DENITRIFIERS DURING GUT PASSAGE

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The earthworm gut is an anoxic microzone that emits denitrification derived nitrous oxide (N₂O). Conditions in the anoxic gut might selectively stimulate ingested soil bacteria. Thus, the main objective of this work was to identify denitrifiers and dissimilatory nitrate reducers in the earthworm gut and surrounding soil at both gene and gene expression levels of three earthworm feeding guilds (epigeic [*Lumbricus rubellus*], anecic [*Lumbricus terrestris*] and endogeic [*Aporrectodea caliginosa*]). The structural gene markers *narG* (encodes for a subunit of nitrate reductase) and *nosZ* (encodes for a subunit of N₂O reductase) were detected in the earthworm gut and surrounding soils at both gene and gene expression levels. Gut derived sequences had high similarity to those of cultured and uncultured soil bacteria and to soil derived sequences. Gut derived *narG* sequences and terminal restriction fragments (TRFs) were mainly affiliated with gram positive organisms at both gene and gene expression levels. Most expressed gene sequences were closely related to those of the genus *Mycobacterium*. Sequences similar to gram negatives were dominant in mineral soil. *nosZ* sequences and TRFs at both gene and gene expression levels belonged mostly to Alphaproteobacteria with high similarity to *Bradyrhizobium japonicum* and uncultured soil bacteria. Differences between gut and mineral soil were large at the gene expression level but minimal at the gene level. Feeding guilds affected the detected *nosZ* community at the gene level and to an even greater extent at the gene expression level. Feeding guilds also affected the detected *narG* sequences at the gene level but had minimal impact on *narG* expression. These collective observations indicate that (i) denitrifiers and dissimilatory nitrate reducers in the earthworm gut are soil derived, (ii) these functional groups are selectively activated during gut passage, and (iii) the feeding guilds affect this selective activation.

Abstract Category

25 The Gut Microbiome

PS.25.015 GUT MICROBIAL COMMUNITY DYNAMICS IN IMMUNOCOMPROMISED MICE DURING T CELL INDUCTION OF COLITIS

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Colitis is the third leading cause of colorectal cancer in North America in both men and women with respect to both incidence and estimated death rates. Although the aetiology of the condition is not well understood, the interplay between the host's immune system and the gut microbiota may play a key role in its development. In this context, we characterized compositional shifts over time of the gut bacterial community in immunocompromised mice after T cell-mediated colitis induction. Two knockout mice, one lacking a key component of the adaptive immune system (RAG-deficient) and the other with defective adaptive and innate immune systems (CD45-RAG-deficient), were injected with naive T cells to induce the onset of intestinal colitis. Stool samples were collected over a 34-day period post-colitis induction. Changes in stool bacterial community composition were studied with DNA fingerprinting (T-RFLP) of 16S RNA genes followed by multivariate statistical tests. While mouse genotype exerted the strongest influence on community composition, RAG-deficient mice with colitis had a significantly different gut microbiota compared to untreated controls; two restriction fragments - 180-bp and 251-bp-long - associated to the control group appeared to have driven these differences. While the temporal variability was high among CD45-RAG-deficient mice, a 251-bp fragment was significantly associated both with untreated RAG-deficient relative to treated animals and with CD45-RAG-deficient mice after 4 days of colitis induction. We identified 70 phylotypes across all animals and collection dates - but only six dominated the sampling space. Thus, our results suggest (i) a non-random distribution of dominant bacteria among immunosuppressed mice, and (ii) that the host's immunological response to colitis may be modulated by the presence of few abundant (specialist) bacteria.

Abstract Category
25 The Gut Microbiome

PS.25.016 CLOSTRIDIACEAE AND ENTEROBACTERIACEAE AS ACTIVE FERMENTERS IN GUT CONTENTS OF THE EARTHWORM *LUMBRICUS TERRESTRIS*

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The feeding habits of earthworms have profound impact on the structure and fertility of aerated soils. The earthworm gut is pH neutral, oxygen-limited, and rich in mucus- and plant-derived saccharides; thus, this microzone in aerated soils is favorable for ingested heterotrophic soil bacteria capable of anaerobiosis. Indeed, high concentrations of molecular hydrogen and organic acids in the gut are indicative of ongoing fermentations. The central objective of this study was to resolve potential links between fermentations and active fermenters in gut content of the anecic earthworm *Lumbricus terrestris* by 16S rRNA-based stable isotope probing (SIP) with [¹³C]glucose as a model substrate. Glucose consumption in anoxic gut content microcosms was rapid and yielded soluble organic compounds and gases, products indicative of diverse fermentations in the alimentary canal. *Clostridiaceae* and *Enterobacteriaceae* were dominant utilizers of glucose-derived carbon. Based on the detection of 16S rRNA, active phyla in gut contents included *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Cyanobacteria*,

Firmicutes, *Gemmatimonadetes*, *Nitrospirae*, *Planctomycetes*, *Proteobacteria*, *Tenericutes*, and *Verrucomicrobia*. These taxa are common to soils. Based on a 16S rRNA gene similarity cutoff of 87.5%, 82 families were detected, 17 of which were novel. These findings (a) illustrate the large diversity of soil taxa that might be active during gut passage, (b) demonstrate that *Clostridiaceae* and *Enterobacteriaceae* (fermentative subsets of this taxa) are selectively stimulated by glucose and might therefore be capable of consuming mucus- and plant-derived saccharides during gut passage, and (c) indicate that ingested obligate anaerobes and facultative aerobes from soil can concomitantly metabolize the same source of carbon.

Abstract Category
25 The Gut Microbiome

PS.25.017 BACTERIAL COMMUNITY ANALYSIS OF AVIAN AND MAMMALIAN SOURCES OF FECAL CONTAMINATION IN COASTAL CALIFORNIA

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Wild and domestic animals that inhabit coastal areas deposit fecal microorganisms that impact water quality. The extent to which various animal and human wastes impair coastal waters is hard to determine with single biomarkers and low-resolution profiling methods. High-throughput sequence analysis of gut microbial communities has potential to reliably identify fecal sources and resolve contentious water quality issues. We characterized bacterial communities from a variety animal feces and human wastes to identify taxa that distinguish common contamination sources. We then tested the utility of our findings during water pollution events. Fresh feces were collected from at least four different populations each of gulls, geese, pinnipeds (seals, sea lions), cows, horses and elk. Human wastes were gathered from sewage treatment plants, septic tanks and holding tanks. We analyzed bacterial 16S rRNA gene composition using the PhyloChip microarray, which is capable of quantifying differences in the relative abundance of both rare and abundant bacterial taxa from the entire targeted pool of 16S rRNA gene amplicons. Fecal bacterial communities strongly clustered by animal species/type. We identified thousands of taxa that distinguished human wastes from animal feces, and different animals from each other. Human wastes clustered together despite differences in the scale and type of processing. Bacterial communities from ruminants (cows, elk) clustered closely with little variation among populations, while horses were distinct within the grazers. Bacteria in bird populations were more variable than in mammals. Analysis of water pollution events demonstrated that groups of indicator taxa found in our source characterization could successfully identify or exclude causes of contamination. Different animals harbor distinct fecal microbial communities that can be exploited for source tracking in spite of intra-source variability due to diet, location or waste processing.

Abstract Category
25 The Gut Microbiome

PS.25.018 COMPARISON OF CATTLE FECAL COMMUNITIES FROM ANIMALS FED DIETS CONTAINING CORN AND DISTILLER'S GRAIN

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Distiller's grain is a byproduct of ethanol production, and is considered a good source of protein for cattle. However, there is concern that use of distiller's grain may create a gastrointestinal environment that allows the growth of fecal pathogens, and the specific changes that occur in beef cattle in response to distillers grains have not yet been well characterized. Our goal was to explore the effects that wet distiller's grain with solubles (WDGS) has on the bacterial communities of beef cattle feces. Feces was collected from twelve steers, randomly assigned to WDGS or control diets. All animals were culture negative for *E. coli* O157:H7. A total of 4,537 clones were examined. Operational taxonomic units were assigned using DOTUR, and taxonomies were assigned using the Classifier tool available through RDP. There were significant differences in the taxonomic distribution of genera in the animals fed WDGS, compared to those on the traditional corn diet. Richness and evenness indices were calculated. Based on taxonomy, the most striking difference was in the *Faecalibacterim* and *Prevotella*, which were underrepresented in feces from the animals fed WDGS. There were no differences observed in number of *Enterobacteriaceae* clones observed between the two diets. Feeding of WDGS results in significant changes in both the Gram-positive and Gram-negative bacterial flora. The changes in the communities are complex, and it is possible that microbial community composition could affect survival of fecal pathogens, although no clone-based differences were observed in these samples in the taxonomic groups to which *E. coli* O157:H7 belongs.

Abstract Category

25 The Gut Microbiome

PS.25.019 THE IMPACT OF PET OWNERSHIP ON BACTERIAL COMMUNITIES IN HOUSEHOLD DUST AND THEIR INFLUENCE ON SHAPING INFANT GUT MICROBIOTA

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Furred pet ownership has been shown to be protective against the development of allergic diseases in children. We hypothesized that pet ownership is associated with a distinct microbial profile in house dust, which influences neonatal gut microbiota composition and immune response development, resulting in infants "primed" against allergy and asthma. We examined the bacterial and fungal communities from 16 household dust samples from homes with dog(s), cat(s) or no pets using the 16s rRNA PhyloChip (high density bacterial microarray) and fungal automated rRNA intergenic spacer analysis coupled with sequencing. T-test and Pearson's

correlation were performed to determine differences in community structure and across treatment groups and relationships between bacterial diversity and fungal richness respectively. Based on PhyloChip data, dust bacterial communities from homes with dogs were significantly richer ($p=0.04$) and more diverse ($p=0.04$) than those without pets; these samples also contained significantly fewer fungal ribotypes ($p=0.05$). A significant inverse relationship existed between bacterial diversity and fungal richness ($r=-0.65$; $p=0.02$). Sequence identification of fungal species revealed many known allergenic species such as *Alternaria alternata*, *Mucor plumbeus*, *Cladosporium cladosporioides* and *Malassezia restricta*. Preliminary data indicate that factors such as pet behavior may be important in interpreting the impact of pet ownership on the house dust bacterial diversity. To determine if microbial communities detected in dust influenced infant gut microbiota, parallel dust and infant stool samples were collected from 8 households with either dogs or no pets. Analysis of this data is on-going. Pet-ownership and more specifically, pet behavior, appears to influence the bacterial and fungal communities present in house dust. Infants in homes with or without pets may be exposed to different microbiota during the critical period of immune development.

Abstract Category

25 The Gut Microbiome

PS.25.020 VARIATIONS IN *VIBRIO* SPP. & OTHER GUT MICROFLORA ASSOCIATED WITH *FUNDULUS HETEROCLITUS* AS A CONSEQUENCE OF CHANGES IN DIET & TEMPERATURE

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In fish, both the gills and intestinal tract contain a significant bacterial population. A fish's gut microflora is critical to digestive processes, and can potentially affect nutrition, growth, and vulnerability to disease. Increased occurrences of certain bacteria within the fish intestine may impact public health via food transmission and wound infection. We used 16S rRNA genes in applications including denaturing gradient gel electrophoresis, 16S rDNA clone libraries, and quantitative PCR to determine whether changing food type and environmental conditions result in modifications of fish gut microflora.

Preliminary studies indicated that *Fundulus heteroclitus* gut microflora is dominated by gamma-proteobacteria ribotypes (43.9%), mainly family Vibrionaceae (20.6%), including some closely related to potential pathogens. Two large-scale manipulation experiments were performed focusing on the effects of changes in diet and water temperature on the gut microflora community. A feeding study examined the effect of differing food quality on the microflora composition. An additional temperature study assessed how differing temperature regimes impacted relative abundance of *Vibrio* spp.

Results from these experiments will provide insight into the effect of food quality on fish gut microflora and help assess the role that direct waterborne transmission across trophic levels plays in the ecology of pathogenic *Vibrio* spp. The goal of the temperature study is to test if increased temperature raises the relative abundance of *Vibrio* spp. within the gut. This links changes in

water temperature with climate change and fish health. Experimental data will be used to delineate the matrix of environmental conditions most favorable for the presence of pathogenic *Vibrio* spp. in fish. Changes in gut microflora, especially increases in the presence of pathogens, can potentially affect the commercial fishing and aquaculture industries, as well as ocean ecosystem and human health.

Abstract Category
25 The Gut Microbiome

PS.25.021 COMPARATIVE ANALYSES OF FOREGUT AND HINDGUT MICROBIAL COMMUNITIES IN HOATZINS AND COWS

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Bacteria are present in the different anatomical regions along the vertebrate gastrointestinal tract. In ungulates, bacteria ferment the dietary fiber in the rumen, and in the colon other symbiotic bacteria produce vitamins for the host. The hoatzin (*Opisthocomus hoazin*) - a strict folivorous bird-, is a case of evolutionary convergence with ungulates due to its enlarged fermentative foregut and additional fermentation in the paired ceca. Our aim was to compare the community structure between each functional environment (foregut and hindgut) of hoatzins and cows using a high-density microarray (PhyloChip). We extracted genomic DNA from the foregut and hindgut of 4 adult hoatzins and 4 pasturing cows and the 16S rRNA genes were amplified by PCR. The pooled amplicons from each bird were then hybridized to the PhyloChip. Our results show that the microbial communities cluster strongly by functional environment (foregut cluster separately from hindgut) and also by host, with the bird samples being significantly more dispersed than those of the cow. The cow samples were richer and more diverse than those of the hoatzin in both gut sections and the hoatzin crop is particularly even. Overall, the most abundant taxa were Firmicutes, Bacteroidetes and Proteobacteria with lesser representation of 33 additional phyla. The foreguts were richer and more even than the hindguts, and the core foregut community had higher proportion of Bacteroidetes (Flavobacteria) as well as Firmicutes (Clostridiaceae), while the core hindgut had higher proportion of Proteobacteria (Gammaproteobacteria). We found that Bacteroidetes, Acidobacteria, and Spirochaetes are more prominent in the foregut specific communities and the cow rumen has a higher proportion of Proteobacteria and Spirochaetes compared to the Hoatzin crop. These results indicate that organ function leads to convergence of the microbial community structure in phylogenetically distant hosts.

Abstract Category
25 The Gut Microbiome

PS.25.022 EFFECTS OF DIETARY FIBERS ON MICROBIAL FERMENTATION AND COMMUNITIES FROM LARGE INTESTINAL DIGESTA OF PIGS

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Dietary fibre has been shown to benefit human health through diversified mechanisms including its physical effect on the digestion, fermentation as a major factor influencing large bowel function and bowel habit, and prebiotic effect to selectively enrich beneficial gut bacteria. The data on the ability of dietary fibre to act as prebiotics, however, is still limited, and much of early studies were performed on pure bacterial cultures, which does not reflect a true picture of population changes of microbiota. In the present study, the effects of dietary fibers on microbial fermentation and communities from large intestinal digesta of pigs were investigated. Oat β -glucan, flaxseed gum, and fenugreek gum were shown to be fermented to short-chain fatty acids (SCFAs) in batch cultures by the digesta. Lactate in the β -glucan-grown cultures had the highest level among SCFAs produced, whereas flaxseed or fenugreek gum-containing cultures generated a significant amount of acetate, propionate and butyrate over three subcultures tested ($p < 0.05$). Denaturing gradient gel electrophoresis (DGGE) analysis of 16S rRNA genes in the initial and subcultures revealed that types of fibers can have a significant impact on the bacterial community structure. Identified DGGE DNA bands were mainly affiliated with Bacteroidetes, and Firmicutes, in which a number of butyrate-producing species were widely distributed. The fermentability of dietary fibers to SCFAs and their selection towards the bacterial communities suggest their potential application in promoting animal/human health.

Abstract Category

25 The Gut Microbiome

PS.25.023 PHYLOGENETIC DIVERSITY OF PROCARYOTIC COMMUNITIES BY 16S rRNA AND METHYL COENZYME M REDUCTASE GENES ASSOCIATED WITH *CORNITERMES VILLOSUS* GUT

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Termites are an important component of tropical soil communities and have a significant effect on the structure and nutrient content of soil. The termite gut microbiota can include a variety of microorganisms from the three domains: Bacteria, Archaea and Eucarya. Digestion in termites is

related to gut physicochemical conditions, structure, and symbiotic microbiota. Termites are excellent research subjects for studying the interactions among microbial species and the numerous biochemical functions they perform to the benefit of their host. However, culture independent molecular studies have revealed that the majority of these microbial gut symbionts have not yet been identified. Accordingly, the aim of this study was to analyze the Bacteria and Archaea gut associated microbiota of the termite *Cornitermes villosus* collected at Juiz de Fora, Minas Gerais, Brazil to increase our knowledge on the diversity of these microorganisms in the termite gut. The total genomic gut DNA extracted was PCR-amplified using Bacteria, Archaea, and methyl coenzyme M-reductase selective primers, and the amplicons were cloned into *Escherichia coli* for sequencing. Analysis of the bacterial clone library showed a remarkable high diversity of lineages within the Bacteria. We identified predominantly members of the phyla spirochetes, as well as bacteroidetes, firmicutes, actinobacteria, fibrobacteres and planctomycetes. In the Archaea clone library we observed new phylotypes (clones sharing > 97% sequence identity were grouped in the same phylotype) from the analyzed clones. To detect and differentiate the methanogens localization, DNA samples extracted from three different gut segments were PCR amplified using mcrA specific primers. Phylogenetic analysis indicates that most of the clones belonged to unclassified euryarchaeota. The application of species richness estimators confirmed the highly diverse nature of bacteria gut samples. Supported by: PETROBRAS,FAPERJ,CNPq.

Abstract Category

25 The Gut Microbiome

PS.25.024 THE AXILLARY MICROBIOME: DEFINING THE MICROBIAL ECOLOGY OF THE HUMAN UNDER-ARM ACROSS TIME AND SPACE.

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In recent years, 16S rDNA-based approaches to characterise and identify bacteria have revolutionised the field of microbial ecology. Similarly, more recent developments in the application of Roche 454 pyrosequencing have made the analysis of complex microbial communities possible. Building the methodologies and capabilities to understand the community composition of microbes is a vital requirement for the future of microbial ecology. Research in 2009 (Costello et al. 2009), revealed that human skin microbial community composition was determined primarily by body habitat, with high interpersonal and minimal temporal variability exhibited. Only by understanding this situation in normal and perturbed states, can approaches to rationally control microbial communities be advanced. This paper presents results of a study carried out to spatially and temporally characterise the human axillary microbiome. Using sample specific, 8-base tagged universal primers, targeted at the 16S ribosomal gene, PCR amplicons were generated from samples obtained from 8 healthy volunteers (4 males, 4 females). Samples were taken on 2 occasions over a 1 week period, at 5 discrete sites in each axilla. One individual was sampled on 4 further occasions to investigate temporal changes in diversity. Following amplicon sequencing (Roche GS-FLX), resulting flowgrams were processed using the PyroNoise algorithm (Quince et al. 2009), to remove inherent noise in the data. Similarly, chimeras were removed and sequences then clustered into operational taxonomic units (OTUs) at

both the 3% and 5% level. Sequences were labelled taxonomically using the ribosomal database project (RDP) classifier. In addition, the data generated has been analysed using a number of other tools including Metastats (White et al. 2009) and Fast UniFrac (Hamady et al. 2009). The output from these analyses, and insights generated, will be presented.

Abstract Category

25 The Gut Microbiome

PS.25.025 COMPARATIVE ANALYSIS OF FECAL MICROBIOTA IN INFANTS WITH AND WITHOUT ECZEMA

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Eczema is a chronic form of childhood disorder that is gaining in prevalence in affluent societies. Previous studies hypothesized that the development of eczema is correlated with changes in microbial population profiles and composition during early life. However, the evidence is contradictory, possibly due to the lack of control and standardization of non-health related confounding factors such as age, antibiotic consumption, diet and mode of delivery. In this study, we recruited seven caesarean-delivered and entirely formula-fed infants with and without eczema, and examined the indigenous microbiota in these infants over the first year of life (i.e., at 1, 3, 12 and 24 months of age). Using 16S pyrosequencing, infants' fecal microbiota were observed to comprise Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes as the four main phyla, and that the presence and absence of specific populations within these four phyla are primarily related to ageing. Quantitative analysis of bacterial targets on a larger sample size (n=36 at 1, 3, and 12 months of age) revealed that the abundance of Bifidobacterium and Enterobacteriaceae differed between caesarean-delivered infants with and without eczema, and these bacterial targets may be potential biomarkers that correlate to the health status of these infants. Our overall findings suggest that the minimization of possible confounding factors is essential prior to comparative evaluation and correlation of fecal microbiota to health status, and that fecal samples collected from caesarean-delivered infants less than 1 year of age may represent a critical cohort to study for potential biomarkers that can distinguish infants with eczema from those without. These findings would greatly facilitate future efforts in a mechanistic understanding of the possible pathogenesis mediated by specific bacterial target groups, and possibly lead to a timely intervention that reduces the occurrence of allergic disorders in later life.

Abstract Category

25 The Gut Microbiome

PS.25.026 ANTIBIOTIC RESISTANCE GENE DYNAMICS IN THE PIG GUT-MICROBIOME AS A RESPONSE TO IN-FEED ANTIBIOTICS (ASP250)

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Increased prevalence of antibiotic resistant pathogenic bacteria has raised concerns about imprudent antibiotic (AB) use. At least 50 percent of ABs produced in the United States are used in agriculture. It is critical to understand how AB use influences antibiotic resistance gene (ARG) abundance and diversity due to the importance of ABs in human and animal health, the volume of ABs used in animal management, and the need to accurately define prudent AB use in animal systems. In this study, ARGs were surveyed in feces DNA, at four time-points spanning 21 days, from 3 treatment pigs and 3 control pigs (same mother, different living quarters), in response to ASP250 feed (g/ton): chlorotetracycline (100), penicillin (50), and sulfamethazine (100). ASP250 is used for growth enhancement and disease prevention. Approximately 200 ARGs were surveyed using quantitative PCR on the BioTrove OpenArray platform; 43 and 40 ARGs were found at least once in control and treatment pigs, respectively. ARG abundance was about 1% and 3% of the 16S rDNA copy number in control and treatment pigs, respectively. ASP250 treatment significantly ($p < 0.05$) increased (1) abundance of Class A beta lactamase, tetracycline efflux, and sulfonamide resistance genes, which confer resistance to the treatment drugs; (2) abundance of three other ARG types (classes of aminoglycoside and multi-drug resistance); and (3) alpha diversity of ARGs (Shannon index). The communities exhibit moderate clustering correlated with treatment using Bray-Curtis as a beta diversity measure. These results suggest that (1) the BioTrove ARG survey can detect effects from ASP250 feed, (2) there is a reservoir of certain ARGs in the pig-gut microbiome, which increases in abundance and diversity with ASP250 feed, and (3) in animal management, small AB doses, like ASP250, can cause ARG shifts in the microbiome. This study, together with similar studies, will shape the definition of prudent AB use in animal management.

Abstract Category

25 The Gut Microbiome

PS.25.027 SIMPLIFYING THE RUMEN MICROBIAL FOOD-CHAIN

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The vast numbers and varieties of microbes present in the rumen make parameterising and computing truly mechanistic models of the rumen near-impossible. Previous work on the rumen food-chain has either focused on two-species syntrophic relationships in laboratory conditions or on the interactions between bacteria and protozoa. In this work, we attempt to develop a model of the microbial food-chain in the rumen, taking into account some syntrophic relationships. We use ordinary differential equations of microbial kinetics to formulate the model. The equations are then simulated in the Python programming language using an LSODA-derived solver.

We show that a minimal system consisting of only two cellulose degraders - each producing a different set of products, one starch degrader and a methanogen, and two polysaccharides, starch and cellulose, representing those polysaccharides that can be digested by host-produced enzymes and those fermented by microbes, respectively, can predict the approximate concentrations of

volatile fatty acids and methane in the rumen.

Since these volatile fatty acids constitute a major part of the ruminant's energy supply, this model, coupled to a model of the digestion in the ruminant's small intestine, can provide a predictive model of how a change in diet will affect a ruminant's energy budget.

Abstract Category

25 The Gut Microbiome

PS.25.028 RIBOSOMAL RNA BASED ANALYSIS OF MICROBIAL COMMUNITIES IN THE RUMEN IDENTIFIES METABOLICALLY ACTIVE GROUPS UNDERREPRESENTED BY GENOMIC DNA DETECTION

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Diversity of microbial communities is commonly ascertained by targeting total community DNA (16S rRNA gene). By focusing on RNA (16S rRNA) we gain an additional insight into the contributions that some bacteria play in these communities. Here we compare total rumen microbial diversity fed different diets using DNA and RNA derived material. Bacterial nucleic acids from three cattle fed 40-70% *Leucaena* legume and Rhodes grass (LR) and two animals fed 100% *Leucaena* (L) were extracted. The extracted DNA, and cDNA synthesised by reverse transcription from the RNA were both amplified using bacterial primers with the modification of a T7 promoter sequence attached to the reverse primer (27F - 1492_T7R) followed by cRNA synthesis and labelling with Cy5. Microbial diversity and abundance were observed by hybridization to a 16S rRNA gene based custom microarray. A total of 117 probes which were different ($P < 0.05$, ANOVA test) in abundance between individual animals were identified. Each individual sample based on diet and nucleic acid (RNA vs. DNA) exhibited distinct microbial profiles that were separated on a principal component analysis (PCA). Evaluation of metabolically active bacteria based on comparative analysis of DNA and RNA showed a significantly higher abundance of some species from the genera *Prevotella* and *Proteobacteria* in RNA samples regardless of diet but some species of the *Firmicutes* phylum were relatively higher for DNA samples. When we compared microbial responses to diets based on RNA, it appeared that *Methylocella* sp. (methanotroph) and *Desulfovibrio* sp., (SRB) may have a higher metabolic activity on the *Leucaena* diet compared to LR diet. The RNA based results also suggest that some of the *Proteobacteria* group which appear to be in low abundance when assessed by contribution to genomic DNA in the rumen might make a greater contribution to rumen metabolism than previously recognized. The functions of this group in the rumen require further investigation.

Abstract Category

25 The Gut Microbiome

PS.25.029 PYROSEQUENCING OF 16S RIBOSOMAL RNA FROM THE GASTROINTESTINAL MICROBIOME OF WINTERING WILD AND FARMED AMERICAN ALLIGATORS (ALLIGATOR MISSISSIPPIENSIS)

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The vertebrate gastrointestinal (GI) microbiome is a largely unexplored ecosystem, particularly for reptiles. Our goal was to characterize GI composition for one of the best-studied extant analogues to extinct reptilian taxa. Over the course of 220 million years, reptile host and endogenous gut microbes have developed complex, yet poorly understood, symbioses to enable digestion and absorption of otherwise inaccessible nutrients from an almost exclusively carnivorous diet. Six GI tract tissue samples from farmed and wild, wintering (non-feeding) American alligators (*A. mississippiensis*) were collected during dissection. Bacterial 16S rRNA genes were generated from amplicon pyrosequencing. The inter-host mouth to colon microbiome differed for each alligator, and fecal bacterial composition did not reflect GI tract communities, missing phylum-level diversity and overrepresentation of other groups; these results call into question the utility of feces to identify internal GI flora for vertebrates. Between farm and wild hosts, 53.4% of the OTUs were found in both GI tracts, suggesting a core alligator microbiome. For instance, the oral cavities in farmed and wild animals were dominated by shared operational taxonomic units (OTUs) from the *Betaproteobacteria* and *Bacteroidetes* (97% sequence identity). However, the farmed alligator stomach and duodenum almost exclusively contained *Clostridia* (97% of all amplicons), compared to *Gammaproteobacteria* (~80% of all amplicons) in the wild. *Bacteroidetes* dominated the farmed host ileum and colon microbiomes, but *Fusobacteria* dominated the wild animal. The dominance by *Fusobacteria* in the wild alligator is unique among vertebrates. Because assessment of GI microbiome diversity allows for comparing the evolutionary development of tetrapod GI tracts from reptiles to birds and mammals, these results indicate that host GI tract microbiome membership could go undetected if only farmed or domestic hosts are evaluated.

Abstract Category

25 The Gut Microbiome

PS.25.030 COMPARIAON OF NUCLEIC ACID EXTRACTION METHODS FOR ANALYZING VAGINAL MICROBIOME

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In order to determine vaginal microbiome using high throughput pyrosequencing such as 454, nucleic acid extraction methods from vaginal swab samples should be properly evaluated and optimized. Efficient and unbiased extraction of bacterial DNA from vaginal swab samples is one of key issues for analyzing vaginal microbiome. Thus, we compared commonly applied commercial nucleic acid extraction kit (AccuPrep® Genomic DNA Extraction Kit, Bioneer) for vaginal swab sample and bead beating methods. Ten vaginal swab samples were obtained from Korean twin cohort and subject to two different nucleic acid extraction methods. These nucleic acid extracts were amplified by PCR using 27f/1492r primers targeting at 16S rRNA gene and subsequently analyzed by denaturing gradient gel electrophoresis (DGGE). Multiple-dimensional scaling (MDS) analysis was performed in order to compare two extraction methods. The results from MDS showed no distinct clustering of each method, which suggests that two extraction methods did not have significant difference. In order to examine bias to specific microorganisms,

unique band identified from each of extraction methods was nucleic acid sequenced and analyzed by BLAST. Microbial species belonging to *Lactobacillus* spp. and Enterobacteriaceae family were dominated in samples from bead beating method. On the other hand, *Lactobacillus* spp. and *Pseudomonas* spp. were dominated in samples from AccuPrep® kit method. More bands were observed in DGGE samples from AccuPrep® kit method. Overall, Gram-positive bacteria were more frequently observed in samples from bead-beating than AccuPrep® method; however, the difference was not significant. Our results suggest that two commonly applied bacterial DNA extraction methods would not significantly bias microbial community for analyzing vaginal swab samples.

Abstract Category

25 The Gut Microbiome

PS.25.031 GENOMIC CHARACTERIZATION OF ENVIRONMENTAL *ESCHERICHIA* LINEAGES AND THEIR COMPARISON AGAINST GASTROINTESTINAL *E. COLI*

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Five distinct phylogenetic lineages within the *Escherichia* genus were recently recovered from a large collection of environmental isolates using multi locus sequence typing (Walk et al., AEM, 2009). Whether or not there are genetic elements that differentiate these environmental strains from their gastrointestinal (GI) counterparts is important for developing more accurate diagnostic tools and for a better understanding of the evolution process of the *E. coli* species. To this end, we sequenced 7 representative isolates of the environmental clades using the latest pair-ended Illumina technology. The environmental genomes share 90 to 95% genome-aggregate Average Nucleotide Identity (ANI) with GI commensal or pathogenic *E. coli* genomes, while ANI values between the GI genomes range from 95 to 99.8%. Although the environmental genomes encode the complete core genome of the GI isolates, a few genes or operons appear to be significantly enriched in each genome set, reflecting ecologically important adaptations (speciation). For instance, many, but not all, GI strains share, to the exclusion of environmental strains, the utilization pathways for pentose and hexose sugars, which are abundant in the mammal GI tract. Despite the differentially shared genes and the genetic discontinuity observed among the groups based on ANI, several cases of primarily phage-mediated inter-group horizontal gene transfer were revealed. The latter findings are consistent with a non-hierarchical genetic network rather than a chronologically ordered hierarchical tree. Quantitative aspects of the genetic cross talking, which have strong implications for the speciation models in *E. coli*, will be also discussed.

Abstract Category

25 The Gut Microbiome

PS.25.032 CORRELATION OF DIFFERENT META-"OMICS" DATASETS FROM THE GUT MICROBIOMES OF TWINS WITH CROHN'S DISEASE

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The cause and etiology of Crohn's disease (CD) are currently poorly understood. To help us to study the possible microbial contribution to disease we focused on a twin cohort including 40 twin pairs (16 individuals with ulcerative colitis (UC), 29 with CD, and 35 healthy) that were profiled by 16S rRNA gene pyrotag sequencing. Additionally, metabolite profiling was performed on a subset of these individuals (15 twin pairs). These two datasets revealed potential populations and metabolites, respectively, that differentiated the disease phenotypes, in particular ileal CD (ICD) from healthy individuals. Therefore, we extended this study to also look at the metaproteome and matched metagenomes for four of the ICD twin pairs. Proteins were mapped to biochemical pathways using iPATH software as a way to visualize differential expression for healthy and ICD disease cohorts. The community structure profiles and metabolite data were correlated to the metaproteomic profiles using Pearson correlation of distance matrices and the significance of these correlations was tested using the Mantel test. We found that several proteins, metabolites and microbes found in healthy individuals were absent in diseased individuals. We also found a high degree of correlation between the different -omics data sets. Some specific metabolites and proteins were positively or negatively correlated to the disease status and to specific microbes previously characterized in the same fecal samples. Together, these results demonstrate that integration of different levels of information using multiple omics approaches allows for a comprehensive view of biological signatures associated with disease.

Abstract Category

25 The Gut Microbiome

PS.25.033 DYSBIOSIS REVISITED: HIGHLIGHTING NEW PHYLOGENETIC SPECIFICITIES OF ACTIVE CROHN'S DISEASE MICROBIOTA

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Crohn's disease (CD) is an inflammatory bowel disease with a still unknown aetiology. Together with host genetic background and environment, gastrointestinal microbes seem to be involved in the onset or maintenance of the pathology. However, no extensive analyses coupling in silico analysis and biological validations have been performed. The present study aimed to characterize

CD microbial population structure and to detect phylogenetic specificities of CD microbiota. The available 16S rDNA sequences were analyzed with existing software and high throughput in house bioinformatics pipeline. The sequences of candidate OTUs were used to design Q-PCR probes. Probes specificities were validated against close cultured bacterial species or uncultured 16S rDNA clones. To validate the in silico predictions, 32 DNA fecal samples from healthy individuals and ileal CD patients were analyzed by Q-PCR and hybridized on a 762 probes hierarchical microarray.

As previously reported, *F. prausnitzii* and *E. coli* were more represented in respectively healthy individuals and ileal CD patients. In addition, several OTUs were highlighted as significantly associated with healthy status and close to *P. distasonis*, *R. bromii*, *O. valericigenes*, *B. bifidum* and *E. rectale* species. Conversely, *E. faecium* and species from the Proteobacteria phylum were over represented in CD patients. Moreover, OTUs not closely related to any known species (*Subdoligranulum* and *Oscillospora* species), appeared associated with healthy status. Whatever the method used, the data highlighted a variability among patients higher than in healthy individuals.

Combining in silico and molecular approaches on a well defined cohort provided a statistically robust new picture of the phylogenetic dysbiosis in active CD microbiota. It for instance pointed out new species from the *C. leptum* group, *R. bromii* and *O. valericigenes*, which inflammatory properties should be investigated.

Abstract Category

25 The Gut Microbiome

PS.25.034 DIET MODULATES GUT MICROBIOTA DYNAMICS IN HEALTHY ADULTS: AN INTEGRATED APPROACH FROM A NUTRITIONAL INTERVENTION TO METATRANSCRIPTOMICS

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It is now recognized that human intestinal microbiota plays an important role in human health. Dietary fibers are metabolized by human intestinal microbiota through hydrolysis and fermentation. However, the impact of dietary fibers on human health and on colonic microbial ecosystem remains not precisely characterized. The goal of this work was to assess the impact of natural dietary fibers on gut microbiota using an integrated approach.

Through a 6 weeks nutritional intervention, 20 healthy adults alternated their own diet with controlled meals periods with 10 or 40g fibers/day. From fecal samples collected at 6 time points, we analyzed the structural and functional dynamics of microbiota by qPCR, and 454 pyrosequencing of 16S rRNA genes and of metatranscriptomes. In addition, SCFA, Comet assays were measured in fecal samples and fecal waters, and statistics performed using R software.

16S rRNA gene approach demonstrated that the main components of the microbiota, such as *Clostridium coccoides* group, were significantly structured by the diet. The abundance of

prevalent species, *Alistipes putridinis*, *Dorea longicatena* and *Eubacterium rectale* also correlated with the different diet periods. SCFA profiles were very specific of a given individual and significantly correlated with the microbiota composition. Our data indicated that a 15 days wash out is not sufficient for the microbiota structure to return to a steady state. Metatranscriptomics showed, as expected, that glycan degradation and methanogenesis genes were over expressed at 40g fiber/day. Strikingly, 18 contigs, among the 1872 unassigned and not close to metagenomic datasets (EU_MetaHIT), were overexpressed, indicating new pathways deserving further characterization.

This study demonstrates that increasing fibers in diet rapidly modulates intestinal microbiota dynamics and provides new insights into fiber catabolism in human digestive tract.

Abstract Category

25 The Gut Microbiome

PS.25.035 MONITORING THE RUMEN MICROBIAL POPULATIONS CHANGES ASSOCIATED WITH FEED-INDUCED LACTIC, BUTYRIC OR PROPIONIC ACIDOSIS IN SHEEP

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Ruminal acidosis is a recurrent problem in ruminant production systems. The prevailing fermentation acid is difficult to predict in practice and it is thought to be the reason for the variable effectiveness of some rumen modifiers. We developed a sheep model of experimentally-induced lactic, butyric or propionic acidosis to describe the associated changes in the rumen microbiota. Twelve rumen-cannulated sheep separated into 3 groups of 4 animals were fed a hay:concentrate diet (80:20) during 3 weeks followed by three consecutive days of acidosis challenge by direct intraruminal supply (1.2% BW) of ground wheat, corn or beet pulp. Rumen contents were analysed for fermentation parameters (rumen pH, volatile fatty acids, lactate) and polysaccharidase activities (amylase, xylanase, carboxymethyl-cellulase). Populations of *Fibrobacter succinogenes*, *Ruminococcus albus*, *ruminococcus flavefaciens*, *Streptococcus bovis*, *Lactobacilli*, *Prevotella* and total bacteria were quantified by qPCR and protozoa were counted under photonic microscope.

At the end of the challenge, rumen pH was lower for wheat (4.87) than for corn (5.17) and beet pulp (5.55). Lactate was predominant for wheat (33.8 mM vs. ~3 mM for other challenges), whereas beet pulp and corn favoured a high molar percentage of propionate (22.5%) and butyrate (16.9%), respectively. *R. albus* and *F. succinogenes* proportions were highest for wheat than corn and beet pulp while *R. flavefaciens* proportions and fibrolytic activities, total bacteria and protozoa were similar among treatments. Amylase activity was highest for wheat, intermediate for corn and lowest for beet pulp. *Lactobacilli* and *S. bovis* proportions tended to be highest for wheat (1.74 vs. ~0.12%; $P = 0.15$) and corn (0.49 vs. ~0.05%; $P = 0.17$) respectively, whereas *Prevotella* was highest for beet pulp (49 vs. ~29%). This study demonstrated that, feed-induced lactic, butyric or propionic acidosis were associated with specific changes in the bacterial population.

Abstract Category
25 The Gut Microbiome

PS.25.036 CAN METABOLIC NETWORK MODELING AND CO-OCCURRENCE ANALYSIS PREDICT SYNTROPHIC RELATIONSHIPS AND METABOLIC NICHE CONVERGENCE AMONG HUMAN GUT MICROBES?

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The human gut hosts a complex community of microorganisms that can harvest nutrients/energy from otherwise undigestible components of our diets (e.g. complex plant polysaccharides). The efficiency of microbial metabolism is influenced by syntrophy, where one microbe produces compounds that the other requires for growth or removes compounds that inhibit the progress of metabolic reactions. Here I explore the extent to which co-occurrence analysis can identify important microbial interactions in the human gut, using the methanogenic archaeon *Methanobrevibacter smithii* as a model. Methanogenic archaea drive bacterial fermentation by preventing the accumulation of metabolic products such as hydrogen and closely associate with specific syntrophic bacteria in other anoxic environments. A preliminary analysis using quantitative PCR for *M. smithii* and > 1 million bacterial 16S rRNA sequences from the stool of 183 individuals, has identified 24 bacterial phylotypes that are positively associated with *M. smithii*, but whether this co-occurrence was driven by syntrophy or by similar environmental preferences is hard to predict. To illustrate whether the underlying cause of co-occurrence patterns can be determined using genome sequences, I have developed metabolic reconstruction techniques that measure similarity in metabolic niche as the % overlap in seed compounds in the network (compounds with no incoming edge that must be acquired exogenously from the environment), and syntrophy as the degree to which seed compounds in one genome can be generated from precursors by the other genome, indicating metabolic complementarity. Application of these techniques to known examples of syntrophy and metabolic niche convergence show its promise for predicting important microbial interactions based on genomic sequences alone.

Abstract Category
25 The Gut Microbiome

PS.25.037 DEVELOPMENT OF A CHEMOSTAT MODEL TO STUDY POPULATION SHIFTS IN THE HUMAN GUT MICROFLORA IN RESPONSE TO NOREPINEPHRINE

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Humans are host to a wide variety of microbes which colonize several body sites, including the GI tract. Experimental and clinical evidence propose the commensal microbial community (microbiota) of the gut as a factor in Inflammatory Bowel Disease (IBD). IBD is a chronic, relapsing and remitting inflammatory disease of the intestine, which includes ulcerative colitis (UC) and Crohn's disease. Interestingly, stress has been proposed to provoke or exacerbate IBD.

The stress hormone norepinephrine (NE) has been shown to elicit wide ranging effects including growth stimulation and upregulation of gene expression patterns of many bacterial species in pure culture. We hypothesize that NE overspill to the GI tract has a direct effect on the balance of the gut microbiota and that the microbial communities from IBD patients are more susceptible to NE-induced dysbiosis than the microbiota from healthy people.

The complex bacterial population from the distal gut contains many bacterial species that are refractive to axenic culture, but as a group these can be modeled in vitro using continuous culture "chemostat" vessels. Being a host-free system, chemostats make ideal vessels in which to study microbial perturbations that directly result from the addition of exogenous stimuli. We set up two identical chemostat vessels using conditions set to mimic the distal gut, and seeded the communities using fresh fecal material from a healthy volunteer. The communities within each vessel were monitored by profiling the 16S rRNA gene content using Denaturing Gradient Gel Electrophoresis. We found that the chemostat communities within each vessel reached steady-state within 7 days of inoculation. Preliminary data suggests the addition of NE can directly affect the community profile by shifting the abundance of distinct sub-populations within the consortium. The use of chemostat models represents a valid approach to study the response of the human gut microbiota to NE stimulation.

Abstract Category

25 The Gut Microbiome

PS.25.038 STRUCTURAL AND FUNCTIONAL ALTERATIONS IN MURINE COLONIC MICROBIOTA ASSOCIATED WITH DEXTRAN SODIUM SULPHATE-INDUCED COLITIS

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Inflammatory bowel disease (IBD) consists, primarily, of two idiopathic intestinal disorders: Crohn's disease and ulcerative colitis. The pathophysiological mechanisms responsible for the development of IBD remain to be elucidated. There is, however, compelling evidence that intestinal microbiota play a role in the development of this disease. Using a mouse model simulating IBD, alterations in the community structure and transcriptional profiles of the hindgut microbiome during the development of colitis were investigated using an integrated 16S rRNA (gene) profiling and metatranscriptomic approach. C57BL/6 wild-type mice and Stat1^{-/-} mice (mice lacking an interferon response) were administered dextran sodium sulphate (DSS) through drinking water, resulting in colitis. Lumen contents were harvested post-mortem from the large intestine and from colon biopsies collected from along the length of the large intestine (allowing analysis of mucosa associated microorganisms and associated gene products). DNA and RNA were simultaneously extracted and pyrosequencing performed on barcoded amplicons of bacterial 16S rRNA genes and reversely transcribed 16S rRNA. Additionally, deep sequencing was performed on cDNA synthesised from total RNA and from mRNA enriched RNA, allowing identification of both microbial populations and associated transcripts. The integration of targeted phylogenetic analyses of 16S rRNA (gene) sequences and metatranscriptomic studies

provides insight into the structure and function of the murine intestinal microbiota during the development of IBD. Marked alteration in the hindgut microbiota was observed in mice with DSS-induced colitis. The phylogenetic structure of microbial communities in wild-type and Stat1^{-/-} mice with and without colitis will be compared, as well as differences in functional transcript profiles associated with the development of colitis will be discussed.

Abstract Category

25 The Gut Microbiome

PS.25.039 DIFFERENCES IN GUT MICROFLORA BETWEEN CAPTIVE AND WILD BIRDS: ARE WE GETTING THE CAPTIVE BALANCE RIGHT?

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The composition of microflora within the gastrointestinal tract exerts an important influence over the overall health of an animal. The microbial community is altered by the presence of foreign bacteria and the consequences of stress. There is no published literature on the difference of enteric microbial communities of captive and wild birds, or on how a deviation from the 'normal' composition affects survival. This study looked at the difference in the structure of enteric bacterial communities of three phylogenetically and ecologically distinct native New Zealand avian species, North Island brown kiwi (*Apteryx mantelli*), brown teal (*Anas chlorotis*) and takahe (*Porphyrio mantelli*). Denaturing gradient gel electrophoresis (DGGE) was used to compare microbial communities. DGGE analyses were conducted on bacterial faecal DNA. A range of statistical diversity indices were used to compare bacterial communities. Captive birds had a greater number and higher diversity of enteric bacterial genera than wild birds. Enteric bacterial diversity was not affected by avian species, captive facility or geographic location. *Captivity status* was the main influence on enteric microflora. What occurs in captivity that alters the diversity of microflora? Diet is not a factor as samples from birds with different captive and wild diets did not affect bacterial diversity. Other possible influences are ingestion of faecal material from other birds or species, stress caused by overcrowding and/or lack of exercise, stimulation and social interactions. These findings have an important bearing on the protocol for release of captive-held species into the wild. Greater emphasis needs to be given to provide captive environments that more closely resemble those of the wild - thus making the adjustment from captivity to the wild less stressful and more successful in terms of survival.

Abstract Category

25 The Gut Microbiome

PS.25.040 CHARACTERIZATION OF FECAL BACTERIAL COMMUNITIES FROM HEALTHY POSTMENOPAUSAL WOMEN

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The gut microbial community composition changes with age and these changes potentially reflect the changing functional needs and host health. Cultivation based reports indicate bacterial

diversity increases after birth, remains relatively stable during adulthood then decreases again with old age. A variety of molecular methods are now being used to confirm and understand these findings. The objective was to determine bacterial gut composition in older women and their possible contribution to health. Pyrosequencing using bar coded primers for the V4 region of the 16S rRNA gene was used to amplify DNA extracted from fecal samples of 19 healthy postmenopausal women. Sequence analysis was carried out using the ribosomal database project pipeline. After processing, an average of 9200 sequences were obtained from each sample for a total of about 176,121 sequences. Eight bacterial phyla found were Firmicutes, Actinobacteria, Verrumicrobia, Proteobacteria, Bacteroidetes, Cyanobacteria, TM7, and Deferribacteres. Relative sequence proportions varied between individuals however, the most dominant phylum in all was Firmicutes (71% to 94%) followed by Actinobacteria (3% to 19%), mainly the genus *Bifidobacterium*. The proportion of Bacteroidetes sequences was on the low side, comprising less than 0.3% of all sequences. To discount the possibilities that these findings resulted from primer bias they were corroborated by qPCR using group specific primers for Bacteroidetes and *Bifidobacterium*. Although the five bacterial phyla commonly reported from the human gut in all ages were present the relative proportions of sequences were skewed to high Firmicutes and low Bacteroidetes. The low proportion of Bacteroidetes follows the trend seen in cultivation-based studies where a decrease in their abundance is seen in the elderly. Since all the individuals in our study are healthy there was no obvious detrimental impact from low Bacteroidetes but this finding requires further research.

Abstract Category

25 The Gut Microbiome

PS.25.041 COMPOSITION OF MICROBIAL GUT COMMUNITIES IN ANTARCTIC MARINE SEALS

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Microbial communities within the mammalian gut provide a variety of services to the host. Mammals are born sterile so gut bacteria are acquired after birth. Community composition is shaped by internal and external factors, and acquiring the "right" community may be an integral part of reproductive success. Due to the inherent difficulty in sampling marine mammals, and wild mammals in general, previous studies have been limited in extent. Here, we aim to tease apart patterns contributing to the composition of the gut microbial community of wild mammals, using two closely related seal species, the leopard (*Hydrurga leptonyx*) and the southern elephant (*Mirounga leonina*) from the family phocidae, which live and forage in the same region. Anal swab samples were collected from sedated southern elephant (n= 14 adult females; 18 adult males; 7 male pups; 3 female pups) and leopard (n= 6 adult females; 8 adult males) seals from the Western Antarctic Peninsula (63° South, 60° West). Further samples were collected weekly from one male and one female juvenile leopard seal at Taronga Zoo, Sydney. Initial patterns in microbial community composition were established using Automated Ribosomal Intergenic Spacer Analysis and taxonomic community composition was then investigated using 454 ribosomal tag pyrosequencing. As seen within a variety of other species, large inter-individual

variation exists. Cross-species comparisons identified significant species-specific relationships. Despite the large phenotypic sexual dimorphism displayed by elephant seals, inter-sexual differences were not apparent for either species. Age-class differences, however, were shown for the southern elephant seal, where young pups, up to three years, were significantly more similar to each other than to adult seals. The significant difference in community composition between the two species may be driven more by behavioral influences. These are preliminary findings from an on-going study.

Abstract Category

25 The Gut Microbiome

PS.25.042 TEMPORAL CHANGES OF BACTERIAL BUTYRATE FUNCTIONAL GENE COMMUNITIES IN ULCERATIVE COLITIS PATIENTS: A ROLE FOR THE GUT MICROBIOME IN HEALTH AND DISEASE

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Ulcerative colitis (UC) is a debilitating form of inflammatory bowel disease that involves ulceration of the colon which may require surgical intervention. Changes in the normal gut microflora have been implicated as a possible causative factor. Specifically, current evidence suggests that butyrate production is markedly reduced in ulcerative colitis. In a normal gut butyrate serves as the primary energy source to and can influence gene expression in colonocytes. However, the lack of a "time zero" makes it difficult to find causative associations between the microbial gut flora and disease progression. When surgery is indicated, the treatment of choice is ileal pouch anal anastomosis (IPAA). The formed artificial pouch is an ideal model for UC, as approximately 50% of patients develop pouchitis, an inflammation of the pouch mucosa. In this study we performed longitudinal studies on several patients that underwent IPAA. We developed barcoded 454 titanium primers for the butyrate production genes butyrate kinase (buk) and butyryl-CoA transferase (but). DNA was obtained from biopsies of the pouch mucosa, pouch lumen, and pre-pouch ileum over an 8 week period. Amplicons generated by PCR amplification were sequenced using the 454 titanium platform. The relative abundances of several butyrate producing groups were found to differ markedly during the eight week period. These changes were most pronounced in the pouch mucosa samples. Sequence clusters most related to Bacteroides and Coprococcus exhibited the highest variation with time.

Abstract Category

25 The Gut Microbiome

PS.25.043 TARGETED METAGENOMICS AND SINGLE CELL GENOMICS OF THE UNCULTURED HUMAN MICROBIOTA

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Only a fraction of microbes that constitute the human microbiome have been cultured so far. Even for cultured organisms, clonal isolates represent only a snapshot of the genomic diversity and physiological potential of the lineages that encompass that species in the human population. Comprehensive genomic characterization of the human microbiome by "shotgun" metagenomic sequencing is limited by the high diversity and uneven frequency of organisms. In parallel with the use of single-cell genomics for uncultured lineages, we are applying a targeted metagenomic approach to specifically separate and sequence taxonomically selected populations of bacteria from the human microbiome, regardless of their abundance. Principles of our approach include specific labeling of target organisms by fluorescent in situ hybridization and isolation of labeled single cells and populations of cells by flow cytometry followed by multiple displacement amplification (MDA) and genomic sequencing. Our targets include oral spirochetes (bacteria associated with disease but with many uncultured species), bacterial taxa from the human gut that have not been studied extensively (*Verrucomicrobia*, *Deltaproteobacteria*) or have no cultured representatives (TM7 and a cyanobacteria-like group). The relative abundance of these organisms varies significantly between human individuals, therefore we used rDNA pyrosequencing to characterize and select samples for isolation. Single cells and cell populations from several of the target lineages were isolated and used for amplification by MDA. Selected amplified libraries will be used to sequence the uncultured single cell bacterial genomes and to characterize the variation present in populations of species for which completed genomes of cultured isolates (*Treponema*, *Desulfovibrio*, *Akkermansia*) are available. This approach fills a niche between sequencing individual cultured organisms and shotgun metagenomic sequencing of an entire microbial community

Abstract Category

25 The Gut Microbiome

PS.25.044 THE EFFECTS OF HOST GENETIC DIVERSITY ON THE COMPOSITION OF MOUSE GUT MICROBIOTA

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Mammalian gut microbial communities are complex and influenced by deterministic and stochastic factors. Distinguishing the nature of those factors and how they impact the host microbiota at individual and population levels is one of the first major steps in understanding the role of specific microbial taxa in human development, health and disease. Cecal contents were obtained from 95 mice representing 10 distinct inbred strains. By using inbred mouse strains, we are aiming at identifying links between host genetic variation and the composition of the gut microbial community. The experimental design allowed not only the comparison between the strains but also allowed studying the effects of gender and cage sharing between sibling and non-sibling mice of the same strain. The microbial diversity in individual mice was analyzed by amplification of two regions of the 16S rDNA, V4 and V1-3, followed by 454 pyrosequencing. Approximately one million sequences were obtained and analyzed using the RDP pyrosequencing pipeline, Mothur, Fast-Unifrac and PrimerE. Non-metric multidimensional

scaling and principal coordinate analysis of Unifrac distances indicate that the microbial communities have mouse strain-specific structure, suggesting that host genetics plays an important role in assembling the adult microbiota. Some mouse strains have closely related community structure, possibly reflecting their genetic history and level of relatedness. Ongoing experiments using mouse strain crosses, genetic mapping and intestinal gene co-expression will provide further insights into heritability and pleiotropic effects of genetic variation on the interaction between the host and its microbiota.

Abstract Category

25 The Gut Microbiome

PS.25.045 THE DIVERSITY OF HUMAN SUBGINGIVAL MICROBIAL COMMUNITY IN HEALTH AND PERIODONTAL DISEASE

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As an ecosystem, the oral cavity encompasses a collection of distinct surface habitats, each colonized by specific, complex microbial consortia assembled in biofilms. These consortia are in contact with each other primarily through the saliva, which contains its own unique community. According to the widely accepted community-based pathogenic theory, the multispecies nature of the oral community results in microbial physiological characteristics and beneficial or pathogenic outcomes for the human host that cannot be simply reconstituted based on the properties of the individual species. Among the oral diseases, periodontitis has a polymicrobial etiology still poorly understood. To identify links between the microbial community structure and periodontal disease, we obtained DNA from subgingival samples from 30 healthy and 30 periodontitis subjects. From the disease group, samples were collected and processed separately from both diseased and healthy sites. We amplified and sequenced two regions of the 16S rDNA, V1-3 and V4, using a 454 FLX pyrosequencing platform. Between 2,500 and 12,000 sequences were obtained for each sample and region, the deepest sequencing level reported to date for samples associated with periodontal disease. The sequence data was analyzed using multiple taxonomic and statistical tools, including the RDP database, Mothur, Fast-Unifrac, Primer E and SAS. For disease sites samples, significant changes in the abundance and/or diversity were found for taxa including *Treponema*, *Porphyromonas*, *Synergistes*, *Megasphaera*, TM7 and others. Healthy sites from individuals with periodontitis displayed a community composition intermediate between that of diseased sites and that observed in healthy individuals. The large population studied and the increase in sequence depth enabled a statistically robust framework for identifying periodontitis-associated taxa and determining heterogeneity among individuals in both health and disease.

Abstract Category

25 The Gut Microbiome

PS.25.046 IN VITRO BIOCONVERSION OF POLYPHENOLS IN TEA BY THE HUMAN GUT MICROBIOTA

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Dietary polyphenols are components of many foods such as fruit, tea and vegetables. In particular, there is epidemiological evidence and experiments supporting a role for flavonoids such as catechin, epicatechin and their gallates found in non-fermented tea with anti-oxidant, anti-inflammatory as well as anti-bacterial properties. The exact mechanisms by which these flavonoids exert their beneficial effects are still unclear and emerging knowledge from bioavailability studies in humans throws doubt on the existing mechanisms. The role of gut microbiota in polyphenol bioavailability is acknowledged but there is limited information on bacterial species capable of such bioconversion as well as gene pathways involved. This is being addressed in the European Union-Transfer of Knowledge project GUTSYSTEM that involves investigation of the complex interaction between the polyphenols, gut microbiota and host. The present study focused on human intestinal microbial bioconversion of tea polyphenols. The Simulator of the Human Intestinal Microbial Ecosystem (SHIME) that mimics the human colon was used and *in vitro* faecal fermentation experiments were performed for tea polyphenols. Bacterial polyphenol metabolites were determined by NMR- and GC-MS-based profiling of phenolics. Molecular tools including qPCR and next generation sequencing are being applied to characterize shifts in the microbial community composition. Simultaneously, an approach for screening a faecal metagenomic library for polyphenol bioconversion genes was investigated. This involved bacterial incubation in the presence of polyphenols under specific conditions followed by HPLC analysis as well as development of negative and positive controls. This assay may now be used for the detection of clones, and eventually gene pathways, capable of tea polyphenol bioconversion. Ultimately, this research will contribute to unravelling the role of bacterial polyphenol bioconversion for particular health effects.

Abstract Category

25 The Gut Microbiome

PS.25.047 DETERMINATION OF MICROBIAL COMMUNITY STRUCTURE IN UNTREATED WASTEWATER FROM DIFFERENT GEOGRAPHIC LOCALES

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Microbial sewage communities consist of a combination of human faecal microorganisms and urban infrastructure-derived microbes originating from infiltration of rainwater and stormwater inputs. Together these different sources of microbial diversity form a unique population structure that may serve as a signature for sewage discharges and a source of candidate alternative indicators specific for human faecal pollution. However, the variability of this unique population structure across geographic space remains uncharacterized. We used massively parallel 454 pyrosequencing of hypervariable regions in rRNA genes to profile microbial communities from

13 untreated sewage influent samples. Sewage samples were collected from different geographic locations in the United States ranging from Hawaii to Florida. We obtained a total of 380,175 high quality sequences for sequence-based clustering, taxonomic analyses, and profile comparisons. All sewage profiles included a discernible human faecal pollution signature made up of several taxonomic groups including Firmicutes (21.7%), Bacteroidetes (11.4%), Actinobacteria (1.47%), and Fusobacteria (1.31%) genera. The most abundant taxonomic groups were from the Proteobacteria phylum (61.9%) and were dominated by Gammaproteobacteria (38.2%) and Betaproteobacteria (18.3%). The taxonomic composition of human faecal- and urban infrastructure-derived microbes remained surprisingly congruent across all untreated sewage samples. Together, these results suggest that untreated sewage bacterial communities maintain a core group of faecal and urban environment microbes across geographically dispersed wastewater treatment facilities, and that alternative faecal indicators targeting multiple hypervariable rRNA gene targets may lead to improved methods for the detection of sewage-derived faecal pollution in ambient waters. Notice - Although this work was approved by EPA for presentation, it may not reflect official Agency policy.

Abstract Category

25 The Gut Microbiome

PS.25.048 THE GUT MICROBIOME OF MICE SELECTIVELY BRED FOR EXCESSIVE EXERCISE

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Bacterial and archaeal cells in the human body outnumber eukaryotic human cells by a factor of 10 to 1, and are estimated to encode 100 times more unique genes than humans. Microbes that reside in mammalian intestines provide crucial benefits to their hosts, including producing vitamins and aiding in digestion. Commensal gut microbes have also been implicated in such diseases as obesity, diabetes, and inflammatory bowel disease. In recent years, studies have shown that obesity alters the composition of gut floral communities. The amount of regular aerobic exercise is also an important factor in determining whether a mammal is obese, and it is unknown how regular exercise affects the ecology of the mammalian gut. Mice that have been experimentally evolved for high rates of wheel running provide an ideal system to study the effects of long-term exercise on gut microbial ecology. Bacterial genomic DNA was isolated from the contents of the ceca of eight mice (four selected, four control) analyzed using a PhyloChip DNA microarray. The microbial community for each sample was profiled. Using a two-sampled t-test and correcting for false discovery rate, 137 operational taxonomic units (OTUs) differed in abundance significantly between the groups, while the majority (~20000) did not. Most of the differences in richness and abundance occurred within the *Firmicutes*, the dominant phylum in mammalian gut flora. 288 *Firmicute* OTUs were unique to the control line, while 185 were unique to the selected line. Additionally, 310 *Verrucomicrobia* OTUs were unique to the control line, while only 3 were unique to the selected line.

Abstract Category

25 The Gut Microbiome

PS.25.049 EFFECT OF SODIUM BUTYRATE ON GASTROINTESTINAL MICROBIAL COMPOSITION OF WEANING PIGLETS

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Sodium butyrate is used as an acidifier in animal nutrition and has been shown to exert a positive effect on body growth and maturation of the gastrointestinal tract in milk-fed pigs, helping them to overcome the post-weaning syndrome. An experiment was designed to evaluate the effect of pre-weaning administration of sodium butyrate on the gastrointestinal microbiota of piglets before and after weaning. Forty eight piglets were assigned to either a control diet or one containing 3 g/kg sodium butyrate, from day 5 of life onward. Animals were sacrificed at the end of the suckling period (day 29) and 11 days after weaning (day 40), and samples from the stomach, jejunum, ileum, caecum and colon were taken. Changes in the gastrointestinal tract microbiota in response to sodium butyrate were characterized using 16S rRNA gene-based analyses including PCR-denaturing gradient gel electrophoresis, global profiling using the Pig Intestinal Tract Chip (PITChip), a phylogenetic microarray, and quantitative PCR using group-specific primers. Sodium butyrate supplementation had an effect on total microbial composition mainly before weaning both in the small and large intestine and only in the ileum after weaning. The PITChip analysis revealed that the administration of the acidifier most strongly affected populations within the *Clostridium* cluster XIVa group and the *Bacteroidetes*. This study reinforces previous reports on the antimicrobial effect of sodium butyrate on piglet gastrointestinal tract microbiota.

Abstract Category

25 The Gut Microbiome

PS.25.050 QIIME ALLOWS INTEGRATION AND ANALYSIS OF HIGH-THROUGHPUT COMMUNITY SEQUENCING DATA

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High-throughput sequencing methods are revolutionizing the field of microbial ecology and leading us to understand the role of microbial community diversity in shaping habitats in our own bodies as well as habitats distributed throughout our planet. However, a major barrier to achieving such insights has been the lack of software that can handle these increasingly massive datasets. Here we report the development of the open-source QIIME (Quantitative Insights Into Microbial Ecology) software pipeline that addresses the problem of taking sequencing data from raw sequences to interpretation and database deposition. QIIME supports error-correcting and denoising of multiplexed 16S rRNA community samples and integration of sequences from different technologies, along with a wide range of microbial community analyses that have been central to several recent high-profile studies, including network analysis, histograms of within- vs. between-sample differences, and analysis of whether 'core' sets of organisms are consistently represented in certain habitats. QIIME also goes beyond these outputs by providing graphical displays that allow users to interact with the data. Our implementation, built in Python with the open-source PyCogent toolkit, makes extensive use of unit testing (the software equivalent of positive and negative controls) to ensure the accuracy of the results, and is highly modular. As part of this modularity, components for specific pipeline functionalities such as choosing Operational Taxonomic Units (OTUs), sequence alignment, inferring phylogenetic trees, and phylogenetic and taxon-based analysis of diversity within and between samples (including integration of third-party applications for many of these steps), can be easily integrated and benchmarked against one another.

Abstract Category

25 The Gut Microbiome

PS.25.051 MICROBIOLOGY OF THE CRITICALLY ENDANGERED NEW ZEALAND PARROT, THE KAKAPO (*STRIGOPS HABROPTILUS*)

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The kakapo is one of the world's rarest species, with only 123 individuals limited to two predator-free islands off New Zealand. It is a remarkable bird, being the world's heaviest parrot, the only flightless parrot and the only parrot to carry out lek breeding. Recovery of the kakapo population is a major conservation priority, with intense management guided by research into kakapo ecology, nutrition and genetics. An additional aspect, also of likely importance but hitherto unstudied, is the role of microorganisms in kakapo biology. In collaboration with the Kakapo Recovery Programme, we have recently initiated a detailed study in order to identify which microbes are associated with kakapo and what impact these may have on kakapo health. Our specific objectives are to (1) apply 16S rRNA- and cultivation-based methods to accurately describe the microbial communities associated with the kakapo GI tract; (2) document variation

in the microbial community among different kakapo individuals, to determine whether certain microbes are common to all healthy kakapo; (3) develop a "probiotic" for kakapo from cultivated GI bacteria. Our initial results, based on 16S rRNA gene library analysis of kakapo faecal samples, indicate a low-diversity community dominated by *Gammaproteobacteria* (mostly *Escherichia coli*) and *Firmicutes* (genera *Clostridium* and *Streptococcus*). In addition to the potential implications for kakapo management, the kakapo's basal position within the parrot tree of life means that elucidating the role of kakapo-associated microbes could enhance our understanding of how microbes may have helped shape the evolution of parrots.

Abstract Category
25 The Gut Microbiome

PS.25.052 CHARACTERIZING THE GUT BACTERIAL COMMUNITY USING ILLUMINA SEQUENCING TARGETING 16S rRNA V3 HYPERVARIABLE REGION

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High-throughput sequencing, such as Illumina(Solexa) and pyrosequencing, allows deep sequencing at an affordable cost, and has been widely used in metagenomics studies. One important application is the characterization of gut bacterial community in relation to health and disease. The objective of our study was to design a pipeline for identifying bacterial species at high resolution in human gut microbial samples. We used Illumina short reads, which provide a higher depth of coverage than pyrosequencing, but have a shorter read length (30-100bp). Using a comprehensive collection of human gut associated bacterial 16S rRNA sequences, we first showed that the V3 hypervariable region (E.Coli reference position 338-536) was an ideal sequencing region given its moderate length and high sequence diversity among different bacterial species. We then developed an efficient method, based on the longest-path finding algorithm, for reassembling short reads. We benchmarked the algorithm against short read datasets simulated from a 3,400 sequence set using different read lengths and coverage, and showed that with a 70bp read length, 1% base error rate, and a coverage that follows a beta distribution (estimated empirically for Illumina and which resulted in a total of 1,020,000 reads), our method recovered 99% of the simulated species with less than 1% chance of chimeric reassembly. In addition, we developed a fast fingerprinting method based on the nucleotide diversity at each position in the sequenced hypervariable region that can be applied without reassembly, and showed that it can differentiate samples from different individuals or patients with different gastrointestinal disease states.

Abstract Category
25 The Gut Microbiome

PS.25.053 MODELING COMMUNITY ECOLOGICAL NETWORK FOR HUMAN GUT MICROBIOTA

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The human gut microbiota, mainly dominated by Bacteroidetes, Firmicutes, and Actinobacteria, play diverse and fundamental roles in human bodies involving hosts' nutrition, health and disease. Recent research also suggests that the microbial community in human intestine was associated with the pathogenesis of obesity. Several recent pyrosequencing projects on human gut microbiota have generated massive amounts of 16S ribosomal sequences, making it possible to study the interactions among microorganisms by reconstructing ecological networks. Here, the 16S pyrosequencing data for 154 individuals from Turnbaugh et. al (2009) was used. Individuals were categorized into four comparable groups, representing different properties including obesity, ancestry, zygosity and age. A 0.03 distance cutoff was used for OTU identification. Microbial ecological networks were then constructed using Random Matrix Theory method and comparisons of network properties were carried out for each group. The results demonstrated that these networks were significantly different from the random networks, indicating that they are biologically meaningful. Some general network properties, such as small world, scale-free, modular, and hierarchical were also detected in all these networks. Besides, network nodes with high connectivity (>5) in the lean microbial ecological network covered more bacteria family/genera. Less inner family links between nodes but more outer family links were observed in the lean microbial ecological network for the main bacteria family, showing that the network structure in lean individuals is more complex than that in obese individuals. Obvious differences were also observed between networks constructed from other two groups, but not between networks constructed from monozygotic and dizygotic twins. These results implied that the microbial networks in human gut are related to human factors like weight, ancestry, and age, but not significantly related with zygosity.

Abstract Category

25 The Gut Microbiome

PS.25.054 CULTURING THE HUMAN MICROBIOME

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We are attempting to culture novel bacteria from the human gastro-intestinal tract to facilitate interpretation of the large amount of metagenomic data from this microbiome and to directly study the causal role of specific organisms in health and disease. Our strategy is to use information about the chemistry and physiology of the human gut, as well as the microbiome metagenomic data, to design selective growth conditions that have not been used previously. The first isolations were targeted at bacteria positioned for direct communication with the host by virtue of their close association with the colonic mucosa. Biopsies were collected from healthy volunteers and plated on medium containing nutrients common to the human gut. Plates were incubated in an hypoxic atmosphere with low (2%) oxygen and high (5%) carbon dioxide to mimic the mucosal environment between the aerobic host cells and the anaerobic gut lumen. The communities that grew under these conditions were compared with parallel platings incubated in ambient air and under anoxia. Over 500 isolates were purified and characterized by partial 16S rDNA sequencing. More than half of the Operational Taxonomic Units identified among the hypoxically-isolated cultures were not found among the colonies from the more traditional ambient or anoxic isolations. These results demonstrate the potential of new enrichment

protocols for culturing many more representatives of the diverse community within the human microbiome.

Abstract Category

25 The Gut Microbiome

PS.25.055 CHARACTERIZATION OF FECAL MICROBIAL COMMUNITIES IN NON-HUMAN WILD PRIMATES REVEALS CORRELATION WITH THE HOST PHYLOGENY

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Diet plays a fundamental role in the evolution, distribution, and social organization of non-human primates. Host-associated microbes comprise an integral part of animal digestive systems and these interactions have a long evolutionary history. It has been hypothesized that the gastrointestinal microbiome of humans and non-human primates may have played significant roles in host evolution by facilitating a range of dietary adaptations. To better understand how these microbiomes relate to the evolution of non-human primate diversity, we have undertaken a comparative microbiome sequencing project to survey the composition of gastrointestinal microbiomes from several non-human primate species. In this study, we analyzed fecal samples from black-and-white colobus, red colobus, red-tailed guenon, Balinese macaque, and howler monkey. Amplicons from eleven samples were subjected to small subunit (SSU) rDNA tag pyrosequencing using primers targeting both ends of the V1-V3 hyper variable region of the SSU rDNA. Consistent with previous reports, *Firmicutes* and *Bacteroidetes* comprised the vast majority of the phyla in each sample. Other phyla present included *Proteobacteria*, *Spirochaetes*, *Actinobacteria*, *Verrucomicrobia*, *Lentisphaerae*, *Tenericutes*, *Planctomycetes*, *Fibroacateres*, and TM7. Bray-Curtis similarity analysis of these microbiomes indicated that microbial community composition within the same primate species are more similar to each other than to those of different primate species and are in line with host phylogeny and dietary preferences. Our analyses support the hypothesis that the fecal microbiomes of wild primates have co-evolved with their hosts, as evidenced by higher intraspecies similarity among wild primate species. These results provide a framework for comparative microbiome analysis between human and non-human primates, as well as a comparative evolutionary understanding of the human microbiome.

Abstract Category

25 The Gut Microbiome

PS.25.056 WOOD WASTE TO BIOETHANOL? - LIGNOCELLULOSE DEGRADERS IN THE HUHU GRUB GUT

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As the source material for bioethanol production, lignocellulosic biomass is superior to cereal grains as it is found in waste materials and does not threaten food supplies. Current procedures for lignin-cellulose separation require uneconomical energy input and have poor yields. Efficient biological mechanisms for lignocellulose hydrolysis have long been sought after, but so far to no avail. The Huhu grub (*Prionoplus reticularis*) is a wood-boring beetle larva native to New Zealand and feeds on lignocellulosic biomass of dead trees. Research into termite and other insect gut flora suggests that microbes capable of hydrolyzing lignocellulose are likely part of the Huhu grub's gut flora, which has not been examined. This research aims to identify microbes involved in lignocellulose metabolism in the Huhu grub gut.

In addition to wild type grubs raised on wood (WT), groups of grubs were fed on artificial diets based on Lignocellulose (L), cellulose (C), and starch (S). DNA was extracted from grub gut content and excreta (i.e., frass) and analyzed using bacterial 16S rRNA gene-based methods. Analysis of terminal restriction fragment length polymorphism (tRFLP) results revealed that bacterial communities from groups WT and L are compositionally different from the non-lignocellulose-fed groups. Major community members were conserved between frass and gut samples, indicating that the frass samples can be used as a proxy for the gut. PCR clone library comparisons showed further diet specific changes as none of the sequences found in group S were found in the significantly more diverse WT group. 16S rRNA gene PCR amplicon pyrosequencing data from all four diet groups are currently under analysis and will provide definitive information on community composition and differences. Once identified, bacteria specific to grubs fed on lignocellulose will be targeted for isolation with the goal of identifying metabolic genes and pathways related to lignocellulose degradation.

Abstract Category

25 The Gut Microbiome

PS.25.057 EFFECT OF ANTIMICROBIAL RESIDUES ON THE HUMAN INTESTINAL MICROBIOTA USING MOLECULAR METHODS

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The ingestion of residues of antimicrobial agents in food of animal origin has the potential risk to human health since changes in the composition of the intestinal microbial community may influence host functions. In this study, we used three molecular approaches including PCR—denaturing gradient gel electrophoresis (DGGE), terminal restriction length polymorphism (TRFLP) and pyrosequencing to detect community changes in the intestinal microbiota after exposure to various levels of the fluoroquinolone antimicrobial enrofloxacin. The diluted human fecal material (10%, 25%, and 50% concentrations) were exposed to 0, 0.1, 1, 5, 15, 50 or 150 µg/ml of enrofloxacin for 24 hours, and the compositions of the microbial communities were analyzed. PCR-DGGE and TRFLP fingerprints were compared with the results of barcoded

pyrosequencing analysis to profile the fecal microbiota. PCR-DGGE and TRFLP analysis gave remarkably similar common bands and fingerprints, with some slightly different intensity. A total of 200,059 sequences (average length 444.2) of microbial 16S rRNA genes from PCR amplicons were generated by using barcoded pyrosequencing. Of the classifiable sequences, 18 phyla were identified across the sample set. The predominant phyla were Firmicutes (59.5% of total sequences) and Bacteroidetes (37.8%) and the minor phyla were Verrucomicrobia(1.1%), Actinobacteria(1.1%) and Proteobacteria (0.3%). The overall bacterial community composition was significantly correlated with differences of enrofloxacin concentration in fecal materials. PCR-DGGE, TRFLP and barcoded pyrosequencing analysis were effective techniques for monitoring and characterizing the diversity of population in the human intestinal microbiota. This molecular approach provides useful information on assessing the risk of the human health concern due to ingestion of antibiotic residues in food.

Abstract Category

25 The Gut Microbiome

PS.25.058 HABITAT ADAPTATION ALTERS GENE CONTENT CONSERVATION IN GUT BACTERIA AND THEIR RELATIVES

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Intensive efforts are underway to relate variation in the phylogenetic composition of the human gut microbiota, as profiled by 16S rRNA surveys, to health and disease. However, making inferences about the ecological function of organisms detected in 16S rRNA surveys is difficult due to the plasticity of bacterial genomes. In order to be able to reliably relate 16S rRNA surveys to ecological function, it is crucial to understand what factors influence the relationship between 16S rRNA sequence similarity and gene content conservation. Therefore we have studied the influence of taxonomy, genome size, habitat adaptation, and pathogenicity on gene content conservation in the genomes of gut bacteria and their non-gut relatives by characterizing the relationship between 16S rRNA gene sequence similarity and gene conservation in genome pairs that varied in these properties. First, 195 bacteria were characterized as either gut specialists, cosmopolitan, or non-gut based on their distribution in 16S rRNA surveys. Second, the gene content conservation of 5,737 pairs of genomes was calculated, and related to the phylogenetic distance separating each genome pair. Both genome size and gut adaptation influenced the relationship between 16S rRNA sequence similarity and genome content. At short phylogenetic distances, specialist or cosmopolitan bacteria found in the gut share fewer genes than is typical for non-gut genomes, but at longer distances gut bacteria are more similar to each other than are non-gut genomes at equivalent distances, suggesting a pattern of short-term niche specialization but long-term functional convergence. Moreover, this pattern is observed in both pathogens and non-pathogens, and can even be seen in the plasmids carried by gut bacteria. The approach developed here could be applied to determine whether the effects of habitat adaptation on genome evolution observed in the gut are universal across other body habitats and environment types.

Abstract Category
25 The Gut Microbiome

PS.25.059 DIET-DISRUPTED GUT MICROBIOTA IN DEVELOPMENT OF THE METABOLIC SYNDROME IN MOUSE MODEL

Zhang, C*¹; Zhang, M¹; Wang, S²; Han, R³; Cao, Y⁴; Hua, W⁴; Mao, Y⁴; Zhang, X⁴; Pang, X⁴; Wei, C⁴; Zhao, G²; Chen, Y⁵; Zhao, L⁴

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Both genetic variations and diet-disrupted gut microbiota can predispose animals to metabolic syndromes (MS). This work assessed the relative contributions of host genetics and diet in shaping the gut microbiota and modulating MS-relevant phenotypes in mice. Together with its wild-type counterpart, the ApoA-I knockout mouse, which has impaired glucose tolerance (IGT) and increased body fat, was fed a high fat (HFD) or normal chow diet (NC) for 25 weeks. DNA fingerprinting and bar-coded pyrosequencing of 16S rRNA genes were used to profile gut microbiota structures and identify key population changes relevant to MS development by Partial Least Square Discriminate Analysis. Diet changes explained 57% of the total structural variation in gut microbiota, while genetic mutation accounted for no more than 12%. All three groups with IGT had significantly different gut microbiota relative to healthy wild-type/normal chow-fed animals. Sixty-five species-level phylotypes were identified as key members with differential responses to changes in diet, genotype, and MS phenotype. Most notably, gut barrier-protecting *Bifidobacterium* spp. were nearly absent in all animals on high fat diet, regardless of genotype. Sulphate-reducing, endotoxin-producing bacteria of the family, *Desulfovibrionaceae*, were enhanced in all animals with IGT, most significantly in the wild-type/high fat diet group, which had the highest calorie intake and the most serious MS phenotypes. Then we developed nutritional interventions in wild-type mice with HFD for 22 weeks followed by 12 weeks NC. The results showed that changing back to NC attenuated HFD induced obesity and imbalance of glucose homeostasis. Thus, diet plays a dominating role in shaping gut microbiota and changes of some key populations may transform gut microbiota of wild-type animals into a pathogen-like entity relevant to development of metabolic syndromes, despite a complete host genome.

Abstract Category
25 The Gut Microbiome

PS.25.060 DIET-DISRUPTED GUT MICROBIOTA IN DEVELOPMENT OF THE METABOLIC SYNDROME IN MOUSE MODEL

Zhang, C*¹; Zhang, M¹; Wang, S²; Han, R³; Hua, W¹; Mao, Y¹; Cao, Y¹; Zhang, X¹; Pang, X¹; Wei, C¹; Zhao, G⁴; Chen, Y⁵; Zhao, L¹

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for Biological Sciences, Chi, Shanghai, China; ⁴Chinese National Human Genome Sequencing Centre, Shanghai, China; ⁵Key Laboratory of Nutrition and Metabolism, Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Shanghai, China

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Abstract Category

25 The Gut Microbiome

PS.25.061 FIRMICUTES AND BACTEROIDETES DOMINATE FIBER DEGRADING BACTERIA ISOLATED FROM PIG FECES.

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Addition of fibrous feedstuffs to pig diets improves feed efficiency due to decreased feed intake without commensurate decrease in weight gain; due to utilization of short chain fatty acids (SCFA) for energy. Bacterially produced SCFA can contribute 10 to 30% of a pig's metabolic energy. Investigation of intestinal bacteria that degrade fiber and produce SCFA can be applied to improve feed utilization and animal growth efficiencies. Continuous culture fermentations (n=12; 2/pig, 6 pigs) of pig feces fed cellulose (6 g/d) or xylan-pectin (4+2 g/d) as sole carbohydrate were used to enrich fiber utilizing bacteria. Fermenters (700 ml, 38°C, pH 6.0-7.5, 0.03%/h turnover, continuous agitation and nitrogen inflow) were inoculated with 350 ml of 1 in 10 (w/w) fecal slurry. After feeding 3 g of substrate, fermenter contents were batch cultured overnight before starting inflow of carbohydrate-free nutrient medium. Carbohydrates were fed twice daily for 8 weeks; contents were sampled for bacterial isolations after 4, 6 or 8 wk.

Replicate plating on single carbohydrate agars (including cellulose and xylan) was used; individual colonies were selected and bacterial isolates obtained. A total of 940 isolates were recovered and 16S rRNA genes sequenced. Sequences were identified using Ribosomal Database Project and identical sequences from the same pig were eliminated, resulting in 360 individual isolates. The dominant bacterial genera of isolates were *Bacteroides* (23%) and *Clostridium* (15%); both *Firmicutes* bacilli and other *Firmicutes* were each 19% with other *Bacteroidetes* only 2% of total isolates. Over 46% of isolates were less than 97% similar to cultured organisms. There was a great deal of strain diversity within species. These isolates will be used to increase understanding of energy supplied to pigs through bacterial production of SCFA and to investigate strain diversity within bacterial species.

Abstract Category

25 The Gut Microbiome

PS.26.001 FUNCTIONAL TRAIT-BASED ANALYSIS OF MICROBIAL COMMUNITY GENOMES

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In the field of community ecology, there is a resurging interest in understanding biogeographical patterns based on functional traits (i.e., biological characteristics linked to fitness). The study of covarying traits in an environmental context is crucial to stress the ecological strategies that operate within natural communities. Recently, the initiative of the Global Ocean Sampling (GOS) Expedition has challenged the scientific community with approximately 8 billion nucleotides present in more than 7 millions of DNA fragments which rise to over 30 million peptide sequences potentially informative. However, this rate of information collection is entirely uncoupled with its ecological interpretation. A synergy between ecology and metagenomics may help bridge this mismatch, by providing theoretical and analytical tools that can reveal microbial community patterns and the processes that underlie them. We have described 53 available GOS metagenomic samples with a set of functional traits that range from nucleotidic to genomic properties using multivariate statistical methods. Community genomics is revealing an unprecedented level of microbial diversity and metabolic novelty in the world's oceans. The approach presented here will greatly benefit the description of metagenomic samples and its functional analysis in a rigorous ecological framework.

Abstract Category

26 Metagenome in Action

PS.26.002 COMMUNITY PROTEOMICS HIGHLIGHTS EXTRA-CELLULAR DIFFERENCES BETWEEN GRANULAR AND FLOCCULAR SLUDGE

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Currently, conventional activated sludge wastewater treatment plants operate as floccular biofilm systems. However, aerobic granular sludge is developing as a novel alternative with numerous operational and economic advantages compared to conventional floccular sludge. Aerobic granules are complex microbial systems, embedded by large amounts of extracellular polymeric substances (EPS). Granules are much larger than flocs (from 1-3 mm diameter) with superior settling capabilities and biomass concentrations. Granules are typically formed from floc biofilms, however there is limited understanding of the granule formation process. We aim to compare protein expression to determine functional differences between floccular and granular sludges.

Two laboratory-scale reactors, one floccular and one granular, were operated for enhanced biological phosphorus removal (EBPR) over a 38-week period. The reactor EBPR performance, biofilm morphology and microbial community composition were closely monitored throughout. Proteomic analyses of the floc and granule sludge were performed by liquid chromatography mass spectrometry, and identifications made using three available EBPR metagenomes.

In both the floc and granule sludges ~1500 putative proteins were identified per sample, this

included a large number of cellular and metabolic proteins. Interestingly, in the granular samples a higher number of membrane associated transport proteins were detected, such as; ABC-type signal transport, TRAP-type compound transport and outer membrane proteins. This suggests there are major differences in the extra-cellular matrices of floccular and granular sludge. Further proteomic investigations are being developed utilizing a quantitative technique not previously used on mixed culture proteomics. This approach will allow us to quantitatively compare and contrast levels of specific proteins to better understand the metabolic and physiological differences between floccular and granular sludge.

Abstract Category
26 Metagenome in Action

PS.26.003 ULTRA-SHORT READ-BASED METATRANSCRIPTOMICS USING A REFERENCE METAGENOME: A CASE STUDY

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Next generation sequencing technologies provide opportunities for rapid acquisition of large sequence datasets at reduced cost relative to other technologies, which makes them attractive for transcript analysis, a technique known as RNA-seq. The transcriptome of an environmental community (*i.e.* metatranscriptome) can reveal ecosystem-related microbial metabolic functions and may offer valuable insights into processes like biogeochemical cycling of carbon. In this study, we carried out proof-of-concept research in which we applied the Illumina sequencing technology to analyse transcripts in mixed microbial communities. Specifically, environmental samples from Lake Washington sediment were interrogated after exposure to different C₁ substrates (methane, methanol, methylamine, formaldehyde), in an attempt to assess the response to these substrates by specific functional types (*e.g.*, methylotrophs). The resulting ultra-short reads (35 and 75 bp) were mapped to a previously generated metagenomic scaffold using several different software tools. We were able to match up to 10% of the 35-mer reads to the scaffold using strict alignment parameters (100% identity match) and up to 19% using relaxed parameters (90% identity match). For the 75-mer transcripts, we found fewer matches, up to 7% using the relaxed approach. A gene expression index was calculated for each gene and after normalization these quantities were compared across the C₁ substrate exposure conditions. We were able to identify differentially expressed genes that fit in the context of pre-existing knowledge of methylotroph C₁ metabolism. Overall, we demonstrated the feasibility of employing the ultra-short read sequencing technologies for metatranscriptomic applications. At the same time, we identified a set of caveats and best practices that need to be considered when using this approach.

Abstract Category
26 Metagenome in Action

PS.26.004 INTERPRETING DIVERSITY IN METAGENOMES USING CANONICAL DIVERSITY ANALYSES WITH A MULTIPLE MARKER APPROACH

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Taxonomic diversity is a complex and multidimensional feature, making it difficult to measure and compare between communities, but metagenomics has opened the opportunity to compare large and complex natural microbial communities. However, the application of traditional community ecology analyses is complicated by partial sampling, sample complexity and an obscured interpretation of fragmented 16SrRNA taxonomic marker. We propose a multiple-marker approach based on the identification of multiple single-copy molecular markers, and its assignation to taxonomic categories via a last common ancestor algorithm. The resulting relative abundance distribution is used to calculate canonical diversity parameters. We test this approach against the simulated metagenomes of nine synthetic communities with contrasting richness and evenness and whose "real" parameters are known. We demonstrate the applicability of this multiple-marker approach for 1) delimiting richness boundaries, 2) identifying meaningful differences across species abundances and 3) comparing diversity across a multidimensional range between samples. Finally we evaluate how this parameters change when sequencing effort and community composition changes. In conclusion, some diversity analysis tools from macroecology can be used to compare metagenomic samples from simple to moderately complex communities when applied with this multiple-marker approach.

Abstract Category

26 Metagenome in Action

PS.26.005 ANALYZING FUNCTIONAL AND TAXONOMIC DIVERSITY OF MICROBIAL MATS FROM CUATROCIENEGAS BASIN FROM A COMMUNITY-ECOLOGY PERSPECTIVE

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Cuatrociengas Basin is an enclosed endovaporitic basin that harbors an extensive system of springs and pools in the heart of the Chihuahuan desert. Despite being an arid system with low available phosphorus, it hosts a large degree of bacterial diversity in a wide array of complex communities forming microbialites and bacterial mats. We analyzed the metagenomes of two microbial mats from two physicochemically contrasting ponds: PG a fairly permanent and stable one and PV a dessication pond. Clone libraries and metagenomic markers show a high diversity in both mats, though PG displays a high diversity at phylum level with little to no dominance, while PV has a large diversity within the genus *Pseudomonas*. This suggests that seasonal disturbances deeply impact diversity at phylum level while it promotes diversification of specific

groups at species/genus level. Community structure comparisons with metagenomes from Guerrero Negro microbial mat and Cuatrociénegas stromatolites reveal that communities cluster by type of community, suggesting a taxonomic "core" for each ecological assemblage with additional variable taxa. The most abundant carbohydrate pathways in PG are all aerobic and related to photosynthesis, while PR is dominated by anaerobic degradation pathways. PR also displays a large potential for the degradation of Aromatic compounds. In both systems phosphate metabolism is largely overrepresented over phosphonate and inorganic phosphorus metabolism. The comparison with other metagenomes reveal that microbial mats from clear water systems are populated by DNA-repair, Chemotaxis/Motility, Cell signaling and Iron-scavenging pathways. The relationship between taxonomic and functional diversity reveals that the functional increase with species richness is not in the raw number of pathways present in a sample but in the variability within individual pathways and the number of pathways diversified, supporting the rivet hypothesis of community assembly.

Abstract Category

26 Metagenome in Action

PS.26.006 METATRANSCRIPTOMIC ANALYSES OF THE CHLOROPHOTOTROPHIC MICROBIAL MAT COMMUNITY OF AN ALKALINE SILICEOUS HOT SPRING IN YELLOWSTONE NATIONAL PARK

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The chlorophototrophic microbial mats of alkaline siliceous hot springs in Yellowstone National Park have been model systems for studies on microbial communities. Recently developed high-throughput RNA sequencing was applied to this model system in order to elucidate both the taxonomic structure of the community and the transcriptional behavior of its members.

Total RNA was extracted from samples collected at 15 timepoints representing a full diel cycle and analyzed by both pyrosequencing and SOLiDTM technologies.

Most of the rRNA and mRNA sequences were derived from chlorophototrophic members of only four kingdoms: *Chloroflexi*, *Cyanobacteria*, *Chlorobi* and *Acidobacteria*. Analyses of 16S rRNA sequences and photosynthesis-related genes confirmed the discovery of two putatively chlorophototrophic organisms; they belong to the taxa *Chloroflexi* and *Chlorobi*, were previously undescribed, and are currently uncultured. Differential transcription patterns inferred from datasets generated by the two technologies were similar and were consistent with previous results inferred from RT-PCR analyses. However, because of ~100-fold greater sequence coverage, the SOLiDTM datasets were superior to pyrosequencing for detecting differential gene expression under the conditions examined. The data revealed differences in the temporal transcription patterns for photosynthesis-related genes for six distinct chlorophototrophs. These differences suggest that mat chlorophototrophs employ different strategies for utilizing solar energy. Transcription patterns of metabolic genes of major community members will also be discussed.

Metatranscriptome analyses revealed that this model microbial community mainly comprises chlorophototrophs, including two novel organisms. It also demonstrated that the transcription patterns of its physiologically distinctive members vary significantly over a diel cycle.

Abstract Category

26 Metagenome in Action

PS.26.007 PROTEOMICS MEASUREMENTS OF FUNCTIONAL REDUNDANCY AND STABILITY TESTING OF CELLULOSE DEGRADING MICROBIAL COMMUNITIES WITHIN ENGINEERED BIOREACTORS

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Understanding the role of functional redundancy within a microbial community is fundamental to predicting the stability associated with key services provided by that community. One provided service of importance is the biological degradation of cellulosic material. While life on earth depends on this service for the material flow of carbon, the underlying enzymatic activities are also of critical importance for the development of second generation biofuels production capabilities. We are developing proteomics and bioinformatics capabilities to measure functional redundancy in a microbial community without the initial requirement of metagenomic sequence information. Activity-based chemical probes targeting endoglucanases [EC 3.2.1.4], exoglucanases including cellodextrinases [EC 3.2.1.74(91)], and β -glucosidases [EC 3.2.1.21] and nitrogen assimilation activity; glutamine synthetase [EC 6.3.1.2], will allow focused analysis on these specific sets of proteins. These probes enable the enrichment of these enzymes for high-resolution, high mass measurement accuracy mass spectrometry analysis (LC-MS). Resulting peptide feature fingerprints are being used to construct a redundancy metric that will ultimately be applied to measure and quantify the response of these enzymes to perturbations applied to cellulose degrading microbial communities established in engineered bioreactors.

Abstract Category

26 Metagenome in Action

PS.26.008 METAMICROBIOME: COLLABORATIVE AND OPEN ACCESS METAGENOMICS FOR ACCELERATED BIOPRODUCT DISCOVERY

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Microbial communities harbor immense genetic diversity with enormous promise for applications in bioproduct synthesis and green chemistry. Metagenomic libraries provide a window into this largely untapped reservoir of nucleic acid diversity and individual libraries have been generated from a variety of terrestrial and aquatic environments. A limitation is that metagenomic libraries are typically project-specific and maintained in isolation. In an effort to

enable the sharing of genetic material from environmental samples for the benefit of the scientific community, we are establishing the Canadian MetaMicroBiome Library. This repository will house composite soil samples that are characterized by extensive 16S rRNA gene and whole-genome sequencing, generating taxonomic profiles and metabolic pathway reconstructions for each sample. The sequence data will be submitted to existing public databases and linked with detailed physical, chemical and geographic characterization of the samples according to established metagenomic standards. Sample DNA will be used to construct high quality libraries in cosmid vectors, allowing for phenotypic screening in a broad range of microbial surrogate hosts. Phenotypic screening of metagenomic libraries provides access to truly novel functions that would otherwise be missed by sequence-based surveys of bulk community DNA or metagenomic libraries. MetaMicroBiome is being initiated with soil samples collected from across Canada, and initial libraries are being screened for novel glycoside hydrolase activities in multiple surrogate hosts. Once MetaMicroBiome is established, contributions of samples from other environments will be accepted for inclusion. The resulting resource will provide the international scientific community with access to a common collection of thoroughly characterized community-contributed genetic material in clone library format for custom phenotypic screens.

Abstract Category

26 Metagenome in Action

PS.26.009 ANALYSIS OF THE COMPOSITION OF MICROBIAL COMMUNITIES IN HYDROCARBON RESOURCE ENVIRONMENTS BY PYROSEQUENCING OF 16S GENES

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Investigations of the microbial communities present in oil and gas reserves have traditionally relied on culture based techniques. However, since the majority of microorganisms are not easily grown in the laboratory, only culture-independent approaches can capture the true indigenous microbial diversity. Next-generation pyrosequencing technologies were therefore used for a 16S rRNA gene-based profiling of microbial communities in a variety of hydrocarbon resource environments. Produced water samples were passed through a 0.2 µm filter with the collected biomass stored at -70°C. DNAs were isolated using the modified Marmur protocol with bead-beating. DNAs from solid samples (e.g. cores or oil sands) were isolated with a FastDNA Spin Kit for Soil. The 16S rDNA genes from the isolated community DNAs were PCR-amplified using universal primers. Bar-codes were then added to the PCR products (representing the resident communities) using a minimal number of PCR cycles with bar-coded primers. Following pyrosequencing, the 16S rDNA sequences determined and used to derive the composition of the microbial communities. Microbial communities in hydrocarbon resource environments differ depending on physical (e.g. temperature) and chemical (electron donor and acceptor, other nutrients) parameters at the sampling site. Main groups identified in most samples included the Archaea (including methanogens), *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Synergistetes* and *Thermotogae*. *Proteobacteria* were

predominant in most of our hydrocarbon environmental samples. Surprisingly, very high fractions of *Firmicutes* were also seen in many of these samples. Pyrosequence data allow a better appreciation of the compositions and variability of microbial communities in hydrocarbon resource environments and will contribute to new and improved ways to detect, monitor, and control microbially-related problems in the oil & gas industry.

Abstract Category
26 Metagenome in Action

PS.26.010 EXPLORATION OF MICROBIAL COMMUNITY DIVERSITY OF A REDUCED PHOSPHATE MANAGED TILLAGE SOIL: A STUDY BY STRUCTURAL AND FUNCTIONAL METAGENOMIC APPROACHES.

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Phosphorous (P) is the most limiting nutrient with respect to availability to crop plants, as most of the applied P gets adsorbed to the soil. Excess supplement of mineral P in soil is a source of environmental pollution problems which results in eutrophication of lakes and estuaries. Phosphate solubilising microorganisms are an important source for P availability to plants with the potential to solubilise adsorbed mineral nutrient in soil. The P solubilising ability of these microbes and community structure is hypothesised to vary in soil with different P levels. In this study we set out to study the microbial community structure of a field site managed over the last 13 years with no phosphate supplements.

The study aimed to analyse overall bacterial community structure by analysis of 16S rRNA genes and genes responsible for organic phosphate solubilisation (i.e. alkaline phosphatase), by PCR-DGGE and by clone library construction from reduced and conventional input sites. A functional based metagenomic library approach was also applied to recognise functional alkaline phosphatase genes in soils with no added P.

The results based on gene libraries and PCR-DGGE suggests a variable microbial community structure. A number of clones were screened and recognised for alkaline phosphatase activity by a functional metagenomic assay.

Conclusively results from the clone library and PCR-DGGE suggest a different community structure. The future characterisation of functional gene from these sites would underpin the link between P management and microbial functional diversity.

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Abstract Category
26 Metagenome in Action

PS.26.011 DIVERSITY, DISTRIBUTION AND VERTICAL COMPOSITION OF THE BACTERIAL COMMUNITY DURING THE RAINY SEASON OF THE LAS CUMBRES

LAKE, PANAMÁ

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Although the tropics are considered the pinnate of biodiversity, their microbial diversity has been poorly documented. In contrast with temperate zone lakes, there are few studies conducted in shallow tropical lakes and even fewer have investigated their bacterial dynamics. In this study, we estimated the diversity, distribution and vertical composition (surface (0-m), 5-m, 10-m and 15-m) of bacterial communities of the Las Cumbres Lake, Panama, during the rainy season (November 2007). We used 16S rRNA gene clone libraries, and the programs DOTUR and LIBSHUFF to estimate the diversity and composition, respectively, of the sequences at different operational taxonomic units (OTU). Twenty percent of the sequences were unique (<97% of similarity). The most abundant phylum was Proteobacteria (50.64%) followed by Cyanobacteria and Bacteroidetes (36.36% and 10.38%, respectively). The Verrucomicrobia and Actinobacteria were the least abundant groups (1.29% for both). The lower depths (10-m and 15-m) had greater diversity than upper depths (0-m and 5-m). However, the species composition as determined by genetic distances differs significantly between depths ($P < 0.0001$, 0-m and 5-m vs 10-m and 15-m). The greater bacterial diversity found at anoxic lower depths (range; 1.8-1.5mg l^{-1}) when compared to oxygen-rich depths (range; 5.9-2.8 mg l^{-1}) suggest an excellent opportunity for niche differentiation. Although we found lower diversity compared to other surveys, both, rarefaction and accumulative species curves show that additional sampling is necessary to estimate true diversity. Our survey is one of the few to contribute data about bacterial dynamics in a tropical freshwater ecosystem.

Abstract Category

26 Metagenome in Action

PS.26.012 MICROBESONLINE PHYLOGENETIC METAGENOMICS DATABASE

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We have added the ability to analyze metagenomic datasets to the MicrobesOnline database (<http://www.microbesonline.org>), which currently also holds over 1000 microbial genomes. Comparison of environmental sequence data with isolate genomes allows for phylogenetic classification and functional characterization of the members of communities. We have developed the FastTree program, which allows us to create phylogenetic trees for all gene families, even those with over 100,000 members, so that genes can be studied within an evolutionary framework. This permits high-resolution analysis beyond simple gene-family counting, allowing an understanding of the evolutionary processes taking place in a community such as gene family expansions, as linking which gene families play the key roles in given communities tells us their particular ecological demands. Additionally, we have determined Microbial Clade-Oriented Sequence Markers (MicroCOSM), gene-families that are phylogenetically informative for given clades, such as at the phylum level, allowing for

increased coverage of reliable phylogenetic classification of metagenomic sequence data. This coupling of metagenomic and isolate genomic data, population structure analysis, sequence binning, and gene-family targeted analysis allows the user to identify the important processes taking place in their environment and the roles played by each member in the community.

Abstract Category

26 Metagenome in Action

PS.26.013 GENOMICS AND FUNCTIONAL ANALYSES REVEAL UNEXPECTED FEATURES AND SUGGEST A COMPLEX EVOLUTION OF NITRITE-OXIDIZING BACTERIA

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Nitrite-oxidizing bacteria (NOB) catalyze the second step of nitrification, the oxidation of nitrite to nitrate, and thus represent a key functional group of nitrogen-cycle organisms. As most NOB are still uncultured and the few available cultures are very difficult to maintain, this functional guild is barely studied and also dramatically undersampled by genomics. NOB are phylogenetically heterogeneous and novel representatives, such as the recently described betaproteobacterial *Nitrotoga* lineage, are still discovered. Interestingly, the different NOB are not equally distributed in major natural and engineered ecosystems. Only few ecophysiological factors determining this niche separation have been identified so far, although deeper insights into this phenomenon would be essential for a better understanding of nitrification ecology. Our study aims at illuminating the microbiology of NOB by a multiphasic approach that comprises genomics and post-genomic analyses, *in situ* techniques to detect NOB and to investigate their physiology, and the specific characterization of functionally important key genes and proteins. Recently, we analyzed the complete genome of "*Candidatus Nitrospira defluvii*" that was assembled from the metagenome of a nitrite-oxidizing enrichment and represents the first genome of *Nitrospira*, which are the predominant NOB in most habitats. Besides unexpected physiological features including a novel nitrite-oxidizing enzyme, the lack of common oxygen defense mechanisms, and the presence of complete heterotrophic pathways in this chemolithoautotroph, the genome of *Ca. N. defluvii* revealed a surprising evolutionary link between these NOB and anaerobic ammonium-oxidizing "anammox" organisms. Comparative analyses including additional new genomic data from *Nitrospira*, and genomes of other NOB, shed light on the evolution and genomic plasticity of this guild and provided explanations for the observed niche differentiation.

Abstract Category

26 Metagenome in Action

PS.26.014 ANALYSIS OF ANAEROBIC LIGNOCELLULOSE DECOMPOSING CONSORTIA FROM PUERTO RICO TROPICAL FOREST SOILS

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Tropical forest soils in Puerto Rico rapidly and efficiently degrade lignocellulose under anaerobic or fluctuating redox potential conditions, which has applications to next-generation lignocellulosic biofuels. While the high complexity of soils precludes genomic assembly and reconstruction and thereby the discovery of novel, full-length sequences, we can use shotgun metagenomic sequencing to survey relevant lignocellulolytic microbial community members and enzymes. To reduce community complexity, select for anaerobic decomposing consortia, and elucidate differences in electron acceptors linked to decomposition, anaerobic consortia derived from Puerto Rican soils were grown in minimal media cultures with the feedstock switchgrass (*Panicum virgatum*, dried and ground) as sole carbon source and various electron acceptors added (NO_3^- , Fe, SO_4^- , and none). The results of the metagenome sequencing of tropical forest soils revealed that they are rich with microbes that possess a wide range of lignocellulose-degrading enzymes, including a number of enzymes that represent potentially novel sequences. Phylogenetic profiling of the feedstock-adapted communities derived from these soils by the SSU rRNA gene microarray PhyloChip reveals that the consortia are significantly simplified compared to the natural communities from which they were derived. Among the different consortia, SO_4^- and Fe additions resulted in the highest metabolic activity, enzyme activity and cell counts per gram of switchgrass. Several members are related to organisms previously characterized as lignin degraders, while others are from less well-characterized phyla, indicating that these cultures are selecting for diverse taxa capable of lignocellulose degradation. The decrease in complexity of these types of communities has enabled reconstruction of full-length genes from metagenomic analysis and thus revealed novel lignin-degrading enzymes or pathways.

Abstract Category

26 Metagenome in Action

PS.26.015 COMPARATIVE GLOBAL METAGENOMICS: A TAXONOMICAL AND FUNCTIONAL COMPARISON OF THE FIRST PIECES OF THE PUZZLE

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Due to various public sequencing projects with metagenomic DNA corresponding to diverse environments, the scientific community possesses significant sequencing data from different ecosystems around the world. We compared at different taxonomical and functional levels 60 metagenomes corresponding to DNA samples extracted from oceans, deep oceans, Antarctic lakes, terrestrial environments (sediments, soil, sludges, acid mine) and animals (human feces, mouse and chicken cecum, and cow rumens). Results show important taxonomical and functional specificities for each environment underlining prokaryote adaptation and evolution capacities. The importance of different functions in the different ecosystems demonstrates both the natural differences between these ecosystems as well as differences in the impact of human

activities. For example, some communities appear to play considerable roles in biogeochemical cycles and in photosynthesis processes; others are clearly influenced by natural pollutions or anthropogenic compounds, such as antibiotics and chloroaromatics. Elements involved in adaptation (integrons, transposons, introns, CRISPRs, plasmids or phages) were also found to vary considerably between ecosystems. Moreover, this comparison provided numerous new hypotheses such as where eukaryotic nucleus, HIV virus and human pathogen species emerged, or still which environments influence global warming. This comparison highlights the interest of metagenomic approaches in microbiology due to the number and broad range of functions and species that can be compared.

Abstract Category

26 Metagenome in Action

PS.26.016 IDENTIFYING KEY GENES AND ORGANISMS IN A BENZENE-DEGRADING METHANOGENIC CULTURE USING A COMBINED PROTEOMIC AND METAGENOMIC APPROACH

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Benzene is a widespread groundwater contaminant that has the potential to be cleaned up in situ via bioremediation. Documenting this cleanup process is a challenge because under the anaerobic conditions that often exist at benzene-contaminated sites, very little is known about the metabolic pathway(s), key organisms and enzymes involved. In this study, a combined proteomic and metagenomic approach is being taken to identify some of these unknowns in a benzene-degrading methanogenic consortium. Samples from different cultures were taken during benzene removal and differential expression experiments were also conducted under fed versus starved conditions. Cellular proteins were extracted using a sonication method; purified samples were subjected to SDS-PAGE and/or trypsin digestion followed by LC-MS/MS analyses. On polyacrylamide gels, 40 kDa and 65 kDa protein bands were consistently observed during benzene degradation. To aid in identifying these and other proteins expressed in the presence of benzene, over 250 Mb of metagenome sequence from the culture is being used as a reference database for comparison to LC-MS/MS data. To date, proteins associated with expected downstream processes such as benzoate metabolism have been identified. Several proteins of unknown function have also been marked for further analysis: a more complete assembly of the metagenome will help confirm their full sequences and allow for a better search of their corresponding gene neighbourhoods for insight into the anaerobic benzene degradation pathway. A novel method for binning metagenome sequences has also been employed and, in the absence of closed genomes, may help to determine which organism encodes the proteins of interest in this study. Key genes and organisms identified through this study could be used as biomarkers for benzene removal at contaminated sites.

Abstract Category

26 Metagenome in Action

PS.26.017 METABOLIC VERSATILITY OF INDIGENOUS DEEP-SEA MICROORGANISMS UNDERPINS BIOGEOCHEMISTRY OF A HYDROTHERMAL VENT PLUME

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Hydrothermal vent plumes are hot spots of microbial activity in the deep sea; hydrothermal energy sources fuel microbial chemosynthesis, and microbes mediate the oceanic fate of hydrothermal inputs. However, little is known regarding the nature of plume microbial communities or the populations that utilize hydrothermal inputs and mediate geomicrobiological transformations. Previous results from Guaymas Basin (GB) plumes showed dramatic enhancement of microbial activity and biogeochemistry in plumes but microbial diversity remarkably similar to the surrounding ambient deep sea. The aim of this study is to identify the key organisms and genes that respond to hydrothermal energy sources and underpin biogeochemical transformations in plumes. We used 454 Titanium pyrosequencing to shotgun sequence community DNA and RNA from four plume and two background samples, generating more than five million reads and one billion base pairs of sequence. Genomic assembly and binning by tetranucleotide frequency was used to reconstruct near-complete genomes from three dominant deep-sea microbial populations: SUP05-like Gammaproteobacteria, SAR324 Deltaproteobacteria, and crenarchaeota. Overall metagenomic content between plume and background was strikingly similar. Common gene expression patterns were also noted; in particular, genes encoding methane oxidation and nitrification are among the most abundant transcripts in both plume and background samples. In contrast, large differences in expression of certain genes and pathways between plume and background were uncovered. Most notably, sulfur oxidation genes and a novel multicopper oxidase gene that may mediate manganese oxidation are over-represented in plume metatranscriptomics datasets. Overall, our results suggest that major biogeochemical reactions in plumes are mediated by microorganisms that are abundant and indigenous in the ambient deep sea, highlighting the active and dynamic nature of deep-sea microorganisms.

Abstract Category

26 Metagenome in Action

PS.26.018 GENOMIC APPROACHES TO EXAMINE THE BACTERIAL DIVERSITY OF ASIAN DESERT SAMPLES AND THEIR POTENTIAL FOR LONG-DISTANCE TRANSPORT BY ASIAN SANDSTORMS

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Arid regions represent nearly 30% of the terrestrial surface of the Earth. They thus correspond to important ecological models, but their microbial biodiversity is not well characterized. As $\geq 95\%$

of environmental bacteria are not cultivable under laboratory conditions, genomic approaches are necessary to discern the bacterial populations in a given environment. Using pyrosequencing of 16S rDNA sequences amplified by PCR from total DNA extracted from surface sand samples, we examined three samples from the Taklemaken desert (China) and two samples from the Gobi desert (Mongolia). The bacterial groups identified from the Taklemaken samples belonged to 52 bacterial families from 11 phyla, while those from the Gobi samples were identified as 65 families from 13 phyla. The dominant groups were *Proteobacteria* (25.10%), *Firmicutes* (24.76%), *Bacteroidetes* (22.65%) and *Actinobacteria* (8.87%) in the Taklemaken samples. In the Gobi samples, the dominant groups were *Firmicutes* (69.88%), *Proteobacteria* (12.18%) and *Bacteroidetes* (8.22%). In Asia, many cities suffer from sandstorms in the spring because of winds that transport sand from these two deserts to distances of several thousand miles. In 2007, approximately 800,000 tons of sand were distributed from China to the rest of the world by sandstorms. We compared the bacterial diversity in the air that can associate with sterile sand in the absence of a sandstorm with that from sand collected during a sandstorm in two Asian cities (Beijing, China and Seoul in South Korea). The results of analyzing the bacterial populations suggest that the percentage of *Proteobacteria* and *Firmicutes* increases, while that of *Actinobacteria* and Non-classified bacteria diminishes, in sand-associated atmospheric samples during a sandstorm in both of the cities. Our results thus suggest that sandstorms may alter the local atmospheric bacterial diversity when deposited and thus represent a potential risk to human and environmental health.

Abstract Category

26 Metagenome in Action

PS.26.019 CAMERA: A CYBER METAGENOMICS RESOURCE

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The mission of the Community Cyberinfrastructure for Advanced Marine Microbial Ecology Research and Analysis, CAMERA, is to enable the microbial ecology community to manage the challenges of experimental metagenomics analysis arising from the vast sequence data and intrinsic complexity of environmental populations. The essential methodologies, the updated release, CAMERA 2.0, involve the integration of environmental metagenomic sequences (with 22 billion bps to date) with annotation and metadata in a semantically-aware environment. Besides incorporating pre-computed annotation, CAMERA provides the users with links between metadata, annotations and terms in popular ontologies such as the Gene Ontology (GO) and Environment Ontology (ENVO). These links allow users to write semantic queries against the database; researchers are able to query on metadata categories such as habitat, sample collection, time, location, and other environmental physicochemical measurements to match their own needs. Compliant with the standards promulgated by the Genome Standards Consortium (GSC), CAMERA integrates tools for sequence quality control, assembly, gene prediction, annotation, and functional and comparative genomics applications. It also provides high-performance networking access and grid-based computing and a large amount of rotating storage. CAMERA organizes tools in a workflow system (with provenance), which allows users

to view and download results, browse the specific details on how results are generated, and upload their own workflows, which enables tool integration from the broader research community. CAMERA also provides data submission tools to allow researchers to share and forward data to other metagenomics sites and community data archives such as GenBank, to ensure wide access to data and annotation. Thus CAMERA provides this cyber resource freely to all members of the scientific community to allow key scientific questions to be addressed.

Abstract Category

26 Metagenome in Action

PS.26.020 METATRANSCRIPTOMIC ANALYSIS OF A SUMMER BACTERIOPLANKTON COMMUNITY IN SOUTHEASTERN U.S. COASTAL WATERS

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Here we describe the first of a series of seasonal metatranscriptomes examining gene expression by bacterioplankton in response to temporal variations in a southeastern U.S. coastal ecosystem. RNA from two replicate summer samples was sequenced to a depth of >1 million reads, resulting in ~500,000 possible protein encoding sequences each. The addition of an internal standard allowed transcript abundance to be quantified on an absolute scale (per liter of seawater), the ideal unit for sample comparisons and for placing expression data in a biogeochemical context. In a BLAST analysis, transcript sequences from this single time point binned to genes from over 1,900 organisms, including both taxa expected to be important components of this ecosystem (Roseobacter, SAR11, flavobacteria), and taxa not previously thought to be highly active in shallow coastal waters (Crenarchaeota and members of the Oligotrophic Marine Gammaproteobacteria). The majority of transcripts binning to sequences in the Refseq database had low counts (1 to 2 counts per category; absolute abundance = 10^6 transcripts per L), with highly expressed transcripts (>100 reads = $>10^8$ transcripts L) making up only 0.5% of the library. Nonetheless, transcripts for genes involved in the major biogeochemical cycles, including the C, N, and P cycles, could be detected. For example, transcripts to 25 of 26 phosphorus cycle genes were identified in the community transcriptome, with highest abundance of transcripts found for low affinity phosphate transporters, alkaline phosphatases, and phosphonate pathways. Transcripts for ammonia uptake and oxidation were also prevalent, correlating with a peak in ammonia concentrations during sampling. Heterotrophs were using a variety of carbon sources as indicated by a diverse array of organic compound transporters, and were supplementing a heterotrophic lifestyle with light driven energy generation as indicated by proteorhodopsin transcripts from a broad array of taxa.

Abstract Category

26 Metagenome in Action

PS.26.021 METAGENOME ANALYSIS AND MICROBIAL GENOME RECONSTRUCTIONS FROM UMTRA SITE SEDIMENT

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We are investigating the microbial community structure and metagenome content of sediments collected from the US DOE ERSP's Old Rifle IFRC site in order to elucidate the mechanisms for biogeochemical transformation of contaminants in the subsurface environment. Previously generated 454 tag sequence libraries from the system, based on the V4 region of the 16S rRNA encoding gene, revealed the dominance of a diversity of *Sulfuricurvum*-like organisms. A large number of shotgun metagenome-derived reads generated with 454 GS-FLX at the time mapped to *Sulfurimonas denitrificans*, the most closely related organism for which a genome sequence was available. We are presently using Illumina 75x2 sequencing reads to assemble long contigs from this subsurface metagenome. The assembly was performed using a combination of the Velvet assembly software as well as Cap3, Maq and other tools. Our current assembly of the data consists of about 20,000 contigs >1000 bp, of which over 200 are >10,000 bp. Some of the contigs have a sequence coverage of over 100x. From the Illumina-generated data we can resolve a high abundance of organisms related to *Sulfurimonas denitrificans*, in addition to genomes representing *Rhodoferrax ferrireducens*, *Dechloromonas aromatica*, *Pseudomonas mendocina*, and a few other species. This confirms the 16S rRNA-based tag sequence inventories performed previously, and now provides an in-depth functional gene complement for the corresponding genomes from a subsurface environment. Ongoing mixed batch laboratory experiments investigating the effects of electron shuttling compounds as well as different electron donors and solid phase iron (hydr)oxide minerals will benefit from an in-depth knowledge of the collective metagenome of the original subsurface sample used as the inoculum.

Abstract Category

26 Metagenome in Action

PS.26.022 IDENTIFICATION OF GENES INVOLVED IN ARSENIC RESISTANCE BY FUNCTIONAL METAGENOMIC APPROACHES

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Arsenic (As) is known as a toxic metalloid, which primarily exists in inorganic form [As(III) and As(V)] and can be transformed by microbial redox processes. Hence microbial transformation has a major impact on arsenic toxicity and mobility which can greatly influence the human health. Our main purpose was to explore the microbial communities of an extreme acidic environment (Tinto river, Spain) to identify novel genes and mechanisms of arsenic resistance, using a functional metagenomic analysis. Metagenomic DNA was obtained from the headwaters of the river. According to the average insert size (fragments ~1-8 kb), small-insert libraries using the high-copy vector pBluescript SKII+ and *E. coli* were constructed. The clones showing As-resistance phenotype were selected by plating on As(V) minimal medium. The As-resistance of

the clones was also confirmed by minimal inhibitory concentration method. The average insert size of each randomly isolated clone (116 clones) and their restriction profiles were determined by restriction analysis using either XbaI or EcoRI and XbaI endonucleases. A sequence analysis of the inserted fragment conferring the As-resistance phenotype was performed (harbouring ORFs at different percentages of similarity to other sequenced genes from other organisms). By this approach, a total of 24 different As-resistant clones [≥ 6 mM As(V)] have been isolated. The sequence analysis of the fragments (~4 kb) revealed the presence of several ORFs, some of them similar to those genes previously described for heavy metal and As-resistance in bacteria. However, others ORFs were not similar to any other sequences deposited in the databases and thus revealed novel insights about unidentified mechanisms of As-resistance. Future molecular analysis (such as sub-cloning and *in vitro* transposon mutagenesis) will be performed to confirm the novel ORFs conferring As-resistance. Acknowledgements: This work was supported by the MEC-Spain grant (CGL-2009-10756/BOS)

Abstract Category

26 Metagenome in Action

PS.26.023 A METAPROTEOMIC APPROACH TO FUNCTIONALLY INVESTIGATE MIXED MICROBIAL COMMUNITIES UNDERPINNING ANAEROBIC BIOREACTORS

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Due to the technological advancement of proteomic research, there has been an increased application in studies characterising proteins expressed in mixed microbial communities (metaproteomics). Applying this approach to the characterisation of anaerobic digestion, a natural process where different microbial trophic groups sequentially convert complex organic molecules into a biogas (55-75% CH₄) in the absence of oxygen may prove desirable. Most industrial application of AD is operated at temperatures exceeding 18°C. However, up to 30% of the produced biogas energy is utilised to heat the bioreactors to the desired temperature of treatment. Thus, low-temperature ($\leq 20^\circ\text{C}$) anaerobic treatment systems offer a very attractive alternative technology if optimal energy production is maintained. In the present study, we applied a metaproteomic approach to investigate temperature dependent protein expression in mixed microbial communities from anaerobic bioreactors. Phylogenetic analysis was also carried out to link key microbial species to function. Samples were taken from three independent expanded sludge bed bioreactors at 37, 15 and 7°C respectively. Each bioreactor was supplied with a synthetic volatile fatty acid influent at an organic loading rate of 3 kilograms chemical oxygen demand m⁻³ day⁻¹. Protein spots excised from 2-D gels showed differential expression as a function of temperature through PDQuest software analysis. In total, 78 relevant protein spots were excised and analysed for protein identification by nanoflow liquid chromatography-electrospray ionization tandem mass spectrometry. This metaproteomic approach gave important functional insights and linked with phylogenetic data provided direct evidence of microbial activities with community structure in anaerobic bioreactors.

Abstract Category

26 Metagenome in Action

**PS.26.024 TECHNICAL CONSIDERATIONS RELATED TO BIASES
ENCOUNTERED DURING METAGENOMIC STUDIES**

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Metagenomic studies require obtaining relatively high quantities of DNA and/or high molecular weight DNA from complex environments. Furthermore, if the interest is only in procaryotic DNA, contamination by eucaryotic DNA may represent an important issue. These technical constraints depend on the type of metagenomic approach: whereas high throughput sequencing (e.g. pyrosequencing) requires several micrograms of DNA without strict size constraints, the construction of fosmid or cosmid clone libraries requires relatively lower amounts of DNA but whose size must be > 25kb. We focused here on two technical aspects: (i) obtaining procaryotic DNA free of eucaryotic contamination, and (ii) obtaining high quantities of DNA. To avoid contamination by eucaryotic DNA, an indirect extraction method is used, requiring a preliminary extraction of bacterial cells on a Nycodenz gradient. A way to circumvent the problem of low DNA recovery yield from complex environments relies on whole genome amplification, a powerful tool to generate micrograms of metagenomic DNA from nanograms of template DNA. Although biases due to whole genome amplification have been shown, an increasing number of metagenomic studies based on such amplified DNA are being published. We evaluated the compositional biases induced by: (i) Nycodenz-based DNA extraction and (ii) amplification of metagenomic DNA by whole genome amplification, in two soil metagenomes. For this purpose, the structures of the bacterial communities obtained with the different protocols were determined by a combination of molecular techniques: sequencing of 16S rDNA clone libraries, fingerprinting, and pyrosequencing. Results show that the biases due to Nycodenz-based extraction or to whole genome amplification depend on the soil and that the former can be more important than the latter. In any cases, it is crucial to check the biases induced in terms of bacterial community structure and technical optimization is highly recommended.

Abstract Category

26 Metagenome in Action

**PS.26.025 DYNAMICS OF FILAMENTOUS BACTERIAL POPULATION IN A
SEWAGE TREATMENT PLANTS DURING THE OCCURRENCE OF SLUDGE
BULKING AND FOAMING**

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Sludge bulking caused by the excessive growth of filamentous bacteria has long been a major operational problem for biological wastewater treatment. Despite of the vast efforts the causes of sludge bulking have remained to be unclear, which makes it difficult to establish bulking controlling strategy. In this study, dynamics of the filamentous bacterial community in a sewage treatment system, in which sludge bulking occurred starting in February to ending in July was investigated using PCR-DGGE and clone libraries with primers specific to filamentous bacteria. According to the DGGE result diverse filamentous bacteria existed in the system and *Microthrix*

parvicella was dominant from February to July showing that this species might be one of the major species causing sludge bulking. On the other hand, Nostocoida limicola was the dominant bulking bacterium in the first three months (February to April) but disappeared then. Three foaming samples (February, March and July) were selected to construct clone libraries for filamentous bacteria. The clone analysis result shows that the diversity of filamentous in the foaming samples was very high. Representative actinobacteria including Microthrix parvicella, Nostocoida sp, Gordonia sp and Mycobacterium sp were the dominant group in clone libraries February (start bulking & foaming) and March (showing most serious bulking & foaming problem) which accounted for 47.1 % and 68.2 % in the whole sequences, respectively. However, these species disappeared in library July (bulking & foaming almost vanished) showing that this group of bacteria might be responsible for the troubles. At the same time, the ratios of bacteroidetes group in libraries February, March and July were 5.88, 0.0 and 38.5% respectively, suggesting that this group might be not responsible for sludge bulking. The above results demonstrated that the representative actinobacteria group should be the major population responsible for the foaming and bulking problem.

Abstract Category

26 Metagenome in Action

**PS.26.026 MASTERING MAJOR CHALLENGES IN METAGENOMICS:
ASSEMBLY OF GENES AND GENOMES FROM COMPLEX MICROBIAL
COMMUNITIES**

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Next generation sequencing technologies have altered the way complex hypotheses in microbiology are tested and valuable insights in the genomic potential of cultivable microorganism are obtained. Whereas the enormous amount of sequence produced during each run represents one of the major reasons for the attractiveness of 2nd generation sequencing technologies, when used for resequencing, RNA-Seq and genome projects, the associated short read length has made the correct assembly a major challenge, when used for metagenomic projects. In the study presented here, we generated more than 130 million reads using Illumina's sequencing technology and performed a de-novo assembly of full-length genes that are encoding cellulases from a very complex switchgrass-adapted rumen microbiome. The identified and assembled genes were cloned, expressed and the obtained recombinant proteins were subjected to activity screening assays to verify our gene assembly and to evaluate the potential of the identified enzymes as industrial biocatalyst for the production of biofuels from lignocellulosic biomass. The strategy developed during this project can be easily adapted for other target enzymes and eventually to the reconstruction of complete metabolic pathways from complex microbial communities.

Abstract Category

26 Metagenome in Action

**PS.26.027 COMPARISON OF MICRODIVERSITY IN CRENARCHAEOTA AND
BACTERIA TRANSCRIPTS FROM A COASTAL PLANKTON ASSEMBLAGE**

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Analysis of 16S rRNA gene sequences in clone libraries led J. Aller & P. Kemp (2008, FEMS Microbiol. Ecol. 65: 74) to pose the question "Are Archaea inherently less diverse than Bacteria in the same environments?" A bacterioplankton transcriptome obtained from a station near Sapelo Island, Georgia as part of the Sapelo Island Microbial Observatory (<http://simo.marsci.uga.edu/>) provided an opportunity to address this problem. Of the ~2 million GS/FLX sequences obtained, 17,386 were assigned (BLASTx hit to ORFs annotated in RefSeq, bit score ≥ 40) to ORFs from Marine Group 1 Crenarchaeota (MG1C). Reads from ORFs that received ≥ 50 hits were assembled against the *Candidatus Nitrosopumilus maritimus* SCM1 genome, then we calculated average identities across the assemblies. We performed the same analysis on comparable assemblies of reads assigned to Bacteria ORFs. Reads assigned to most MG1C ORFs assembled into one contig, with few reads excluded and average identities $>95\%$. These assemblies usually contained 2 or 3 sequence variants, suggesting that the MG1C population contained 2 or 3 strains. Ammonia permease ORFs were more variable, one of them (Nmar_1698) contained 3 or 4 variants. Sequences assigned to Bacteria ammonia permease ORFs were more diverse (identities $<90\%$) than those assigned to MG1C ORFs and not all reads could be assembled. At least 4 sequence variants were detected in populations of reads assigned to ammonia permease ORFs from *Pelagibacter*, *Rhodobacter* sp, *Fulvimarina pelagi*, SAR92 (gamma Proteobacteria) and *Verrucomicrobium*. Our analysis suggests that the MG1C population is genetically more homogeneous than the Bacteria in this sample. This may indicate that we sampled a "bloom" of MG1C, or it may be a general feature of MG1C populations. If inherent to MG1C populations, this property has implications for understanding the ecology and evolution of this group and may bear on their functional responses to changing environmental conditions.

Abstract Category
26 Metagenome in Action

PS.26.028 CHARACTERIZING MICROBIAL COMMUNITIES IN THE SWITCHGRASS RHIZOSPHERE

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The research and development of switchgrass as a biofuel crop plays a critical role in meeting U.S. bioenergy goals by 2030. In addition to minimizing land requirements, research on plant-microorganism interactions could also lead to the identification and optimization of beneficial relationships between rhizosphere bacteria and plant productivity and sustainability. We used a combination of metagenomic and cultivation approaches to study the phylogenetic and functional composition of bacterial communities in the rhizosphere of switchgrass. The cultivar Cave-in-Rock was sampled at the Michigan State University Experimental Farm. DNA extracted from rhizosphere soil was used for whole shotgun sequencing and targeted pyrotag sequencing

of 16S rRNA and nitrogen reductase (nifH) genes. Bacteria were also isolated from roots, shoot, and rhizosphere soil of the same plants. We obtained 642,435 and 15,657,275 75 reads from the Roche 454 FLX Titanium and Illumina Genome Analyzer sequencing platforms, respectively. After trimming, filtering, and alignment of reads, assembled contigs with lengths up to 13,797 bp were identified. Using the MG-RAST pipeline, 63% of the sequences could be matched to proteins in SEED subsystems, and Pseudomonadaceae assignments accounted for 54% of all reads. Functions commonly associated with Pseudomonas were identified in the metagenomic data, and this was further supported by isolations of this and related genera in large numbers through cultivation studies. This work demonstrates the effectiveness of using metagenomic approaches to characterize rhizosphere bacterial communities, specifically by combining the relatively longer reads of 454 sequencing and the shorter, deeper reads of Illumina for assembly.

Abstract Category

26 Metagenome in Action

PS.26.029 METAGENOMICS OF THE U.S. GREAT PRAIRIE - JGI GRAND CHALLENGE SOIL PILOT

Howe, A*¹; Brown, C¹; Tringe, S²; Guo, J¹; Zhang, Q¹; Malfatti, S²; Glavina del Rio, T²; Chain, P³; Wang, Z²; Jesus, E¹; Lamendella, R⁴; Hugenholtz, P²; Jansson, J²; Tiedje, J¹

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The Great Prairie of the Midwestern United States are the largest expanse of the world's most fertile soil, and microbial communities within these soils help drive biomass production as well as carbon and nitrogen cycling. Following community profiling by pyrotag sequencing reported in the accompanying abstract (Jansson et. al), we used whole-metagenome shotgun sequencing to characterize and compare microbial communities in soil samples from paired native prairie and long-term cultivated sites in Iowa, Kansas, and Wisconsin. Soil ecosystems are challenging for metagenomic analysis because of their high microbial diversity and the lack of reference genome sequences. Using both the Roche 454 and Illumina GA sequencing platforms on a total of 8 reference soil samples, we obtained approximately 200 Mb 454 sequence data and 5-10 Gb Illumina data per sample. We conservatively estimated the total sample coverage of our sequencing efforts using a k-mer counting method and simple alignment of 454 and Illumina reads. We also used a protein domain profile search to identify domains in both the short reads of the Illumina datasets and the longer reads of the 454 datasets and evaluated the results for consistency between the two datasets. Our results demonstrate that Illumina metagenomic sequencing is an effective strategy for systematic investigation and comparison of complex microbial communities. Furthermore, we compared protein domain abundances and species abundances with MEGAN analysis at Phylum, Class and Genus levels between locations and different land management strategies. The data were also correlated to existing environmental metadata collected from the sites. These results are the first to deeply explore the soil metagenome and the results suggest that land management has a significant impact on the gene composition of the soil community.

Abstract Category
26 Metagenome in Action

PS.26.030 BACTERIAL COMMUNITY ANALYSIS OF THE TONGUE DORSUM USING A DOMAIN BASED ANALYSIS OF PYROSEQUENCING DATA

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The different surfaces of the oral cavity are colonised by distinct microbial communities. The tongue is heavily colonised by a thick biofilms of bacteria, yet it does not suffer from any bacterial "disease" aside from its role in oral malodour (halitosis). Thus, it is proposed that the tongue represents a stable microbiota-host system and we aim to understand the nature of this stability using a metagenomic approach. Metagenomic DNA has been extracted from samples collected from the tongue dorsum of nine healthy individuals and pooled. One run of pyrosequencing on the Genome Sequencer FLX Titanium Series has been completed yielding 1,182,126 metagenomic sequence reads of approximately 450 bp in length. Analysis of these data has used the MG-RAST annotation service and HMMER 3.0 for domain analysis. A preliminary survey of the taxonomic distribution of reads in the tongue metagenome has demonstrated that 95% of sequences were bacterial, with proportions of the different phyla largely in agreement with a 16S rDNA library previously constructed from the same DNA sample. We identified structural domains within the reads by carrying out a 6-frame translation of each read, and searching the resulting amino acid sequences with the CATH-Gene3D profile HMM library and HMMER 3.0. Overlapping assignments were resolved using the DomainFinder 3 software. Possible functions for assigned domains were extracted from the Gene3D database. The likely pathways of significantly over-represented superfamilies indicated that carbohydrate pathways of di and larger oligosaccharides were likely to be important.

Abstract Category
26 Metagenome in Action

PS.26.031 K-SHUFF: A ROBUST NEW TOOL TO QUANTITATIVELY COMPARE GENE SEQUENCE LIBRARIES

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K-shuff is a powerful computer program designed to identify spatial clustering based on the reduced second moment measure, or *K*-function. We define two types of *K*-functions for comparison of dissimilarity matrices of gene libraries. The intra-*K*-function (or IKF) describes the richness or evenness of genetic diversity within each library and can be used to calculate a diversity index. The cross-*K*-function (or CKF) provides a natural measurement for the

dissimilarity of the composition between libraries and can also yield a distance measure. Both functions can be used for statistical inferences following a Monte Carlo testing procedure. *K*-shuff was used to compare 16S rRNA gene sequence libraries from estuarine seawater, salt marsh sediment, and soil. The IKF-based diversity measure and CKF-based distance measure both remained relatively constant with libraries from 25-100 sequences. The IKF estimates of community diversity also agreed well with conventional measurements of diversity, such as the Shannon (H_0), Simpson (1/D), and Chao1. In a power analysis, *K*-shuff was more sensitive to differences between libraries than either LIBSHUFF or UNIFRAC. When comparing multiple libraries, *K*-shuff yields distance measures as well as test statistics for the overall as well as all pair-wise comparisons. It is implemented in FORTRAN and can be run in either a Windows or Linux environment. In conclusion, *K*-shuff is a robust new tool to quantitatively estimate both structural as well as compositional differences between two or more gene sequence libraries.

Abstract Category

26 Metagenome in Action

PS.26.032 MICROBIAL PROFILING OF NATIVE PRAIRIE AND LONG-TERM AGRICULTURAL SOILS OF THE MIDWEST UNITED STATES

Jansson, J*¹; Hugenholtz, P²; Allgaier, M²; Lamendella, R¹; Jesus, E³; Tringe, S²; Howe, A³; Susilawati, E³; Chavarria, K¹; Jackson, R⁴; Rice, C³; Tiedje, J³

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Native prairie sites in the Great Prairie have been preserved and represent a tremendous resource due to their potential to sequester large amounts of carbon. As part of the JGI Great Prairie Metagenome Pilot project (see accompanying abstract by A. Howe), we surveyed the microbial community compositions in paired native prairie and agricultural sites in Wisconsin, Iowa and Kansas by bar-coded 454 pyrotag sequencing of the SSU rRNA gene (V6-V9 region) using universal primers. Samples were collected from each plot in an expanding spatial scale: 1 cm, 1 m and 10 m in two directions at a 90 degree angle from a reference core, for a total of 64 samples. Read quality filtering, length trimming, dereplication, clustering, classification, and barcode partitioning of pyrotags were analyzed using the PyroTagger computational pipeline (<http://pyrotagger.jgi-psf.org/>). More than 580,000 quality filtered sequences were retrieved in total from the samples with approximately 4000-5000 useful tags per sample. Non-metric multidimensional scaling and hierarchical clustering were performed to visualize the relationship between differential management practices and native prairie. Canonical correspondence analysis was performed to correlate the metadata parameters to community structure derived from all soil samples. Strong correlations were found to some of the environmental parameters, including organic matter content and nutrients. The data revealed that the native prairie communities were distinct from those of the adjacent managed sites in each state. All three native prairie communities clustered together and separately from the rest of the samples when all the data was compared. These results suggest the existence of a core prairie microbiome.

Abstract Category

26 Metagenome in Action

PS.26.033 METAGENOMIC ANALYSIS REVEALS SIMILAR DESULFUROCOCCALES POPULATIONS IN HIGH-TEMPERATURE GEOTHERMAL MICROBIAL COMMUNITIES OF YELLOWSTONE NATIONAL PARK

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Metagenomic analysis of geothermal microbial communities provides direct information regarding the metabolic potential of indigenous populations to mediate reactions central to geochemical cycling. Many thermophiles within the Crenarchaeota (e.g., Orders Desulfurococcales and Thermoproteales) are capable of protein degradation and may couple the oxidation of organic compounds with the reduction of elemental sulfur. One of the primary goals of this study was to determine the role of specific community members in geochemical cycling across different geochemical conditions. Metagenome sequence from four high-temperature geothermal systems in Yellowstone National Park (YNP) was used to dissect community structure using complementary phylogenetic and functional approaches. Approximately 1.4-1.7 Mbase of consensus sequence was obtained for each of four Desulfurococcales populations from different geothermal sites in YNP ranging in pH from 3 to 6 (temperature 70-80 °C). Three of the four samples were obtained from suboxic, elemental sulfur sediments (pH 4 to 6), and one sample from an acidic iron oxide microbial mat containing little to no elemental sulfur (pH 3.3). The GC content of the four Desulfurococcales populations ranged from 56-59 and each consensus sequence contained a single 16S rRNA gene (> 900 bases). Phylogenetic and functional analysis revealed that the four populations are highly related, however, they are significantly divergent from any current Desulfurococcales organisms that have been sequenced. Numerous peptidases were identified suggesting a heterotrophic metabolism based on a diverse substrate range; however, no evidence was obtained in these populations for the reduction of elemental sulfur or the oxidation of hydrogen. Results of this study suggest that highly similar Desulfurococcales-like populations inhabit geochemically variable habitats and may serve a similar functional role in community structure across disparate geochemical conditions.

Abstract Category

26 Metagenome in Action

PS.26.034 STATISTICAL CLUSTERING OF SEDIMENT METAGENOMES

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To what extent different parameters create and change microbial community patterns, and to what degree microbes display similar biogeographic patterns to macro-organisms remain driving questions in microbial ecology. Metagenomic sequencing allows a holistic approach to these questions which considers patterns in the taxonomic and metabolic profiles of entire microbial

communities. Here we have sequenced the metagenomes of four sediment environments located along intense salinity and nutrient gradients in a hypersaline coastal lagoon. When compared to 30 publicly available metagenomes using multidimensional scaling, our samples were most similar to other sediment and biofilm metagenomes from diverse environments suggesting that to some extent surface rich habitats share a characteristic metagenomic profile. Within this clustering, our metagenomes showed a high degree of statistical similarity, indicating that spatial proximity is a factor determining microbial community structure as biogeography theory suggests. Combined, this data indicates that at different levels both the structural nature of the habitat and spatial proximity are powerful forces shaping microbial diversity

Abstract Category

26 Metagenome in Action

PS.26.035 INFLUENCE OF SOIL AGGREGATE SIZE ON MICROBIAL COMMUNITY COMPOSITION AND FUNCTION

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Recent inventories of microbial diversity have shown that soils support an enormous quantity and diversity of micro-organisms. A number of explanations have been raised to explain this incredibly high level of diversity, but the importance of soil structure in the maintenance of soil biodiversity has been largely overlooked. Indeed, the majority of studies have necessarily focused on composite soil samples and large soil volumes (>0.5 g), thereby neglecting the highly structured and heterogeneous nature of soil. However, microbial populations in soil may be spatially separated in many cases, with soil aggregates potentially serving as distinct islands habitats. To investigate the heterogeneity of soil communities at a more relevant scale, we therefore addressed microbial community structure and function via two different approaches, (1) parallel examination of microbial community structure and function of different soil aggregate size classes through meta-transcriptomics, and (2) community analysis of individual soil aggregates. Freshly sampled soil (4 cores, top 5 cm) was quickly dry fractionated (4mm, 2mm, 0.8mm, 0.25mm and 0.053mm sieves), the total RNA content was extracted, cDNA synthesized and pyrosequenced. In addition, individual aggregates (6-180 mg) were picked for subsequent DNA extraction and community analysis via T-RFLP and high throughput 16S rRNA gene tag sequencing. Meta-transcriptomic analysis of the different aggregate size classes showed differences in both community structure and function between fraction classes. Furthermore, we observed that individual soil aggregates often supported highly disparate microbial communities. Taken together, these results suggest that soil structure is important in providing heterogeneous micro-niches across distinct soil particles and that spatial separation of soil particles may contribute to the maintenance of high levels of soil-borne microbial diversity.

Abstract Category

26 Metagenome in Action

PS.26.036 CHARACTERIZATION OF CHONGGAK KIMCHI FERMENTATION USING A BARCODED PYROSEQUENCING AND 1H-NMR SPECTROSCOPY

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Chonggak kimchi is a traditional Korean fermented food using radish. For the characterization of Chonggak kimchi fermentation, we performed samplings every three days during one month. In this study, their bacterial diversities were examined culture-independently using a barcoded pyrosequencing approach. Their bacterial 16S rRNA genes of Chonggak Kimchi samples were PCR-amplified by using a universal bacterial primer set with a unique identifying barcode for each sample, allowing us to analyze all of the amplified samples in a single pyrosequencing run. Phylotypes were identified by using naïve Bayesian rRNA classifier at the Ribosomal Database Project website. The results showed that their complicated bacterial communities were changed into simple ones consisting of mainly three genera, *Leuconostoc*, *Lactobacillus*, and *Weissella*, which were well known as essential bacteria of general Kimchi fermentations, through the fermentation process. Metabolite analysis in fermentation was performed using 1H-NMR spectrometry and principal component analysis (PCA). The NMR data showed that fructose, glucose, and glucitol decreased constantly, but lactate, acetate, manitol, and glutamine were produced during the fermentation. Additionally in this study the relationship between microbial communities and their metabolites will be discussed. This work was supported by EB-NCRC program and Technology Development Program for Agriculture and Forestry.

Abstract Category

26 Metagenome in Action

PS.26.037 PHYLOGENETIC DISTRIBUTION AND COMPARATIVE GENE ANALYSIS OF A KOREA FERMENTED FOOD KIMCHI USING METAGENOMIC APPROACHES

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Kimchi is the most well known Korean fermented vegetable product using various vegetables such as Chinese cabbage, radish, and cucumber and seasoning mixtures of red pepper powder, garlic, and ginger et al. Many bacteria are involved in Kimchi fermentations, but it is well known that lactic acid bacteria (LAB) play prominent roles. In this study, microbial communities and their metabolic processes were investigated during the kimchi fermentation process using metagenomic approaches. For this research, community genomic DNA samples were collected periodically from kimchi soups containing microorganisms. Using the 454-FLX technology, we generated total 701,556 reads with an average read length of 438 bp. Community analysis based on 16S rRNA gene sequences showed that microbiomes during the kimchi fermentation were predominated by members of the three genera *Leuconostoc*, *Lactobacillus*, and *Weissella*, which were well known as essential bacteria of general kimchi fermentations. Comparative function analysis studies about kimchi microbiome showed that gene ratios corresponding to each

metabolic function categories were varied during the fermentation process. Furthermore, genome mapping using random metagenome reads showed that the produced metagenome covered well genomes of previously sequence LAB with very high coverage. Therefore, analyses of the kimchi metagenomes will provide insights into microbial communities of fermented kimchi and also help the understanding of fermentation processes by microbial activities. This work was supported by EB-NCRC program and Technology Development Program for Agriculture and Forestry.

Abstract Category

26 Metagenome in Action

PS.26.038 METAPROTEOMIC ANALYSIS OF THE SOIL SECRETOME: A METHODOLOGICAL APPROACH

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Metaproteomics is the functional analysis of complex communities at a given point in time. The heterogeneity of soil, associated microbial communities, and presence of interfering compounds make the extraction of protein from soil a technical challenge. The soil enzyme pool comprises active enzymes stabilised by sorption to soil compounds, and little is known about the interactions of the many varied interlocking biotic and abiotic components. Our aim is to develop a method for the extraction of the secretome from various soils, allowing key regulatory nodes and enzymatic targets to be identified. By monitoring soil health through the use of biomarkers, the resilience of soil to perturbations can be investigated. A three-phase soil secretome extraction, comprising a gentle enzyme extraction with buffer, dialysis to remove salt, and two-stage concentration by ultrafiltration was developed. Potassium sulfate with EDTA was most efficient in recovering a high quantity of protein with minimal co-extraction of interfering substances. Highly organic soil samples required a further purification step through an insoluble polyvinylpyrrolidone (PVP) column, resulting in loss of protein through humo-protein-PVP complexes. Samples were concentrated using an ultrafiltration system yielding a higher quantity of protein compared to traditional precipitation methods. We have developed a reproducible method for the extraction of the secretome from various soil types. All protein extractions were reproducible and analysed using conventional SDS-PAGE gels, with individual bands sequenced using MS/MS Maldi-TOF. Enzymatic activity was confirmed using 4-methylumbelliferone-labelled substrates. Additional steps for highly organic soils resulted in lower protein yields, this limitation is currently being addressed by improving throughput to allow processing of larger quantities of soil and by targeting purification steps for optimisation.

Abstract Category

26 Metagenome in Action

PS.26.039 INFERRING THE SPATIAL DISTRIBUTIONS OF MICROBES FROM METAGENOMIC DATA

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Assessing the spatial distributions of uncultured microbes has strong implications for understanding the distributions of diseases and processes of microbial community assembly. However, assessing these distributions is difficult, given the limits of metagenomic data. Here, we show that distance-decay relationships -- which characterize the tendency of proximate communities to share more taxa than distant ones, and which can be constructed from existing metagenomic data sets -- carry detailed and useful information about the shape of spatial distributions. Specifically, we begin by developing a model that predicts that distance-decay relationships are essentially quadratic polynomials; the model is based on the assumption that spatial distributions can be approximated by polygons. We validate the model using simulations based on six standard macroecological ecological data sets. We then show that the parameters in the model can be interpreted geometrically, being proportional to the area, perimeter, and angularity of an underlying spatial distribution. We fit the model to two microbial data sets, where the spatial distributions of the taxa are unknown. We infer that the distributions of microbes have highly variable shapes, potentially reflecting different dispersal processes and environmental requirements. Our approach presents a powerful means for characterizing spatial distributions of microbes.

Abstract Category

26 Metagenome in Action

PS.26.040 THE ROLE OF ARCHAEA IN ANAEROBIC CARBON CYCLING IN A MODEL ACIDOPHILIC MICROBIAL COMMUNITY

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Developing a predictive understanding of carbon-cycling in an ecosystem requires a detailed knowledge of community composition and species' functional contributions. We studied the degradative portion of the carbon cycle in an acidophilic model community by examining biofilms that have undergone an aerobic to anaerobic shift. This shift occurs when a biofilm floating on the air-solution interface sinks to the bottom of an acidic flow. We evaluated *in situ* change in community structure using fluorescent *in situ* hybridization (FISH) and 454 pyrosequencing, and analyzed function with 2D LC tandem mass spectrometry.

Upon onset of anaerobic conditions, FISH and proteomic data indicate that the community transitioned from dominance by bacteria to archaea. However, compared to FISH data, the community proteome showed a more modest change than expected, likely due to persistence of bacterial proteins from dead cells. Thirteen archaeal species were identified in the sunken biofilms. These belong predominantly to the order *Thermoplasmatales* (referred to as A through I-plasma), but also include a deeply branched Euryarchaeotal clade (referred to as ARMAN).

Genomic analyses of the archaea indicated that they are likely obligate heterotrophs, but that inorganic electron donors may play also an important role. Indeed, genotypic differences suggest varied roles in community functioning. In order to better understand these different roles, methods were developed to cultivate anaerobic communities in the laboratory, enabling isotope labeling, proteomic, and metabolomic experiments that probe the physiology, function, and competitive interactions amongst these different archaeal species under a variety of conditions.

The community structure change accompanying the aerobic to anaerobic transition indicates that the archaeal members are facultative anaerobic heterotrophs that play a key role in carbon cycling under acidic conditions.

Abstract Category

26 Metagenome in Action

PS.26.041 FLUCTUATION OF GENE POOLS OF SOIL MICROBIOTA INDUCED BY CONTAMINATION WITH AROMATIC HYDROCARBONS

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Background and aims: Perturbations in soil environments such as chemical pollutants are known to drastically influence structures and functions of microbial community. The aim of presented study is to investigate the sequential metagenomic snapshots of gene pools of microbiota in contaminated soil with aromatic hydrocarbons. Methods: 3-Chlorobenzoate (3CB), phenanthrene, biphenyl, and carbazole were added to experimental soil. Metagenomic DNA samples were periodically prepared from the contaminated and non-contaminated control soils, and approximately ten-million 75-base reads per soil sample (9.5 Gb in total) were obtained by the SOLEXA sequencing. The reads were analyzed for the homology searches (BLASTN and X) using public databases such as Ribosomal Database Project, Kyoto Encyclopedia of Genes and Genomes (KEGG), and the databases for aromatic dioxygenases and other related enzymes. Results: Bacterial community analysis on the basis of 16S rRNA genes indicated the immediate domination of particular betaproteobacterial groups in the contaminated soil, suggesting the important role of these bacterial groups in the catabolism of the contaminants. The community structures of the contaminated soils tended to return to those of the non-contaminated soils. Analysis of metabolic potentials revealed that (i) the genes for aromatic dioxygenases as well as their downstream catabolite enzymes were accumulated in the gene pools during the degradation of the contaminants, and (ii) the synchronization of fluctuation patterns was observed for several gene sets. Conclusions: A vast amount of short sequences of the metagenomic DNA samples periodically prepared from the contaminated soil allowed us to observe the fluctuation of gene pools of the soil microbiota induced by the contaminants.

Abstract Category

26 Metagenome in Action

PS.26.042 METAGENOMIC EXPLORATIONS OF COMMUNITY STRUCTURE AMONG PHOTOTROPHIC MICROORGANISMS INHABITING DIVERSE GEOTHERMAL HABITATS IN YELLOWSTONE NATIONAL PARK

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Metagenomics is enabling microbial ecologists to gain insight into the relationship between microbial community composition and the functional repertoire of a community's constituents. We obtained 2,355 16S rRNA clone sequences and 320 Mbp of whole genome shotgun (WGS) sequence from six high-temperature microbial communities dominated by phototrophic bacteria in Yellowstone National Park, WY USA. Community structure differences among springs were determined by UNIFRAC analyses of 16S rRNA sequences, and these differences correlated with geochemical parameters. Metagenomic sequences were assembled into scaffolds with CELERA, and predicted genes were annotated. Phylogenetic marker genes were identified using AMPHORA, and annotations for genes putatively involved in phototrophy and sulfur cycling were manually curated. Metagenomic sequences were taxonomically grouped according to their alignments to reference genomes coupled with the phylogenetic signatures inherent in the oligonucleotide frequencies of scaffolds. Many 16S rRNA-defined clades within the *Chloroflexi* exhibited site-specificity, suggesting that some organisms are uniquely associated with particular springs. There was correspondence between community profiles obtained from the 16S rRNA and WGS metagenomes, as shown by the abundance of particular 16S rRNA-defined taxonomic groups compared to the abundance of phylogenetic marker genes. Scaffolds containing genes putatively involved in phototrophy and sulfur cycling provide support for functional differences among guilds of anoxygenic and oxygenic phototrophs in these springs and provide further evidence for the existence of currently undescribed and uncultured chlorophototrophic organisms. The combination of 16S rRNA and WGS sequencing has revealed the relative abundance of uncultivated microorganisms and their respective functional roles in these communities, leading to hypotheses regarding the primary factors controlling community structure.

Abstract Category

26 Metagenome in Action

PS.26.043 COMPARATIVE METAGENOMIC ANALYSIS PROVIDES CLUES ABOUT THE EVOLUTION, FUNCTION AND COMPLEXITY OF FRESHWATER MICROBIAL COMMUNITIES

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Lake Lanier is an important freshwater lake for the South East US as it represents the main source of drinking water for the Atlanta metropolitan area and is extremely popular for recreation activities. To better characterize the microbial communities associated with freshwater ecosystems and provide quantitative insights into how these communities are impacted by human activities, we have initiated an integrated omics study of the microbial community of Lake Lanier. Here, we report on the first metagenomic dataset from the Lake, which was obtained using the latest 454 technology and is composed of ~560 Mbp of random whole-genome shotgun (WGS) sequence data. Comparison of this dataset against available WGS datasets from soil, coastal and open ocean waters, and the deep sea, revealed, as expected, that the Lake community was functionally more related to other lakes or the coastal ocean compared to the deep-sea or the soil. However, the Lake was as different from the ocean as it was from the soil in terms of the phylogenetic diversity and abundance of the bacterial genera composing the corresponding communities. The apparent convergence in metabolism (but not phylogeny) of the water-associated communities may be attributable to extensive ancient (historical) horizontal gene transfer (HGT) during the transition from the salt to the freshwater lifestyle or environment-driven convergent evolution in metabolic pathways. The Lake community consists of sequence-discrete populations (species?) and we are following these populations over time to provide quantitative insights into the magnitude of *in-situ* HGT. We will report on the latter analyses as well as on the shifts in the composition of the Lake community over a strong summer storm as revealed by time-series metagenomic data.

Abstract Category
26 Metagenome in Action

PS.26.044 CURATED LSU rRNA AND ITS DATASETS AND A FUNGAL CLASSIFIER FOR SHORT READ SEQUENCES

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Historically, fungi have been classified using morphological features in culture or fruiting bodies. More recently, taxonomic and phylogenetic fingerprinting, based on sequence analysis of gene fragments from the large subunit rRNA gene (LSU) or the internal transcribed spacer (ITS) region, has become widespread. With the advent of high-throughput sequencing methods, it is now possible to generate in-depth environmental surveys of fungal communities. The lack of an accurate sequence database for fungal rRNA genes is a severe limitation in interpreting these environmental surveys. We are generating a validated, curated sequence datasets for the D1/D2 region of the LSU gene and the upstream ITS region by evaluating database sequences for taxonomic accuracy and quality. To include sequences from uncultured fungi, we have

conducted extensive high-quality Sanger sequencing of fungi from several different soil environments. The databases are being used as training sets for a fungal classifier based on a naïve Bayesian model. The LSU and ITS databases contain about about 10,000 sequences each, encompassing over 1500 different genera (March 2010). A more diverse group of over 30,000 fungal LSU sequences from soils have been aligned and are being used to identify gaps in our datasets. A comparison of classifier performance was conducted using a 'leave-one-out' strategy over a wide phylogenetic range. Four hundred base pair reads were more reliable than 75 bp reads, and depended on starting position. Our curated LSU and ITS databases are a rapidly expanding, valuable resource for the microbial ecology community. We have also provided a tool-a fungal classifier-that fills a significant technology gap, enabling accurate taxonomic identification and binning of fungi from 454 and Illumina sequence surveys.

Abstract Category

26 Metagenome in Action

PS.26.045 ISOLATION OF TWO NOVEL ESTERASES POSSESSING CHLORAMPHENICOL ACETATE ESTERASE ACTIVITY FROM CONTAMINATED SOIL METAGENOMIC LIBRARY

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Function-driven metagenomic analysis has a high potential for obtaining novel biocatalysts or molecules for biotechnological applications. Esterase/lipase is one of the fascinating biocatalysts because of its unique characteristics, such as stereoselectivity and co-factor independence. In this study, we constructed a metagenomic library from waste contaminated soil and selected fosmid clones with lipolytic activity based on tributyrin hydrolysis. Analysis of the culture supernatant extracts from *E. coli* carrying the lipolytic clones by thin layer chromatography resulted in the isolation of two clones, pDL26 and pDL136, which produced a metabolite that was not present in extracts from *E. coli* carrying fosmid vector. Instrumental analysis of the metabolite profile revealed that pDL26 and pDL136 contained genes encoding an enzyme for chloramphenicol acetate esterase activity. Two genes, *estDL26* and *estDL136*, were identified from the original clones responsible for the chloramphenicol reactivation activity. Deduced amino acid sequence analysis showed that EstDL26 and EstDL136 contained a putative catalytic triad characteristic of esterase/lipase, and a consensus motif HGGG found in hormone sensitive lipases.

Chloramphenicol acetate esterase activity analysis with purified EstDL26 and EstDL136 demonstrated that EstDL136 completely converted 1- and 3-monoacetyl chloramphenicols and 1, 3-diacetyl chloramphenicol to chloramphenicol. However, EstDL26 could not completely deacetylate 1-acetyl chloramphenicol probably due to stereoselectivity. Both EstDL26 and EstDL136 exhibited strong hydrolysis activity towards short chain lengths (< C10) of *p*-

nitrophenyl-acyl esters with the highest activity towards *p*-nitrophenyl-butyrate, suggesting that both are esterases. Here, we report two novel esterases identified from environmental genomes that possess chloramphenicol acetate esterase activity.

Abstract Category

26 Metagenome in Action

PS.26.046 IDENTIFICATION OF CADMIUM RESISTANCE GENES FROM INDUSTRIALLY CONTAMINATED SOIL METAGENOMIC LIBRARY

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Cadmium is a toxic heavy metal found in the environment. To date, cadmium resistance genes have been reported from culturable microorganisms. Besides, the discovery of putative cadmium resistance genes from the Sargasso Sea metagenome, there are no reports of the isolation of cadmium resistance genes from a soil metagenome. The discovery of novel cadmium resistance genes will not only shed light on the cadmium detoxification mechanisms but the knowledge gained can then be used to plan out appropriate remediation strategies. We collected soil samples from a site located in Busan, Korea, which had been contaminated with industrial waste for over 40 years. First, terminal-restriction fragment length polymorphism analysis was performed to find out the microbial community inhabiting the contaminated site. Organisms belonging to phyla *Proteobacteria*, *Firmicutes*, *Acidobacteria*, *Bacteroidetes* as well as uncultured bacteria were found to be residing in this site. Majority of the microorganisms inhabiting the contaminated site belonged to phylum *Proteobacteria*. Then, a metagenomic library was constructed in *E. coli* EPI-300 using pCC1FOS fosmid vector to screen out heavy metal resistance genes. Approximately 66,200 metagenomic clones were screened to find clones carrying genes responsible for imparting resistance to cadmium. A total of 12 unique clones were found that were able to grow on LB agar containing cadmium. Using transposon mutagenesis, we identified metal resistance genes encoding proteins which exhibited similarity to heavy metal translocating P-type ATPases. Defining the DNA fragment length for cadmium resistance and further DNA sequence analysis will not only reveal genes encoding for heavy metal translocating P-type ATPases, but also regulatory proteins. Further testing of the selected clones for metal-specific resistance towards other heavy metals is in progress.

Abstract Category

26 Metagenome in Action

PS.26.047 METAGENOMIC APPROACH FOR THE IDENTIFICATION OF DIVERSE LIPOLYTIC ENZYMES FROM UNCULTURED BACTERIA OF MARINE SEDIMENTS

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Metagenomic libraries were constructed using marine sediments from the deep sea of the Edison Seamount, the intertidal flat of Ganghwa Island and the seashore of the Arctic station. Seventeen positive clones were identified by screening for lipolytic activity on tributyrin plates. Based on the phylogenetic analysis, 13 enzymes were classified as belonging to Families I, IV, and V, Feruloyl esterase group or Patatin-like protein group, and 4 other enzymes were assigned to four new groups that have never described. By employing a combination of approaches such as removing the hydrophobic region in the N-terminus, co-expression of chaperone genes and low temperature induction, we obtained soluble proteins encoded by 14 genes. The optimum activities of the enzymes were determined in the temperature range of 20-45 °C and the pH range of 7.5-9.5. Cold-activity was observed for 7 enzymes. While 13 enzymes showed esterase activity by hydrolyzing short-chain fatty acid esters (C2-C10), EM3L4 showed lipase activity by hydrolyzing long-chain fatty acid esters (C16-C18). In this study, we were able to use a metagenomic-based approach to discover novel lipolytic enzymes in terms of primary sequence and enzyme activity from three marine sediments.

Abstract Category

26 Metagenome in Action

PS.26.048 ASSEMBLY OF THE GENOME OF *CALOTHRIX RHIZOSOLENIAE* (CYANOBACTERIA) STRAIN SC01, A MARINE NITROGEN FIXING CYANOBACTERIUM

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Calothrix rhizosoleniae is a symbiotic heterocystous cyanobacteria which was isolated in Fall 2004. This species was found growing attached to the spines of a *Chaetoceros* species (diatom) cells. Several chains of the *Chaetoceros* diatom with associated *Calothrix* were hand-picked from a 5m water sample collected at Station ALOHA (22° 45'N, 158° 0'W) in the North Pacific Ocean. The *Calothrix* continued to grow in the absence of the original host diatom cells. This is the first genome assembly for an open ocean nitrogen (N₂) fixing cyanobacterial symbiont. The culture of *C. rhizosoleniae* contained contaminating *alpha-proteobacteria*, which is a common problem with recently isolated cyanobacterial cultures. The assembly of one half of a high throughput pyrosequencing (454 Life Sciences) run on the *C. rhizosoleniae* DNA was complicated by the need to remove the contaminating sequences. In order to differentiate the cyanobacterial genome from the contaminating bacteria, the contigs were parsed into similar to alpha-proteobacteria and similar to cyanobacteria bins. MegaBlast, Blastn and tBlastx were used to classify contigs into these two phyla. This allowed the mapping of sequences and then re-assembly with only cyanobacteria sequences. The combination of Blastn and tBlastx yielded the best results by largest contig (72,904 bases) and genome length of 8.52x10⁶ bases in 10,600 contigs. An additional 2.5 plates of new 454 sequencing data has been completed and will refine the current assembly. The Python scripts used to classify contigs and improve assemblies may be

generally applicable for analyzing metagenomic sequencing data reads coming from a mixture of a few organisms.

Abstract Category

26 Metagenome in Action

PS.26.049 THE TRANSCRIPTIONAL RESPONSE OF COASTAL BIOFILMS TO EXOGENOUS AUTOINDUCER-2

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Many microbes produce signaling compounds (autoinducers) that diffuse out into the medium. When the autoinducer reaches a particular concentration, the population responds as a whole. Autoinducers are especially important in biofilms, where they can accumulate to detectable levels without being washed away. In marine environments, particle- or surface-attached bacteria have an increased supply of carbon and other nutrients and are more protected from environmental challenges than planktonic cells. Here, we characterize the global transcriptional response of coastal marine biofilms to exogenous 4,5-dihydroxy-2,3-pentanedione (autoinducer-2, AI2), a signaling molecule to which a wide variety of microbial species respond. Glass wool was incubated in seawater for ~60 hours to allow bacterial attachment, then transferred to bottles of sterile-filtered water that were either untreated or treated with AI2. Samples were collected immediately before treatment, then after 30 minutes, 90 minutes, or 24 hours of incubation. Total RNA was extracted from these samples and used as templates for cDNA synthesis, and the cDNA was sequenced by 454-FLX. Until now, the transcriptional response to exogenous autoinducers has only been monitored in monocultures, using microarrays. In contrast to microarray analysis, the pyrosequencing method does not require a priori knowledge of the genes in a population and is sensitive enough to identify very small changes in transcript abundance with statistical significance. We applied biclustering analysis to the sequence data to identify genes whose transcript abundances change in a coordinated fashion in response to AI2. This combined bioinformatic and statistical analysis has enabled identification of genes and pathways that respond to exogenous AI2 on different time

Abstract Category

26 Metagenome in Action

PS.26.050 MAPPING INTERSPECIES HYDROGEN FLUX IN ANAEROBIC MICROBIAL COMMUNITIES USING A CUSTOM-DESIGNED HYDROGENASE DNA MICROARRAY

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Molecular hydrogen is a key metabolic intermediate in many anaerobic microbial communities. Hydrogen flux in anoxic environments is determined by the rates of hydrogen formation by

microbes that use H⁺ as electron acceptor and of hydrogen consumption by microbes coupling hydrogen oxidation to reduction of various electron acceptors. Understanding the fate and flux of hydrogen in complex anoxic microbial communities can reveal relative metabolic activities between different anaerobic functional groups in processes such as reductive dechlorination, hydrogen production in phototrophic microbial mats, and the role of hydrogen in intestinal diseases. In order to comprehensively interrogate H₂ flux, we have developed a DNA microarray to characterize the diversity, abundance, and expression status of hydrogenase genes in a variety of anaerobic microbial communities. An oligonucleotide microarray with 44,000 tiling 60mer probes was designed based on 1316 hydrogenase and reductive dehalogenase gene sequences obtained from the IMG/M database and other sources. We also designed and implemented a novel analysis method to rank the likelihood of gene presence based on the length of gene fragments showing significant fluorescence. This "hydrogenase chip" was subsequently used to interrogate H₂ metabolism and flux in diverse anaerobic ecosystems, including bench-scale reductive dehalogenating microbial communities, cyanobacterial microbial mats, and rat intestinal microbiota. Dominant producers and consumers of hydrogen in these environments were identified. In fluctuating environments, differential gene expression was shown to correlate with different hydrogen fluxes. These results were consistent with other methods applied to the same environments, including 16S rRNA gene clone libraries and quantitative PCR. The hydrogenase chip is a novel and useful tool for understanding the dynamics of hydrogen production and consumption in anaerobic microbial communities.

Abstract Category

26 Metagenome in Action

PS.26.051 INFLUENCE OF ENVIRONMENTAL VARIATION ON THE GENOME CONTENT OF *PROCHLOROCOCCUS* AND THE BIOGEOCHEMICAL IMPLICATIONS

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The marine cyanobacterium *Prochlorococcus* is found in many ocean regions and is a significant contributor to global ocean primary productivity. Adaptation to specific light or temperature levels is strongly associated with specific phylogenetic groups. In contrast, the presence of nutrient uptake genes is quite variable within *Prochlorococcus* lineages and these genes are often located in genomic clusters. Using metagenomic analyses, it is demonstrated that *Prochlorococcus* cells living in regions characterized by less than 0.1 μM phosphate contain genes involved in phosphate uptake, regulation and utilization of organic phosphates whereas most of these genes are absent in cells from high P areas. This includes a new family of alkaline phosphatases named *phoX*. Interestingly, the same pattern is observed among P acquisition genes in SAR11 cells. Furthermore, the alkaline phosphatases *phoA* and *phoX* in *Prochlorococcus* and SAR11 share high sequence similarity despite the distant relationship of these two lineages based on 16S rRNA or other core genes. Finally, *Prochlorococcus* cells found primarily in low nitrogen regions contain genes enabling them to assimilate nitrite and nitrate whereas other *Prochlorococcus* cells (and most available cultures) are restricted to reduced nitrogen species.

These results show that gene gain and loss among ocean bacteria is important for adaptation to low nutrient environments.

Abstract Category

26 Metagenome in Action

PS.26.052 BACTERIAL INTEGRON METAGENOME IN MICROBIAL COMMUNITIES INHABIT A DEEP GAS HYDRATE CORE - ANCIENT ORIGIN OF INTEGRON AND ITS ROLE IN THE DEEP BIOSPHERE

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Behind any anthropogenic activities, believed to be concerned with the origin of integron, the integron metagenome has been discovered in the deepest bacterial communities. Integron metagenomes were picked from bulk microbial DNA that was extracted from a gas hydrate-bearing core, depth 202 m collected from Cascadian margin through IODP expedition 311. The bacterial communities within the core were dominated by Gammaproteobacteria of the genus *Pseudomonas*, with the respect of occurrence of phylotypes related to other genera such as *Caulobacter*, *Sphingopyxis*, *Brevundimonas*, *Acidovorax*, *Desulfovibrio*, and *Microbacterium*. Each of the recovered integron sequences consisted of an integrase gene, *intI*, gene cassette promoter, *Pc*, integron attachment site, *attI*, and integrated gene cassettes, a feature of known integron structure. The *intI* phylotypes had unique phylogenetic lineages but showed conservation in catalytic sites with those recovered previously from deep-sea hydrothermal vents, and terrestrial forest soils, an implication of conservation of the recombinant function of *intI* through evolution. A pool of gene cassettes was recorded and harboured a wide range of unique adaptive genes with functions, mostly signal proteins, related with deep biosphere dynamic mechanisms. Occurrence of integron in bacterial communities represented the early life implicated that this mobile DNA element constituted a functional part of the ancient bacterial genome.

Abstract Category

26 Metagenome in Action

PS.26.053 PRESSURE ASSISTED "OMICS" ANALYSES OF RESIDENT MICROBES IN PETROLEUM RESERVOIRS

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Globally, only about 30-50% of oil contained in reservoirs is recovered. In the United States alone, approximately 300 billion barrels of oil is unrecoverable using current technologies. In an effort to develop technologies that utilize indigenous microorganisms in enhancing oil recovery, we are using a systems biology approach to fully characterize the microbial communities in oil reservoirs. This multi-omics (metagenomic, transcriptomic, proteomic, and lipidomic) approach is hampered by the low cell abundances in these extreme environments. To address this issue, we

are employing pressure, in combination with and in comparison to, conventional extraction techniques, to enhance cell lysis thereby increasing the yield of nucleic acids, proteins, and lipids from concentrated biomass. Thus far, we have developed a protocol that utilizes pressure to successfully extract proteins from biomass concentrated on filters. Future work will be directed at using pressure to increase the yield of DNA, RNA, proteins and lipids from environmental samples and enrichment cultures, allowing for unprecedented insights into the *in situ* microbial processes in oil reservoirs.

Abstract Category

26 Metagenome in Action

PS.26.054 IDENTIFICATION OF POLYSACCHARIDE HYDROLASES FROM A SOIL METAGENOMIC DNA LIBRARY USING HIGH THROUGHPUT FUNCTIONAL SCREENING

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With the increasing focus on sustainable production of fuels from cellulosic materials, mechanisms for efficient conversion of complex polysaccharides to digestible mono- or disaccharides are in demand. Our research seeks to identify genes from metagenomic DNA libraries that may be of value in conversion of cellulosic material to fuels or fine chemicals. Here we describe a high-throughput functional screen designed to rapidly identify recombinant metagenomic *E. coli* clones encoding enzymes that efficiently cleave polysaccharide bonds. We describe research in which multiple polysaccharide proxy substrates were analyzed and multiple screening strategies were used. Genetic analysis of recombinant metagenomic *E. coli* clones that give positive results will be described, and implications for rapid functional screening of metagenomic DNA libraries will be discussed.

Abstract Category

26 Metagenome in Action

PS.26.055 VERTICAL AND HORIZONTAL SHAPINGS OF MICROBIAL AND CHITINASE DIVERSITIES IN TERRESTRIAL SYSTEMS

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Soil is a huge reservoir of microbial diversity that supports key ecosystem services such as biogeochemical cycles. Microorganisms are for example actively involved in chitin degradation, one of the most abundant organic compounds in nature that play central roles in both C and N cycles. Thus, chitinases constitute interesting functional markers through environments and organisms. The objective of this study was to analyse the total microbial and the chitinase diversities in soil samples from different area and at different depths in order to investigate the connection between microbial phylogeny and function in these different habitats. We also determined the major shaping factors responsible of these microbial and functional distribution

patterns in both horizontal and vertical gradients. Soil cores were harvested around the Lake Erken in Sweden from soils with different histories and covers (agricultural, conifer forest, flooded and unflooded soils) (horizontal gradient). DNA was extracted from the upper and lower organic layers as well as from the mineral layer of these soil cores (vertical gradient) and bacterial, fungal and archaeal diversities were determined by 454 sequencing of 16S rRNA or ITS. Chitinase genes were also sequenced and soil pH, C and N contents were determined. Phylogenetic analyses were performed to determine i) the structure of the microbial communities across the different soil areas (horizontal shaping) as well as according to the depth (vertical shaping), ii) the associated structure of chitinolytic communities. Microbial diversity and functional phylogeny across environments were compared and analysed to determine if they are shaped by the same factors and if they respond in the same way to changing environments. This study takes part in the Uppsala Microbiomic center (UMC) project investigating the microbial communities and functioning along a gradient from terrestrial to aquatic areas.

Abstract Category

26 Metagenome in Action

PS.26.056 METAGENOMIC ANALYSIS OF MICROBIAL COMMUNITIES IN DEEP KARST SINKHOLES IN YUCATAN PENINSULA, MEXICO

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Little is known about the extent of pollution in the natural water systems in Yucatan Peninsula. Although it is believed that the lack of industry in the area has kept the pollution levels relatively low, the rampant growth throughout the peninsula has sharply increased the threat of water pollution as well as raised the question about the treatment of wastewater produced by the increasing population of eastern Quintana Roo. One of the sewage disposal methods is injection of raw sewage into the saline groundwater. The impact of such treatment has not been thoroughly studied, and this research aims to offer some insight to its effects. We are investigating the composition and interaction of microbial communities in selected deep karst sinkholes (cenotes), which occur in the highly permeable carbonate rocks of the northern Yucatan Peninsula. Microbes are essential participants in the global cycling of sulfur and carbon, but characteristics of such communities are relatively poorly known because of their habitat in deep anaerobic waters. Characterization of these systems will offer a valuable 'fingerprint' of the natural microbial communities present, and allow monitoring of changes in the community composition as a response to change in water quality.

We are using RAPD PCR and ARISA in addition to 16S rRNA-based phylogenetic analysis to assess the population diversity and change through space and time. We are also investigating the microbe-mineral interaction in these systems to see whether providing limiting anions and cations (Fe^{3+} , Fe^{2+} , Mg^{2+} , Zn^{2+} , PO_4^{3-} , NO_3^-) and a surface for biofilm formation would affect the growth of the microbes. It may also offer some insight to natural microbial communities' ability to perform bioremediation to address the contamination problem. Current results have shown sufficient difference between the microbial community composition depending on the light and organic matter input as well as the distance from the coast.

Abstract Category
26 Metagenome in Action

PS.26.057 METAGENOMIC SEQUENCING OF AN *IN VITRO*-SIMULATED MICROBIAL COMMUNITY

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Sequencing DNA directly from the environment, a technique commonly referred to as metagenomics, is an important tool for cataloging microbial life. This culture-independent approach involves collecting samples that include microbes in them, extracting DNA from the samples, and sequencing the DNA. A sample may contain many different microorganisms, macroorganisms, and even free-floating environmental DNA. A fundamental challenge in metagenomics has been estimating the abundance of organisms in a sample based on the frequency with which the organism's DNA was observed in reads generated via DNA sequencing. We created mixtures of ten microbial species for which genome sequences are known. Each mixture contained an equal number of cells of each species. We then extracted DNA from the mixtures, sequenced the DNA, and measured the frequency with which genomic regions from each organism was observed in the sequenced DNA. We found that the observed frequency of reads mapping to each organism did not reflect the equal numbers of cells that were known to be included in each mixture. The relative organism abundances varied significantly depending on the DNA extraction and sequencing protocol utilized. We describe a new data resource for measuring the accuracy of metagenomic binning methods, created by *in vitro*-simulation of a metagenomic community. Our *in vitro* simulation can be used to complement previous *in silico* benchmark studies. In constructing a synthetic community and sequencing its metagenome, we encountered several sources of observation bias that likely affect most metagenomic experiments to date and present challenges for comparative metagenomic studies. DNA preparation methods have a particularly profound effect in our study, implying that samples prepared with different protocols are not suitable for comparative metagenomics.

Abstract Category
26 Metagenome in Action

PS.26.058 MICROBIAL CANNIBALISM IN EXTREME STARVED ENVIRONMENT

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Due to their geochemical characteristics, some soils can be considered as extreme. This is the case with some New Caledonia soils, such as ultramafic soils, that are characterized by deficiencies in essential elements and overabundance of heavy metals, particularly nickel. This phenomenon is amplified in mine spoils where carbon and nitrogen concentrations are extremely low, thus, classifying mine spoils as starved environments. Our objective was to determine which adaptive strategies enable bacterial populations to adapt to extreme environments. A comparative

metagenomic analysis applied to partial metagenomic sequences of two ultramafic soils (pristine ultramafic soil, mine spoils) and two "classical" soils (prairie soil and corn field soil) was developed to detect genes and bacterial populations specific to each soil. The new program and the graphical interface that we developed specifically for this study helped highlight the effect of the ultramafic origin, the "human" influence (mining, farming), and the starvation conditions on bacterial community function. Our results highlight the originality of the bacterial community structure and its metabolism in the mine spoils. To adapt to these extreme edaphic conditions, bacterial populations tend to minimize nutrient loss and to enhance biosynthetic pathways reaction by using dead bacterial cells as nutrients

Abstract Category

26 Metagenome in Action

PS.26.059 METAGENOMICS EXPLORATION BY MICROARRAY-BASED OPERON CAPTURE

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The emergence of massively parallel DNA sequencing platforms has made sequencing an affordable approach to study various ecosystems by metagenomics approach. However, the cost and sequencing run time required for such endeavors remain too prohibitive for an individual investigator or a small group wishing to undertake a survey of complex environmental samples. It would be desirable to focus on individual genomic subsets of interest by reducing the sequence complexity of the sample. Recently, the targeted selection of sequences by hybridization to microarray capture probes has been demonstrated to have the potential for efficient and selective enrichment of high-complexity sequences from large genomes.

Therefore, the ability to fractionate a complex metagenome for sequencing specific target regions provides a potential approach to a variety of biological questions. The highly parallel in situ oligonucleotide synthesis characteristic of current microarray printing technologies has enabled the production of complex capture systems. With our new probe design algorithms (PhylArray, MetabolicDesign, HiSpOD) we developed capture microarrays to target prokaryotic and fungi rDNA operon, gene clusters involved in polycyclic aromatic hydrocarbons degradation and methane production.

We report a flexible method for selective capture of sequence fragments from complex metagenomic DNA that enable the complete diversity evaluation of ribosomal operons and gene clusters by next-generation sequencing.

Abstract Category

26 Metagenome in Action

PS.26.060 EXPERIMENTAL AND COMPUTATIONAL APPROACHES FOR MICROBIAL DIVERSITY CHARACTERIZATION USING ARTIFICIAL COMMUNITIES

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The use of 16S ribosomal RNA gene sequencing (rDNA) to characterize the taxonomic diversity and abundance of organisms plays an important role in microbial ecology studies. Microbial diversity characterization by pyrosequencing dramatically increases the scale at which environmental samples can be analyzed both in number and sequence depth. Nevertheless, the approach often has limitations for which it is difficult to control or account. Such limitations include unequal efficiency of DNA isolation, unknown ribosomal gene copy number, biases in DNA amplification and sequencing errors. Previous studies aimed at improving the techniques and overcoming some of those limitations but were limited by sequencing depth, as well as the diversity of completed reference genomes. To determine the effects of experimental and computational steps involved in characterization of microbial diversity by 454 sequencing, we constructed artificial cellular and genomic DNA communities using cultivated organisms that have known genomic sequence and rDNA copy number. More than 70 species representing 14 bacterial and 3 archaeal phyla were included in the analysis. Genomic DNAs were mixed at known concentrations and hypervariable regions of the 16S rDNA gene (V1-3 and V4 for bacteria and V4 for archaea) were amplified and sequenced with the 454 FLX system. For a subset of the organisms, we also mixed known cell numbers and tested two DNA extraction methods. The data was analyzed in terms of PCR/sequencing errors, chimeras, abundance of individual alleles and species. For many taxa, the inferred abundance matched relatively well the composition of the assembled communities. However, significant primer-dependent biases were observed for particular species or even phyla. Some biases were not related to sequence but possibly to gDNA modification. A single set of 16S rDNA primers may, therefore, incompletely represent the diversity present in natural microbial communities.

Abstract Category
26 Metagenome in Action

**PS.26.061 TARGETED METAGENOMICS OF METHANE COLD SEEP
SYNTROPHIC ASSEMBLAGES USING MAGNETO-FISH**

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We are investigating the well-studied syntrophy of anaerobic methane oxidation (AOM), i.e., coupling archaeal methanotrophy with bacterial sulfate reduction, through targeted metagenomics. Although the organisms involved in AOM have yet to be cultivated, several culture-independent techniques have identified methanogen-like archaeal groups (ANME) as key players. Direct evidence of some ANME groups associating with various sulfate reducing bacteria (SRB) belonging to the δ -proteobacteria suggest a syntrophic partnership between the two groups, but the precise nature and degree of importance of the association is unknown. We used Magneto-FISH, the selective immunomagnetic capture of fluorescently hybridized cells, to select specific assemblages in their entirety in order to study the physical and metabolic interactions in an ecologically-relevant context. Genomic DNA preparations were acquired from

Magneto-FISH experiments targeting the aggregates associated with Desulfosarcina (DSS) SRB, Desulfobulbus (DSB) SRB, and the ANME-2c subgroup in sediment samples from Eel River Basin. Prior to pyrosequencing, DNA preparations were checked for enrichment of target groups using qPCR and T-RFLP and comparison to bulk sediment community structure. Comparative metagenomics of the different probe-based captures was used to characterize the diversity and specificity among the archaeal/bacterial assemblages involved in AOM and to examine the metabolic potential (i.e., nutrient acquisition, carbon utilization, and electron transfer) within these consortia. Among the genes identified in the metagenomes were those involved in various carbon assimilation pathways, vitamin synthesis and uptake, and chemotaxis, motility, and quorum sensing by both bacteria and archaea. Elucidating the interspecies interactions and molecular dynamics of the AOM syntrophy is crucial to understanding the controls on and specificity of interspecies syntrophic associations.

Abstract Category

26 Metagenome in Action

PS.26.062 NOVEL FUNCTION AND GENE DISCOVERY FROM NON-ANNOTATED METAGENOMIC SEQUENCES

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Metagenomic approaches involve the use of high throughput sequencing that lead to a large collection of less-than-gene-length sequences. Subsequent sequence analysis can eliminate a large proportion of these sequences due to their non-similarity with known sequences and related microbial functions. In an attempt to increase our understanding of ecosystem functioning, these non-annotated sequences were studied both in relation to those sequences that were annotated and in terms of their variation from one ecosystem to another. Different ecosystems (e.g., oceans, sediments, soils and mammal intestinal tracts) perform significantly differently in percentage of sequences associated with those from known functions (ranged from 5% to 80%). Clearly, the method of associating function to sequences derived from metagenomic approaches influences the number and type of sequences left out. Although these differences are of methodological interest, the sequences that cluster apart from sequences of known function provide insight into potential undiscovered genes and functions. Depending on the relative similarity of these non-annotated sequences to those for which we hypothesize their function, two possible relations were considered: the sequences are functionally similar but evolutionarily separated or functionally different. In order to differentiate between these two possibilities, the presence of specific characteristics of functional gene classes can be used to categorize the sequences. Our efforts to unveil potential novel functional genes can help understand functional gene evolution in similar terms as ecological pressure and selection.

Abstract Category

26 Metagenome in Action

PS.26.063 METAGENOMIC ASSESSMENT OF A SULFUR-OXIDIZING ENRICHMENT CULTURE DERIVED FROM MARINE SEDIMENT

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The biological oxidation of reduced sulfur compounds is a critically important process in global sulfur biogeochemistry. In this study, we enriched chemolithotrophic sulfur oxidizers from marine sediments under denitrifying conditions, which could oxidize a variety of reduced sulfur compounds: thiosulfate, tetrathionate, sulfide, and polysulfide. In order to characterize sulfide oxidation, we analyzed sulfide:quinone reductase gene (sqr)-containing fosmid clones prepared from the cultures. One of the group I sqr-containing fosmid clones (43.6 Kb) was sequenced and shown to harbor four putative rhodanese genes. Comparative alignment with the closest genome of *Thiomicrospira crunogena* XCL2 revealed that three genes were shared within the vicinity of sqr. Fosmid clones harboring carbon fixation (cbbL and cbbM) and denitrification (narG) genes were screened, and the phylogeny of the functional genes was analyzed. Along with the dominance of the 16S rRNA gene and sqr-containing fosmid clones associated with γ -*proteobacteria*, the results of our phylogenetic study of the functional genes in the fosmid clones points to the prevalence of chemolithotrophic γ -*proteobacteria* in the denitrifying cultures. The findings of this study imply that a combination of cultivation and metagenomic approaches might provide us with a glimpse into the characteristics of sulfur oxidizers in marine sediments.

Abstract Category

26 Metagenome in Action

PS.26.064 EFFECTS OF DISTANCE AND SELECTIVE ENRICHMENT ON ALKANE MONOOXYGENASE GENE DIVERSITY IN PASSAIC RIVER SEDIMENT

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The Passaic River in Newark, New Jersey, is a historically polluted site due to the presence of industry that dates back to the 1800's. Many petroleum refineries and chemical manufacturing plants still operate along the river severely impacting and polluting the waterway. The aim of the present study was to examine the bacterial population diversity and degradative gene distribution in sediment at different points along the river and how this may affect the microbial response to a hydrocarbon challenge. Triplicate sediment samples were collected in the middle of the Passaic River from three sites in a heavily industrialized area (0 m, 100 m, and 1 km in relationship to the start point). Each of the nine sediment samples were enriched in minimal media with hexadecane as the sole carbon source. Total DNA was extracted at the beginning of the enrichment and after 4 days. Cell growth was monitored by DAPI staining. PCR was performed on the extracted DNA using primers for the 16S rRNA gene and primers for two different families of alkane monooxygenase encoding genes (P450 and AlkB type). In order to obtain a complete picture of the genetic diversity of each sample, the 54 PCR products were subjected to pyrosequencing. Gene sequences unique to each of the sample locations were detected, and parallels between each of the sample populations were observed. As expected, a shift in the

hexadecane enrichment cultures was observed in both the overall microbial community and the populations of the two different families of alkane monooxygenase genes.

Abstract Category

26 Metagenome in Action

PS.26.065 GENOME SIGNATURES THAT REFLECT ADAPTATION OF *S. ONEIDENSIS* MR-1 TO ITS ENVIRONMENT.

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Background and aims: An organism's genome sequence reveals its genetic composition including functions and metabolic pathways essential for survival in its environment. Comparing genomes of related microorganisms from different environments may be used to identify functions that reflect adaptation to specific surroundings. We have compared the genome sequences of *S. oneidensis* MR-1 to 18 other *Shewanella* strains from diverse environments, with the goal of identifying gene products that can be linked to environmental conditions (nutrients, temperature, salinity).

Methods: We analyzed proteins encoded by the strains and determined their orthologous relationships. Assigning gene product functions, metabolic pathways and cellular roles, were done using sequence similarity and the BioCyc or SEED classification schemas. The proteins were grouped into families according to Pfam domains. Significant protein family membership differences were calculated using Multi Experiment Viewer.

Results: Of the 631 proteins unique to *S. oneidensis* MR-1 450 were plasmid or mobile genes. The remaining were clusters of 2-12 proteins for nucleoside uptake and utilization, chemotaxis, surface antigen biosynthesis, and respiration. Functions absent from *S. oneidensis* MR-1 included nitrate reduction, oxaloacetate decarboxylation, detoxification, arabinose utilization, and sensors/regulators. The presence or absence of functions was evaluated against the phylogenetic distance and environments of the strains. Comparing protein family sizes between the genomes showed an increased in the membership of some *S. oneidensis* MR-1 families (lactate transport, potassium transporter) and a decrease in others (ion channel, biotin/lipoic acid binding proteins).

Conclusions: Through comparative genome analyses we are identifying proteins and protein families that differ in their distribution among related genomes. Functions encoded by these proteins suggest functional constraints of specific environments.

Abstract Category

26 Metagenome in Action

PS.26.066 CYANIDIALES TRANSCRIPTOMICS IN ACIDIC GEOTHERMAL ENVIRONMENTS

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The unicellular, asexual, eukaryotic algae belonging to the order Cyanidiales are unique in that their environment is defined by acidic pH (0.5-3.5) and high temperature (38-56°C), where they dominate microbial communities in the cooler reaches of acidic geothermal outflows. This study focuses on *in situ* algal gene expression dynamics as a function of UV exposure, a keystone ecological factor for these algae in Yellowstone.

Pyrosequencing-based cDNA libraries were constructed from flash-frozen algal biomass sampled at low (January) and high (June) UV irradiance periods. Limited proteomics was also used to compare transcription with translation to more fully understand regulatory activities.

Quality-filtered singletons were annotated using the *Cyanidioschyzon merolae* genome. Of the 107,900 singletons, ~60% had high identity (> aa 95%) to *C. merolae* genes. Transcripts exhibiting significant allelic variation will be incorporated into environmental microarrays comparing UV-protected vs. UV-exposed algal mats. A gene coding for a hypothetical protein was highly represented in both libraries, comprising 21 and 33% of low and high irradiance singletons, respectively. cDNA and protein profiling suggested major differences between pure cultured cells and *in situ* mat cells. Analysis of cDNA structures suggests a post-transcriptional regulatory step may be involved *in situ*.

Our in-depth look into Cyanidiales ecology provides an opportunity to link *in situ* gene expression with measurable and experimentally controlled environmental changes. We hope to identify cellular changes that will help show how (or if) these algae are adapting to major environmental changes such as UV irradiance. Lastly, although transcriptomics can theoretically tell us much about microbial activity in nature, proteomics will be key in understanding cellular activities gene expression at the functional level.

Abstract Category
26 Metagenome in Action

PS.26.067 BACTERIAL PHYLOGENETICS IN A HYPERSALINE MICROBIAL MAT

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The overarching goal of this work is to characterize a portion of both the genetic diversity and microbial diversity that exists in a complex microbial mat. Sea salt is crystallized by solar evaporation at North America's largest saltworks, the Exportadora de Sal, in Guerrero Negro,

Baja California Sur. Seawater flows through a series of evaporative basins with an increase in salinity until saturation is reached and halite crystallization begins. Several of these ponds are underlined with cm-thick microbial mats. Here, we report a survey of the stratified microbial communities for the distribution of representatives of Bacteria in the mats that was done through a community sequencing project of the Joint Genome Institute (JGI). Samples of microbial mat from Pond 4 near 5 were obtained and sectioned into mm increments in the field and frozen in liquid nitrogen. DNA extraction was performed by a phenol:chloroform method and quantitated. JGI utilized both a metagenomic approach to search for genes present, and cloning and sequencing of full-length, Sanger sequenced 16S rRNA genes for Bacteria only. More than one-hundred thousand, full-length 16S rRNA genes were obtained which describe stunning microbial diversity from a single environment. Of the approximate 105 known phyla of Bacteria, 60 were found in the layers of these mats, with as many as 30 previously unknown bacterial phyla discovered. This study reveals that the extent of bacterial diversity remains largely unexplored, and that full-length rRNA gene sequence studies are needed to better understand bacterial diversity-in any environment. The metagenomic shotgun sequenced data revealed millimeter-scale functional gradients, and through isoelectric point profiles, indicate that hypersalinity provides an overriding selective pressure on the entire mat community. Collectively, the results indicate that the diversity of life is extensive even in this seemingly inhospitable "extreme" environment.

Abstract Category

26 Metagenome in Action

PS.26.068 SELECTION IN COASTAL *SYNECHOCOCCUS* (CYANOBACTERIA) POPULATIONS EVALUATED FROM ENVIRONMENTAL METAGENOMES

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Metagenomics has provided an unprecedented resource of information ripe for population genetics analyses. Current analytical methods, however, are poorly equipped to handle metagenomic data, particularly of short, unlinked sequences. A custom analytical pipeline was developed to examine the role of selection in *Synechococcus* populations by calculating dN/dS ratios using data derived from 454 metagenome sequences. Genome-wide polymorphism data were analyzed based on sequence alignments to *Synechococcus* genomes representing the dominant clades in the Southern California Bight. The large majority of genes have evolved under purifying selection (dN/dS < 1). The metagenome sequence coverage of the reference genomes was not uniform and a negative correlation was observed between the depth of read coverage per gene and dN/dS ratios. These results suggest that genes that were highly represented in the environment (i.e. high read coverage) tended to be more evolutionarily conserved. Genes that were more rare had higher dN/dS and 59% were hypothetical genes. In contrast, hypothetical genes comprised only 31% of the highly represented genes in the environment with low dN/dS ratios. Although the functions of these hypothetical genes are not known, examining the role of selection in the evolution of these genes provides additional clues for investigating their roles in defining the ecological niches of *Synechococcus* populations.

Abstract Category
26 Metagenome in Action

PS.26.069 IMETASYS: AN INTEGRATED METAGENOMIC AND MICROBIAL SYSTEMS ANALYSIS WORKBENCH

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Metagenomics has recently emerged as a powerful culture-independent approach for studying the complexity of microbial genomes in their natural environments. Several metagenomic projects, generating enormous amounts of sequence data, have either been recently completed or are currently in the progress and planning phases. Due to the limited number of computational resources available for analyzing such complex data, there is an urgent need for the development of high-throughput computational methods and resources. To meet these computational challenges, we developed a computational resource "iMetaSys" which provides an integrated environment for the comprehensive analysis of metagenomic and single-bacterial genomic data. The iMetaSys platform integrates the best of the publicly available bioinformatics applications and database resources, along with novel tools developed at RIKEN, for carrying out various analyses. The available services and programs are packed as modules, including options for sequence cleaning and validation, compositional analysis, taxonomic binning and classification of sequences, comprehensive gene prediction, functional annotation, mapping of genes to biochemical pathways, and comparative analysis. These services are freely provided through a publicly accessible web portal; however, to run iMetaSys users are required to create a secure login account. MetaBrowser, a supplementary integrated application of iMetaSys, enables users to access the publicly available metagenomic data which has been analyzed and annotated using iMetaSys, and provides options for carrying out comparative analysis with their own data. iMetaSys has already been successfully used for the analysis and functional annotation of four bacterial genomes, most of the publicly available metagenomes, and for construction of the MetaBioME database.

Abstract Category
26 Metagenome in Action

PS.26.070 METAGENOMIC CHARACTERIZATION OF NITRATE REDUCING MICROBIAL COMMUNITIES

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In the past two decades evidence accumulated that the so called nitrogen cycle is in fact a network of pathways. Many of the dissimilatory and respiratory pathways are performed only by bacteria and archaea. Early research on bacterial nitrogen metabolism focused on the examination and manipulation of pure cultures. Later on, gene sequences of (uncultured) microorganisms were obtained by sequencing DNA regions amplified by PCR. To date, metagenomic sequencing is the most promising approach for the detection of yet unknown gene

variants in order to obtain a comprehensive picture of the diversity of functional genes present in the environment. It has the advantage that it avoids PCR bias and is cultivation independent. This study aims to characterize the "network" of functional genes associated with the reduction of nitrate. Microbial denitrification, dissimilatory nitrate reduction to ammonium and anaerobic ammonium oxidation all compete for nitrate. Information on presence, abundance, and sequence diversity of the genes involved can help evaluate the detected (and the potential) nitrate reducing activity in the habitat under observation, and give further insight into functional versatility of environmental microbial communities.

DNA was extracted from sediment samples collected at a site on a Wadden sea tidal flat (Janssand, Germany) where high denitrification rates were observed. Total extracted DNA was sequenced by 454 (Titanium) pyrosequencing. Sequence data analysis was performed by automated region annotation followed by manual verification and annotation refinement of regions coding for proteins associated with the three pathways of nitrate reduction. Comparison of selected functional genes detected in the tidal flat metagenome with homologous gene sequences found in databases showed to what degree the currently existing reference sequences and hidden Markov models (Pfam profiles) appropriately represent the total diversity of environmental functional genes.

Abstract Category

26 Metagenome in Action

PS.26.071 A METAGENOMIC ANALYSIS OF *PROCHLOROCOCCUS* IN THE RED SEA

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The Red Sea has among the highest irradiance, temperature, and salinity of any body of water in the global ocean and as such serves as a natural laboratory for the study of microbial adaptation to extreme environments. Marine cyanobacteria of the genus *Prochlorococcus*—the predominant primary producer in oligotrophic waters like the Red Sea—have adapted to thrive under high irradiance and high temperature, but the particular adaptations involved remain poorly understood. The Red Sea presents a unique opportunity to study these adaptations, yet the *Prochlorococcus* community there is likewise not well characterized. To begin to address these gaps in our knowledge, we have sequenced the microbial metagenome of a 50-meter sample from an open-ocean site in the Red Sea. The metagenome exhibits common features of the oligotrophic, euphotic ocean. *Pelagibacter*, *Prochlorococcus*, and cyanophages comprised 88% of all identifiable sequences. The predominant *Prochlorococcus* ecotype was eMIT9312 (clade HLII), comprising 97% of all *Prochlorococcus* sequence reads, consistent with previous clone libraries from the Red Sea. Fragment recruitment plots of these *Prochlorococcus* reads to sequenced genomes showed two regions of poor sequence coverage (i.e., genomic islands). Comparison of the *Prochlorococcus* gene content of the Red Sea metagenome to that of a comparable metagenome from the Mediterranean Sea showed over-representation in the Red Sea

sample of genes for DNA repair, which may be indicative of adaptation to high irradiance in the Red Sea. This snapshot of the *Prochlorococcus* community in the Red Sea raises questions about adaptations to the extreme conditions of this environment, in particular which genes these *Prochlorococcus* may have acquired that have not been found in genomes from other oceanic regimes. Cultivation and genome sequencing of new *Prochlorococcus* strains from the Red Sea will be required to address these questions.

Abstract Category

26 Metagenome in Action

PS.26.072 MICROBIAL EFFECTS ON SOIL PHYTATE UTILIZATION BY LEGUMINOUS PLANTS

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Though *myo*-inositol hexakisphosphates (phytate), is major components of soil organic P and highly stable in soil, the utilization of soil phytate by plants is generally limited. However, some plant species, such as *Lupinus albus* and *Lotus japonicus*, grow better than other species when phytate is applied for P source, although they lack extracellular activity of phytase. Therefore, phytate-utilizing microbes in rhizosphere is considered to be played an important role in acquisition of P by such plants from soil phytate. To elucidate mechanisms of this type of soil phytate utilization, we try to analyze in a comprehensive manner by culture and unculture based studies.

To understand the key metabolic processes in the phytate utilization, we analyzed rhizosphere soil microbial communities with metagenomic approach. Our analysis revealed that the abundance ratio of gene clusters, considered related to soil phytate status, such as alkaline phosphatase and citrate synthase, changed with phytate utilization availability of plant, and the abundance ratio of a class of *Betaproteobacteria* was increased along with soil phytate utilization. Therefore, we have isolated over 300 strains screened from rhizosphere of *L. albus* by using phytate as a sole applied C and P source, and one of them; *Burkholderia* sp. FpRpG4 demonstrated particular high plant growth promoting effects, and caused cluster root formation of *L. albus*.

The cluster root, which is bottlebrush-like clusters of rootlets, is thought to contribute to *L. albus*'s vigorous growth when phytate is the only source of P, which results in abundant exudation of organic acids and proton to mobilize the nutrients chemically. Utilization of soil phytate by microbes is thought to be inhibited mainly because free phytic acid eventually forms insoluble salts, so our results implies a sort of symbiotic relationship between plant, which solubilize phytate, and bacteria, which make phytate P available for themselves and plant.

Abstract Category

26 Metagenome in Action

PS.26.073 ASSESSMENT OF MICROBIAL COMMUNITIES IN OIL SANDS OUTCROPS IN ALBERTA, CANADA

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One of the causes of the world's energy crisis is the realization that we do not yet have a good alternative for fossil fuels. Many alternative energy sources, such as biofuels, are being developed. However, present-day society remains heavily dependent on fossil fuels. The continuous exploitation of these resources also leads to a significant increase in carbon dioxide emissions that contribute to global warming. As the world's reserves of conventional oil and gas decrease, the attention has turned to oil sands. The Energy Returned on Energy Invested (EROI) index for oil production from oil sands is relatively low with the current technologies compared to other fossil fuels. On top of the carbon dioxide emission, the tailings waste from oil sands production also creates major ecological challenges. Under these economical and environmental pressures, development of greener oil sands extraction and processing are needed. Little information on the composition of microbial communities found in oil sands is available. We have focused on natural oil sand outcrops in river valleys in the Fort McMurray area, Alberta, Canada. Here bitumen hydrocarbons are naturally degraded by exposure to air and sunlight. Samples from various outcrops were obtained in August 2007, mixed and stored at -80 °C. Aliquots were incubated aerobically at room temperature in defined medium under daylight or in the dark for over two years. Analysis by culturing indicated the presence of aerobic, but also of anaerobic bacteria (nitrate-reducers, sulfate-reducers, methanogens). DGGE analysis indicated the presence of *Chromatiaceae* in day light-, but not in dark-incubated microcosms. More detailed analyses by pyrosequencing and GC-MS of residual oil are currently ongoing.

Abstract Category
26 Metagenome in Action

PS.26.074 CLUSTER ANALYSIS OF A METAGENOME ASSEMBLY FROM ALKALINE SILICEOUS HOT SPRINGS IN YELLOWSTONE NATIONAL PARK.

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The composition of the upper layers of 60°C and 65°C regions of the Octopus and Mushroom Spring microbial mat communities was described using (i) Celera assemblies of metagenomic sequences, (ii) analysis of the frequency distribution of all possible tri-, tetra-, penta-, and hexa-oligonucleotides in assembled scaffolds and (iii) the k-means clustering algorithm. Analysis of assemblies >20 kb in length resulted in identification of 6-8 clusters, which varied in composition in terms of the percentage of sequences that can be confidently associated with a reference genome. For instance, in cyanobacterial clusters corresponding to *Synechococcus* spp. A-like and B'-like populations, 87.6% and 80.6% of the sequences could be confidently associated with the *Synechococcus* spp. A and B' genomes, respectively. However, 7.8% and 13.5% of the remaining sequences were from the other of the two genomes, suggesting imperfect assembly and/or cluster demarcation. Furthermore, theory-dependent analysis of *Synechococcus* A-like and B'-like populations, suggested that each of these two clusters may contain >10 populations of ecologically distinct species. We are exploring multiple approaches to improving our ability to discern clusters in terms of both number and quality by (i) relaxing the assembly

size from >20 kb to >5 kb to increase the amount of sequence that can be used in cluster analysis, (ii) evaluating a refined version of k-means, called g-means, which searches for Gaussian shaped clusters, (iii) focusing on limited subsets of closely related populations, such as A- and B'-like *Synechococcus* instead of all metagenomic sequences, (iv) optimizing the phylogenetic signal in oligonucleotide frequency data upon which clustering algorithms depend, (v) optimizing Celera assembly and (vi) examining other assembly algorithms. Our aim is to find the limit of resolution of metagenomic approaches for detecting ecological species of *Synechococcus*.

Abstract Category
26 Metagenome in Action

PS.26.075 METAGENOME-BASED FINGERPRINTING ANALYSIS OF PLANKTON COMMUNITY IN A SHALLOW EUTROPHIC LAKE (LAKE DONGHU, WUHAN)

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The plankton community is one of the most important target communities for ecological study due to its structural and functional roles in the aquatic ecosystem. Traditional plankton investigation with morphological identification has historically been a difficult task due to the lack of obvious distinguishing features. Target the plankton community (including bacterioplankton, phytoplankton and zooplankton) as a whole, the present study investigated the plankton in relatively independent lake areas (showed a gradient in trophic state from mesotrophic to hypertrophic) with metagenome-based fingerprinting methods to explore the relations between community genetic diversity and environmental factors. Results suggested that DNA fingerprints of plankton communities were generally correlated with species composition and physiochemical factors. The factors strongly correlated with the distribution of plankton community were seasonally different, and concentration total phosphorus (TP) was the main factors related to the PCR-denaturing gradient gel electrophoresis (DGGE) fingerprints of plankton communities. Simulated experiment also suggested that TP was the most important environmental predictor of PCR-DGGE fingerprints of plankton community. Heavy metals also exhibited strong relations to the PCR-DGGE fingerprinting patterns. These and other observations suggested that metagenome-based fingerprinting analysis was promising tool for ecological investigation of plankton community.

Abstract Category
26 Metagenome in Action

PS.26.076 EVOLUTIONARY AND FUNCTIONAL IMPLICATIONS OF CORRELATED CHANGES IN GENE SEQUENCE AND GENE ORDER IN CULTIVATED AND UNCULTIVATED BACTERIA AND ARCHAEA

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Gene order rearrangements are key components of genome evolution. It is known that genome rearrangement increases with increasing sequence divergence, but the form and universality of the relationship remain unclear. We aim to test the generality of this relationship by including the genomes of uncultivated co-occurring acid mine drainage (AMD) Archaea in an evolutionary analysis with 100 published genomes. We explore reasons for the strong trends and model them to develop a probabilistic method for synteny-based gene function annotation.

Genomes of Archaea that populate AMD biofilms were reconstructed and compared in a pairwise manner using BLASTP. We modeled rearrangements of genes not in operons that we presumed accumulated by chance. Probabilities of gene synteny due to chance were obtained from these models. If the probability of synteny due to chance was < 0.10 , annotations were improved based on annotations of syntenous orthologs or adjacent genes.

We demonstrate a general relationship between gene evolution and whole genome evolution. Genes that have their regulatory structure (operons) disrupted by genome rearrangement experience faster rates of evolution. We used the relationship between synteny and evolutionary distance to develop a new method to refine functional annotation. The method was applied to show that AMD Archaea have the genes necessary for molybdopterin guanine dinucleotide synthesis, a cofactor used in anaerobic energy conservation.

A fundamental finding is that evolution in Bacteria and Archaea involves highly correlated rates of genome rearrangement and gene sequence evolution that we attribute to relaxed and positive selection on different classes of rearranged genes. Thus, preservation of synteny at large evolutionary distances can be used to constrain gene functional prediction.

Abstract Category

26 Metagenome in Action

PS.27.001 THE NEVADOS NATIONAL PARK (COLOMBIA) AS SOURCE OF EXTREMOPHILIC LIPOLYTIC ENZYMES

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Esterases and lipases are two of the most utilized enzymes in organic synthesis, and obtaining a thermostable esterase or lipase with the desired stereoselectivity or regioselectivity would have an immediate impact in industrial processes. The aim of this research was to isolate and characterize strains of thermophilic, psychrophilic and acidophilic bacteria with lipolytic activity, from the Nevados National Natural Park, between 3400 to 5200 m above sea level. We sampled water at 8 sites (acidic thermal springs located in High Andean Forest (HAF) ecosystems). We also sampled soils at 13 sites included HAF ecosystem. We have established several culture collections obtained from the samples collected in the Nevados. Many of the water and soil samples were subjected to enrichments in media supplemented with lipid substrates (such as Tween 80, hexadecanoic oil, dodecanoic acid, trioleine, tributyl citrate). From these enrichments, 15 thermoacidophilic and psychrophilic strains have been isolated, most of them can grow with Tween 80 as sole carbon source. These strains have been characterized at the phenotypic level (growth conditions and substrate utilization) and some have been identified by analysis of the 16S rRNA gene. Assays for lipases were performed using p-nitrophenyl acetate (pNPA). Two isolates (USBA-GBX-490 & 491) are closely related to *Achromobacter* spp., one was identified as an *Acidocella* sp. and three isolates are psychrophilic strains closely related to *Pseudomonas* spp. In addition, these strains showed esterase activity using pNPA. The isolates presented lipolytic activity between 3 to 24 U/min/ml. This report presents the results obtained with the study: the preliminary description of the isolated strains, the results of the enzymatic activities and, based on this, the identification of the most promising strains that deserve further studies.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.002 DIMORPHISM IN METHANE SEEP INHABITING *THIOMARGARITA*

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Dimorphism is rare in the bacterial world - occurring primarily in the prosthecate alpha proteobacteria. Here we report on a dimorphic life cycle in *Thiomargarita*-like bacteria that inhabit methane seeps associated with mud mounds and fault scarps along the Costa Rica margin. Microscopic examination of samples recovered by submersible show that a dimorphic life cycle begins with the attachment of spherical *Thiomargarita* to stabilized substrates such as authigenic carbonates, mussel byssal threads, and the integuments of diverse seep fauna. The attached cells elongate to lengths of up to 700 micrometers, before budding spherical daughter cells. These daughter cells are morphologically-indistinguishable from free-living *Thiomargarita*

that populate surrounding sediments. Furthermore, 16S rRNA gene sequencing of individual free-living and attached morphotypes exhibit >99% sequence similarity, suggesting that budded daughter cells of attached *Thiomargarita* constitute at least one sub-population of free-living *Thiomargarita* found in surrounding sediments. Gene sequencing also shows that these bacteria are closely related to previously known *Thiomargarita* ecotypes from Namibia and the Gulf of Mexico. The attachment of *Thiomargarita* to stabilized substrates and the elongation of individual cells may serve as a novel mechanism for nutrient acquisition. Dense mats of attached *Thiomargarita* covering the shells of gastropods and limpets may play a role in the ecology of methane seep fauna. Morphologically-similar bacteria have also been observed at methane seeps at Hydrate Ridge, suggesting that elongate *Thiomargarita* may be widespread in seep settings.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.003 ACIDOBACTERIA ISOLATES FROM THE BRAZILIAN CERRADO

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The Cerrado is a savanna-like environment that comprises approximately 22% of central Brazil. As revealed by 16S rRNA gene clone libraries, the phylum Acidobacteria is dominant in these soils. Acidobacteria is present in most 16S rRNA libraries derived from different soils sources; in the Cerrado this predominance is above 50% in all soils tested. The goal of this work was to isolate Acidobacteria members in order to study its physiology and investigate their role in Cerrado soils.

Soil samples were obtained from an area located 130 km south of Brasilia. Three samples were selected, one associated with cultivation of eucalyptus and the others obtained from two distinct cerrado native vegetations. Acidobacteria specific primer amplification using metagenomic DNA revealed that all samples harbored acidobacteria. Soil was collected from the upper 5 cm and 1 g was suspended in 50 mL of VL55 medium salt solution. Samples of 300 µl were spread on VL55 solid medium containing xylan as the sole carbon source and incubated at 23°C for 8 weeks. The number of colonies was scored weekly. Acidobacteria was detected by colony-PCR using group specific 16S primers. Colonies that yield positive amplification, as well as randomly selected colonies, were sequenced for 16S rRNA gene phylogenetic analysis. An average of 100 colonies per plate was observed after 3 weeks of incubation. Although the highest bacteria count was obtained from the eucalyptus soil, these samples yield no acidobacteria. All the confirmed acidobacteria isolates were from native soils and belonged to GP1 presenting similarity between 90 and 97 % with known species. The majority of the non-acidobacteria colonies were related to Proteobacteria. This study indicates the presence of new species of Acidobacteria that were for the first time cultivated from the Cerrado biome. The characterization of these bacteria will provide crucial information on their role in the nutrient cycling in the Brazilian Cerrado soils.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.004 MICROBESONLINE ISOLATE *OMICS DATABASE

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The MicrobesOnline database (<http://www.microbesonline.org>) currently holds over 1000 microbial genomes and is updated semi-annually, providing an important comparative and functional genomics resource to the community. New functionality added recently includes the addition of fungal and algal genomes, metabolic map and *omics data visualization tools, an updated user interface for the phylogenetic tree based genome browser that allows users to view their genes and genomes of interest within an evolutionary framework, improved tools to compare multiple microarray expression data across genes and genomes, phylogenetic profile searches using our high quality species tree, and addition of external microarray data from the Many Microbial Microarrays Database for bacteria and Yeast. MicrobesOnline continues to provide an interface for genome annotation, which like all the tools reported here, is freely available to the scientific community. To keep up with the rapidly expanding set of sequenced genomes, we have begun to investigate methods for accelerating our annotation pipeline. In particular we have developed the FastBLAST method to speed up the most time consuming process of our analysis pipeline, homology searching through HMM alignments and all against all BLAST. Our FastTree program allows us to create phylogenetic trees for all gene families, even those with over 100,000 members, so that all genes can be studied within an evolutionary framework.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.005 ISOLATION AND IDENTIFICATION OF THERMOTOLERANT FUNGI FROM HOT SPRING IN TURKEY

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In this study we aimed to the detection of thermotolerant fungi from hot spring water in Kütahya province and than identification of this fungi.

Hot water samples which are the material of this study have taken from the springs of Gediz, Simav, Tavşanlı, Emet, Hisarcık and Yoncalı Districts. These samples brought to the laboratory quickly while this, we have provided to stabilized the temperature of samples. We were carried the isolation of filamentous fungi from these water samples by using the membrane filtration method. The isolated molds that incubated at 45 °C, were tested for temperature. After these tests, they were decided to be thermotolerant fungi. Macroscopic and microscopic properties of the fungi isolates is examined to identification with traditional methods. And carbohydrate tests of the isolates were done by biolog microstation system.

14 hot water samples were used in isolation. As a result of the isolation, 50 mold isolates were obtained. Applied temperature tests were revealed that these molds are thermotolerant. As a

result of the identification of isolates were determined that all of them are *Aspergillus fumigatus* species.

As a result of the isolation and identification from hot spring in Kütahya province; it was determined that *A. fumigatus* species were found widely in these waters.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.006 DIVERSITY OF A COLLECTION OF IRON OXIDIZING BACTERIA FROM VARIOUS SULFIDIC MINE WASTE DUMPS

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About 80 cultures of acidophilic iron oxidizing bacteria from all over the world have been cultivated in the BGR culture collection for many years. Described acidophilic iron oxidizing bacteria belong to the genera *Acidithiobacillus*, *Acidimicrobium*, *Alicyclobacillus*, *Ferrimicrobium*, *Ferrithrix*, *Leptospirillum* and *Sulfobacillus*. To explore the phylogeny of the cultivated bacteria, DNA was extracted and 16S rDNA was amplified with universal bacterial primers. The phylogenetic analysis based on 16S rDNA sequences was carried out with ARB. 42 nearly full 16S rDNA sequences were obtained. Twenty 16S rDNA sequences belonged to the genus *Acidithiobacillus*, they were all closely related to *At. ferrooxidans* ATCC 23270T. The described 16S rDNA variability of *Acidithiobacillus ferrooxidans* (less than 98 % [1]) was not represented in the BGR culture collection. This was not expected because *At. ferrooxidans* is very common in mine waste dumps [2]. This may be explained by the long cultivation period in a selective medium. Six 16S rDNA sequences belonged to the genus *Leptospirillum* and are closely related to the described two species *L. ferrooxidans* and *L. ferriphilum*. Sixteen 16S rDNA sequences formed three clusters in the phylum *Actinobacteria*. Cluster 1 and 2 belonged to two not described species. Cluster 3 included 16S rDNA sequences of the BGR culture collection and *Ferrimicrobium acidiphilum*.

Two new 16S rDNA sequences of the phylum *Firmicutes* could be affiliated to the genera *Sulfobacillus* and *Alicyclobacillus*. Some 16S rDNA sequences belonged to the genus *Acidiphilium* which is often found in co-culture with acidophilic iron oxidizing bacteria. A similarity of more than 97 % to *Acidiphilium* sp. PK 48 was detected which may represent a novel species.

[1] A.P. Harrison, Archives of Microbiology. 131, page 68-71, (1982). [2] D. Kock, A. Schippers, Applied and Environmental Microbiology, Volume 74, page 5211-5219, (2008).

Abstract Category

27 Novel organisms and Novel Functions

PS.27.007 TOW NOVEL STRAINS BELONGING TO A NOVEL GENUS IN THE FIRMICUTES AND A NOVEL SPECIES IN THE TISSIERELLA, ISOLATED FROM DESALINATION PROCESS

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In order to determine microbial communities of reverse osmosis (RO) based desalination process, culture-dependant and -independent approaches were performed. In this study, two isolates, strains R7 and R4 were isolated from intake seawater and the strains belonged to a novel species in Tissierella (R7) and a novel genus in the Firmicutes (R4), respectively. mPlate Count Broth (mPCB) and R2A medium were modified by addition of sodium chloride (3.5%) and filter sterilized seawater (5%). Samples from RO plant (intake seawater, particle suspensions from prefilter and RO membrane) were serially diluted and plated on modified mPCB and R2A agar media. The 16S rRNA sequences of the two strains were phylogenetically analyzed and physiological and biochemical characteristics were also determined. Strain R4 is distinguished from other members of the Firmicutes by ca. 10% 16S rRNA sequence dissimilarity. Phylogenetic trees showed the placement of strain R4 in the Firmicutes as a novel genus. Strain R7 is Gram-staining-negative and non-sporulating bacterium. 16S rRNA gene sequence analyses indicated that strain R7 is closely related to other species of the genus Tissierella, but the sequence divergence was more than 5%. Phenotypic characteristics of strain R7 also showed clear distinction from other Tissierella species. Based on 16S rRNA sequence analysis and phylogenetic evidence, a novel genus in the Firmicutes and a novel species in the Tissierella, are proposed. [This research was supported by a grant (07SeaHeroB01-03) from Plant Technology Advancement Program funded by Ministry of Construction & Transportation of Korea.]

Abstract Category

27 Novel organisms and Novel Functions

PS.27.008 CHARACTERIZATION OF THE LIGNOCELLULOLYTIC GENE REPertoire IN THE GENOME OF ANAEROBIC GUT FUNGI

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Anaerobic fungi are highly fibrolytic microorganisms that produce a wide array of cell-bound and cell-free cellulolytic, hemicellulolytic, glycolytic, and proteolytic enzymes. As such, anaerobic fungi represent a yet-untapped resource that could be exploited in biofuel research. Therefore, discovery and categorization of the anaerobic fungal repertoire of lignocellulolytic machinery would not only greatly expand our knowledge of their unique physiology, but also potentially need to the discovery of novel lignocellulolytic enzymes. We here present our efforts in mining the genome of Neocallimastix strain 4s, isolated in a cellobiose and switchgrass-based media in our laboratory. Within the genomic fragments obtained (0.5Mbp with a GC content of 32.7%) we identified a total of 1140 open reading frames, 132 (11.5%) of which contained genes coding enzymes potentially involved in plant biomass degradation process. These included alpha and beta glucosidases, endogluconases, exogluconases, glycoside transferases, xylanases, galactosidases, mannases, pectinases, and multiple carbohydrate esterases. We also identified a cellulose dockerin domain gene, various components of hydrogenosome machinery, a cyclosporine synthetase gene, and multiple chitin metabolism genes. Current research involve sequencing additional three strains from different anaerobic fungal genera (Piromyces, Anaeromyces, Orpinomyces) using an Illumina sequencing platform with special sample

preparation to allow for unbiased sequencing of AT rich genomes. Such sequencing effort will facilitate the ability to conduct a more extensive analysis of the order Neocallimastigales lignocellulosic degradative potential and allow for comparative genomics studies within the order as well as between Neocallimastigales and aerobic Fungi.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.009 NEW SYNTROPHIC BACTERIA POPULATION FOR ACIDIC PHOSPHORUS RECOVERY FROM SEWAGE SLUDGE INCINERATION ASH

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The recovery of phosphorus from sewage sludge incineration ash was investigated by using the biotechnological process of bioleaching and bioaccumulation of released phosphorus by a newly developed syntrophic consortium of bioleaching-bacteria, *Acidithiobacillus* sp., and polyphosphate accumulating organisms. The aim of the project is to generate a phosphorus recovery by biotechnological techniques as well as the characterization of the bacterial community involved in this complex process.

Bacterial enrichment was conducted with sewage sludge and additional sulfur. Identification of microorganism was done by biochemical tests, FTIR-spectroscopy and 16S-rRNA-gene sequence analysis using both cultivation dependent and - independent methods. Polyphosphate granules were observed microscopically. Analytics of metals and phosphorus was done by ICP-mass spectrometry and ion chromatography.

By using the acidic syntrophic bacteria population, 66-80% of phosphorus is liberated from the ash. After 3 days, the phosphorus amount in the liquid is decreasing with the appearance of a precipitate. In comparison to the ash, the phosphorus amount in the precipitate is increased by about 30-50%. The amount of toxic metals is decreased by about 80-96%. On the basis of 16S-rRNA-gene sequence analysis the 4 most abundant polyphosphate-accumulating isolates were assigned to the class Actinobacteria representing most probably new species of the genera *Gordonia* and *Rhodococcus*. With the cultivation-independent approach, *Acidithiobacillus ferrooxidans* was found to be the main actor in the phosphorus recovery process. By bacterial oxidation of ferrous to ferric iron, iron phosphate can precipitate even at low pH.

The use of bacteria for both phosphorus release and recovery in acidic environment is a promising technique for resource reclaiming. For the first time, members of the class Actinobacteria were detected to be involved in acidic phosphorus recovery.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.010 ISOLATION AND CHARACTERIZATION OF BIOSURFACTANT-PRODUCING ALKALIPHILIC ARCHAEA ISOLATED FROM SODA LAKES OF THE WADI AN NATRUN, EGYPT

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Biosurfactants are amphiphilic compounds produced by microorganisms, which either adhere to cell surfaces or are excreted extracellularly in the growth medium. In recent years, interest in biosurfactants has generated due to their possible applications in environmental protection, crude oil drilling, and in the pharmaceutical and food processing industries. The search for biosurfactants in extremophiles seems to be particularly promising since the biosurfactants of these organisms have particular adaptations to increase stability in adverse environments that can potentially increase their stability in the harsh environments. The objective of this study was to screened for biosurfactant production in standard medium using the qualitative drop-collapse test and emulsification activity assay using an alkaliphilic achaea, isolated from soda lakes of the Wadi An Natrun, Egypt. The Wadi An Natrun lakes are populated by dense communities of alkaliphilic microorganisms. Biosurfactant production was detected in some of these isolates. Sixteen of the alkaliphilic strains reduced the growth medium surface tension below 40 mN m⁻¹, and exhibited high emulsion stabilizing capacity. Diesel oil- water emulsions were stabilized over a broad range of conditions, from pH 7 to 11, with up to 35% sodium chloride and up to 25% ethanol in the aqueous phase. Emulsions were stable to three cycles of freezing and thawing. The components of the biosurfactant were determined; it contained sugars, proteins and lipids. The strains with enhanced biosurfactant producers, were selected to identify by phenotypic, biochemical characteristics and by partial 16S rRNA gene sequencing.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.011 NEURAL NETWORK-BASED TAXONOMIC CLUSTERING OF NOVEL ORGANISM READS OBTAINED FROM METAGENOMIC SAMPLES

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Metagenomic studies inherently involve sampling genetic information from an environment potentially containing thousands of distinctly different microbial organisms. This genetic information is sequenced producing many short fragments (<500 base pair); each are tentatively a small representative of the DNA coding structure. Any of the fragments may belong to any of the organisms in the sample, but the relationship is unknown a priori. Furthermore, most of these organisms have not been identified and correspondingly are not represented in any of the publicly available search databases. Our goal is to be able to predict the taxonomic classification of an organism based on the fragments obtained from an environmental sample that may include many (some previously unidentified) organisms.

To elucidate the diversity and composition of the sample, we first use a supervised naïve Bayes classifier to score the fragments of known genomes, followed by an unsupervised clustering to group fragments from similar organisms together. We are then free to analyze each cluster separately. This is challenging since we are not interested in similar sequences, but sequences

that come from similar genomes which are known to vary widely intra-genomically. Our dataset comprises of an extremely challenging scenario involving clustering fragments at the phyla level, where none of the phyla have been previously seen or identified.

We present two variations of our proposed approach, one based on ART and K-means. We show that ART can cluster 500bp fragments from 17 novel phyla at an overall isolation/grouping that is 10% better than K-means and nearly 7 times over chance.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.012 SALT DEPENDENCE OF PIGMENTATION AND MOTILITY IN *VIBRIO* SP. ISOLATED FROM THE SALAR DE ATACAMA, NORTHERN CHILE

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Currently, only a small percentage of extremophile microorganisms present on the earth have been isolated and characterized. Although some halotolerants and halophiles microorganisms have been identified and isolated from saline lakes, Northern Chile, little is known about their physiological mechanisms used to face the osmotic stress. In previous work, some microorganisms of *Vibrio* genus were isolated, which are able to grow at low temperatures and high salt concentrations. In addition, we developed strategies to grow these microorganisms in different salt concentrations. The aims of this study were to determine the effect of salt concentration in growth, pigmentation and motility of *Vibrio* sp. isolated from the Salar de Atacama. We observed that the microorganism *Vibrio* sp. was able to grow in absence of sodium chloride, and can also tolerate up to 150 g/L of sodium chloride. These results were observed at the optimum growth temperature (30 °C). In addition, a red pigment, presumably Prodigiosin, was detected in the microorganism. The pigment concentration shows a direct relationship with increasing salinity of the growth medium. On the other hand, *Vibrio* sp. has swimming motility dependent on salt concentration, and we observed the highest motility ratio at 25 g/L of sodium chloride. In addition, only *Vibrio* sp. cells grown in the presence of sodium chloride showed a single polar flagellum, as was evidenced by transmission electron microscopy. In conclusion, our results support the hypothesis that the *Vibrio* sp. is a halotolerant microorganism able to grow in a wide range of salt, and that the ability to choose a mobile or sessile cellular state maybe involved in the stress response.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.013 DISTRIBUTION OF *nifH* GENE IN LEPTOSPIRILLI

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Acidic environments are characterised by low concentrations of bioavailable N-containing compounds. Therefore, the ability of indigenous microorganisms to fix nitrogen, represents an important trait and a key role for the community. *Acidithiobacillus ferrooxidans*, *Acidithiobacillus ferrivorans*, *Leptospirillum ferrooxidans* and *L. ferrodiazotrophum* are considered to be the only lithoautotrophic acidophiles that are able to fix dinitrogen (Norris et al, 1995; Tyson et al, 2005, Hallberg et al, 2010). Analysis of the genome of *L. ferriphilum* strain Sp-Cl revealed the presence genes involved in the nitrogen fixation, though previously, *L. ferriphilum* has not been considered to be a diazotroph. To assess the ability of members of the *Leptospirillum* genus to fix nitrogen, some strains of all three known species were screened for the presence of the *nifH* gene by PCR. Thirteen strains of *Leptospirillum* spp. were grown in autotrophic basal salts medium (pH 1.5), 50 mM of ferrous iron as energy source, at 37°C. Cell lysates were prepared to be used as template in 16S rRNA and *nifH* amplification as previously described (Hallberg et al., 2006; Ueda et al., 1995). All strains of *L. ferrooxidans* and *L. ferrodiazotrophum* yielded positive PCR products for *nifH* gene, with amplicons of the expected molecular weight (290 bp) being obtained. In contrast, 3 out of 5 strains of *L. ferriphilum* (including type strain) gave appropriate-size PCR products. The sequences of the amplified *nifH* fragments showed high similarity amongst all strains examined. This study suggests that some strains of *L. ferriphilum*, like other *Leptospirillum* spp., have the potential to fix dinitrogen. While this important trait appears to be characteristic of all strains of *L. ferrooxidans*, it appears to be more sporadically distributed among strains of *L. ferriphilum*. Given the importance of *L. ferriphilum* in commercial mineral bioprocessing this ability may have important consequences for biomining operations.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.014 CHARACTERIZATION OF MICROBIAL COMMUNITIES FROM A THERMOALKALINE SPRING IN YELLOWSTONE NATIONAL PARK AND ENRICHMENT CULTURES FOR HYDROGEN PRODUCTION

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The objective of this project is to characterize the inhabitants of a thermoalkaline spring in Yellowstone National Park and to enrich for hydrogen production at elevated temperatures. Cultures were enriched with acetate and yeast extract from spring PKF-11 which is a thermoalkaline spring in the Heart Lake Basin of Yellowstone National Park. Spring PKF-11 has an approximate temperature and pH of 75°C and 8.6, respectively, and contains elevated levels of arsenic (804 ppb). Based upon pyrosequence and clonal library determination of SSU rDNA gene sequences, sediment slurry samples were predominated by *Deinococcus-Thermus* (37%), Unknowns (24%), *Firmicutes* (18%), and *Aquificae* (14%). The clonal libraries also detected *Thermodesulfobacteria* sequences that were not detected in the pyrosequencing determinations. Theoretically, 1 mole of acetate can be converted into 4 mole of hydrogen; however, the reaction is not energetically favorable at standard (25°C, 1 atm pressure, 1 mol/kg concentration, pH 7.0) biochemical conditions. Acetate conversion to hydrogen is favorable in equilibrium with atmospheric CO₂ and H₂ and becomes more energetically favorable with increasing temperature (Amend and Shock, 2001). Enrichments with acetate and yeast extract grown at 75°C produced

hydrogen gas and were predominated by sequences most closely related to *Acidobacteria* with approximately 6 major phylotypes. To date, our attempts to isolate hydrogen-producing strains have not been successful and might be a result of interdependent population interactions.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.015 FUNCTIONAL DIVERSITY OF ALPHA-ARHD GENE IN AMAZONIAN DARK EARTH AND BLACK CARBON

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Amazonian Dark Earth (ADE) soils have high concentrations of nutrients, humic substances, organic matter and stable presence of higher amount of black carbon (BC) in relation to adjacent and background soils, and may be a niche of high microbial diversity and a specific environment for biosynthesis and bacterial degradation as well. Therefore, the characterization of functional genes from ADE soil can provide basis on the role of how adaptive microorganisms may influence the balance of fertility of these soils and their involvement in the biogeochemical cycles. This study aimed the evaluation of the functional diversity of catabolic genes for bacterial degradation of aromatic hydrocarbons in ADE soils and black carbon from "Hatahara" site (Iranduba, Manaus-AM), one of the most studied archaeological ADE sites, through cultivation-independent molecular techniques. Two libraries were constructed for ADE soil and BC containing the α -ARHD bacterial gene, in a total of 400 clones. Based upon the results generated by MOTHUR (cut-off 0.05), twenty-seven OTUs (Operational Taxonomic Unit) were defined for the ADE library and thirty-two for BC. Venn Diagram showed twenty-four unique OTUs from BC, suggesting that specific microbial processes may be occurring in this environment. In addition, several bacterial genera related to biodegradation activities were detected solely in the BC library. The richness values determined by *Jackknife*, *Chao1* and *ACE* estimates, as well as the heterogeneity measures *Shannon-Wiener* and *Simpson* indices presented greater diversity in the BC library, with values of 3.05 and 0.05, respectively, and the rarefaction curve approaching to a maximum for both libraries. These results allow to access information about the important role of black carbon as a microhabitat for maintenance of bacterial potentially degraders, at high levels of microbial diversity in Amazonian anthropogenic soils. Support: FAPESP, CNPq

Abstract Category

27 Novel organisms and Novel Functions

PS.27.016 DIVERSITY OF DENITRIFYING ALKALIPHILIC BACTERIA FROM ALKALINE SODA LAKES OF ETHIOPIA

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Denitrifying bacteria were isolated and characterized from four different alkaline soda lakes in Ethiopia. The Ethiopian alkaline soda lakes are well known for their high primary productivity where *Spirulina* is the main primary producer. High primary productivity requires adequate supply of inorganic nitrogen in the form of nitrate. Because some of the highly productive soda lakes have no inflow and outflow it was assumed that the nitrogen form a closed loop between organic and inorganic forms. This means that when *Spirulina* cells die the protein is hydrolyzed and the ammonia released from the deamination of amino acids is converted to nitrate by ammonia oxidizing bacteria and the nitrate is then used by *Spirulina* to synthesize nitrogen containing organic compounds. However, in all the four soda lakes studied, different groups of denitrifying alkaliphilic bacteria were isolated. Some of the isolates are extremely fast denitrifiers. Molecular characterization of these alkaliphilic denitrifying isolates showed that some of them are common in all the lakes while others are unique to each particular lake. In situ measurement of oxygen concentration showed that a few meters or centimeters below the surface the oxygen concentration approaches to zero. Thus the presence of highly efficient denitrifying bacteria indicates that the nitrate released by ammonia oxidizers is rapidly converted to nitrogen gas. Therefore, to support high primary productivity in these soda lakes there must exist some means to fix atmospheric nitrogen. Currently work is in progress to understand the nitrogen economy of these lakes.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.017 ALKALIPHILIC CYANIDE DEGRADING BACTERIA FROM ALKALINE SODA LAKES OF ETHIOPIA

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Cyanide is a potent and fast acting toxic chemical to almost all organisms. A large quantity of cyanide containing wastewater is released into the environment from mining, electroplating, and metal finishing industries. Although physical and chemical have been tried for cyanide removal biological treatment offers several advantages. But, at pH values less than 9.2, cyanide is converted to gaseous hydrogen cyanide and its availability for microbial action is reduced. Thus biological treatment at high pH using alkaliphilic microorganisms is considered to have huge potential. In this study we investigated the diversity of cyanide degrading alkaliphilic bacteria from alkaline soda lakes in Ethiopia. Water and sediment samples were used as inoculum in a reactor continuously fed with cyanide as the sole nitrogen source. The pH of the system was maintained at 10.0 using sodium carbonate. The reactor was run for six months and large quantity of biomass was formed during this period. At steady state a cyanide removal efficiency of 100% was achieved. In addition to cyanide degrading bacteria different groups of alkaliphilic ammonia oxidizing and denitrifying bacteria were isolated from the reactor. To check the diversity of cyanide degrading bacteria in all the alkaline soda lakes pure culture strains were isolated directly from water and sediment samples using cyanide as sole nitrogen source. Some of the cyanide degrading isolates belong to the genera *Halomonas* and *Bacillus* and work on characterization of the remaining isolates is currently in progress. This indicates that naturally

occurring alkaline habitats contain a diverse group of cyanide degrading microorganisms with huge potential to develop biological treatment processes for cyanide containing wastewaters.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.018 DETERMINATION OF THE PHYLOGENETIC AFFINITIES AND ECOLOGIES OF NOVEL FUNGAL PHYLA IN THE BOREAL FOREST

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A fungal clone library spanning partial ribosomal large subunit (LSU) and internal transcriber spacer (ITS) gene-regions constructed by Taylor et al (2007) from soils at the Bonanza Creek LTER (BNZ-LTER) yielded five novel fungal DNA sequences that are divergent from known Phyla, and whose relationships to other fungi are uncertain. Our aims are 1) To determine the phylogenetic affinities of these fungal taxa and 2) To determine the habitat preferences and spatial distributions of these taxa across the boreal forest.

Phylogenies were constructed using the sequences originating from Taylor's fungal clone library. Primers were designed to selectively target each novel taxon. These primers were then used to conduct a PCR survey of organic soil extracts from a single black spruce site. The Boreal Forest Fungal Metagenomics Database was used to search for similar sequences from other BNZ-LTER sites.

The authenticities of four of the sequences were confirmed by re-amplification. Two of these novel sequences appear to be representatives of the recently discovered soil clone group one (SCG1; Schadt et al 2003), while the other three appear to fall within the basal fungal lineages. The PCR survey showed that two representatives of SCG1 and one other novel taxon are abundant throughout the floodplain black spruce (*Picea mariana*) site. The other two taxa were far less frequent and occurred predominantly in one corner of the black spruce site, although one of these taxa was also represented by a single clone in an upland deciduous forest site.

The placements of the three taxa in the basal fungal lineages have little statistical support and remain questionable, suggesting that they represent highly divergent lineages. The results of the PCR survey suggest that SCG1 is widely distributed throughout the boreal forest while the patchy spatial structure displayed by two of the other taxa suggests they have narrow habitat preferences.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.019 CHARACTERIZATION OF SULFIDE DEHYDROGENASE, A THERMOSTABLE ENZYME IN A NON-CULTURABLE LARGE SAUSAGE-SHAPED BACTERIUM

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A white filamentous microbial mat, called a "sulfur-turf", is often found in sulfide-containing neutral to alkaline hot springs at 50-70°C. The sulfur-turf is mainly formed by a large sausage-shaped bacterium that has not been succeeded in culture yet. However, the presence of a large amount of elemental sulfur in this sulfur-turf implies the large sausage-shaped bacterium grows by oxidizing hydrogen sulfide in hot spring water. A sequence-based metagenomic approach was adopted in order to know physiological features of the large sausage-shaped bacterium. Up to the present, it has been revealed that this bacterium possesses the potential lithoautotrophic metabolism such as CO₂ fixation and oxidation of sulfur compounds. The aim of this work is to investigate enzymatic characteristics of sulfide dehydrogenase in the bacterium found by the genome sequence analysis. XanaGenome (Xanagen, Japan), a comprehensive database software for microbial genome analysis, was used to identify the putative genes coding sulfide dehydrogenase. Two putative sulfide dehydrogenase genes (*dhsU1* and *dhsU2*) were found in the genome. To amplify one of these putative sulfide dehydrogenase gene fragments (*dhsU1*: 1392 bp), PCR primers were newly designed and used to amplify. A heterologous expression system of the amplified gene was constructed in *Escherichia coli* BL21(DE3), and a recombinant his-tagged gene encoding sulfur dehydrogenase (MW = 77 kDa) was overexpressed. Sulfide dehydrogenase activity (in a crude extract) was measured by sulfide-dependent horse heart cytochrome c reduction. The recombinant enzyme was significantly thermostable and showed the maximum activity at 70°C and pH 8. This enzymological investigation decisively demonstrated that the non-culturable large sausage-shaped bacterium certainly has sulfide dehydrogenase activity and is able to acquire energy for the growth by oxidizing sulfide.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.020 ISOLATION AND CHARACTERIZATION OF A NOVEL SULFATE-REDUCING BACTERIUM CAPABLE OF DEGRADING *p*-XYLENE

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Crude oil contains various compounds which are toxic to most organisms, and causes significant issue which affects ecosystem seriously, when it is released to environment. Among various constituents of crude oil, monoaromatic hydrocarbons are particularly toxic and have relatively high solubility in water. Because of these characteristics, monoaromatic hydrocarbons are regarded as important pollutants. It has been reported that monoaromatic hydrocarbons are degraded anaerobically by microorganisms under nitrate-reducing, Fe(III)-reducing, sulfate-reducing and methanogenic conditions. Although biodegradation of *p*-xylene, one of monoaromatic hydrocarbons, has been confirmed, there is no pure culture of bacterium capable of degrading *p*-xylene under anaerobic conditions. In this study, *p*-xylene-degrading bacterium was isolated from the highly enriched *p*-xylene-degrading sulfate-reducing culture established with an inoculum of crude oil-contaminated sediment. Isolation was performed by the agar shake dilution using *p*-xylene as a substrate. The isolate could utilize *p*-xylene as a sole source of

carbon and energy, but could not utilize benzene, toluene, ethylbenzene, *o*-xylene, *m*-xylene, and *n*-hexane. The isolate was closely related to *Desulfosarcina ovata* which is *o*-xylene-degrading bacterium and belongs to the family *Desulfobacteraceae* in the class *Deltaproteobacteria*. In addition, we determine the sequence of aromatic hydrocarbon-degrading gene, *bamA*. The *bamA* gene encodes 6-oxocyclohex-1-ene-1-carbonyl-coenzyme A hydrolase which involved in the anaerobic degradation pathway of benzoyl-coenzyme A. The deduced BamA sequence of novel isolate was related to that of *Desulfosarcina ovata*, with similarity of 91%. To our knowledge, isolated strain PP31 is the first anaerobic bacterium degrading *p*-xylene.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.021 PSEUDOMONAS VS PSEUDOMONAS: BIOCONTROL IN ARABIDOPSIS

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The aim of this study was to investigate the potential of cytokinins producing locally isolated bacteria in biocontrol against aerial pathogen of Arabidopsis, *Pseudomonas syringae* pv tomato DC3000. Focus was on the interactions of cytokinins and auxins in such systems. Methodology and Results: Two wild strains of *Pseudomonas aeruginosa* (Am3 and Am7), producing different quantity of phytohormones (cytokinins and IAA) in their cultures as determined via ultra-performance liquid chromatography-electrospray tandem mass spectrometry, were selected. These strains showed variable potential to control disease manifestations in Arabidopsis leaves challenged with the pathogen. While Am3, conferred resistance by increasing cytokinins concentration in the localized area as evident from Arabidopsis ARR5::GUS system, Am7 was ineffective in controlling the pathogen activities. Exogenous cytokinins were also effective against the pathogen in localized tissues of the leaves. Reactive oxygen species (ROS), salicylic acid (SA) and Jasmonic acid (JA) were irrelevant in Am3-pathogen or cytokinins-pathogen interactions. However 13-15 folds increase in the accumulation of phytoalexins (camalexins) was recorded with Am3 and cytokinins as compared to their concentration in mock inoculated leaves. Growth of the pathogen was significantly reduced in Arabidopsis tissues supplied with microbial or authentic cytokinins shown by GFP assay. Introduction of auxins (IAA and 2,4-D) to the system nullified the effect of cytokinins as well as biocontrol bacteria. Conclusions: It may be concluded that *P. aeruginosa* Am3 controls the growth of challenging pathogen in Arabidopsis by increasing cytokinins concentration associated with enhanced camalexins production which can be reversed with exogenous auxins.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.022 DISCOVERY AND VALIDATION OF BIOLOGICAL HYPOTHESES BY ASSOCIATIVE SEARCHES OF FUNCTIONAL GENOMICS DATA

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Functional genomics confronts researchers with a deluge of functional genomic experiments and technologies aimed at understanding biological function at the level of whole cells and genomes. For example, the Environmental Stress Pathway Project 2 (ESPP2) generates gene expression, gene fitness, proteomic, metabolomic, and protein-protein interaction data. In light of this it has become advantageous to: a) simultaneously query multiple data types, b) jointly determine confidence across data layers, and c) systematically form hypothesis from multiple types of evidence. Two dimensional clustering, i.e., biclustering, of gene expression data aims to reveal experimentally verifiable gene co-regulatory associations or regulons. A few bicluster search methods overcome limitations of basic biclustering, such as assigning one module per gene, however a statistical framework for integration of diverse gene and protein data and features remains a challenge. We have developed a random-walk algorithm to search for gene associations that maximize a probabilistic combination of statistical criteria across data types. The algorithm models four data archetypes: gene-by-experiment, gene-by-gene, experiment-by-experiment, and gene-by-feature. We evaluate module discovery using a series of simulated 'biclustering challenge' datasets. We show that the algorithms' performance surpasses existing methods by more accurately identifying multiple, potentially overlapping biclusters while providing statistical associations across data types. To ascertain biological significance we use a diverse *Saccharomyces cerevisiae* functional genomics data compendium including many gene expression conditions and genome-wide data on transcription factor binding sites. Our method provides a novel framework for enhanced discovery of the regulation and consequences of gene transcription. This framework can also be used to search for associations within other biological contexts.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.023 REGULATION OF METHYL-TERT-BUTYL ETHER DEGRADATION PATHWAY IN *METHYLIBIUM PETROLEIPHILUM* PM1

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With low biodegradation rate under oxygen-limited conditions, MTBE is the primary groundwater contaminant in California. Its downstream metabolite, tert-butyl alcohol (TBA), a potential carcinogen is being increasingly encountered at sites historically contaminated with MTBE. *Methylibium petroleiphilum* PM1 is a methylotrophic bacterium capable of completely degrading MTBE and TBA under aerobic conditions. It is imperative to understand the regulation of the pathway to develop efficient approaches to improvise current bioremediation and monitoring strategies. This study aims to (1) determine if the MTBE-degradation pathway in PM1 is inducible and (2) construct a green fluorescent protein (GFP) based reporter vector to identify promoters of *mdpA* and *mdpJ* genes that code for the enzymes involved in the degradation of MTBE and TBA, respectively. To accomplish this, (1) PM1 cells were grown on four substrates: MTBE, TBA, 2-hydroxy Butyric Acid (HIBA) and pyruvic acid. First three are

metabolites in MTBE degradation pathway; pyruvate served as control. mRNA was extracted from cells and analyzed for expression of *mdpA* and *mdpJ* by RT-qPCR using gene-specific primers. (2) Reporter vector to identify promoters of *mdpA* and *mdpJ* was constructed to incorporate a multiple cloning site, *gfp* and streptomycin resistance gene as the selectable marker. It was observed that (1) Cells grown on MTBE had higher expression of *mdpA* than those grown on other substrates. Cells grown on TBA had higher expression of *mdpJ* but not *mdpA*, indicating that MTBE and TBA degradation by PM1 is induced in the presence of respective substrates. (2) Cells transformed with reporter vector did not express *gfp* unless supplemented with a promoter known to be recognized in PM1, establishing that design of the reporter vector is sufficient for subsequent promoter discovery experiments in PM1. This study will further drive our future work on biosensor development for MTBE detection in environmental samples

Abstract Category

27 Novel organisms and Novel Functions

PS.27.024 DIVERSE AND UNIQUE ARCHAEA IN YELLOWSTONE LAKE

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Background and aims: Decades of studies indicated that Yellowstone Lake was a "simple" ecosystem. However, recent evidence of underlying geothermal activities suggested the lake may contain archaeal diversity analogous to terrestrial hot springs and distinct from that found in the very different environment of the photic zone. Therefore, this study aimed to explore archaeal diversity in Yellowstone Lake and relate their distribution to significant geochemical signatures. **Methods:** A remotely operated vehicle was used for lake reconnaissance and sampling from active hydrothermal vents and photic zone waters. Coupled with geochemical profiling, archaeal communities were analyzed by PCR-DGGE, cloning, pyrosequencing, qPCR, FISH, and phylogenetic analysis.

Results: In contrast to photic zone waters, archaea were significant components of vent microbial communities. West Thumb Otter Vent and Deep Vent contained a high diversity of archaeal groups, and 78.1% of the observed OTUs from pyrosequencing reads were found exclusively in vent waters. The major archaeal groups fell within the Deep Hydrothermal Vent Euryarcheotic Group (DHVEG)-6, Deep Sea Euryarcheotic Group (DSEG), *Crenarchaeal* Marine Group I, and *Nitrosocaldus*. Detailed phylogenetic analyses demonstrated that novel Euryarcheotic and Crenarcheotic clades were retrieved from both clone libraries and 454-pyrosequencing. In addition, novel Nanoarcheotic groups were found in West Thumb Deep Vent. These were more closely related to a sequence from the Yellowstone hot spring Obsidian Pool than they were to sequences from Iceland or Kamchatka.

Conclusions: Extend microbial diversity analyses suggest that Yellowstone Lake contains diverse and novel *Nanoarchaeotes*, *Euryarchaeotes*, and *Crenarchaeotes*. From photic zones, shallow vents to deep vents, archaeal groups adapt to localized ecological niches and their distribution correlates to geochemical signatures in the lake.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.025 FUNCTIONAL GENE DIVERSITY OF ANAMMOX BACTERIA

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In the last decade, anaerobic ammonium oxidizing (anammox) bacteria have been recognized as an important sink of fixed nitrogen in the world's oceans and a sustainable way to remove ammonium from wastewater. The ever-growing list of sites where the presence and activity of anammox bacteria have been detected include world's primary production sites (Peruvian and Namibian upwelling zones and the Arabian Sea), lakes, estuaries and virtually any place where oxygen-limitation, ammonium and nitrate co-occur. The first studies on anammox bacteria in these very different ecosystems reported a limited diversity, generally restricted to one genus (*Scalindua*). On the other hand, it was also shown that a single wastewater treatment plant could harbor several anammox species albeit at very low abundances. Dedicated enrichment culture studies revealed that under laboratory conditions, niches that would favor one strain of anammox bacteria could be created. This approach led to the cultivation of "specialized" species such as *Anammoxoglobus*, *Brocadia*, *Jettenia* and *Scalindua* by adding organic acids or sea salt to these enrichment cultures. Besides nitrite and ammonium, the enriched anammox bacteria could oxidize organic acids and Fe(II) and reduce nitrate, Fe(III) or Mn(IV). Physiological experiments illustrated that differences (growth rate, yield, etc.) between species were small and their affinities for different compounds may be the basis for natural selection. Recently the genomes of several anammox bacteria were sequenced. Compared to the previously available genome sequence of *Kuenenia stuttgartiensis*, most of the genes predicted to be involved in the nitrogen and energy metabolism were conserved in the *Scalindua*, *Brocadia* and *Jettenia* genomes. Based on a thorough analysis of the available genomes and comprehensive sequencing efforts, two of the genes/gene products involved in hydrazine metabolism have emerged as targets for detection of anammox bacteria in nature.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.026 ENRICHMENT AND ISOLATION OF ANODE — RESPIRING BACTERIA FROM ENVIRONMENTAL SOURCES USING A LOW — POTENTIAL POISED ANODE

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Anode-respiring bacteria (ARB) are of interest due to their special ability to produce electrical current from organic substrates and wastes in microbial electrochemical cells. Presently, few ARB capable of producing high current densities ($> 5 \text{ A/m}^2$ anode) have been isolated and characterized. The objective of this study was to enrich for ARB biofilms using environmental samples, characterize these ARB biofilm-communities, and isolate novel ARB capable of producing high current densities. We collected soil and sediment from various environmental

sources and tested them as inoculum in a single-chamber microbial electrolysis cell (MEC) using a defined bicarbonate medium with acetate as electron donor. The MEC anode was poised at -0.30 V vs. Ag/AgCl, a potential shown to select for ARB that form a solid conductive matrix and therefore produce high current densities. Three ARB enrichments from our environmental samples show high current density production. The highest current densities observed (11 A/m²) were obtained from mangrove sediments following a two-step enrichment. These current densities are higher than those exhibited by previously characterized ARB communities under similar medium compositions, suggesting the presence of novel ARB or unique microbial interactions. Cyclic voltammograms of the mangrove ARB community show a combined Nernst-Monod curve with two midpoint potentials (E_{KA}). This suggests the presence of at least two ARB that produce current at two different optimal anode potentials. In an effort to identify and characterize these promising novel ARB, we have successfully obtained isolates from the MEC anode biofilm of the three enrichments. For isolation, we used agar plates and/or serial dilutions with the same defined medium containing iron citrate or anthraquinone-2,6-disulfonate as electron acceptor. We are currently testing and characterizing the capability of individual isolates from environmental samples to produce current in MECs.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.027 DIVERSITY, ENRICHMENT AND MECHANISM OF ANAEROBIC NITRITE-DEPENDENT METHANE OXIDIZING 'NC10' BACTERIA FROM WASTE WATER SLUDGE

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Anaerobic sewage treatment has potential advantages compared to conventional aerobic sewage treatment, like lower production of biosolids, lower costs, and lower energy consumption. Anaerobic sewage treatment is possible at low temperatures but high concentrations of dissolved methane and ammonium present in the effluent of domestic waste water currently prohibit application. Recently discovered microorganisms affiliated to the bacterial phylum 'NC10' named '*Candidatus Methylopirabilis oxyfera*', perform nitrite-dependent anaerobic methane oxidation. In order to effectively apply '*M. oxyfera*' in sewage treatment, their metabolism and regulation must be understood. For this purpose (meta)genomic, proteomic and physiological studies were carried out on enrichment cultures of '*M. oxyfera*'. The genome, transcriptome and proteome contained a mixture of anaerobic and aerobic pathways (Ettwig, K. F. et al. Nature 2010). These findings gave new insights in methane oxidation under anaerobic conditions and the potential application of this process in waste water treatment. Ten different waste water treatment plants (WWTP's) were screened for the endogenous presence of '*M. oxyfera*' using molecular diagnostic methods. We could identify 'NC10' bacteria with a 98% similarity to '*M. oxyfera*' in nine out of ten WWTP's tested. Furthermore, sludge from one selected WWTP was used to start a new enrichment culture of 'NC10' bacteria. After ten months, this enrichment

culture showed nitrite-driven activity coupled to anaerobic methane oxidation. These data will help to implement the application of 'NC10' bacteria in future waste water treatment.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.028 IDENTIFICATION AND CHARACTERIZATION OF A NOVEL DENITRIFYING PHOSPHORUS-REMOVAL BACTERIUM, PSEUDOMONAS STUTZERI B204

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To study the mechanism of simultaneous removal of the phosphorus and nitrogen simultaneous removal, a denitrifying phosphorus-removal bacterium (DPB) named b204 was isolated and identified, its characteristics of growth and P/N-removal were investigated. Strain b204 belongs to *Pseudomonas stutzeri* according to 16S rRNA gene analysis. The generation time and specific growth rate were 4.5h and 0.154/h, respectively. It could grow significantly faster than plenty of phosphate-accumulating organisms (PAOs). After 26h cultivation, more than 60% of P, nitrate and nitrite were removed. In different electron acceptor conditions, such as nitrite, oxygen and nitrate, the phosphorus uptake rate were almost identical. The results indicated that strain b204 had an excellent potential for eliminating phosphorus and nitrogen.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.029 NOVEL ACTINOBACTERIA RECOVERED FROM MARINE SEDIMENTS TAKEN FROM THE SEA OF CORTES

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Little is known about the diversity of *Actinobacteria* in marine ecosystems though the description of the first sea-water dependent actinobacterial genus (ie. *Salinispora* Maldonado *et al.* 2005) supports the view that the marine habitat still constitutes an untapped source of microbial diversity. Hence, novel approaches that could enhance the recovery rate of this microbial group and improve our current understanding of the true diversity of these microbes are urgently needed. Success in recovering members of new actinobacterial taxa is crucially dependent on the use of improved selective isolation strategies, sample pretreatments and the dereplication of isolate collections. This work focused on the selective isolation of *Actinobacteria* from marine sediments collected in the Sea of Cortes at a depth of 300 and 900

meters. Media of known formulation were prepared either with natural seawater or normal water in 1:10, 1:100 and 1:1000 dilutions to selectively isolate marine *Actinobacteria*. 300 strains were selected on the basis of their genetic and morphological properties to establish their precise taxonomic position. Recovery rates for "rare" actinobacteria were higher when diluted media (1:100 and 1:1000) were employed and members of the genera *Actinomadura*, *Arthrobacter*, *Micrococcus*, *Micromonospora*, *Nocardia*, *Nocardiopsis*, *Nonomuraea*, *Pseudonocardia*, *Saccharomonospora*, *Saccharopolyspora*, *Salinispora*, '*Solwaraspora*', *Streptomyces*, *Streptosporangium*, *Verrucosipora* and three putative novel genera were found after 16S rRNA gene sequencing of representatives of the resulting clades. A reappraisal of the '*Micromonospora-Rhodococcus-Streptomyces*' unique marine grouping is needed since it seems likely that this actinobacterial-marine category does not fully represent the extent of this group in such ecosystems.

Part of this work was presented at the Marine Natural Products Gordon Research Conference in Ventura California, February 28th - March 5th 2010

Abstract Category

27 Novel organisms and Novel Functions

PS.27.030 DIVERSITY OF BACTERIAL LACCASE-LIKE GENES IN DRAINED PEAT SOILS

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Laccases of fungal origin have been intensively studied due to their importance in various biotechnological applications. There is constant demand for new laccases with improved properties such as stability at higher temperatures or at an alkaline pH. Growing molecular evidence suggests that laccases may also be widespread in bacteria. While only a handful of bacterial laccases have been purified and characterized, several novel traits have already been discovered (e.g., pH-stability and 2-domain organization of the enzyme as opposed to the usual 3-domain structure of the fungal laccases). The aim of this study was to examine the diversity of bacterial laccase-like genes in two types of high organic peat soil using a cloning and sequencing approach. Gene libraries of small fragments (150 base pairs) revealed an amazing diversity of bacterial laccases. The fragments clustered in 11 distinct lineages, and one third of 241 sequences resembled laccase-like genes of Acidobacteria. Additionally, a new primer was used to retrieve several large fragments of the putative bacterial laccase genes that spanned all four copper-binding sites. Both "conventional" 3-domain laccases and the newly discovered 2-domain laccases have been obtained using this approach, demonstrating the potential usability of the primer. The present study thus contributes to the understanding of the diversity of bacterial laccases and provides a new tool for finding laccase-like sequences in bacterial strains and environmental samples.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.031 GENETICS AND BIOCHEMISTRY OF THE GLUTAMATE-MEDIATED METHYLAMINE UTILIZATION PATHWAY IN METHYLOTROPHIC PROTEOBACTERIA.

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The ability of some microbial species to oxidize monomethylamine via glutamate-mediated pathways was proposed in the 1960s, however, genetic determinants of the pathways have never been described. In this study we describe a gene cluster essential for operation of the N-methylglutamate pathway in the methylotrophic beta-proteobacterium *Methyloversatilis universalis* FAM5. Four major polypeptides from protein fractions displaying high activities of N-methylglutamate synthetase, N-methylglutamate dehydrogenase and gamma-glutamylmethylamide synthetase were selected for mass spectrometry-based identification. The activities of enzymes were associated with the presence of peptides identified as ferredoxin-dependent glutamate synthase (GltB2), large subunit of putative heterotetrameric sarcosine oxidase (SoxA), and glutamine synthetase type III (GSIII), respectively. A draft genome of this methylotrophic beta-proteobacteria was generated, and corresponding genes were subjected to mutational analysis, functional and metabolic profiling. We demonstrated that these genes play a key role in methylamine utilization and encode N-methylglutamate synthetase, N-methylglutamate dehydrogenase, and gamma-glutamylmethylamide synthetase. Homologous genes were identified in a wide variety of sequenced bacterial genomes, implying a wide distribution of the methylamine utilization pathway in nature. Purification and biochemical characterizations of the enzymes are currently on the way.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.032 THIOSULFATE-DEPENDENT CHEMOLITHOAUTOTROPHIC GROWTH OF *BRADYRHIZOBIUM JAPONICUM*

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Thiosulfate-oxidizing *sox* gene homologues were found at four loci (I, II, III, and IV) on the genome of *Bradyrhizobium japonicum* USDA110, a symbiotic nitrogen-fixing bacterium in soil. In fact, *B. japonicum* USDA110 can oxidize thiosulfate and grow under a chemolithotrophic condition. The deletion mutation of the *soxY₁* gene at *sox* locus I, homologous to the sulfur-oxidizing (Sox) system in *Alphaproteobacteria*, left *B. japonicum* unable to oxidize thiosulfate and grow under chemolithotrophic conditions, whereas the deletion mutation of the *soxY₂* gene at *sox* locus II, homologous to the Sox system in green sulfur bacteria, produced phenotypes similar to wild-type USDA110. Thiosulfate-dependent O₂ respiration was observed only in USDA110 and the *soxY₂* mutant, not in the *soxY₁* mutant. In the cells, 1 mol thiosulfate was stoichiometrically converted to approx 2 mol sulfate, and consumed approx 2 mol O₂. *B. japonicum* USDA110 showed ¹⁴CO₂ fixation under chemolithotrophic growth conditions. The CO₂ fixation of resting cells was significantly dependent on thiosulfate addition. These results show that USDA110 is able to grow chemolithoautotrophically using thiosulfate as an electron

donor, oxygen as an electron acceptor and carbon dioxide as a carbon source, which likely depends on *sox* locus I including *soxY₁* gene on USDA110 genome. Thiosulfate oxidation capability is frequently found in members of the Bradyrhizobiaceae, which phylogenetic analysis showed to be associated with the presence of *sox* locus I homologues including *soxY₁* gene of *B. japonicum* USDA110.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.033 DIETZIA SP., A NOVEL TYROSINASE PRODUCING HOT SPRING ISOLATE

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Tyrosinases are ubiquitously distributed oxidative enzymes that utilise monophenols, aromatic amines and *o*-aminophenols as substrates, catalysing their *ortho*-hydroxylation and oxidation reactions. Actinobacteria, mycelious Gram-positive bacteria that are involved in the formation and degradation of complex biopolymers, are known to produce tyrosinases. The aim of the study is to identify and characterise novel actinobacteria that exhibit tyrosinase activity. Eight Actinobacterial isolates from thermophilic environments were tested for tyrosinase activity using L-dihydroxyphenylalanine and *p*-cresol as substrates. After preliminary kinetic data confirmed tyrosinase activity, the optimal pH and temperature conditions as well as the extracellular melanogenic properties were determined. A Zambian hot spring isolate showing was selected for further study due to its rapid generation time and nocardioform morphology. It was identified by 16S rRNA PCR as belonging to the recently identified genus *Dietzia*. Phylogenetic analysis showed 96 % and 97 % homology to the genus type strain *D. maris* and *D. cinnamea*, respectively. These data, together with the morphological and chemical properties suggest that the isolate is a novel species. The eight currently known members of this genus exhibit industrially relevant traits such as biodegradative activity and biodemulsifying properties, as well as being considered as candidates in the commercial production of carotenoids. The isolate is the first tyrosinase-positive member of the genus *Dietzia*. The isolate showed broad pH and temperature profiles, with the growth rate comparable to those of other *Dietzia* species. PCR primers were designed to conserved regions of actinobacterial tyrosinase genes. The amplified product generated with the isolate genomic DNA was labelled and applied as a Southern hybridisation probe to facilitate the construction of a small insert library in an *E. coli* — *Streptomyces* shuttle vector, which will be used for the cloning and expression of this novel *Dietzia* tyrosinase gene.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.034 ISOLATION AND GENOME SEQUENCING OF A NOVEL ANTARCTIC TEMPERATE PHAGE

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Bacteriophages have been shown to be the most numerous and diverse biological entities in the biosphere. Phage sequence space represents a large and, as yet, unexplored source of novel genetic tools and enzymes with potential application in fundamental biological research, medicine and biotechnology. Of increasing interest is the isolation and characterisation of microbes from extreme environments as adaptation to these conditions can be exploited for biotechnological and industrial purposes. Antarctica is considered one of the most extreme environments on earth with a range of highly specialised microbial biotopes. In this study a novel temperate phage, termed C2, was isolated from a psychrotrophic Antarctic continental soil bacteria, *Psychrobacter antarcticus*. Prophage induction was achieved through Mitomycin C treatment. TEM analysis of the induced phage revealed an isometric head with a long noncontractile tail placing the phage in the Siphoviridae family. The genome sequencing and annotation of phage C2 is near completion. The C2 genome has a GC content of 44.3%, with 43 protein-coding genes and 1 tRNA gene predicted. The genome is organised in a modular fashion: host specificity, tail assembly, head assembly, packaging, replication, regulation and integration. Several of the predicted ORFs within the head and tail modules had significant similarity (64-94% identity) to a predicted prophage within the sequenced genome of *P. arcticus*. Other predicted proteins show low similarity to proteins of *Pseudomonas*, *Burkholderia* and *Acinetobacter* phages. At least 40% of the predicted proteins encoded by C2, however, showed no homology to proteins with known functions and could therefore not be assigned functions in silico. These results support the current view that the genomes of many tailed phages are mosaics that arise through genetic exchange from a diverse pool of phages.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.035 DISCOVERY OF NOVEL MICROORGANISMS AND ANTIMICROBIAL TRAITS IN NATURAL DISEASE SUPPRESSIVE SOILS

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Soil ecosystems represent an enormous untapped resource for discovering novel microorganisms, traits and bioactive genes. Specific soils have been identified worldwide in which beneficial microorganisms guard plants against infections by soil-borne pathogens. However, the microbiological and molecular mechanisms underlying this soil immune response are largely unknown. In the present work, the microorganisms and mechanisms involved in natural suppressiveness of soils to the fungal pathogen *Rhizoctonia solani* were investigated by culture-dependent and independent approaches. Culture-based analyses revealed quantitative and qualitative differences in bacterial populations between *Rhizoctonia* suppressive and conducive

soils. Subsequent genotyping and functional analyses led to the identification of novel *Pseudomonas* species producing yet unidentified chlorinated peptide antibiotics. The cultivation-independent approaches included PhyloChip-based community analysis and activity profiling by metagenomic analysis of rhizosphere RNA. The Phylochip analysis distinguished the bacterial communities from soils with different levels of disease suppressiveness and specifically pointed to the Proteobacteria as an important rhizosphere competent phylum and dynamic group associated with disease suppression. Although the richness observed in either suppressive or conducive soils was not significantly different, the relative abundance for some groups correlated well with the different levels of soil suppressiveness. While the cultivation-dependent approach resulted mainly in isolates of the γ -Proteobacteria class, the Phylochip analysis revealed bacteria belonging to several phyla, including Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, Acidobacteria, Planctomycetes and Chloroflexi. In conclusion, this study provides new insights into the microbial diversity and fundamental mechanisms underlying multitrophic interactions in natural disease suppressive soils.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.036 CHARACTERIZATION OF AN ELECTROCHEMICALLY ACTIVE BACTERIAL STRAIN ISOLATED FROM A MICROBIAL FUEL CELL ELECTRODE

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Following recent advances in microbial fuel cell (MFC) technology, the study of electrochemically active bacteria is imperative. Electrochemically active bacteria are able to grow by transferring the electrons produced from organic substrate degradation to a solid electrode in an MFC. Exocellular electron transfer has been shown to be an important factor in anaerobic respiration of microbial communities that degrade organic matter. In order to gain a better understanding of these microbial exocellular electron transfer processes in MFCs several bacterial strains were isolated from a high current-density electrode. An electrochemically active strain that was isolated was chosen for detailed physiological and phylogenetic studies. The strain detected on the surface of the electrode, as shown by denaturing gradient gel electrophoresis, was isolated from an Fe(III)-reducing, acetate-oxidizing enrichment of biofilm obtained from the anodic electrode. Phylogenetic analyses of the electroactive strain were performed based on 16S rRNA, %G+C content and DNA-DNA relatedness to closest phylogenetic relatives. Physiology studies included utilizable electron donor and acceptors and comparison of protein and cytochrome content of the strain between cells grown *in-vitro* and cells forming the electrochemically active biofilm on the MFC anode. In order to further characterize the strain in its electroactive state we used different voltammetric techniques and determined maximum power output.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.037 EVOLUTIONARY FATE OF GENE DUPLICATES IN THE GENOMES OF CHLOROPHYLL *D*-PRODUCING CYANOBACTERIA: IMPLICATIONS FOR THE ECOLOGICAL NICHE

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Gene duplication is a potentially important mechanism for the ecological tuning of an organism to its environment via dosage effects and/or for the evolutionary innovation of novel functions. Here, we analyzed the fate of gene duplicates for two strains (*Acaryochloris* strains MBIC11017 and CCMEE 5410) of a novel group of cyanobacteria that produces the far-red light absorbing chlorophyll *d* as its main photosynthetic pigment. The genomes of both strains contain an unusually high number of gene duplicates for bacteria. As has been observed for eukaryotic genomes, we find that the demography of gene duplicates can be well-modeled by a birth-death process. Most duplicates have a comparatively recent origin and are unique to individual strains. Analyses of selection on duplicates of different age classes suggest that paralogous copies exhibit near neutral evolutionary dynamics immediately following duplication but that duplicate pairs which have been retained for longer periods are under strong purifying selection against amino acid change. We conclude that most duplicates are quickly purged from the genome and that those which are retained likely confer fitness benefits to the organism primarily via dosage effects. It was also possible to infer potential ecological differences between the strains based on their differences in retained recent duplicates. For example, the amplification of several iron transport and metabolism genes in *Acaryochloris* strain MBIC11017 that exist as single copies in *Acaryochloris* strain CCMEE 5410 likely reflects differences in the nutrient status of their respective environments.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.038 GLOBALLY-DISTRIBUTED LINEAGES OF PUTATIVE SULFATE-REDUCING FIRMICUTES FROM VARIED DEEP SUBSURFACE HABITATS

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Candidatus *Desulforudis audaxviator* is a novel predicted bacterial genus and species, first reported as indigenous to the continental deep subsurface accessible via the ultradeep mines of South Africa. Here this organism appears to dominate rock-hosted and fracture water microbial communities at depths greater than 2 km below land surface from multiple mines across a large

geographic area. Owing to its dominance at depth (analogous to a nearly pure culture), a complete genome of *D. audaxiator* was recently obtained; an examination of which supports an inferred lifestyle based upon the utilization of hydrogen and sulfate produced by natural lithospheric radioactivity. More recently, relatives of this microorganism have been noted in SSU rRNA gene libraries by our group from other continental settings, including deep wells which sample carbonate aquifers in the US' Mojave Desert and in fluids from underground nuclear detonation cavities at the Nevada Test Site (NTS). The NTS clones in particular are of possible significance, sharing about 98% SSU rRNA gene sequence identity with *D. audaxiator* and inhabiting an anthropogenic analog of this organism's original reported habitat. These clones, along with others recently detected from flowing boreholes at off-axial marine settings and a geothermal heating system in Europe hint at a globally-prominent role in hydrogen-rich subsurface settings for members of this group.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.039 DIVERSITY OF AZO DYE DEGRADING ALKALIPHILIC BACTERIA FROM A NATURALLY OCCURRING AKLAINE HABITAT

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Different alkaliphilic bacteria capable of decolorizing azo dyes released from textile industry wastewater were isolated and characterized. Out of a total of 120 strains tested six showed dye decolonization efficiency of 90-100% within 48 h when grown under anaerobic or anoxic condition. However, under anaerobic condition no dye decolonization was observed indicating that the azo dye is used as final electron acceptor. Based on 16S rRNA sequence the six dye decolorizing alkaliphilic isolates found to belong to the genera *Bacillus* and *Halomonas*. As textile wastewater has high pH our data indicates the potential usefulness of alkaliphiles from naturally occurring habitats to develop efficient treatment processes. To further test the potential of alkaliphiles from alkaline soda lakes for textile dye removal an oxic-anoxic reactor continuously feed with textile industry wastewater was inoculated with water and sediment sample from one of the soda lakes in Ethiopia, L Chitu, and run for three months. The dye removal efficiency of reactor was 100%. The diversity of alkaliphilic microorganisms which brought about complete degradation of the dye is currently under investigation.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.040 PHYSIOLOGICAL FUNCTIONS OF FRESHWATER *THIOPLOCA* DEDUCED FROM GENE ANALYSIS

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The filamentous sulfur-oxidizing bacteria of the genus *Thioploca* accumulate nitrate and elemental sulfur in their cells. It was reported that marine *Thioploca* species contribute

significantly to nitrogen cycling in the sediment and overlaying water because of their large biomass and capability to accumulate nitrate in their cells. Further it can be expected that the contribution of *Thioploca* to local material cycling in their habitat is more significant in lakes than in ocean since the lakes are closed water area. However, the knowledge about freshwater *Thioploca* is fewer than about marine *Thioploca*. In this study, we estimated physiological functions of freshwater *Thioploca* with the basis of gene analysis on samples from four lakes, including a newly found habitat, Lake Okotanpe, Japan. PCR were performed with DNA extracted from the *Thioploca* samples from four lakes. The primers for 16S and 23S rRNA genes were used for phylogenetic analysis. The primers for *soxB* and *aprA* genes were tested to deduce sulfur oxidation pathway, and *nrfA*, *nirS*, *nirK* and *nosZ* genes were investigated as genes involved in nitrate respiration. Several pairs of primer to amplify RubisCO gene were also used to examine carbon fixation pathway. The PCR products obtained were subjected to cloning analysis or direct sequencing. The morphology of *Thioploca* from L. Okotanpe was similar to that of freshwater *Thioploca* from other lakes. The phylogenetic analysis based on 16S and 23S rRNA gene revealed that *Thioploca* of L. Okotanpe was closely related to that of other lakes. Based on phylogenetic analysis, it was suggested that the obtained sequences of *soxB* and *aprA* genes originated from *Thioploca*. In the cloning analysis of *soxB* gene, phylogenetically closely related sequences were obtained from the DNA samples from all lakes. In conclusion, it seemed that freshwater *Thioploca* species have the oxidation pathways of thiosulfate and sulfite.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.041 NOVEL PHENAZINE-PRODUCING FLUORESCENT *PSEUDOMONAS* SPP. ISOLATED FROM DRYLAND WHEAT THROUGHOUT THE INLAND PACIFIC NORTHWEST

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Certain strains of the rhizosphere bacterium *Pseudomonas fluorescens* produce phenazine antibiotics that suppress soilborne root pathogens of cereal crops. Phenazine producers contain the operon *phz*ABCDEFG and the essential biosynthesis gene *phzF* is conserved across all strains. In 2007 and 2008, we discovered abundant populations of *phzF*⁺ strains on roots of dryland cereals grown in the Inland Pacific Northwest, U.S.A. The aim of the present study was to determine the diversity within these populations and to identify biogeographical factors influencing their distribution. To examine population diversity, 397 *phzF*⁺ strains of *Pseudomonas* were isolated from the roots of dryland wheat and barley grown in central Washington State. Genotyping alongside *P. fluorescens* 2-79, a *phzF*⁺ reference strain isolated originally from the same region, revealed 31 genotypes via whole genome BOX-PCR. Near complete 16S rDNA sequence analysis revealed close relationships to *P. orientalis*, *P. synxantha*, *P. libanensis*, and *P. gessardii*. A comparison of phylogenies derived from 16S rDNA + *recA* and from *phzF* showed group-specific sequence conservation and no evidence of inter-population horizontal gene transfer. Phenotypic characterization using Biolog GN-2 plates

indicated group and genotype-specific profiles that differed from those of the related species. Geographically, an area of about 22,000 km² was sampled for *phzF*⁺ populations. Large populations and high colonization frequencies dominated in non-irrigated fields with low (150-400 mm annually) precipitation over a region encompassing 1.56 million cropland hectares, while the opposite was found in irrigated fields and those with higher precipitation. The robust diversity and specific, yet widespread, biogeography indicate that the capacity to produce PCA is broadly distributed among diverse strains of fluorescent *Pseudomonas* species endemic to non-irrigated dryland cereal crops.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.042 INCREASE OF BIOACTIVE SECONDARY METABOLITES IN STREPTOMYCES SCABIEI THROUGH UV MUTAGENESIS

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The actinomycete genus *Streptomyces* produces a wide array of secondary metabolites, many of them having important biological activities. The production of bioactive microbial secondary metabolites is often controlled by non-ribosomal peptide synthetases (NRPSs). The production of thaxtomin A, the main phytotoxic compound produced by a common potato scab, *Streptomyces scabiei*, is controlled by a bimodular NPRS, designated TxtA and TxtB. A series of UV mutagenesis experiments were conducted to induce changes in the bimodular NPRS system and to increase the titre of thaxtomin A (4-nitroindol-3-yl-containing 2,5-dioxopiperazine) in a liquid culture. A selection program for *S. scabiei* was carried out through six cycles of mutation and selection for increased thaxtomin A production, measured using liquid-liquid extraction and HPLC quantification. For the initial cycle, a conidial suspension was treated with UV light, the survivors were plated and about 10 colonies were picked for subsequent liquid cultures. In each cycle, the five best candidates with the highest thaxtomin A titres were picked and carried forward to the next cycle. Highly significant differences in thaxtomin A titre were detected in each cycle, and a near threefold increase in productivity compared with the wild-type was achieved over the six cycles.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.043 EXPLOITABLE STREPTOMYCETES DIVERSITY ON THE BASIS OF BIOSYNTHETIC AND SYSTEMATIC TOOLS

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Members of the genus *Streptomyces* are an extremely diverse group of economic importance as a biotechnological source for novel natural compounds. *Streptomyces* can be found in a wide diversity of ecological sources where they play an important ecological role. Isolation of *Streptomyces* has been of importance as part of drug discovery programs and has focused on terrestrial sources. Despite their importance, there are no reports of the intrinsic diversity, distribution and ecology of *Streptomyces* in Mexican soils that serve for agricultural purposes. This work concentrated on the selective isolation, characterization and detection of biosynthetic clusters in *Streptomyces* isolated from Mexican agricultural sites. Fifty-three *Streptomyces* were isolated from soil samples collected from different agricultural locations. The assignment of the isolates to the genus level was achieved by using chemotaxonomic and molecular techniques, namely cell wall composition and genus-specific primers. To evaluate the diversity of the isolates, molecular fingerprinting methods focused on random amplification of repetitive sequences, modular polyketide synthases, iterative polyketide synthases, and non-ribosomal peptide synthetase primers to group the strains into clades on the basis of their unique genomic information. Fingerprinting using outwardly facing primers with the molecular techniques suggested high genomic heterogeneity within the isolates and this was also supported by fingerprints obtained with the biosynthetic genomic information. A consensus dendrogram grouped the strains into several subclades, and 16S rRNA gene sequencing of representatives of the subgroups showed putative novel species among the nearly 600 *Streptomyces*. The antimicrobial activity of the isolates against pathogens of both human and agricultural importance supports the view that such soils may represent a good source of unknown species that could be studied for biotechnological purposes.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.044 CHARACTERIZATION OF A BACTERIUM FROM THE RARELY-CULTIVATED PHYLUM GEMMATIMONADETES

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Bacteria belonging to phylum Gemmatimonadetes are frequently detected in a variety of environments using culture-independent methods. Despite their ubiquity and prevalence, almost nothing is known about their physiology or ecology due to lack of cultured representatives: To date, only one bacterium in this phylum has been characterized (*Gemmatimonas aurantiaca* T-27, isolated from wastewater). The objective of this study was to culture and characterize a novel Gemmatimonadetes strain (KBS708) in order to determine its functional or ecological niche. KBS708 was randomly isolated from agricultural soil after 36 days on minimal media (VL55) with peptone. Culture purity was confirmed by 16S RFLP analysis. 16S rRNA gene sequencing indicated KBS708 belongs to phylum Gemmatimonadetes with less than 94% similarity to other uncultivated Gemmatimonadetes and only 90% similarity to *G. aurantiaca* T-27, indicating it is

the first cultured representative of a new family within this phylum. KBS708 is a gram negative chemoorganoheterotroph which forms a biofilm when grown in static liquid cultures. Liquid culture growth is flocculated and occurs at a pH range 4-7, with an optimum of pH 6 and at a temperature range of 20-31°C, under light and dark conditions. Slow growth on the surface of plates and rapid growth when embedded within agar suggests a sensitivity to oxygen. KBS displays a unique pleomorphic morphology and resistance to ampicillin and lysozyme suggesting it may lack a cell wall. Other interesting morphological features revealed by SEM and TEM include evidence of an S layer, abundant membrane vesicles, and exopolysaccharides. Cells range from 0.5-0.7 µm in diameter and 1-16 µm in length, though it is unclear if these longer objects are elongated individual cells or chains of multiple cells encased within an extracellular sheath. Genomic sequence of KBS708 is in progress and will provide further insight into its physiology and ecological niche.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.045 SYSTEMATIC AND GENOMIC ANALYSIS OF *PLANOCOCCUS* SP. UTK ISOLATED FROM AN ANTARCTIC SOIL SAMPLE

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An orange-pigmented bacterial strain, UTK, was isolated from an Antarctic soil sample taken from the edge of an ephemeral coastal pond. This isolate grew optimally at pH9.0, 0.1M NaCl and 37°C. The cells were Gram-positive cocci that form pairs, tetrads and aggregates. Membrane lipid analysis showed that anteiso-C15:0 was the major fatty acid. The G+C content of the genomic DNA was 46.8%. 16S rRNA gene sequence analysis suggested this isolate was a member of the genus *Planococcus* and was closely related to *P.maritimus*, *P. rifietoensis*, and *P. maitriensis*. Notably, this isolate exhibited some growth in the minimal medium using cellulose as sole carbon and energy source at alkaline pH, that suggested it might possess a commercially valuable enzyme system in the biorefinery. To rapidly identify the cellulolytic enzymes in this bacterium, the genomic DNA was sequenced by current 454 GS FLX sequencer with Titanium series reagents. A single run produced a total of 200,966 reads and 75,398,539 bases with an average read length of 375 bases, providing an estimated 22 fold average coverage depth of the genome. The 454 assembly software, Newbler, yielded unordered contigs, which consisted of 102 large contigs (maximum length 228,615 bp). Initial genome annotation by RAST revealed a total of 3366 predicted gene products in which 1192 were classified in to 23 functional subsystems. One potential cellulose gene was identified in the genome. Further genomic and biochemical analysis will elucidate the potential of this strain for biomass conversion, and also aid our understanding of the adaptations that allow this organism to survive in the extreme Antarctic soil environment.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.046 ISOLATION AND CHARACTERIZATION OF A SULFATE-REDUCING BACTERIUM FROM U(VI)-CONTAMINATED GROUNDWATER

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Desulfovibrio FW-101-2B was isolated from contaminated groundwater at the Field Research Center at Oak Ridge National Lab (ORNL-FRC) near Oak Ridge, Tennessee. The ORNL-FRC is comprised of four unlined waste disposal ponds which were used for approximately 30 years, and contain approximately 85 million gallons of nitric acid mixed with uranium, technetium, and other heavy metals and organic compounds. Seepage of these wastes into the surrounding bedrock has created one of largest mobile subsurface uranium plumes in the United States. Biostimulation with ethanol as a carbon and electron source yielded reduction of soluble uranium from the groundwater in monitored wells, and community analysis indicated a predominance of *Desulfovibrio* species in the groundwater. Ethanol enrichments for sulfate-reducing bacteria taken from well FW-101 were plated on a solid medium, upon which *Desulfovibrio* FW-101-2B arose as a single dark-brown hemi-spherical colony. *Desulfovibrio* FW-101-2B utilized several three- and four-carbon organic acids and three-carbon diols as electron donors and sole carbon sources. Sulfate and thiosulfate were used as electron acceptors. Presence of sodium nitrate did not affect growth at 20mM, however, growth did not occur when nitrate was the only nitrogen source. Uranium(VI) at concentrations up to 100µM did not affect growth, whereas chromate induced a carbon source-dependent lag phase of at least 150 hours. Whole genome sequencing has been completed at the Joint Genome Institute. Genome sequence determination revealed a single circular chromosome of 4.1 MB and a G+C content of 66.5%. The genome is predicted to contain 3,737 protein-encoding genes. Based on KEGG predictions, the closest taxonomic matches of fully-sequenced organisms are *D. vulgaris* Miyazaki, *D. desulfuricans*, and *D. vulgaris* DP4. However, based upon 16S rDNA sequencing, the closest cultured relative is *Desulfovibrio carbinoliphilus*.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.047 DETECTING NOVEL SPECIES AND GENERA FROM SHORT NEXT-GEN READS

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High-throughput, next-generation sequencing technologies enable us to obtain DNA sequence information from the mass amount of organisms that have not been previously sequenced. The most accurate solutions to the read-to-taxa classification problem require supervised methods, which are limited by the size of the database. Every database is highly incomplete since there are only thousands of genome sequencing projects, while there are millions of species that exist. We now address the problem of identifying "unknown" taxa and demonstrate a detector on species- and genus-level ranks. We show that it possible to use a naive Bayes classifier (NBC) and an

interpolated markov model-method, PhymmBL (and implicitly BLAST), to detect genomes of novel species and genera from short reads (25-500bp). While NBC, BLAST, and PhymmBL can classify novel genomes to their nearest class in the database, they currently lack the capacity to detect if these genomes belong to a novel taxa. We demonstrate that by developing a detector, we can increase accuracy rates of classification by introducing the "novel" class. For example, on a test set, for which NBC/BLAST/PhymmBL can only achieve 21-28% accuracy for species classification, they improve by over 50% by filtering out the novel genomes before classifying the rest. Also, we show on a test-set that the detectors can detect known/unknown species with an accuracy of 85% for the species-level and 65-70% for the genus-level. While the PhymmBL detector is not optimal for the genus-level, it outperforms all other methods on a test set.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.048 MOLECULAR CHARACTERIZATION AND PHYLOGENY OF NOVEL HERBICIDE-DEGRADING *STENOTROPHOMONAS* SP. STRAINS ISOLATED FROM CHILEAN AGRICULTURAL SOIL

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Simazine has been one of the most extensively used s-triazine herbicides for weed control in agricultural soils. Native s-triazine-degrading microorganisms have been used for bioremediation of polluted sites. The aim of this study was to characterize strains of *Stenotrophomonas* spp. and their relevance for simazine degradation. Diverse *Stenotrophomonas* strains, isolated by enrichment from two agricultural soils with histories of simazine application and from a control soil of central Chile, were able to grow on simazine as the sole nitrogen source. A Multi-Locus Sequence Analysis (MLSA), using 16S rRNA genes and *gyrB* and *rpoD* house-keeping genes enabled differentiation and species-level identifications for the isolates as a species of *Stenotrophomonas*. All simazine-degradation genes, *atzA*, *atzB*, *atzC*, *atzD*, *atzE* and *atzF*, responsible for s-triazine-mineralization, were detected by PCR-amplification and sequence analyses in four strains. Simazine-degradation by resting cells was studied in two strains that possess all *atz* genes, showing the ability to degrade at least 50% of the initial simazine in less than one hour. All other strains of the *Stenotrophomonas* sp. possessed one or more of the *atz* genes and demonstrated varying degrees of simazine-degradation. The 16S rRNA gene sequence as well as *gyrB* and *rpoD* sequence analyses indicate that the isolated strains probably comprise a distinct and novel species of *Stenotrophomonas*. The effectiveness of a MLSA strategy has been demonstrated for reliable identification of bacterial strains isolated from environmental samples. s-Triazine-degrading bacteria isolated from contaminated soils could be applied as biocatalysts for bioremediation of these herbicides. Acknowledgements: Milenio-P04/007-F (Chile) (MS), USM 130836 and 130948 (MS), ALF, Västra Götlandsregionen Sweden FOU-72241 (EM), Health Canada 4500118123 (LS, EM), INCO ICA4-CT-2002-10011, European Union (MS, EM), CONICYT fellowship (MH).

Abstract Category

27 Novel organisms and Novel Functions

PS.27.049 MESOPHILIC FACULTATIVE METHANOTROPHY: CHARACTERIZATION OF A NOVEL METHANOTROPH THAT CONSTITUTIVELY EXPRESSES PMMO WHEN GROWN ON ACETATE

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An aerobic methanotrophic bacterium was isolated from a spring bog in southeast Michigan, and designated strain SB2. This bacterium was found to be a Gram-negative, non-motile, slightly curved rod, with intracytoplasmic membranes aligned along the periphery of the cell. These cells expressed the membrane-bound or particulate methane monooxygenase (pMMO) as well as a chalkophore with high affinity for copper and did not express the cytoplasmic or soluble methane monooxygenase (sMMO). Phylogenetic comparisons based on both *pmoA* and 16S rRNA sequences indicated that this cell belonged to the *Methylocystis* genus, and was closely related to *Methylocystis rosea* SV97T. Unlike *M. rosea* SV97T, strain SB2 was able to utilize not only methane for growth, but also ethanol and acetate. The particulate methane monooxygenase was found to be constitutively expressed in the absence of methane on cells grown on acetate using real time RT-PCR. The capability of these cells to degrade chlorinated ethenes was examined to further verify the presence and activity of pMMO under acetate-growth conditions. It was found that acetate-grown cells when exposed to trichloroethylene, trans-dichloroethylene, and vinyl chloride, were able to degrade these compounds, but such activity was lost when acetylene was added, indicating that pMMO was expressed and responsible for chlorinated ethene degradation. This is the first finding showing that active pMMO can be maintained and expressed by methanotrophs in the absence of methane.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.050 RESOLVING THE HIDDEN BIOSPHERE: A HIGH-THROUGHPUT PROCEDURE THAT IDENTIFIES OPERATIONAL TAXONOMIC UNITS FROM METAGENOMIC DATA

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Microbial diversity is typically characterized by hierarchically clustering SSU-rRNA sequences into operational taxonomic units (OTUs). Targeted sequencing of environmental SSU-rRNA markers via PCR may fail to detect OTUs due to various biases in priming and amplification. Analysis of shotgun sequenced environmental DNA, known as metagenomics, avoids amplification bias but is frustrated by the fragmentary, non-overlapping nature of the shotgun sequence data. To circumvent these limitations, we developed a computational workflow that

identifies OTUs from metagenomic SSU-rRNA sequence data via the use of phylogenomic principles and probabilistic sequence profiles. Methodological accuracy was verified through tests of simulated metagenomic data. Comparisons of PCR and shotgun sequenced SSU-rRNA markers derived from two different environments - the human distal gut and the global open ocean - reveal that while PCR libraries identify more OTUs per sequenced residue, metagenomic libraries can recover the vast majority of PCR-identified OTUs. In addition, metagenomic libraries contain OTUs missed by targeted sequencing, many of which correspond to novel phylotypes at the species, genus and even family level. Taken together, these results suggest that our methodology provides resolution into the part of the biosphere currently hidden from PCR based surveys of diversity.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.051 ISOLATION AND CHARACTERIZATION OF THERMOPHILIC AMMONIA-OXIDIZING BACTERIA IN COMPOST

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Thermophilic ammonia-oxidizing bacteria (T-AOB) isolated from compost are needed for biological deodorization of ammonia in compost. AOB such as *Nitrosomonas* sp. and *Nitrospira* sp. have been isolated from activated-sludge and soil. These AOB were found to be autotrophic and mesophilic, and were amenable to growth on defined mineral media at 20-37°C. Of these autotrophic AOB, *Nitrosomonas europaea* was found to grow on organic matter, and some heterotrophic bacteria were shown to have nitrification activity. Accordingly, media containing organic matter has come to be used in autotrophic and heterotrophic AOB research. In contrast to mesophilic AOB, T-AOB have been identified from hot springs and deep-sea hydrothermal vents, but have not been isolated from compost. Two new types of media were designed to culture and detect T-AOB from compost (Shimaya and Hashimoto. 2008. *Soil Sci. Plant Nutr.*). C-1 medium was designed to reflect compost composition. C-2 medium was modified synthetic organic wastewater for the cultivation of T-AOB. Compost suspensions were added to flasks containing various media and the flasks were incubated at 50-65°C with shaking. T-AOB did not increase in the conventional medium. However, T-AOB increased by an order of magnitude by six weeks in C-1 and C-2 media. The cultures were inoculated onto each medium (2% agar) and incubated at 50-65 °C. Colonies were isolated and investigated for the ability to carry out nitrification. Among a total of 4800 colonies isolated by using each medium, 66 colonies showed nitrite production. In particular, one strain isolated on C-1 medium showed the highest nitrite production. A laboratory-scale composting test was done to evaluate the effects of adding microorganisms on ammonia emission during composting of animal waste. Ammonium emissions were less than when no strain was added. These results suggest that ammonia emissions from composting of animal wastes can be decreased by adding the newly isolated strain.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.052 COMPARATIVE GENOME ANALYSIS OF TWO BIOTYPES OF *VIBRIO VULNIFICUS*

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Vibrio vulnificus is a gram-negative marine bacterium which can cause severe human infection when acquired through wounds or in contaminated seafood. *V. vulnificus* strains are divided into three biotypes (BTs): BT1, BT2 and BT3. Among these, BT1 and BT3 are known human pathogens, whereas BT2 is virulent to eels. The complete genome sequences of two strains of BT1, YJ016 and CMCP6, are currently available, and we have recently sequenced a BT2 strain. The aim of this study is to annotate the BT2 genome and to perform a comparative analysis among the three strains to identify possible virulence mechanisms in the *V. vulnificus* genome. On the assembled finished sequence of the BT2 CECT4999 strain, and the two BT1 strains, our computational pipeline iMetaSys was applied for ORF prediction, functional annotation, and biochemical pathway mapping. The unannotated proteins were annotated using fold analysis, and virulence-related genes were identified using virulent protein databases. The three genomes were compared to identify conserved, diverged, and unique proteins in each strain. We could annotate more than 90% of all the predicted ORFs with known functions, COGs category, or biochemical pathway information in all three strains. Out of the remaining unannotated ORFs, 23-25% could be annotated at high confidence using functional motifs and predicted folds. Approximately 79% of the ORFs were common to the two biotypes, and 12% and 14%, respectively, were found unique to BT1 and BT2. Of all the ORFs, 19-21% are candidates encoding virulence factors, with a majority of them involved in chemotaxis and motility. Interestingly, the BT2 strain carries proteins involved in alternative pathways for virulence, as compared to the two BT1 strains. Our analysis revealed some useful insights about conserved sequences in the two biotypes, and those which are biotype specific. This study also highlights differences in the possible virulence mechanisms of the two *V. vulnificus* biotypes.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.053 *CANDIDATUS NITROSOSPHAERA VIENNENSIS*, AN AMMONIA OXIDIZING ARCHAEON FROM SOIL

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Ammonia oxidizing archaea have recently been discovered to be widespread in marine and terrestrial environments indicating that they play a major role in the global nitrogen cycle. Even though they are ubiquitous and dominate over ammonia oxidizing bacteria in many environments, the isolation and cultivation of these chemolithoautotrophs is very challenging. We report here on the first ammonia oxidizing archaeon cultivated from a terrestrial habitat,

tentatively named *Candidatus Nitrososphaera viennensis*, which has been isolated from a garden soil in Vienna (Austria). Based on its 16S rRNA gene sequence it belongs to the soil group of ammonia oxidizing archaea and its closest cultivated relative is the moderately thermophile *Candidatus Nitrososphaera viennensis*. Growth experiments showed that several features clearly distinguish *Ca. N. viennensis* from its closest relative *N. gargensis* and from the more distantly related marine ammonia oxidizing archaeon *Nitrosopumilus maritimus*, as e.g. its adaptation to higher concentrations of ammonium (optimal concentration about 3mM; inhibition >10mM). The doubling time of *Ca. N. viennensis* is about 40 hours and it reaches cell densities of at least 5×10^7 cells/ml. Various cultivation approaches are under way to explore the metabolic capacity of soil archaea, and to compare it to their bacterial counterparts, the ammonia oxidizing bacteria. Our physiological investigations are supported by the draft genome sequence of *Ca. N. viennensis* which gives many indications for distinguishing features in these organisms.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.054 EFFICIENT EXTRACELLULAR PRODUCTION OF FUNCTIONAL RNAS BY MARINE PHOTOTROPHIC BACTERIUM *RHODOVULUM SULFIDOPHILUM*

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Functional RNAs, such as ribozyme, RNA aptamer, and small interfering RNA, can act as regulators of gene expression. These are expected to be potential candidates for RNA therapeutic agents. However current techniques for RNA syntheses are too costly for industrial production of these RNAs for use as drugs. Previously, we developed a new method for extracellular production of streptavidin RNA aptamer, as artificial RNA, using engineered plasmids and the marine phototrophic bacterium *Rhodovulum sulfidophilum*, which produces extracellular nucleic acids in nature [Suzuki, H. *et al.* (2010) *Appl. Environ. Microbiol.*, 76, 786-793]. However, the produced RNA aptamer having 5'-hydroxyl terminus seemed to be unstable compared to natural RNAs having 5'-terminal-phosphates. In order to solve this problem, we tested the method of tRNA scaffold [Phonchon, L. *et al.* (2007) *Nat. Methods*, 4, 571-576], in which an artificial sequence is included in tRNA structure.

We constructed the plasmid for production of the RNA aptamer of tRNA type. This plasmid was introduced into *R. sulfidophilum*. The bacterium was cultivated under anaerobic light conditions. The amount of products in culture medium was determined by quantitative reverse transcription PCR.

Extracellular production of the RNA aptamer of tRNA type by *R. sulfidophilum* was succeeded. The product was maintained stably until the stationary phase, although the products having 5'-hydroxyl termini were unstable during stationary phase. Moreover, the yield of RNA aptamer was improved approximately 7 fold.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.055 A NEW HALOALKALIPHILIC SULFATE-REDUCING BACTERIUM FROM SEARLES LAKE, CA THAT GROWS AT SALT SATURATION

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Searles Lake, California is a salt-saturated, arsenic rich environment, the anoxic sediments from which lack detectable sulfate reduction activity. Nonetheless, we detected the presence of sulfate reduction in an enrichment culture obtained from these sediments. A pure culture of a sulfate reducing microorganism, strain SLSR1, was obtained using serial dilution. Culture growth was determined using direct counts, and metabolic products and reactants were measured using HPLC, IC, and spectrophotometric methods. Culture purity was verified and phylogeny determined by sequencing of 16S rRNA with DGGE followed by cloning and sequencing. SLSR1 cells grew by oxidizing lactate to acetate plus CO₂ while reducing sulfate to sulfide with a specific growth rate of 0.008/hour. If arsenate was present cells grew first on arsenate with a specific growth rate of 0.002/hour. Sulfate reduction did not commence until all of the arsenate was consumed. Killed controls showed no activity, and lactate was not fermented. The pH optimum for growth on sulfate was 9.4, while the optimum for growth on arsenate was 8.4. Sulfate and arsenate reduction occurred at salinities between 130 and 340 g/L ($A_w = 0.8$), with optimal reduction at 180 g/L. No growth occurred below a salinity of 80g/L. Borate, which is prevalent in Searles Lake (0.46 molal), is not required for sulfate reduction, but concentrations above 60 mM are inhibitory. Taxonomic analysis characterizes strain SLSR1 as a new species within the order Haloanaerobiales. A respiratory arsenate reductase gene (*arrA*) has been isolated and sequenced, and has a unique sequence as compared to presently characterized *arrA* genes. Strain SLSR1 represents a pure culture of a bacterium able to respire sulfate at salinities previously thought to exclude such a metabolism.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.056 ISOLATION AND CHARACTERIZATION OF AEROBIC BUTANOL-TOLERANT BACTERIA FROM NATURAL ENVIRONMENTS

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Biobutanol is expected to play a major role in the next generation of biofuels. The major limitation in butanol fermentation is its high toxicity toward organisms. So far, anaerobic butanol-tolerant microbes such as clostridia have been extensively investigated. However, little is known about aerobic butanol-tolerant microbes in natural environments. Aerobic butanol-tolerant microbes are promising candidates as production hosts for genetic manipulation to develop butanol-fermenting mutant with higher butanol-tolerance. Our aim of this study is to newly isolate aerobic butanol-tolerant bacteria from natural environments and to investigate their taxonomic traits and mechanisms to tolerate butanol. For enrichment of butanol-tolerant

microbes, environmental samples such as freshwater sediments, soils, and composts were exposed to 2~4% (v/v) of 1-butanol for 2 days or 3~9 months. Ten aerobic microbes able to tolerate >2% of 1-butanol were successfully isolated using modified GYT medium at 30°C under aerobic conditions. All strains were Gram-positive bacteria belonging to the genera *Bacillus* or *Enterococcus* in the *Firmicutes* or the genera *Cellulomonas* in the *Actinobacteria*. Among those isolates, strain CM4a isolated from grease-contaminated soil showed the highest degree of butanol tolerance (up to 3.5% of 1-butanol). The 16S rRNA gene sequence of strain CM4a was related to those of *Enterococcus* spp. with less than 96.7% similarity, indicating that strain CM4a is novel at the species level. The mechanism of butanol tolerance of this strain is now under investigation.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.057 NITROGEN FIXATION IN THE FILAMENTOUS, TERMINAL HETEROCYST FORMING CYANOBACTERIUM CYLINDROSPERMOPSIS RACIBORSKII CS-505.

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Cyanobacteria are photosynthetic organisms of great importance in carbon sequestration and atmospheric nitrogen (N₂) fixation. Because nitrogenase is irreversibly inactivated by O₂, diazotrophic filamentous cyanobacteria separate N₂ fixation temporally (fixing in the dark phase of their photoperiod), or spatially (differentiating specialized cells called heterocysts) from photosynthesis. A $\Delta patA$ mutant of the cyanobacterium *Nostoc* sp. PCC7120, which normally forms a regular pattern of intercalated heterocysts, is reported to differentiate only terminal heterocysts. Although the terminal heterocysts of the $\Delta patA$ mutant are functional, they can not satisfy the nitrogen requirements of the whole filament. What mechanisms allow cyanobacteria that normally differentiate terminal heterocyst to survive in diastrophic environments? Do they fix N₂ in the vegetative cells during the dark phase of their photoperiod?

We studied *Cylindrospermopsis raciborskii* CS-505, to determine when and where N₂ fixation occurs in a terminal heterocyst differentiating cyanobacterium: light/dark nitrogenase expression profile was determined by qPCR (*nifH* expression) and nitrogenase activity was measured by gas chromatography. We separated heterocysts and vegetative cells by sonication and a two-phase aqueous polymer system (Dextran 500 and Polyethylene glycol 3500), and used these cell fractionates to measure nitrogenase activity by gas chromatography and determine the expression of the *nifH* gene in each cell type by qPCR. Our results point to a diurnal expression of *nifH* and nitrogenase activity restricted only to the terminal heterocyst.

We discarded N₂ fixation in the vegetative cells of the filaments and set a baseline so further studies can center in the mechanisms that distribute the fixed nitrogen from the terminal heterocysts to the rest of the filament's cells.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.058 TARGETED CULTIVATION OF ENDEMIC MICROORGANISMS FROM TRAMWAY RIDGE, MOUNT EREBUS, ANTARCTICA

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Geothermal soils found at Tramway Ridge, Mt. Erebus, Antarctica are isolated from similar environments by hundreds of kilometres of ice. Genetic surveys suggest that most microbes present in the hot (65°C) subsurface soils of Tramway Ridge are endemic and only remotely related to known bacterial divisions. In order to overcome the inherent challenges in the cultivation of such a unique community, we have begun a multi-year targeted cultivation attempt that combines genetic data, geothermal soil characteristics and metabolic profiling in order to isolate and characterize endemic organisms. We began a series of experiments using five different media obtained from the literature. These media varied in nutrient composition and carbon source. During the 2009/2010 field season, we tested the ability of each media to grow Tramway Ridge organisms under anaerobic, aerobic, solid, and liquid conditions with fresh soil inoculums. All media show growth with aerobic solid conditions showing the best response. Sixty pure-culture isolates are being prepared for genetic characterisation. At least four of these are Gram-negative which is important as only Gram-Positive bacteria have been isolated from Tramway Ridge prior to our study. As a supplement to our media experiment, we used Biolog phenotypic microarray plates to provide additional information about the microbial community in regards to the utilization of various carbon, nitrogen, sulfur and phosphorus compounds. We found high rates of microbial activity for several compounds such as inulin, 2,3-Butanone, urea and several amino acids which differ substantially from the carbon and nitrogen sources commonly used for enrichment of geothermal soil isolates. This large scale targeted media approach has so far proved successful in isolating organisms never before cultured from Erebus while guiding the rational design of Tramway Ridge-specific media for future field work in 2010/2011.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.059 ULTRA-SMALL METHYLOTROPHIC BACTERIA FROM ACIDIC SPHAGNUM PEAT BOG

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Sphagnum-dominated acidic peat bogs represent one of the most extensive wetland types in the Northern Hemisphere. Up to 80% of all microbial cells in acidic peat are represented by ultra-small forms. The phylogenetic affiliation and the metabolic potential of these microorganisms remain poorly understood. In a course of a study on bacteria involved in the process of methanol oxidation in Obukhovskoye peat bog, Yaroslavl region, Russia (58°14'N, 38°12'E), the novel strain of ultra-small methylotroph was isolated and designated strain ESY. This study was initiated in order to characterize this organism and examine the potential diversity of ultra-small methylotrophs in this habitat by 16S rDNA and *mxoF* gene clone library analyses of the cell fraction that passed through 0.22-µm-pore-size filters. Cells of strain ESY were thin rods measuring 0.25-0.6x0.4-1.5 µm. The corresponding cell volumes ranged from 0.02 to 0.15 µm³.

Methanol was the preferred growth substrate. The specific growth rate of this methylotroph under optimal growth conditions was 0.02 h^{-1} . The 16S rRNA gene sequence of strain ESY displayed 98.5-99 % similarity to that of the acidophilic methylotroph *Methylovirgula ligni* (Vorob'ev et al., 2009). 16S rDNA and *mxoF* gene clone libraries were prepared from 0.22- μm filtered peat water incubated under methane. The 16S rDNA clone library was dominated by *Methylovirgula*-related sequences (98-99 % similarity). The parallel analysis of *mxoF* gene sequences (encoding the large subunit of methanol dehydrogenase) indicated that most inferred peptide sequences of *mxoF* clones were closely affiliated with the MxoF sequences of *M. ligni* and strain ESY (90% similarity). In summary, we characterized the novel bacterium involved in the process of methanol oxidation in acidic wetlands and showed that methylotrophs related to this organism can pass through 0.22- μm -pore-size filters, therefore, comprising a part of ultra-small bacteria detected in these ecosystems.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.060 ISOLATION AND CHARACTERISATION OF THE MODERATELY THERMOPHILIC BACTERIA FROM BEPPU HOT SPRINGS, JAPAN

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During recent attempt to isolate thermophiles from many Japanese hot springs, we have isolated novel moderately thermophilic bacterium from Beppu hot springs by culturing at various temperatures.

Sample was taken from Kamado-Zigoku, Beppu hot springs in May, 2009. The temperature and pH of the sample were 95°C and 4.3, respectively. A 100- μl of sample was plated onto the modified Brock's basal salts (Kurosawa *et al.*, 1998) supplemented with 0.1 % (w/v) yeast extract, pH 4.0, solidified by 0.7 % (w/v) Gelrite, and incubated at 55°C for 2 days. Colonies appeared on the plates were purified by single-colony-isolation. Genomic DNA of the isolates were extracted for PCR amplification of 16S rRNA genes using bacterial universal primers, B27F (5'-AGAGTTTGATCCTGGCTCAG, *Escherichia coli* position 9-27) and U1492RM (5'-GGYTACCTTGTTACGACTT, *E. coli* position 1512-1492). The following thermal cycle was used for 25 cycles: 95°C for 30 sec, 60°C for 30 sec, and 72°C for 1.5 min. PCR products were sequenced and the obtained data were compared with the available sequences in the DDBJ database using BLAST.

Total five strains were successfully purified and taxonomically classified into three groups in species level. They showed 16S rRNA gene similarities with *Geobacillus stearothermophilus* (99%, 1 strain), *Meiothermus ruber* (99-100%, 3 strains) and *Paenibacillus ehimensis* (91%, 1 strain). The result of BLAST indicated that the strain BEP15 is a novel species. Strain BEP15 was pink-pigmented, rod-shaped and Gram-negative bacterium. Catalase- and oxydase-test were negative. In this paper, we will present the results of physiological, biochemical and chemotaxonomic analysis of this isolate.

(Reference)

Kurosawa *et al.* (1998) *Int J Syst Bacteriol.* 48: 451-456.

Abstract Category

27 Novel organisms and Novel Functions

PS.27.061 BIODIVERSITY AND PURIFICATION OF BARELY CULTURABLE ACIDOBACTERIA FROM DIFFERENT FOREST SOILS

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We have hitherto collected large number of novel bacteria using improved culture method from various environments. We have reported that forest soil is interesting sources for discovery of novel bacteria. In the present study, we investigated the biodiversity of *Acidobacteria* by cloning and sequencing from soil community DNA including basal soil of pine mushroom, different layers of pine and quercus forest soils. PCR-amplified inserts were digested with the restriction enzyme *HeaIII* and *AluI* to examine the diversity of clone libraries. The RFLP patterns and dendrogram were constructed using the UPGMA method in the GelComparII. Thirty-one, 101, and 153 distinct RFLP patterns were obtained from basal soil of pine mushroom, different layers of pine and quercus forest soils, respectively. PCR products from a clone representing each restriction pattern were sequenced. *Acidobacteria* are abundant members of bacterial populations in each soil; eighty-five percent of the basal soil of pine mushroom clone libraries, 50% of the pine forest soil clone libraries, and 14% of quercus forest soil clone libraries were belong to the *Acidobacteria* phylum. Members of the phylum *Acidobacteria* have been very difficult to isolate and culture in the laboratory, and cultured representatives of only 4 of *Acidobacteria* 25 subclass currently exist in the Ribosome Database Project (RDP release 10, update 18). The acidobacteria strains isolated in this study were classified; the isolates which grew in the subculture in DNB medium were 'barely culturable acidobacteria' and those that formed an initial colony on the plate but did not grow in the subculture were 'temporarily culturable acidobacteria'. Eleven of the temporarily culturable acidobacteria were belonged to Gp1 and Gp6 subclass, three of the barely culturable acidobacteria were Gp6 and Gp14 subclass. Here, strain KH42 belong to Gp14 was distinct from previously isolated acidobacteria (Gp1, Gp2, Gp4 and Gp6).

Abstract Category

27 Novel organisms and Novel Functions

PS.27.062 CHARACTERIZATION OF ACTINOMYCETES-LIKE BACTERIA BELONGING TO THE CLASS *KTEDONOBACTERIA* IN THE PHYLUM *CHLOROFLEXI*

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Many uncultured bacteria belonging to the class *Ktedonobacteria* have been detected in geothermal soils, volcanic deposits, *etc.*, meanwhile the cultured species with validly published is only one species *Ktedonobacter racemifer*, which was isolated from soil in Italy. Recently, we have isolated 3 strains in the class *Ktedonobacteria* isolated from compost (strain SK20-1) and fallen leaves on geothermal soil (strain ONI-1 and ONI-5), and are proposing strain SK20-1 as *Thermosporotrichaceae* fam. nov., *Thermosporothrix hazakensis* gen. nov., sp. nov. (Yabe *et al.* Int J Syst Evol Microbiol 2009 doi: 10.1099/ijms.0.018069-0). Results of phylogenetic and morphological analysis of these isolates showed that, although strain ONI-1 and ONI-5 are different from *K. racemifer* and *T. hazakensis* at order level, these species have similar morphological features, which formed branched substrate and aerial mycelia with spores like actinomycetes. Therefore, the species belonging to the class *Ktedonobacteria* may present typical actinomycetes morphology, and we recommend that strain ONI-1 and ONI-5 should be placed in a novel genus in a novel order within the class *Ktedonobacteria*. In the present study, we will describe phylogenetic, morphological and physiological characteristics of *T. hazakensis* SK20-1, strain ONI-1 and ONI-5.

Abstract Category

27 Novel organisms and Novel Functions

PS.28.001 VIRAL GENOMICS AND EVOLUTION IN SUBSEAFLOOR DIFFUSE FLOW MICROBIAL COMMUNITIES IN THE MAIN ENDEAVOUR HYDROTHERMAL FIELD, JUAN DE FUCA RIDGE

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Hydrothermal vents are thought to be one of the most ancient continuously inhabited ecosystems on Earth, and may have been a site for the origin and early evolution of life. Yet despite an intense research focus on hydrothermal vent microbiology, little is known about their viral ecology. In the dynamic vent environment, transduction may play an important role in the evolution of microbial communities. To explore these concepts, we are investigating genetic characteristics of seafloor diffuse flow viral communities at Hulk vent in the Main Endeavour Field of the Juan de Fuca Ridge. Viruses were concentrated and purified from 170 liters of diffuse flow fluid that was collected at the base of Hulk vent. DNA was extracted from this sample, and the viral metagenome will be obtained through 454 pyrosequencing. The target of our analysis will be metabolic genes that appear to have been laterally transferred in the virome: it has been hypothesized that since microbes inhabiting dynamic ecosystems such as hydrothermal vents are subject to rapidly changing temperature, pH, and nutrient conditions, selection is likely to favor the lateral transfer of metabolic genes that facilitate adaptation to changing conditions. This may include genes related to sulfur and iron reduction, nitrogen fixation, and biofilm formation. As it has been hypothesized that a primary transducing agent in the virosphere may consist of gene transfer agents (GTAs), known GTA-like sequences will be searched in the metagenome as well. Additionally, matches to CRISPR spacer sequences from known vent organisms isolated from nearby vents may give a sense of viral range in the vent system. Finally, as viruses are increasingly thought to have been present at the earliest stages in the evolution of life, studying viruses in an environment that was present on the ancient earth may elucidate their role in the evolution of life in its earliest stages.

Abstract Category

28 Viral Ecology

PS.28.002 TIME SERIES STUDY REVEALS REGULAR TEMPORAL AND VERTICAL VARIABILITY OF VIRAL ABUNDANCE IN THE SUBTROPICAL NORTH ATLANTIC

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Marine viruses play an important role in controlling prokaryotic production, community structure, and biogeochemical cycling of life's essential elements. Despite their importance there is a paucity of data describing temporal and vertical dynamics of marine viral abundance, especially in open ocean systems. Here we present a decade-long, high resolution time-series of viral distribution in the surface 400 m in the northwestern Sargasso Sea. The time series of viral abundance was placed in the context of physical parameters, specific prokaryotic lineages, and ocean biogeochemistry measured through the Bermuda Atlantic Time Series (BATS). The data reveal open ocean viral dynamics in unprecedented detail showing regular annual patterns in

viral abundance related to mixed layer depth, including significant inter-annual variability in the magnitude of the annual viral peak and systematic temporal and vertical variability in the virus to bacteria ratio. The timing and vertical positioning of these patterns are related to variability in water column stability and the dynamics of specific picophytoplankton and heterotrophic bacterial lineages. The high correlation between viral and Prochlorococcus densities suggest that a significant fraction of the viruses at BATS are cyanophage.

Abstract Category
28 Viral Ecology

PS.28.003 EXPLORATION OF FRESHWATER ECOSYSTEMS IN THE HIGH ARCTIC: PHYLOGENETIC DIVERSITY OF T-4 TYPE BACTERIOPHAGES

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Tailed bacteriophages, viruses that infect bacteria, are the most abundant biological entities in aquatic environments, numbering approximately 10-100 million per millilitre. They have a significant impact on biogeochemical and ecological processes; they facilitate nutrient cycling, help maintain bacterial biodiversity, and mediate microbial mortality and genetic transfer. They are also believed to be the greatest reservoir of genetic diversity; yet, little is known about their presence and diversity in polar freshwater systems. The present study characterizes the genetic diversity of tailed bacteriophages (T-4 type tailed bacteriophages) in high-arctic freshwater systems. Gene homologs to a major capsid gene (g23) found in T-4 type tailed bacteriophages were amplified from diverse high-arctic freshwater systems, confirming their prevalence in polar regions. In addition, phylogenetic analysis of g23-related sequences from different polar inland waters showed a great and unexplored diversity. Sequences from the polar freshwater systems were distinct from those of marine environments. The majority of polar sequences belonged to two groups that were closely related to environmental sequences retrieved from tropical rice-paddy soil. These results suggest that T4-like phages from tropical and arctic freshwaters are genetically more similar to each other than they are to those from marine environments.

Abstract Category
28 Viral Ecology

PS.28.004 COMPARISON OF THE RESPONSES OF BACTERIOPHAGES AND BACTERIOVORAX TO A COMMON PREY BACTERIUM IN A SEA WATER MESOCOSM

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Bacteriophages, viruses of bacteria, are considered by many to be a major contributor to bacterial mortality in the environment. The potential roles of *Bacteriovorax*, a halophilic predatory bacterium, and other *Bdellovibrio*-And-Like-Organisms that attack and lyse Gram-negative bacteria in a manner similar to bacteriophages, have been largely ignored. In contrast to bacteriophages that are more efficient at attacking rapidly growing prey cells, *Bacteriovorax* is highly active in reducing bacteria that are in stationary phase. Since many bacteria are in a slow or non-growing stage in oligotrophic environments, we hypothesized that *Bacteriovorax*, rather than bacteriophages, play the major role in bacterial mortality in those conditions. We therefore designed a mesocosm study to simultaneously examine the population dynamics of the two predators to the same prey, *Vibrio vulnificus*. Equal numbers of active *Bacteriovorax* and bacteriophage were inoculated into flasks containing *V. vulnificus* suspended in autoclaved natural sea water at a predator: prey ratio of 10:1. Control mesocosms were used to monitor growth of *V. vulnificus* with *Bacteriovorax* and bacteriophage respectively or no predators. Over a 40 hour period, samples were examined every 4 hours by electron microscopy, and the two predators and prey were enumerated by their respective plating methods. Results showed that *Bacteriovorax* was the first and greatest responder to the prey increasing 100 to 1,000-fold in plaque-forming units with a simultaneous 99% reduction in CFU of *V. vulnificus*. The bacteriophage response was weak, as evidenced by no increase in plaque-forming units. Electron microscopy confirmed that the *Bacteriovorax*, not bacteriophage, was most frequently observed to infect the prey *V. vulnificus*. Based on this in vitro mesocosm experiment, we conclude that *Bacteriovorax* can have an important role in controlling and shaping bacterial communities in marine systems.

Abstract Category
28 Viral Ecology

PS.28.005 MONTHLY VARIATION OF MARINE VIRAL COMMUNITIES AT THE SAN PEDRO OCEAN TIME SERIES

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The Microbial Observatory program at the San Pedro Ocean Time-series station (SPOT) off Los Angeles, CA has been ongoing since 2000 and seeks to characterize temporal variation in microbial communities and how it relates to the environment. Fingerprinting methods can quickly and inexpensively survey shifts in community structure and have been previously applied with great success to the bacterial and protistan communities at this location. Community fingerprinting techniques can also allow for identification and targeting of divergent microbial communities to increase sequencing coverage from pyrosequencing. We developed and applied a terminal restriction fragment length polymorphism (TRFLP) method to detail changes within the T4-like virus superfamily community in surface water at SPOT. Restriction enzymes were selected to maximize the number of detectable terminal restriction fragments (TRFs) while minimizing the number of putative operational taxonomic units (OTUS, as defined by sequence similarity of environmental clone libraries) within each observed TRF. Results from SPOT shows a monthly shift in viral community structure and that while some viral TRFs were common throughout the year, others were restricted to certain time points. Viral TRFs also

varied in relative abundance between months. We anticipate comparing our fingerprint analyses with sequence-derived OTUS from our ongoing study of pyrosequenced gp23 amplicon libraries from the same sample set to determine an ecologically-relevant framework for the TRFs. Additionally, both T4-like viral datasets in conjunction with the bacterial and eukaryotic community data from the MO program will allow for a better understanding of the complex interactions within the marine microbial loop.

Abstract Category
28 Viral Ecology

PS.28.006 THE COLD-ACTIVE MOLECULAR CHARACTER OF *COLWELLIA PSYCHRERYTHRAEA* STRAIN 34H PHAGE 9A

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Much of the planet is perpetually cold, and inhabitants must tolerate if not thrive at temperatures close to and often below 0 C. The role of viruses in shaping and controlling marine microbial communities in these extensive cold environments has received disproportionately little attention. We continue work on the most cold-active phage host system available: lytic bacteriophage 9A and psychrophilic bacterium *Colwellia psychrerythraea* 34H, established by Wells and Deming. In collaboration with the Betty and Gordon Moore Foundation and the Broad Institute at MIT and Harvard, we have obtained the genome sequence of phage 9A and analyzed its molecular character to gain insight into bacteriophage ecology in the cold. High titer phage stocks were acquired by culturing host cells with 9A on silica-gel overlay plates at 2C. Phage were purified and DNA was extracted and submitted to the Broad Institute for 454 sequencing and annotation. Within the 28 identified (of 148) coding sequences of the 105kb 9A genome are signatures of its cold activity, indicators of host specificity and clues to its phylogeny. The 37% GC content of 9A speaks to the flexibility required for function at cold temperature, thermolability and an intimate relationship with 34H (GC 38%). In contrast, the presence of a homologous region of 9A within repeating CRISPR spacer regions of mesophilic anaerobe *Syntrophus aciditrophicus* indicates a broader geographic distribution and potential functional range of at least this ORF. A DNA adenine methylase in the 9A genome, despite the lack of 34H restriction endonucleases, also suggests a broader host range than has been demonstrated *in vitro*. The replicative enzymes of 9A may be the most cold-active viral enzymes of this class sequenced to date. Analyses of the complementary genomes of this phage host system begin the genomic exploration of cold active viral ecology and present provocative implications for viral gene activity, defense strategies and host range.

Abstract Category
28 Viral Ecology

PS.28.007 ISOLATION OF MYCOBACTERIOPHAGE FROM SPHAGNUM PEAT BOGS

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Sphagnum moss (peat) bogs are oligotrophic, acidic environments characterized by reduced amounts of bacteria. Historically these sites have been identified as suitable ecological niches for mycobacteria with at least 26 different known species previously associated with bogs. These represent up to 1/3 of the total number of known mycobacterial species. Our aim was to use these sites to search for mycobacteriophage since we hypothesize phage specific for mycobacteria would naturally be found where the bacteria are most abundant in the environment.

Mycobacteriophage have been used for purposes as diverse as testing for mycobacterial drug sensitivity, diagnosing mycobacterial infections and contaminations of milk, and genetically modifying mycobacteria. Currently over 250 species of mycobacteriophage have been identified, but this has been primarily accomplished using lab strains of fast-growing, saprophytic *Mycobacterium smegmatis* and the slow-growing, pathogenic *Mycobacterium tuberculosis* as host cells. Soil, water, and vegetation from four northern Minnesota peat bogs were obtained and native mycobacterial species were isolated using selective media. Over 700 mycobacterial candidates were identified by acid-fast staining, PCR analysis using mycobacterial-specific primers, restriction digestion patterns of PCR products, several biochemical tests, and finally 16S rRNA sequencing. From this pool we identified a number of mycobacteria previously not associated with bogs. Pools of fast-growing and slow-growing mycobacterial species were used to enrich for phage from bog environmental samples, and plaques were obtained on lawns of pure cultures of individual mycobacterial species. We identified many potentially novel mycobacteriophage with differing host ranges, phage shapes, and phage genomic sequences. We conclude that sphagnum moss bogs are environments particularly rich in mycobacteriophage.

Abstract Category
28 Viral Ecology

PS.28.008 AN ARSENAL OF ANTIQUES: COMPARING CRISPR SPACERS FROM AXENIC ISOLATES AND ENVIRONMENTAL VARIANTS

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A novel microbial defense system against viral attack, with analogies to gene silencing via RNAi in eukaryotes, has recently been uncovered. Although many details remain elusive, fundamental components of this prokaryotic "immune system" are Clustered Regularly Interspaced Palindromic Repeats (CRISPRs). CRISPR arrays consist of small tandemly repeated genomic segments (21-47 bp) separated by non-repetitive spacers (20-50 bp). New spacers are integrated in a sequential manner, can range anywhere from 2 to 728 repeats, and are suggested to serve as a "memory" of previous viral insult. However, basic questions of CRISPR distribution and diversity have yet to be addressed in naturally occurring populations and to the best of our knowledge, no model system has yet been developed to explore these fundamental aspects. To elucidate this we compared the CRISPR spacer content of two thermophilic cyanobacteria variants *Synechococcus* sp. OS-A and OS-B' originally isolated from Octopus Spring mat community in Yellowstone National Park with CRISPR spacers amplified from Octopus Spring mat samples taken across subsequent years. Primers were generated to CRISPR repeat regions with Primer3 using the sequenced genomes OS-A and OS-B. Regions of interest were amplified

via PCR, and cloned into a TOPO plasmid for Sanger sequencing. In the absence of viral selective pressure CRISPR arrays in isolates maintained under laboratory conditions differed from those found in naturally evolving populations.

Abstract Category
28 Viral Ecology

PS.28.009 METAVIROMICS OF AN EXTREME ENVIRONMENT

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We are investigating virus-host relationships and virus diversity in Boiling Springs Lake (BSL): a large, acidic hot spring (pH 2.5, 55C-95C) located in Lassen Volcanic National Park, USA. Viruses appear to be the "top-down" regulator of this purely microbial community comprised of archaea, bacteria, several species of unicellular eukarya [1][2]. No extremophilic eukaryotes have yet been identified. BSL offers a unique opportunity for their discovery. Recent bioinformatic analysis has identified so-called "virus hallmark genes" that are prevalent in large groups but have few, if any, cellular homologues [3]. We will analyze hallmarks as a means of classifying uncultivated viruses present in the ecosystem and to infer virus host-range.

20 liters of BSL sediment was collected in September 2009. Virus-like particles were concentrated by ultrafiltration. Virus metagenomic DNA was amplified with Phi29 DNA polymerase and pyrosequenced. Individual clones derived from the metaviromic sample were sequenced by dye-termination.

A DNA metavirome of approximately 100,000 sequences has been generated from BSL sediments to address virus diversity, prevalence, identity, and the presence of hallmark genes in the BSL ecosystem. To date, none of the BSL-sediment sequences have yielded significant hits in the public databases.

The BSL-sediment metavirome is highly diverse and provides many opportunities for virus discovery and detailed characterization of the ecosystem.

[1] Wilson, M.S., et al. (2008) *Microb Ecol.*, 56(2), 292-305. [2] Brown, P.B., Wolfe, G.V. (2006) *J Eukaryot Microbiol*, 53(6), 420-31. [3] Koonin, E.V., Senkevich, T.G., Dolja, V.V., (2006) *Biol Direct*, vol 1.

Abstract Category
28 Viral Ecology

PS.28.010 CHARACTERIZATION OF SULFOLOBUS SPINDLE-SHAPED VIRUS 1 REPLICATION

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Fuselloviridae are globally-distributed ubiquitous viruses infecting hyperthermoacidophilic Archaea. The first to be discovered was *Sulfolobus* spindle-shaped virus 1 (SSV1). The virus genome integrates into the host genome and produces virus at low levels. Release of SSV1 virions appears not to cause cell lysis, although it does retard cell growth. Although SSV1 was confirmed as a virus in 1984, fundamental understanding of its replication cycle is lacking. Almost all available data are on infected cells after induction by ultraviolet irradiation. To better understand the process of infection and natural virus production we performed a one-step growth curve following infection of *Sulfolobus solfataricus* with SSV1.

SSV1 stocks were made from infected *Sulfolobus* cultures by centrifugation and filtration. Viruses were concentrated by tangential flow filtration. Adsorption experiments were performed to determine growth curve parameters. Virus stocks were added to growing cell culture at a multiplicity of infection of 3 and further incubated at 75°C. Samples were taken regularly. Virus titer was determined by spotting dilutions of cell-free supernatant on lawns of *Sulfolobus solfataricus* followed by incubation. Halos of growth inhibition indicated the presence of infectious virus.

Preliminary results indicate a steep drop in virus titer after incubation for two hours and a nearly complete loss after six hours. This decrease is due to both the incubation temperature and adsorption of viruses to host cells. Virus titer rises slowly between seven and ten hours, indicating that the release of virions has begun, with less than 1 virus per 1,000 cells. These results differ significantly from virus production by induced cells, in which virus production begins by the fourth hour, rapidly reaching a much higher titer. Our results should reflect virus production under natural conditions more closely than artificially induced virus production.

Abstract Category
28 Viral Ecology

PS.28.011 ENRICHING DESCRIPTIONS OF MARINE PHAGES USING THE "MIGS" STANDARDS: A CASE STUDY ASSESSING THE CONTEXTUAL DATA FRONTIER

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In any sequencing project, the depth of comparative analysis possible is dictated by the degree of accompanying contextual data. The structure, content, and storage of this contextual data must be standardized to ensure consistent coverage and facilitate comparisons. Such standardization efforts are now emerging as a rapidly developing field through a range of grass-roots efforts, most notably by the Genomic Standards Consortium (GSC), which has published the "Minimum Information about Genome/Metagenome Sequences (MIGS/MIMS)" checklist.

A subset of genomes (30 sequenced marine phages) was collected and its compliance with the MIGS specification checked. This analysis confirms that current International Nucleotide Sequence Database Collaboration (INSDC) submissions are deficient in most MIGS fields. To

counter the missing data, contextual information was manually curated and stored in MIGS-compliant reports for each sequenced marine phage. These reports are available in Genomic Contextual Data Markup Language (GCDML) format, where they will persist in structured machine-readable form for future re-use.

This case study presents descriptive exploratory data analyses based on contextual data automatically extracted from the machine-readable reports, including trends in isolation and sequencing dates, distribution of genome sizes and %G+C, phage and host taxonomy, and patterns in sample site environmental parameters. Furthermore, manual curation of georeferencing data made it possible to put 27 of the 30 phage genomes "on the map", facilitating future analysis based on their environmental context.

This work emphasizes how the integration of contextual data at the time of sequencing or database submission will minimize downstream manual curation, maximize integration of data (e.g. environmental parameters) from emerging resources, and streamline biological knowledge generation

Abstract Category
28 Viral Ecology

**PS.28.012 NOVEL CIRCOVIRUS IDENTIFIED IN THE CALANOID COPEPOD
LABIDOCERA AESTIVA FROM TAMPA BAY, FLORIDA**

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Mesozooplankton are critical components of marine food webs as the primary consumers of phytoplankton, as prey for upper trophic level predators including commercially important fish, and by mediating carbon transfer from the atmosphere to the ocean interior through fecal matter deposition. Thus, the factors that control population dynamics of zooplankton are critical to understanding the structure and function of marine food webs. Two decades of research on marine viruses have demonstrated their profound effects on all levels of marine life from bacteria to whales. Despite the ecological importance of zooplankton, virtually nothing is known about the impact of viruses on the most abundant zooplankton group - the copepods. Here we present the complete genome of a circovirus identified using viral metagenomics of a calanoid-copepod-dominated zooplankton tow from Tampa Bay, Florida. The single-stranded DNA copepod circovirus genome is 1764 nt long, and displays only weak amino-acid level identities to known avian circoviruses. The copepod circovirus is similar in genome organization and replication gene sequence to a circovirus recently identified from a virioplankton metagenome from the Chesapeake Bay. Quantitative PCR for the circovirus replication gene demonstrated 100% prevalence with viral loads of 10^5 - 10^6 copies per *Labidocera aestiva* individual. Much lower viral concentrations were detected in bulk zooplankton tow samples from Tampa Bay, and the circovirus was not detected in zooplankton collected from Shoals Marine Lab in Maine. Further studies are required to understand the relationship between the circovirus and *Labidocera*, including the route of infection, prevalence, and viral effects on the host.

Abstract Category
28 Viral Ecology

PS.28.013 LYSOGENIC BACTERIOPHAGES IN DEEP-BIOSPHERE POPULATIONS

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Throughout the last years, we have obtained interesting insights into the microbial community composition of the marine deep biosphere while the viral inventory of deep-subsurface sediments was not studied so far. Viruses might be the main "predators" for indigenous microorganisms and probably have a major impact on deep biosphere populations in providing labile organic compounds for this generally nutrient depleted habitat.

About 70% of known bacterial genomes contain prophages. These can be induced to become free phage particles if the host cells are set under certain stress situations such as a treatment with DNA-damaging antibiotics. Therefore, culture collections from a respective habitat represent an archive of its viral diversity.

Representative deep-biosphere isolates from our culture collection obtained during Leg 201 of the Ocean Drilling Programm (ODP) were screened for the presence of lysogenic phages. Six out of thirteen strains from various phylogenetic groups, sites and sediment depths harbour mitomycin C inducible prophages. Different morphotypes were identified, all with variations in size and shape.

Further studies focus on forty isolates of *Rhizobium radiobacter* from the same culture collection. We could show that this species is a highly abundant inhabitant of different ODP sites and sediment depths of the Equatorial Pacific and the Peru margin. Therefore, *R. radiobacter* was chosen as a model organism to identify the intra-species diversity and the biogeography of phages that infect deep biosphere populations of this species.

We want to test whether our isolates contain the same viruses or if they exhibit distribution patterns that are correlated to certain sampling sites or sediment layers. All of the investigated strains were tested positively for inducible phages. A further molecular study on the phage diversity by pulsed-field gel electrophoresis revealed the presence of several phages with varying morphotypes and genome sizes.

Abstract Category
28 Viral Ecology

PS.28.014 VIRUSES, SIGNFICANT DRIVERS OF SOUTHERN OCEAN CARBON CYCLING?

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Viruses are implicated in carbon cycling as infection of unicellular marine organisms releases organic matter to the dissolved pool. The Southern Ocean is an important carbon sink as it is responsible for approximately one third of oceanic CO₂ absorption. It comprises two main zones, the Antarctic and sub-Antarctic, and is further subdivided into a number of regions over which little is known about the relative significance of viruses. Viral activity was investigated using

incubation experiments over a range of Southern Ocean provinces from the northern boundary to coastal Antarctic sites. Highest rates of viral-mediated mortality (all given as percentage standing stock) of both bacteria ($71\% \text{ d}^{-1}$) and phytoplankton, particularly cyanobacteria ($150\% \text{ d}^{-1}$), were detected in the sub-Antarctic. The comparably lower activity of viruses recorded at sites in the Antarctic Zone may have resulted from reduced availability of bacterial and algal hosts there. Throughout the different regions surveyed in the Antarctic Zone the significance and apparent role of viruses varied; at sites along the Zero Meridian viral-mediated bacterial mortality was $13.8\% \text{ d}^{-1}$ but lysis of phytoplankton was rarely detected, conversely, in the Drake Passage, viral-mediated bacterial mortality was only $2.2\% \text{ d}^{-1}$, whereas, viral lysis of phytoplankton was significant ($38\% \text{ d}^{-1}$). A transect of the Weddell Sea indicated that bacteria in this region were also subject to significant levels of lytic viral infection ($26.7\% \text{ d}^{-1}$). Literature values indicate that the carbon content of Southern Ocean bacteria is higher in the south so despite lower infection levels in the Antarctic Zone, viral lysis of bacteria was estimate to release 25 to $125 \mu\text{g carbon l}^{-1} \text{ d}^{-1}$, which was greater than the $3.3 \mu\text{g carbon l}^{-1} \text{ d}^{-1}$ estimated for the sub-Antarctic. Thus the virus community is active and distinct over the various provinces of the Southern Ocean and is important for carbon cycling, potentially influencing CO_2 absorption.

Abstract Category
28 Viral Ecology

PS.28.015 ABUNDANCE AND DIVERSITY OF VIRUSES THROUGHOUT THE WATER COLUMN IN THE NORTHWESTERN SARGASSO SEA

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Viruses play a key role in the marine environment because they regulate the transfer of energy between trophic levels and influence global carbon and nutrient cycles. Studies have revealed tremendous diversity of marine viruses. However, most previous studies take a snapshot of the viral community, rather than examining how viral communities change either in time or in space. The goal of this project is to gain a better understanding of the abundance and diversity of viruses in the NW Sargasso Sea. The study site is the Bermuda Atlantic Time-series Study site, allowing us to analyze the viral parameters in the context of a long history of physical, chemical and biological measurements. Epifluorescent microscopy reveals a recurring seasonal pattern of viral abundance, where during the winter, virus concentrations are low and uniformly distributed in the mixed layer, and during the summer, a strong subsurface maximum in viral abundance develops at 80-100 meters. Diversity analyses have therefore focused on comparing the viral community composition from the surface and the subsurface maximum during the different seasons. Viral diversity was examined through detailed analysis of specific viral groups, as well as complete community fingerprinting using randomly amplified polymorphic DNA (RAPD) PCR. Cyanomyophage are present both at the surface and at 100m depth, with significant overlap in diversity between the two depths and seasons. Marine single-stranded DNA phage exhibit a narrow depth distribution, and were consistently detected between 80m-120m, but not at the surface or deeper. RAPD analysis conducted on surface samples and 100m samples collected during the winter overturn and again during summer stratification suggest that there

may be greater differences in viral communities between the two depths during times of greater water column stratification. Future work will determine whether patterns in viral diversity are maintained in repeated seasonal cycles.

Abstract Category
28 Viral Ecology

PS.28.016 TEMPORAL DYNAMICS OF THE GENOTYPIC AND PHENOTYPIC DIVERSITY OF COASTAL MARINE CYANOPHAGE

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Phages that infect cyanobacteria are important components of marine food webs because they act as a top-down control over the major primary producers in the oceans. To investigate the dynamics of the genotypic and phenotypic diversity of cyanophage communities over time, we isolated cyanophages on *Synechococcus* host strain WH7803 each month for 15 months from three coastal locations in southern California. We then genetically characterized over 900 of these isolates by amplifying and sequencing the *g20* gene, a capsid assembly gene commonly used to characterize cyanophage phylogenetic diversity. We also characterized the host range phenotypes of over 200 of the isolates by assaying their ability to infect four additional *Synechococcus* host strains. We found that both the phylogenetic composition and the host range phenotypes of the isolates varied significantly by month, with marginal variation attributed to site. Specifically, we observed particular phylogenetic clades dominating the community at distinct times of year, with accompanying shifts in host range phenotypes. For example, isolates from winter months belonged to different clades and had significantly different host range phenotypes than isolates from summer months. Further, isolates within a single taxon ($\leq 1\%$ sequence divergence) often differed in host range when they originated from different months, suggesting that cyanophage host range is not entirely constrained by phylogeny and may be fluctuating rapidly in the environment based on changing host composition and availability. Overall, our findings suggest that cyanophage community composition, in terms of both phylogeny and host range phenotype, varies seasonally, and therefore may be driven by seasonal dynamics such as host composition and/or temperature. Seasonal variation in cyanophage communities could in turn lead to temporally dynamic patterns of cyanobacterial mortality in marine ecosystems.

Abstract Category
28 Viral Ecology

PS.28.017 CRISPR/CAS SYSTEM DRIVES DIVERSITY IN AN ISOLATED *SULFOLOBUS ISLANDICUS* POPULATION

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Models of host-virus interactions suggest that host diversity in a population under predation by viruses may be higher than without predation due to tradeoffs of viral resistance with resource utilization. The CRISPR (clustered regularly interspaced short palindromic repeats) microbial immune system has been shown to provide host resistance to viruses and plasmids. This system is in the crenarchaeon *Sulfolobus islandicus* and can be used as a marker for diversity as it is quickly changing, acting in response to viral predation. A population of *S. islandicus* from a single hot spring in Kamchatka, Russia is examined by using sequences from 39 isolates. Multi locus sequence analysis (MLSA) of 12 core genes from around the *S. islandicus* genome are used to examine the overall genomic diversity present in this population. MLSA sequences suggest an epidemic structure in which a large clonal group with identical MLSA genotypes coexists with a diverse set of recombinant strains. The CRISPR spacer arrays from the isolates are examined in order to determine the diversity present as a result of interactions with viruses from the environment. In dramatic contrast to MLSA, the CRISPR arrays are very diverse and do not match the relationships described by MLSA. The CRISPR spacer sequences split the large clonal group of isolates from MLSA into two very different groups that show little evidence of selective sweeps. We show that the isolates share a common pool of viruses, because independently acquired spacers from different isolates match one another. Additionally, 12 of the isolates are fully sequenced. These show gain and loss of sets of *cas* genes and whole CRISPR loci. Relationships between *cas* genes suggest some of the gene sets derive from more distantly related organisms, but are still functional, at least in expression and processing of the CRISPR RNA. Based on these data, we present a model in which CRISPR immunity promotes diversity in natural populations.

Abstract Category
28 Viral Ecology

PS.28.018 GENOME SEQUENCES OF TWO SYNECHOCOCCUS SIPHOVIRUSES INDICATE DIVERGED EVOLUTION OF CYANOPHAGES

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Picocyanobacteria *Synechococcus* are major primary producers in diverse marine habitats, and their distribution is greatly influenced by their co-existing viruses. Genomic studies on cyanobacterial viruses (cyanophage) have provided new insight into biological and ecological features of cyanophages. We report here the genome sequences of two cyanophages (S-CBS2 and S-CBS3) that infect two different marine *Synechococcus* strains (CB0204 and CB0202) isolated from the Chesapeake Bay. S-CBS2 and S-CB3 are the members of Siphoviridae, and no genome of *Synechococcus* siphoviruses has been reported prior to this study. The 72-kb genome of S-CBS2 comprises ca. 102 predicted open reading frames (ORFs), and 37 of them are homologous to the ORFs on P-SS2, a siphovirus infecting marine *Prochlorococcus*. The genome architecture of S-CBS2 is most similar to that of P-SS2 among all the known phage genomes, but the genome size of S-CB2 is only two third of P-SS2. The genome of S-CBS3 (33 kb) shared little homology with those of S-CBS2 and P-SS2. No photosynthesis genes were identified in the S-CBS2 and S-CBS3 genomes. The terminase-based phylogeny shows that S-CBS2 and P-SS2 belong to a separate phyletic group, while S-CBS3 is more closely related to lambda-like phages.

Comparative genomics showed that the genome evolution of cyanosiphoviruses is more diverged compared to that of other cyanophage groups (myoviruses and podoviruses). Metagenomic analysis exhibited that cyanosiphoviruses are present in relatively low abundance in the ocean compared with cyanomyoviruses and cyanopodoviruses. Based on the low occurrence of cyanosiphoviruses in the ocean and the lack of intact prophage in all sequenced genomes of marine picocyanobacteria, we hypothesize that the vast majority of picocyanobacteria in the sea are not lysogenized, and lytic infection on highly productive picocyanobacteria is the driving force for diverged genome evolution of cyanophage.

Abstract Category
28 Viral Ecology

PS.28.019 HOST-LIKE GENES CARRIED BY MARINE CYANOPHAGE REVEAL DIFFERING SELECTIVE PRESSURES BETWEEN ECOSYSTEMS

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Marine viruses affect the population dynamics and genetic diversity of marine bacteria, and as such play an important role in ocean ecosystems. Cyanophage, viruses infecting cyanobacteria, are known to harbor and express host-like genes such as *PsbA* and *hli*, during infection of their hosts. We hypothesized that host-derived genes found in phage from different environments should reflect selective pressures that have shaped the evolution of host populations, and the co-evolution of phage and host. Using the genomes of 27 cultured marine cyanophage as reference genomes, we recruited cyanophage genes from pyrosequenced metagenomics datasets derived from samples collected at two sites in the Atlantic and Pacific Oceans. We then compared the relative frequencies of genes at the two sites and found 17 cyanophage genes with statistically significant overrepresentation at either one site or the other. Of particular interest were two phage-encoded genes involved in phosphate metabolism, PstS and a putative alkaline phosphatase, that were over-represented in the Atlantic relative to the Pacific. This is consistent with the much lower phosphorus concentrations in the Atlantic, which are known to have driven the evolution of P-metabolism in cyanobacteria at this site. Furthermore, phylogenetic analysis of the cyanophage encoded phosphate-binding protein PstS from the Global Ocean Survey database reveals that phage carry distinguishable subtypes of this gene. PstS sequences from phosphate-limited environments, like the Sargasso and Caribbean seas, are more sequence similar than PstS genes from ocean regions with higher P concentrations. This suggests there is selective pressure for cyanophage to maintain particular PstS sequences under conditions of extreme phosphate limitation. These patterns were not seen in cyanophage core genes, which is consistent with the expectation that phage core genes are not likely to be subject to ecosystem-specific selective pressures

Abstract Category
28 Viral Ecology

PS.28.020 A REPLACEMENT FOR ANODISC MEMBRANES FOR VIRUS ENUMERATION

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Whatman Anodisc 0.02 μm filter supported membranes have been extensively used for determining stained virus abundance using epifluorescence microscopy (EFM). Their small pore size, flat surface, ease of use, cost, and minimal background fluorescence has made them a vital part of viral ecology research. Unfortunately, these membrane filters have been on perpetual backorder with current stocks nearly depleted leaving a methodological hole in the field of viral ecology. The aim of this research was to find a replacement technique with available materials. Polycarbonate membranes with 0.01 and 0.03 μm pore sizes were found to be good replacements as long as the filter membranes were dyed prior to use as the white membranes exhibit high background fluorescence. Irgalan black and Sudan black B dyes both decrease background fluorescence enabling fluorescent SYBR Gold stained T4 virus particles to be easily identified. Protocols for dyeing the filter membranes with Irgalan black and Sudan black B were found in Hobbie et al. 1977 and Zimmermann et al. 1978, respectively, with only minor adjustments in dye concentration required. Irgalan black (0.2g) was dissolved in 1 L of 2% acetic acid. For Sudan black B, the dye was dissolved in 100% ethanol and then diluted to 50% ethanol with distilled virus-free water resulting in a final concentration of Sudan black B of 1:375. Filter membranes were dyed for at least 2 hours, rinsed 4X with distilled virus-free water, and incubated at 75°C to dry the membranes. The electrostatic nature of the membranes required eliminating the static prior to and throughout handling the filters. Once dyed, membranes used for determining T4 virus abundance were found to decrease filtration rates at least 8-28X. In addition, mounting membranes on a glass slide required 8 μL of antifade solution as greater volumes caused excessive wrinkling of the filter.

Abstract Category
28 Viral Ecology

PS.28.021 METAGENOMIC CHARACTERIZATION OF VIRAL COMMUNITIES AND FUNCTIONS IN WASTEWATER TREATMENT PROCESSES

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Wastewater treatment plants (WWTPs) are reported to contain 10^8 - 10^{10} per ml of viral particles and thus can be considered as one of the important reservoirs and sources of viruses. However, little is known about the identity, diversity and function of viruses in WWTPs. Using metagenomic analysis, this study characterizes the viral communities in samples taken from the influent (IF), activated sludge (AS), anaerobic digester (AD) and effluent (EF) throughout a municipal WWTP. Using pyrosequencing, ~ 616 Mb of sequence reads were obtained from those four samples. Based on blast comparison ($E < 0.01$), the fraction of unknown sequences was unexpectedly high (>70%) in those four viromes. Among those identifiable sequences in individual viromes, 92-94% was affiliated with double-strand DNA bacteriophages from *Siphoviridae*, *Myoviridae*, and *Podoviridae*. Large contigs (>5 kb) were successfully obtained within individual WWTP viromes after sequence assembly, but none were related to known viruses. Moreover, < 3% of sequences (BLASTn, $E < 10^{-3}$) in individual WWTP viromes were

found in those viromes obtained previously from natural environments. The analysis of functional profiles reveals that those four WWTP viromes were overrepresented by sequences related to DNA metabolism, cell division and cell cycle, motility and chemotaxis, and cell signaling ($p < 0.05$). Such differences are likely attributed to the higher biomass concentration, activity, and turnover rate of those microbial communities found in WWTP. Comparing those four WWTP viromes based on phylogenetic and functional profiles, GC distributions, and PCA analysis indicated that AS and EF were similar to each other, and distinctly different from IF and AD. Overall findings reveal important insights into the largely-unknown viral communities in WWTP, and suggest that these WWTP viromes can have potential impacts on the surrounding environments after discharge.

Abstract Category
28 Viral Ecology

PS.28.022 BIOGEOGRAPHY OF COASTAL CYANOPHAGES IN SOUTHERN NEW ENGLAND, USA

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Viruses infecting cyanobacteria are abundant and genetically diverse in coastal environments. To assess the possible impact of viruses on marine *Synechococcus* communities, we have been tracking changes in the genetic diversity and relative abundance of cyanophages in Narragansett Bay, Rhode Island, USA for ten years. The cyanophage community in this location is dynamic; however, there are predictable temporal changes in genotypic composition. It is not known if these temporal patterns are unique to the Narragansett Bay community or are representative of other nearby cyanophage communities. To address this question, we examined the spatial and temporal patterns of cyanophage diversity at ten sites along the coast of Southern New England. In July 2009, September 2009, January 2010, and March 2010 seawater samples were collected from coastal sites along Cape Cod Bay, Nantucket Sound, Buzzards Bay, Rhode Island Sound, Narragansett Bay, and Block Island Sound. Viruses were isolated via extinction dilution enrichment using *Synechococcus* strain WH7803. A segment of the DNA polymerase gene was amplified and sequenced for 20 to 40 viral isolates from each location at each sampling date. At present, over 500 cyanophage isolates have been characterized. These isolates can be grouped into 57 distinct DNA polymerase genotypes. While over 90% of the isolates were myoviruses, the September 2009 Narragansett Bay samples were dominated by podoviruses. Both spatial and temporal variations in community composition were observed. In particular, the cyanophage communities at the two Cape Cod Bay sites were strikingly different than the communities observed at the other sites. We are continuing to analyze additional isolates and also are examining the biotic and abiotic factors that may influence the composition of cyanophages at these sites.

Abstract Category
28 Viral Ecology

PS.28.023 POPULATION DYNAMICS AND GENETICS OF AN ABUNDANT COASTAL CYANOPHAGE, RHODE ISLAND MYOVIRUS 2

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Marine viral communities are notably diverse with metagenomic studies revealing thousands of co-occurring viral types. Despite the tremendous amount of viral diversity, we have repeatedly isolated a cyanophage, Rhode Island Myovirus 2 (RIM2), from Southern New England coastal waters. In this study, we characterized the temporal and spatial population dynamics of RIM2 and then assessed the allelic diversity of the host-derived photosystem II *psbA* gene in RIM2 isolates. Cyanophages capable of infecting marine *Synechococcus spp* were isolated via extinction dilution enrichment from Narragansett Bay, Rhode Island from 1999 to 2010 and from eight additional coastal sites in Southern New England in 2009 and 2010. Isolates from the extinction dilution were assigned to a RIM strain based on *g20* portal protein and *g43* DNA polymerase gene sequences. All isolates of RIM2 have identical or nearly identical *g20* and *g43* gene sequences (>99.5% nucleotide sequence identity). Over the past 10 years, RIM2 isolates were routinely isolated from July, August, September, and October Narragansett Bay seawater samples. In July 2009, RIM2 accounted for 40% to 80% of the isolates characterized from each of eight sites along the Southern New England Coast, suggesting that this viral type is extremely abundant over a wide spatial range. In contrast, RIM2 has only rarely been observed in seawater samples collected between November and June. Although all RIM2 isolates have nearly identical *g20* and *g43* gene sequences, host-derived photosystem II *psbA* genes are very variable. Multiple *psbA* alleles (differing from one another by up to 10% in nucleotide sequence) were observed among RIM2 isolates from the same water sample. Preliminary data suggest that *psbA* allelic diversity is not spatially or temporally partitioned. These results show that there can be significant allelic diversity of host-derived genes within a single viral population.

Abstract Category

28 Viral Ecology

PS.28.024 VIRAL AND CELLULAR METAGENOMIC ANALYSIS OF ACIDIC HOT SPRINGS IN YELLOWSTONE NATIONAL PARK

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We present a metagenomics approach to assess viral diversity and the cellular defense response to viral interaction. Metagenomes for paired cellular and virus-enriched fractions were obtained from high temperature acidic hot springs from Yellowstone National Park (YNP). These low diversity host communities are comprised of as few as two dominant archaeal genera while analysis of the viral community by metagenomics revealed previously unknown viruses. Sub-sampling of the viral metagenomes has resulted in enhanced assembly of the putative viral genomes. Most of the assembled viral genomes are not related to known viruses. Analysis of the cellular metagenome CRISPR-spacer (Clustered Regularly Interspaced Short Palindromic

Repeats) sequences associated with the hosts' viral defense matched sequences present in the viral metagenomes. A microarray containing CRISPR spacers has been used to follow viral populations over time. The results support the concept that CRISPRs are actively evolving cellular loci that reflect past and current virus encounters. The study demonstrates that paired cellular-viral metagenomics studies can expand our understanding of virus diversity and the cellular mechanisms contributing to this diversity.

Abstract Category
28 Viral Ecology

PS.28.025 VIRAL PRODUCTION ON MARINE AGGREGATES: EVIDENCE FROM EXPERIMENTS USING TWO DIFFERENT SEAWATER CULTURES

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Marine aggregates act as scavengers of viruses or are reservoirs which sustain high viral abundance. Interactions of viruses and aggregates may influence biogeochemical cycles in marine environments; however little is known about the rate of viral production and decay and viral induced mortality of bacteria on aggregates. In this study, microcosm experiment was performed to investigate the rate of viral production on aggregates which were produced from eutrophic and oligotrophic waters. Water was collected at a station in the bay and outside the reef barrier in New Caledonia. Organic aggregates were from seawater and incubated in viral-free water with continuous flow for 12 days. Changes in viral production and abundance in the aggregate and ambient water were measured. In the bay water treatment, low viral production rate at the beginning of incubation on aggregates increased about 24-fold at Day6 and remained constant until the end of incubation. Relatively similar rate of viral production were observed on aggregates and in ambient water. Contrary, in the offshore treatment, viral production rate was high on aggregates at the beginning of the incubation, and increased about 4-fold from Day6 to the end of incubation both on the aggregate and in ambient water. In this treatment, viral production on aggregates was higher than ambient water throughout incubation. Interestingly, initial viral production rates and variations during incubation were different in the bay and the offshore treatments; however variations of viral production rate between aggregates and ambient water were similar for both treatments. This indicates that aggregates released viruses to ambient water which may influence free-living community and its rates may differ depend on trophic condition. Our result suggests that viruses-aggregates interactions influence biogeochemical cycles in marine environments.

Abstract Category
28 Viral Ecology

PS.28.026 MITOMYCIN C INDUCTION OF METHANOGENIC ARCHAEA TO DETECT LYSOGENIC ARCHAEAPHAGE

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The majority of methane generated by ruminant digestion may be attributed to the activity of methanogenic Archaea. Phage therapy has the potential to reduce methane emissions from ruminants by either directly reducing archaeal numbers, or by helping to establish alternative rumen microbial populations, for example, reductive acetogens, which may out-compete methanogenic archaea for hydrogen. Very little is currently known about phage which may infect the archaeal strains dominant within the rumen, such as *Methanobrevibacter ruminantium*. To gauge the extent to which temperate phages exist in ruminal methanogens, cultures of known methanogens were treated with the inducing agent mitomycin C and DNA from extracellular particles extracted.

Small (9.6 ml) and large (100 ml), 2 d cultures of archaea, including isolates of *Methanobrevibacter* sp. (YE301, YE303), *Methanobacterium bryantii* (YE299), *Mbb. smithii* (DSM861), and *Mbb. ruminantium* (ATCC35063) were treated with mitomycin C (1 µgml⁻¹) and incubation continued for a further 5 d at 39°C. Control cultures, to which no mitomycin C was added, were also incubated for a total of 7 d. Cells were then removed by centrifugation and the supernatant used for phage DNA extraction. Extracted DNA was examined for phage genetic material using standard and pulsed field gel electrophoresis (PFGE).

Treatment of five different methanogens with mitomycin C did not appear to result in increased concentrations of extracellular DNA. PFGE analysis indicated that some strains, for example, *Methanobrevibacter* sp. YE303, produced high molecular weight extracellular DNA but whether this is of viral origin requires clarification.

The presence of viable temperate phages may be a rarity amongst rumen methanogens, such as *Methanobrevibacter* sp. The archaeal strains examined may be useful for the isolation of lytic phages from the environment as they do not appear to contain viable lysogenic phage, which may potentially inhibit lytic phage infection.

Abstract Category
28 Viral Ecology

PS.28.027 METAGENOMIC COMPARISON OF PROVIRAL AND FREE VIRAL ASSEMBLAGES IN FOREST AND AGRICULTURAL SOILS

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Paired metagenomes of extractable (free viruses) and mitomycinC (mitC)-inducible viruses (proviruses) were analyzed from agricultural and forest soil from the Kellogg Biological Station, MI. Comparative analysis from the forest soil revealed that free viral and proviral assemblages differed significantly. In the free viral assemblage 71% and 19% of the predicted ORFs had

either no known match or were identified as hypothetical proteins, respectively. In contrast, the proportion of 'no match' ORFs was substantially lower, 38%, in the induced viral assemblage and 31% of ORFs matched hypothetical proteins. Among the subset of ORFs from the proviral library that could be assigned taxonomic affiliation, approximately 38% possessed homology to *gamma-proteobacteria* (or prophage within their genomes), while less than 2% of ORFs from the free viral library could be assigned to this class. This finding suggests that mitC-mediated induction may be primarily restricted to *gamma-proteobacteria* or reflect bias towards this class in sequence databases. Some bacterial proteins such as ATPases with chaperone activity (ATP-binding subunit), 3-phosphoglycerate kinase, and NAD-dependent epimerase/dehydratase family proteins were only detected within the free viral library. Interestingly, the well-known SOS or recA-dependent phage proteins such as cro repressor, some integrases, bacteriophage CII protein, and some terminases responsible for regulating the lytic-lysogenic switch in lambda-like phage were only detected in the proviral metagenome. However, homologs of site-specific recombinases, phage integrase family, and helix-turn-helix domain-containing proteins (cro-like DNA binding protein) within the free viral library indicates that temperate phage, possibly with a different regulatory mechanism governing the lysogenic-lytic switch, also occurred within the free virus fraction. Similar comparative analysis of viral assemblages in agricultural soils will also be presented.

Abstract Category
28 Viral Ecology

PS.28.028 CHARACTERIZATION OF HALOPHILES AND THEIR VIRUSES FROM THE CARGILL SALT WORKS, NEWARK, CA

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Extremely halophilic microorganisms inhabit environments between 10% and 30% salt concentration and have been studied extensively. Much has been learned regarding their physiology and ecology. However, very little is known about the viruses (phages) that infect these microorganisms and how they impact the ecology of their hosts. In marine and aquatic systems phages have been shown to have great impact on their host populations by affecting population dynamics and exerting evolutionary pressure. Salterns provide ideal systems for studying halophiles and their phage as they contain ponds of varying salinity allowing for comparative studies among the different saline habitats. Water samples were collected in the Cargill Solar Salt Works (Newark, CA). Hosts were initially enriched in modified growth medium at 15% and 23% salinity. Individual strains were isolated by streak plating and identified using 16S rRNA gene sequencing. Phages in source water were concentrated by centrifugation and filtration and then isolated via top agar overlays with susceptible hosts. Hosts have been isolated from both the bacterial and archaeal domains. Halophiles were identified as *Halomonas*, *Salicola*, *Alkalibacillus* (bacterial) and *Halorubrum* (archaeal). Eleven phages have been isolated to date from five different isolates, four of which are from the archaeal genus *Halorubrum* and one from the bacterial genus *Salicola*. One *Halorubrum* phage-host pair and one *Salicola* phage-host pair were studied to determine the tolerance ranges of both hosts and viruses to variations in salinity, pH, and temperature. The *Halorubrum* host showed salinity, pH,

and temperature optima higher than those of the *Salicola* host. Substrate usage differences were detected between hosts using BIOLOG phenotypic micro array plates. This study will expand the knowledge of halophilic host-phage pairs in this saltern and allow for comparisons with the host-phage systems of other saline environments.

Abstract Category
28 Viral Ecology

PS.28.029 THE ROLE OF FRESHWATER PHYCODNAVIRUSES IN PHYTOPLANKTON MORTALITY

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An initial effort to characterize algal virus communities in a variety of North American freshwater environments was conducted by sequencing clone libraries of PCR-amplified phycodnavirus DNA polymerase gene fragments. The results suggested that freshwater environments were home to diverse communities of phycodnaviruses, yet all PCR fragments sequenced were related to DNA polymerase genes from cultivated marine Prasinoviruses; surprisingly, sequences related to other types of phycodnaviruses were not observed. To address whether PCR could be used to detect other types of freshwater phycodnaviruses, new primers were designed and different environments were sampled over several seasons. Ultimately, these efforts resulted in the recovery of sequences related to DNA polymerase genes from freshwater Chloroviruses and marine Prymnesioviruses. While these results confirmed that diverse phycodnaviruses exist in freshwater environments, they did not establish their importance as agents of phytoplankton mortality. Hence, a modified version of previously described dilution-based mortality experiments was conducted to compare the mortality of phytoplankton due to viruses or grazers for an entire phytoplankton community (via chlorophyll a), and for an individual phytoplankton taxa, *Chlorella* sp. (via quantitative PCR). This experiment demonstrated that viruses were an insignificant source of mortality for both the entire community and for *Chlorella* sp., whereas grazers were a significant source of mortality for the whole community, but not for *Chlorella* sp. Thus, although the experiment failed to demonstrate the importance of viruses in phytoplankton mortality, it did suggest that whole-community determinations of mortality can miss important details of carbon flow through aquatic food webs since different primary producers within the same community can be subjected to different sources and amounts of mortality.

Abstract Category
28 Viral Ecology

PS.28.030 NOVEL DNA VIRUSES FROM CARIBBEAN CORAL

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Coral reefs provide critical habitat to tropical marine life and are fundamental in maintaining the immense diversity of organisms found in tropical marine waters. In recent years, coral reefs have

been declining, in large part due to increased sea surface temperature, diseases and increased anthropogenic inputs. These stressors can be exacerbated by viral infection which has been demonstrated but has not been well characterized in corals. This is the first study to identify DNA viruses associated with the Caribbean coral *Montastraea cavernosa* both before and after acute heat stress. *M. cavernosa* fragments removed from a seawall in the Florida Keys were acclimated to aquarium conditions for over 5 months. Fragments were heat stressed in an incubator for 12 hours at 31-32°C. Using an extraction protocol modified from Vega Thurber et al., (2009) viral DNA was extracted from the fragments and cloned using random hexamer primers into competent *E.coli* cells. Cloned DNA was then isolated for sequencing. We found a greater diversity of viral types in heat stressed *M. cavernosa* than controls, suggesting that a consequence of increased sea temperature is greater viral stress. Characterizing viruses from control and heat stressed *M. cavernosa* is the first step into understanding the role that viruses in combination with environmental stress have on coral decline.

Abstract Category

28 Viral Ecology

PS.28.031 ANALYSIS OF VIRUS-CRISPR INTERACTIONS IN A NATURAL SYSTEM

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CRISPRs (clustered regularly interspaced short palindromic repeats) are rapidly evolving adaptive microbial immune systems that contain short sequences (spacers) that confer immunity through recognition of viral or other foreign DNA. Here we simultaneously analyzed virus populations and CRISPRs of targeted microbial populations to study virus-host interaction dynamics in five relatively low diversity acid mine drainage (AMD) microbial communities collected over a two-year period from one location. We tracked microbial community composition, reconstructed CRISPR loci from the dominant archaeon (G-plasma) and bacterium (*Leptospirillum* Group II), and assembled genomes of the dominant viral populations. A major bloom of a virus that appears to replicate in G-plasma correlates with a strong decline in abundance of G-plasma. Though we detect extensive locus diversification, there is no record of a single spacer acquisition event that led to rebound of the G-plasma population after the viral bloom. Notably, the deeply sampled viral population (~800X) is highly non-clonal. We infer a stochastic event in which many viral types proliferated, with many host sub-populations establishing resistance simultaneously. Unlike G-plasma loci, the *Leptospirillum* Group II CRISPR did not exhibit addition of new spacers over time. Strains that share spacer complements shift in abundance, suggesting selection among coexisting sub-populations. The first ~2/3 of spacers are shared across all locations and time points examined previously, indicating a common ancestry and preferential loss of newly acquired spacers. CRISPRs and viruses are co-evolving rapidly, but virus-host interaction dynamics may not always be described by locus expansion followed by strong selection. Instead, the spacer age distribution across the

locus is inferred to be highly non-linear and locus diversification patterns may reflect different levels of immunity and infectivity in host and virus sub-populations.

Abstract Category
28 Viral Ecology

PS.28.032 VIRUSES INFECTING MARINE CYANOBACTERIA EXPRESS A CALVIN CYCLE INHIBITOR AND ALTER HOST REDOX POISE

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Marine viruses shape the diversity and biogeochemical role of their microbial hosts. Cyanophages that infect the cyanobacteria *Prochlorococcus* and *Synechococcus* often carry metabolic genes not found in other bacteriophages. The proteins encoded by these "auxiliary metabolic genes" are thought to increase phage fitness by altering host metabolism during infection. Dominant among the suite of auxiliary metabolic genes carried by cyanophage are genes involved in photosynthesis and the pentose phosphate pathway (PPP). Cyanophage genomes contain genes for the PPP enzymes transaldolase, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase. Most significantly, cyanophages also encode an inhibitor of the Calvin cycle, CP12, which in cyanobacteria is thought to direct carbon flux away from the Calvin cycle and toward the PPP. Measuring their expression during infection in model phage—host pairs, we observed that phage-encoded PPP enzymes and CP12 were co-expressed with photosystem II genes while the NADPH/NADP ratio increased two-fold, consistent with increased activity of the NADPH-producing light reactions and PPP. Phage ribonucleotide reductase, which produces nucleotides using reducing equivalents from NADPH, was co-expressed with this set of genes. We propose, therefore, that phage carry these genes to boost the host PPP and light reactions to produce NADPH for phage genomic DNA production. No Calvin cycle genes have been found in cyanophage, supporting the hypothesis that the selection pressures molding phage genomes involve fitness advantages conferred through mobilization of host energy stores and not through carbon fixation.

Abstract Category
28 Viral Ecology

PS.28.033 ISOLATION AND CHARACTERIZATION OF PBCV-1-RESISTANT CHLORELLA NC64A

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In aquatic systems, viruses play several major roles which can impact the larger community. Elucidating interactions with host cells is critical to understanding their large-scale influences. Resistance to infection is one factor that must be considered in models of viral effects. Analyzing the differences between susceptible and resistant hosts can lead to better comprehension of key steps in the infection process. PBCV-1, a member of Phycodnaviridae, and its host, *Chlorella*

NC64A, have been a model for studies of virus-host interactions for ~30 years. Recently, we isolated a confirmed PBCV-1-resistant variant of *Chlorella* NC64A. Cells are not chronically infected, as has been reported previously, and Southern hybridizations show no sign of lysogeny. Also, the growth rate of the resistant isolate is not affected by addition of PBCV-1 to actively growing cultures. Fluorescently labeled virus particles do not bind to resistant cells as compared to susceptible cells. This finding is further supported by an assay designed to quantify particle attachment, which demonstrated no significant attachment as compared to a non-host microalga and susceptible NC64A controls. In addition to single cells, resistant NC64A cultures have multicellular aggregates, a phenomenon not observed with susceptible cells. SDS polyacrylamide gel profiles of surface proteins are consistently very different between susceptible and resistant cells. These two observations suggest resistance is due to a change in surface properties. However, lysin, a crude enzyme preparation from PBCV-1 lysates, which degrades susceptible cell walls, also degrades the cell walls of resistant cells. Further characterizations are underway while future studies may include the comparison of transcriptomes of resistant, susceptible, and infected NC64A. Overall, the expansion of this virus-host model to include resistance can add more pieces to our understanding of the global importance of aquatic viruses.

Abstract Category
28 Viral Ecology

PS.28.034 METAGENOMIC EXPLORATION OF VIRUSES THROUGHOUT THE INDIAN OCEAN

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The characterization of global marine microbial functional and taxonomic diversity is a primary goal of the Global Ocean Sampling Expedition. Nineteen water samples were collected aboard the *Sorcerer II* sailing vessel from the Indian Ocean in an effort to more thoroughly understand the lifestyle strategies of the microbial inhabitants of this nutrient poor region. Five samples, collected from the western Indian Ocean, were selected for comprehensive metagenomic exploration; and sequencing was performed on all size fractions including viral (<0.1 μ m), 0.1 μ m-0.8 μ m, 0.8 μ m-3.0 μ m and 3.0 μ m-20 μ m. A total of 228,633 Sanger and 3,189,942 454 reads were produced from the viral samples, resulting in a combined 3,896,751 predicted ORFs. Furthermore, phylogentic approaches were used to identify predicted proteins of viral origin in the larger fractions of data (85,063) from all samples, which were included in subsequent metagenomic analyses. The predicted viral proteins, contained within protein clusters, were mapped to a diverse array of pathways involved in metabolic and other cellular processes. In an effort to assess functional diversity, the richness and evenness of protein clusters containing viral sequences was determined and the Shannon's diversity index was calculated for all sampling locations. Functional diversity was generally highest among the virus-specific libraries and lowest among the largest size fractions. Additional analyses further revealed fundamental differences between the categories of viral sequences. Virus-containing protein clusters grouped according to size fraction and the most abundant protein clusters also differed between the viral fraction and viruses identified within the larger size fractions. These observations suggest that

distinct populations of viruses exist within Indian Ocean waters, differing in particle size, diversity, total gene complement and metabolic potential.

Abstract Category
28 Viral Ecology

PS.28.035 FOSMID TALES: CURIOUS VIRUS SIGNATURES WITHIN BACTERIAL METAGENOMIC DATA

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Over the past decade, viral metagenomic sequencing has revealed a vast array of genetic diversity harboured within aquatic viruses. Most viromes are composed of thousands of short (100-700 bp) reads, few of which assemble into contigs or share homology with known sequences. While this ocean of novel genetic sequence is astonishing, it remains largely inaccessible to science, filling databases with sequences of unidentified taxonomy and function. Fosmid-based sequencing could bridge the gaps between short-read viromes and genome sequencing projects by serving as scaffolds to stitch together virome sequences. During the fosmid library sequencing of the 0.2 μm size-fraction collected from Saanich Inlet, British Columbia, six fosmids ranging in size from 33.1 to 36.2 Kbp were mapped as complete circles and identified as potential viruses or prophages. Characteristic of viral sequences, 70% (219) of the 313 fosmid ORFs lacked a significant homolog in GenBank nr (nr), and 57% (178) shared significant homology with sequences from previous viral metagenome sequencing efforts. Of the ORFs with significant homologs in nr, 22% (21) shared similarity with viral proteins, with a predominance of siphoviridae sequences. The majority of identifiable ORFs shared similarity with bacterial proteins, but 49% (36) of these occurred in probable prophage regions of a bacterial genome. To discern patterns in geographic distribution, the fosmids were compared to 14 publicly available viral metagenomes and additional microbial fosmid and shotgun libraries from Saanich Inlet. Fosmid coverage by virome sequences ranged from 1.4 to 19.7%, and unique temporal and spatial patterns of presence and abundance emerged for each of the fosmids. In conclusion, viral sequences are amenable to fosmid-based cloning and sequencing, are occasionally captured during bacteria-directed studies, and can provide a new approach to examine viral biogeography and viral-host connections.

Abstract Category
28 Viral Ecology

PS.28.036 VIRAL COMMUNITIES OF THE CALIFORNIA CURRENT EXPOSED USING METAGENOMICS AND SINGLE VIRUS GENOMICS

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Viruses play an important role in fueling the marine microbial loop through viral lysis of bacteria and phytoplankton; thus releasing energy-rich dissolved organic matter for bacterial consumption

and increasing overall CO₂ concentrations through respiration. Therefore, understanding the metabolic potential of marine viroplankton through genomic analyses will increase our knowledge surrounding their dynamic role in marine ecosystems. We have combined metagenomics and single virus genomics (SVG) approaches to bridge the gap between viral community and whole genome analysis. Samples across a coastal upwelling mosaic were collected from the California Current (July 2007). Three of the seven stations sampled were selected for viral metagenomic sequencing using 454-Titanium (~1.3 million peptides) with isolation and sequencing of viruses (70 particles) from one site using SVG methods. Many of the known genes comprising the 'accessory genome' carried within marine viroplankton communities were present [psbA and pebA (photosynthesis), cobS (cobalamin synthesis), hsp20 (heat-shock)], with the addition of unprecedented genes involved in nitrate assimilation. Further analysis is being conducted to evaluate if these genes can be recovered from viral molecules through genomic assembly and PCR experiments. Metagenomic sequencing of larger microbial size classes was conducted and viral sequences classified using a phylogenomics pipeline. Results suggest that the viral peptides from these larger size classes have a unique metabolic repertoire compared to viruses that passed through 0.1 μm filters. In addition, viral sequences from the largest size class (3.0 μm-20 μm) were the least diverse, which may be due to viral infection of microalgae. The production of an extensive environmental viral reference genome library from sites with complementary metagenomic data will result in a better understanding of marine viroplankton genotypic and functional diversity.

Abstract Category
28 Viral Ecology

PS.29.001 VARIATION ON CHEMICAL COMPOSITION AMONG MICROBIAL BIOFILMS FROM FURNAS HOTSPRINGS, S. MIGUEL, AZORES

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Most termal microbial communities constituted by biofilm formers promote direct or indirect chemical nucleation. It is possible to encounter a wide chemical and physical variety of hydrothermal fluids at Furnas hotspots. These ecological parameters enhance the high biological diversity found within these habitats. It is possible to find not only acidic and alkaline hotspots within close proximity but also a wide range of temperatures ($\geq 35^{\circ}\text{C}$ until 98°C). The purpose of our work was to compare major elemental chemical composition among microbial biofilms were dominant microbial communities vary. Microbial biofilms from environmentally distinct hotspots (based on hydrothermal fluid characteristics) were collected during spring and summer. The major microbial components were identified based on descriptions found in published data, and by optical and electron microscopy. Scanning electronic microscopy coupled to element percentage determination was carried out using energy dispersive X-ray spectroscopy (EDX) in order to identify major elemental chemical composition and quickly screen for chemical variations among different biofilms (JEOL scanning electron microscope with an elemental composition detection limit of 0.1%). Major chemical elemental differences among microbial biofilms with distinct compositions were observed. Biofilms from different ecological thermal habitats (niches) within the same thermal pool present distinct elemental chemical composition profile. Since the thermal fluid source is the same one may conclude that these variations are due to specific microbial community composition. The particular microbial community composition is responsible for particular metabolic strategies that lead to a differential mineral nucleation or mineral concentration.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.002 QUORUM SENSING IN A PHOTOSYNTHETIC *BRADYRHIZOBIUM*: PRODUCTION OF CINNAMOYL-HOMOSERINE LACTONE

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Many Proteobacteria use acyl-homoserine lactones (HSLs) as quorum-sensing signals. Signal is generated by members of the LuxI family of acyl-HSL synthases. With one known exception these signals are fatty acyl-HSLs. The exception, p-coumaryl-HSL (pC-HSL), is an aromatic HSL produced by the phototrophic bacterium *Rhodospseudomonas palustris*. The phototrophic *Bradyrhizobium* ORS278 generates a signal that can substitute for pC-HSL in bioassays. We purified the signal substitute from ORS278 and identified it chemically. High-resolution mass

spectrometry showed the purified material had a molecular mass of 254.0778, consistent with the mass of the Na adduct of cinnamoyl-HSL (cinn-HSL). By using radiotracer methods, we found that when fed a variety of fourteen related aromatic acids (including cinnamate and p-coumarate) ORS278 cells produced material that co-eluted with synthetic cinn-HSL in HPLC. Similar results were seen when the ORS278 LuxI protein was expressed in a heterologous host bacterium. The *Rhodopseudomonas* bioassay was used to show that cinnamic acid stimulates production of cinn-HSL by ORS278. Both the HPLC fractionated material and synthetic cinn-HSL were active in the bioassay. The genome of ORS278 has a single luxI homolog, and a mutation in this gene resulted in loss of cinn-HSL production. We do not yet know what role cinn-HSL plays in the biology of ORS278, but it seems likely that there are many aryl-HSLs yet to be discovered.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.003 COMPARISON OF STAINING AND COUNTING FOR MICROSCOPIC ENUMERATION OF BACTERIA IN BIOFILM

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Background: Bacteria associated with leaves and root surfaces of plants form assemblages as aggregates, microcolonies, or biofilms. Biofilms are known to be micro-habitats with conditions markedly different from surrounding water environments. Therefore, understanding dynamics of bacterial flora in biofilm is an important and basic study to characterize the unique and active micro-habitat. Methods: Three kinds of samples were prepared : cultures, water and biofilms (leaves and root). Each of AO, DAPI and BacLight staining methods were used as described by Hobbie et al.,(1977), Porter et al.,(1980) and Haugland (2002), respectively. Bacteria were enumerated by visual sight and an image analyzer (i-Solution version 6.3; IMT-iSolution Inc., Vancouver, Canada) with an Olympus BH2 microscope with UV (DAPI), or WB (AO, BacLight) dichromatic filter sets and Fluorite 100 x objective. A statistical software package, SPSS version 14.0 (Chicago, IL, USA), was used for variance decomposition by analysis of variance (ANOVA). The relationship between visual and image analysis was determined using regression analysis. Results: Mean values of bacterial counts for culture, water and biofilm samples were highest with BacLight, AO, and BacLight methods, respectively. In most cases, visual counts were slightly higher than the counts by image analyzer. ANOVA at significance level of 95% indicated that BacLight was suitable to detect cultures of *Lactobacillus* sp. and the bacteria in biofilms of plant leaves and roots. In case of *E. coli*, the average counts were similar between BacLight and AO methods. In water samples, AO showed significantly better sensitivity than the other two methods. The linear analysis revealed significant agreements between visual and image analysis counts for all staining. Conclusions: Culture of *Lactobacillus* sp. and biofilm samples were best analyzed with BacLight while AO staining resulted highest validated counts for culture of *E. coli* and water samples.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.004 USING STREAM BIOFILM MICROBIAL COMMUNITIES AS AN INDICATOR OF FRESHWATER ECOSYSTEM HEALTH.

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Stream biofilms are a complex aggregation of microorganisms embedded in a polymer matrix and cover almost every surface in freshwater environments. Because of their sedentary way of life, microorganisms associated with biofilms are affected by past and present environmental conditions and therefore constitute a potential integrative indicator of stream health. A wide range of experiments was conducted in both flow chamber microcosms and natural stream environments to investigate the main drivers of microbial community structure and composition and evaluate the potential use of biofilms as a bio-indicator of freshwater ecosystem health. Using community fingerprinting techniques such as terminal-Restriction Fragment Length Polymorphism and Automated Ribosomal Intergenic Spacer Analysis as well as 16S rRNA gene clone libraries we investigated variations occurring in biofilm bacterial and ciliate protozoan communities. Initial experiments conducted in flow chamber microcosms showed that significant differences in microbial community structure could be detected within only a few days of exposure to common water contaminants and remained detectable weeks after transfer to uncontaminated water. Further research investigating biofilm of more than 60 stream sites variously impacted by urbanization revealed a strong separation between rural and urban streams and confirmed the potential use of stream biofilm as a bio-indicator of stream health. Environmental monitoring techniques developed in this project were then successfully tested to investigate the efficacy of an enclosed stormwater treatment system, where traditional biological indicators such as macro-benthic invertebrates were not available. We are now extending our research to 300 different streams in order to define a general Bacterial Community Index characterising stream ecosystem health based on the structure of biofilm bacterial communities.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.005 ENVIRONMENTAL TRIGGERS AND INTRACELLULAR SIGNALING OF BIOFILM DISPERSAL

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Biofilm dispersal is a crucial event that determines the lifestyle of bacteria and this process is usually finely regulated in response to both environmental and physiological cues. Previous studies of the natural life cycle of biofilms revealed an important role for nitric oxide (NO), a by-product of anaerobic respiration and a signaling molecule used broadly in biological systems, in inducing the switch from a sessile to a free swimming, planktonic mode of growth. Moreover, exposure to low, non-toxic levels of NO by using NO donor molecules was found to stimulate phosphodiesterase activity, decrease intracellular levels of the second messenger cyclic di-GMP

(c-di-GMP) and enhance dispersal in the model organism *Pseudomonas aeruginosa*. These findings suggested that NO signaling is part of a global regulatory network that involves c-di-GMP and controls lifestyle transitions in bacteria. To further investigate the regulation of biofilm dispersal, *P. aeruginosa* biofilms were grown in continuous-flow culture microfermentors that allowed for the study of dispersal in response to a range of environmental stimuli, including exposure to NO or NO-related compounds, changes in oxygen and nutrient levels and other putative dispersal factors. The results show that, when induced, biofilm dispersal occurred within minutes of changing the environmental conditions and was characterised by a rapid release of bacterial cells in the bulk liquid flow. Addition of NO, sodium nitrite at neutral pH but not at acidic pH, decreases in nutrient (glucose) and oxygen levels, as well as increases in temperature were found to be potent inducers of biofilm dispersal. Because the onset and duration of the dispersal events closely resembled the effects of NO, these data suggest that the regulatory mechanisms implicated in the response to such diverse environmental triggers may converge towards global regulatory pathways, possibly involving c-di-GMP.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.006 IDENTIFYING QUORUM SENSING ACTIVITY IN HYPERSALINE MATS USING A FUNCTIONAL METAGENOMIC APPROACH

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Quorum Sensing (QS) is a cell-density dependent phenomenon whereby bacteria produce and detect small diffusible signaling molecules called autoinducers. This signaling pathway results in changes in gene expression, ultimately regulating important biochemical processes that play a role in determining the structure and function of microbial communities in a range of natural environments. While the QS systems of many 'model' bacteria have been described, little is known about QS in natural environments. To understand the role of QS in microbial community structure, function, and stability more information is needed regarding the breadth and depth of QS genes in natural ecosystems. The complex metabolic interactions within microbial mat communities make these systems ideal models for examining quorum sensing activities under natural conditions. Our objective was to screen for QS activity in hypersaline mats using a functional metagenomic approach to understand putative QS in such mats. High-molecular-weight (HMW) DNA was extracted from microbial mats (San Salvador, Bahamas) using a DNA extraction method optimized in our lab. The DNA was sheared to 40 kb, ligated into CopyControl fosmid vectors, and transformed into *Escherichia coli* (*E. coli*) TansforMax EPI300 cells. The transformants were grown on Luria Bertani (LB) agar and selected with 12.5 µg/ml chloramphenicol. Clones were cultured in liquid LB amended with CopyControl fosmid autoinduction solution to induce production of high fosmid copy number according to the manufacture's instructions. Using *Agrobacterium tumefaciens* NTL4 QS biosensor, we screened each fosmid clone for putative QS activity in 96-well plates. To date, we have obtained more than 1200 clones out of which approximately 35 show putative QS activity. We are currently using mass spectrometric and NMR methods to identify the autoinducers produced by the active clones and are sequencing fosmid inserts to identify the QS genes.

Abstract Category
29 Biofilms and Sociomicrobiology

PS.29.007 MICROSCALE CHEMICAL HETEROGENEITIES IN SEDIMENT BIOFILMS

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Most uranium bioremediation studies have improved our understanding at the macroscale level, but not at the microscale. The goal of this research was to quantify microscale physicochemical heterogeneities in Hanford 300A sediments and to understand metabolic responses of *Shewanella oneidensis* MR-1 biofilms to U(VI).

Chemical conditions inside sediments from Hanford 300A were analyzed using microelectrodes. Temporally- and spatially-resolved metabolic responses of live *S. oneidensis* biofilms to U(VI) were elucidated using NMR.

Our results showed that there were significant differences in microscale and macroscale chemical conditions in the sediment-groundwater system. Under anoxic conditions, redox potentials in sediments were significantly lower than those in oxic conditions. Dissolved oxygen in the sediment decreased with depth. We observed anoxic zones in the sediments while the bulk phase was saturated with oxygen. Significant amounts of hydrogen was found in certain locations inside the sediment under anoxic conditions. pH inside the sediment was relatively constant.

Uranium caused a temporary inhibitory effect on *S. oneidensis* MR-1 biofilms. We found that surface-averaged relative effective diffusion coefficients decreased towards the bottom of the biofilms and varied by biofilm growth.

Microscale physiochemical conditions in the sediment were heterogeneous and significantly different from those at the macroscale, suggesting the importance of microscale studies towards the understanding of U mobility in the subsurface.

Abstract Category
29 Biofilms and Sociomicrobiology

PS.29.008 PSEUDOMONAS AERUGINOSA USES A CYCLIC-DI-GMP-REGULATED ADHESIN TO REINFORCE THE BIOFILM EXTRACELLULAR MATRIX.

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Pseudomonas aeruginosa, the principal pathogen of cystic fibrosis patients, forms antibiotic-resistant biofilms promoting chronic colonization of the airways. The extracellular matrix is a crucial component of biofilms that provides the community multiple benefits. Recent work in the field suggests that the secondary messenger cyclic-di-GMP promotes biofilm formation. An analysis of factors specifically expressed in *P. aeruginosa* under conditions of elevated c-di-GMP revealed functions involved in the production and maintenance of the biofilm extracellular matrix. We have characterized one of these components, encoded by the PA4625 gene, as a putative adhesin and designated it *cdrA* (cyclic diguanylate-regulated TPS partner A). CdrA shares structural similarities to extracellular adhesins that belong to two-partner secretion systems. The *cdrA* gene encodes a 220 KDa protein that is predicted to be a rod-shaped protein harboring a β -helix structural motif and is part of a two gene operon that also encodes an outer membrane transporter, CdrB. We verified that *cdrAB* expression increases with elevated c-di-GMP and decreases when c-di-GMP is depleted. We further demonstrated that *cdrA* expression is minimal in liquid culture, but is elevated in biofilm cultures. Overexpression of CdrAB was also sufficient to produce autoaggregation in liquid culture. Aggregation mediated by CdrA is dependent on the Psl polysaccharide and can be disrupted by adding mannose, a key component of Psl. In co-immunoprecipitation assays, CdrA binds the Psl polysaccharide. A mutation in *cdrA* caused a decrease in biofilm biomass and resulted in the production of biofilms with decreased structural integrity. Psl-specific lectin staining of biofilms suggests that CdrA either crosslinks Psl polysaccharide polymers and/or tethers Psl to the cells, resulting in increased biofilm stability. Thus, this study identifies the first protein structural component of the *P. aeruginosa* extracellular matrix.

Abstract Category

29 Biofilms and Sociomicrobiology

**PS.29.009 ENHANCED BIOFILM GROWTH OF ENTEROHEMORRHAGIC
ESCHERICHIA COLI BY INDIGENOUS BACTERIAL COMMUNITIES**

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Enterohemorrhagic *Escherichia coli* (EHEC) include a diverse group of Shiga toxin-producing *E. coli* that cause severe disease including hemolytic uremic syndrome. *E. coli* O157:H7 (EcO157) is the most common EHEC serotype and contributes significantly to human infections and outbreaks. Biofilm growth of EcO157 can greatly enhance its environmental survival and persistence. Here we examined the interaction of EcO157 with indigenous bacterial communities from field water collected in Salinas, California, and from bagged spinach (commercial), and explored the impact of the native bacterial communities on the survival of EcO157. A spontaneous *rifR* mutant of the EcO157 strain linked to the 2006 US outbreak associated with spinach was used. The biofilm communities were analyzed by 16S rDNA sequencing and their interaction with EcO157 in mixed biofilm were examined by confocal microscopy. When grown in field water, EcO157 appeared unable to compete with indigenous microorganisms. However, supplementation of the field water with 5% rich medium (TSB) promoted rapid proliferation of EcO157, which then became the dominant member of the community. Interestingly, attachment of EcO157 to abiotic surfaces increased significantly and strong biofilms developed under the

latter conditions. Furthermore, the attachment of EcO157 to leaf disks was the greatest when grown in the field water supplied with 5% TSB, which was 10.9- and 15.5 fold greater than when grown in 5% TSB alone, or in field water alone, respectively. Analysis of the biofilm community structures revealed that despite the great species diversity observed in both biofilm communities, the dominant members shifted from *Comamonas* spp. and *Exiguobacterium* spp. to *Aeromonas* spp. when the attachment surface changed from glass to spinach. Our data suggest that indigenous bacterial communities can enhance the biofilm growth of EcO157, and subsequently, their survival in natural environments.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.010 THE IMPROVEMENT OF *RHODOCOCCUS ERYTHROPOLIS* STRAINS AND APPLICATIONS IN INDUSTRIAL BIOFILM—BASED REMEDIATION PROCESSES

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Rhodococcus erythropolis disposes a wide biodegradation potential to aliphatic and aromatic organic compounds. The presence of long—chain mycolic acids provides the cell wall with hydrophobic envelope. The cell surface hydrophobicity was found to play an important role in the biofilm formation. The aim of this work was to prepare industrially applicable strains showing high biodegradation activities towards phenol and aniline, and forming a stable biofilms.

The strains *R. erythropolis* able to utilize aniline and phenol were prepared by way of physiological adaptation. The recombinant strain with higher and stable level of phenol hydroxylase activity was constructed by cloning of the phenol hydroxylase operon pheA2A1R in the multicopy plasmid pSRK21. Cell surface hydrophobicity was assayed by method of microbial adhesion to hydrocarbons. Biofilm formation was monitored using image analysis. As biofilm carriers were used hydrophilic (glass) and hydrophobic (polyethylene, polypropylene, silicon rubber) materials.

Biodegradation rates of phenol in the course of exponentially growth phase of the suspension populations was 6.0 mg l⁻¹ h⁻¹ for the adapted strain and 13.0 mg l⁻¹ h⁻¹ for the recombinant strain. The strain adapted to aniline reached stable growth in the basic salt medium containing 2.0 g l⁻¹ of this pollutant. The all strains were able to colonize hydrophobic and hydrophilic carriers. Prepared biofilm catalysts reached up to twice biodegradation rates in comparison with suspension populations. Results were verified in continuous pilot plant reactor. The efficiency of the process (biofilm fluidized bed reactor 100 l) expressed as biodegradation of dissolved organic compounds was 90 % at residence time of 6 days.

The acquired data was used for design of industrial facilities. The realized process of wastewater treatment consists of 2 biofilm fluidized bed reactors (each 260 m³) enabling variable control.

Abstract Category
29 Biofilms and Sociomicrobiology

PS.29.011 THE INVOLVEMENT OF GENE *FLIA* IN THE INTERACTION OF *ESCHERICHIA COLI* WITH *STENOTROPHOMONAS MALTOPHILIA* IN DUAL SPECIES BIOFILMS

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Microorganisms in natural environments seldom live alone and commonly share the same ecological niche with other species. Behaviors of one species such as growth, metabolism, and/or life style change in response to the presence of other species. Our previous study found that biofilm formation of *Escherichia coli* K-12 PHL644 in a static culture was significantly inhibited when co-cultured with *Stenotrophomonas maltophilia*. *E. coli* cells switched to a less adhesive life style in co-culture. The transcriptional response of *E. coli* to *S. maltophilia* was studied by microarray analysis of *E. coli* in co-culture, in order to identify the genes that were involved in species interactions resulting in decreased biofilm formation of *E. coli*. One of the identified *E. coli* genes, *fliA*, was highly up-regulated (14.4-15.5 fold) in both biofilms and planktonic co-cultures with *S. maltophilia*. Its up-regulation was confirmed using an *E. coli* transcription reporter strain. The role of *fliA* on cell motility, curli expression, cell aggregation, and biofilm formation was evaluated using a wild type strain, a *fliA* knockout mutant, and an overexpression strain. FliA is an alternative sigma factor responsible for transcription of class 3 genes in flagella synthesis and motility. However, expression of class 3 genes, such as *fliC* and *motA*, was unchanged in co-culture. Overexpression of *fliA* substantially reduced the ability of cell aggregation by reducing curli production of *E. coli*. As a result, biofilm formation of *E. coli* with induced *fliA* overexpression in co-culture was significantly reduced, while a knockout mutant formed more microcolonies in mixed-species biofilms. In conclusion, up-regulation of *E. coli* gene *fliA* in response to *S. maltophilia* contributed to decreased biomass of *E. coli* in mixed species biofilms by reducing adhesion curli production.

Abstract Category
29 Biofilms and Sociomicrobiology

PS.29.012 CHEMICAL EQUILIBRIUM AMONG MICROBIAL ORGANISMS IN HOT BIOFILMS

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Equilibrium relates the Gibbs energies and relative abundances of chemical species and therefore offers a framework for predicting the responses of microbial communities to physical-chemical gradients in their environments. We describe a thermodynamic model for the relative abundances of coexisting organisms in chemotrophic and phototrophic biofilms in Bison Pool, an alkaline hot spring in the Lower Geyser Basin of Yellowstone National Park. We used chemical equilibrium calculations to quantify microbial community structure at five sites from the boiling source pool to 22 meters down the outflow channel that differ in temperature and chemical

composition of the effluent. Protein translations of metagenomic sequences were classified taxonomically using the Basic Local Alignment Search Tool (BLAST) and the National Center for Biotechnology Information's RefSeq database. We analyzed the data in three ways: 1) For each site, proteins were binned at the rank of phylum. The relative stabilities of the composite proteins were calculated as a multidimensional function of temperature, pH and activity of hydrogen, which resulted in a progression of stability fields for the proteins that mirrors their actual spatial distribution. 2) The numbers of protein sequences in each phylum were used to quantify empirically the taxonomic makeup of each site. 3) Overall compositions of proteins in organisms identified in the BLAST search were subjected to equilibrium chemical speciation calculations to generate theoretical predictions of the taxonomic makeup of each site. The results of step (1) are consistent with Gibbs energy minimization in the adaptation of proteins to prevailing geochemical gradients, especially oxidation-reduction state, in the outflow channel. The close correspondence between the results of steps (2) and (3) provides evidence that some natural microbial communities are sustained in a metastable chemical equilibrium state among the microbial cells themselves.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.013 DEVELOPMENT AND CONTROL OF *PSEUDOMONAS AERUGINOSA* AND *STAPHYLOCOCCUS AUREUS* BIOFILMS

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In order to monitor the development and control of biofilms formed by *Pseudomonas aeruginosa* and *Staphylococcus aureus*, stainless steel disks were exposed to spectrophotometrically calibrated inocula in growth chambers containing nutrient broth. The chambers were incubated at 37°C and 135 revolutions per minute in an orbital shaker. Following a 24 hour period for biofilm development, duplicate disks were rinsed to remove planktonic organisms then scraped to remove sessile organisms. The fluids surrounding the disks and the scrapings were serially diluted and spread plated on nutrient agar for quantification. A second set of duplicate disks was rinsed, exposed to varying concentrations of phenol (0.5% to 5.0% by weight) for 15 minutes, rinsed again, and scraped. Samples from the surrounding fluid were also exposed to the varying concentrations of phenol for 15 minutes. Following the same procedure, the fluids surrounding the disks and the scrapings were serially diluted and spread plated for quantification. Both organisms were consistently retrieved from the untreated fluid samples indicating supportive growth conditions; both organisms developed large, robust populations. The counts of sessile organisms retrieved from untreated disks were also indicative of supportive growth conditions and the organisms' abilities to form biofilms. The counts of planktonic organisms exposed to phenol indicated all concentrations above 0.5% by weight were able to reduce bacterial populations by 99.99%. The counts of sessile organisms exposed to phenol indicated that concentrations above 3.0% by weight were able to reduce bacterial populations by 99.99%; the results indicated that significant increases in concentration of phenol were required to control organisms protected by biofilms. Further studies will analyze development and control of mixed population biofilms.

Abstract Category
29 Biofilms and Sociomicrobiology

PS.29.014 A NEW BIOSENSOR FOR QUORUM SENSING: BROAD DETECTION ON A FINE SCALE

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Bacteria that communicate through quorum sensing produce, detect, and/or respond to small chemicals or proteins called autoinducers, and the study of these processes is aided greatly by the use of biosensors. To date, the TraI/R quorum sensing system of *Agrobacterium tumefaciens* can detect the broadest range of autoinducers called acyl-homoserine lactones (AHLs) and has been used successfully in biosensors. However, *A. tumefaciens* TraI/R-based biosensors have been constrained to their natural host and by the reporter used to show detection. Here, we describe a TraI/R-based biosensor containing a green fluorescent protein (GFP) reporter within an *E. coli* host. Our goal was to produce a biosensor with a wide range of detection (TraI/R), a sensitive reporter with broad screening applications (GFP), and an easily manipulated host (*E. coli*). The biosensing plasmid construct includes the promoter region of *traI* and the *traR* gene, amplified from *A. tumefaciens* C58 (NC-003065), as well as a commercially available plasmid backbone containing a GFP gene from *Aequorea coerulea* (belt jellyfish). Addition, removal, and/or overexpression of the following genetic elements were studied for their effects on biosensor performance: varying ribosome binding sites, *E. coli sdiA* (*traR* homolog), *A. tumefaciens rpoA* (RNA polymerase α -subunit), and *E. coli groESL* (foreign protein folding). While the biosensor requires further optimization, it is able to detect the presence of a broad range of AHLs (C4-C12) and has potential to be a sensitive tool in studying quorum sensing.

Abstract Category
29 Biofilms and Sociomicrobiology

PS.29.015 REAL-TIME IMAGING OF GENE EXPRESSION WITHIN A CURRENT PRODUCING GEOBACTER SULFURREDUCTENS BIOFILM

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Previous real-time analysis of pH within anode biofilms of *Geobacter sulfurreducens* microbial fuel cells demonstrated significant acidification deep within biofilm pillars and near the anode surface, which might be expected to inhibit microbial activity in those zones. Furthermore, it has been proposed that cell metabolism might decrease as distance from the anode surface increases. In order to better understand metabolism within *G. sulfurreducens* anode biofilms a short half-life green fluorescent protein reporter system was developed to examine spatial and temporal gene expression patterns within current producing biofilms. A C-terminus tag reduced the fluorescent

half-life of the reporter protein to ~90 mins, enabling temporal gene expression reporting. Evaluation of expression of the reporter protein under control of the promoter for the nitrogen fixation gene, *nifD*, clearly demonstrated induction of gene expression under ammonium-limiting conditions and repression when sufficient ammonium was provided. When expression of the reporter was under control of the promoter for the gene for the key metabolic enzyme, citrate synthase, expression was high when acetate was available, but fluorescence was rapidly lost after acetate additions were stopped. Under normal operating conditions the citrate synthase gene was actively expressed throughout the biofilm, suggesting that cells were metabolically active both within low pH zones near the anode as well as at substantial distance from the anode surface. The genes for PilA, the structural protein for electrically conductive pili, and OmcZ, the mobile outer-surface c-type cytochrome required for optimal current production, were also continuously expressed throughout the biofilm. These results demonstrate that it is possible to monitor the expression of key genes throughout anode biofilms in order to gain insight into the factors influencing current production in microbial fuel cells.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.016 MOLECULAR CHARACTERIZATION OF MICROBIAL COMMUNITIES IN BIOFILMS FROM DRIP IRRIGATION SYSTEMS OPERATING WITH TREATED WASTEWATER AND AGRONOMIC WATERS

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Almería is located in the Southeast of Spain and constitutes one of the driest areas in this country, with an economy which relies in the agricultural activity carried out in greenhouses. In order to address water shortage, reuse of treated wastewater or utilization of agronomic waters has been long proposed and used as a viable option for irrigation. Utilization of treated wastewater for irrigation rises a number of issues related both to the safety of the process, as well as to the appearance of hydraulic problems due to the formation of microbial biofilms. The aim of this work was the molecular characterization of microbial communities in biofilms from irrigation systems (drippers and tubes), using agronomic water as well as treated wastewater. Molecular characterization of microbial communities from the water and the biofilms was carried out using Denaturing Gradient Gel Electrophoresis (DGGE). The fingerprinting obtained from biofilm samples showed a reduced number of bands, indicating that diversity was low. Virtually all the retrieved bands, independently of water origin, corresponded to esporulated and thermophilic members of the phylogenetic group Firmicutes (Gram positives with low G+C content), suggesting that only microorganisms able to grow at high temperatures were selected.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.017 ANALYSIS OF THE MICROBIAL COMMUNITY DEVELOPING IN DRIP IRRIGATION SYSTEMS OPERATING WITH REGENERATED WASTEWATER IN GRAN CANARIA (SPAIN)

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The traditional scarcity of hydric resources in Spain is gradually shifting water management practices towards the use of regenerated water obtained from wastewater treatment plants. Since drip irrigation systems operating with regenerated water have obturation problems produced by biofilms. For this reason we decided to study the type of microorganisms involved in their formation. The aim of this study was the molecular characterization of microbial communities in biofilms from irrigation systems, which used treated wastewater from two WWTPs (Bocabarranco and Cardones) of Gran Canaria (Spain).

Molecular characterization of microbial communities from the biofilms was carried out using Denaturing Gradient Gel Electrophoresis (DGGE) of 16S rRNA genes. The resulting bands were sequenced, and statistical analyses of DGGE band patterns were carried out.

The gel shows the presence of a large number of bands in all the samples indicating that the microbial community was rather complex. Also, regarding the effect of pressure cleaning in the microbial diversity patterns, the results indicate that in the case of the Cardones WWTP this pattern did not change as a consequence of the cleaning process. In samples from the Bocabarranco WWTP the band patterns were significantly affected as a result of cleaning. The results of the cluster analysis indicate the lack of consistent clustering around the WWTP variable. This probably indicates that the characteristics of the water used were not the main determinant of the microbial diversity patterns observed. Most of the retrieved DGGE bands belonged to the phylogenetic groups β -proteobacteria, Bacteroidetes, Actinobacteria, Deinococcus-Thermus, but sequences from γ -proteobacteria, α -proteobacteria, Chloroflexi and Acidobacteria were also found.

Preliminary analysis of recovered 16S rRNA sequences, indicate that the biofilm has an heterogeneous composition with representation of groups usually found in aquatic environments.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.018 REMOVAL OF BIOFILM IN HOUSEHOLD DEVICES

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Ecofriendly cleaning (e.g. low temperature) can favor the formation of biofilm in household washing devices and serve as reservoir of potential human pathogens. Therefore, manufactures develop strategies to prevent cell attachment and/or remove formed biofilms. Our aim was to develop a kit for evaluating biofilm removal in household washing machines comprising single-species model biofilms which could be implemented directly in the machine for testing.

Washing machines (n=11) from different countries were investigated in terms of microbial diversity. Among the 90 isolates, *Pseudomonas putida* and *Rhodotorula mucilaginosa* were selected for their ability to form thick biofilms. Model biofilms were grown in fermenters for up to 13 days. The biofilm was evaluated by quantification of polysaccharides, proteins, optical density and viable cell count. The repeatability of the bioprocess was tested during 3 independent trials. Storage tests were conducted with 4 preserving solutions at 3 temperatures for up to 2 months. The ability of the model biofilms to evaluate biofilm removal in washing processes was tested in washing machines. Biofilm coupons were placed on 3 locations and washed at temperatures ranging from 30 to 60°C in the presence of various detergents. The remaining biofilm was quantified (as previously described) or color intensity after crystal violet staining was measured.

The process of biofilm formation was repeatable (e.g. 4% variability of protein content in the yeast biofilms). An appropriate storage procedure (e.g. 0.1 M trehalose at 4°C) allowed the storage of biofilm up to 2 months. Crystal violet staining showed that the biofilms were selectively removed by *in situ* washing tests (e.g. liquid and powder detergents removed, 1 and 2 log of viable cells of bacterial biofilm, respectively).

The model biofilm kit allows the estimation of biofilm removal efficiency in household devices such as washing machines.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.019 INFLUENCE OF BIOFILMS ON IRON AND MANGANESE DEPOSITION IN DRINKING WATER DISTRIBUTION SYSTEMS.

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Discoloured water events are a persistent cause of customer dissatisfaction. A water utility in Australia received 227 discoloured water complaints (during 2001-2004), which represented 86% of their customer complaints. This study investigated the contribution of pipe wall biofilms towards iron (Fe) and manganese (Mn) deposition, and discoloured water events. Eight reactors were operated to test 4 treatments in duplicate. Four reactors were exposed to low Fe and Mn concentrations and the remaining 4 were exposed to higher concentrations. Two of the 4 reactors that received low and high Fe and Mn concentrations were chlorinated and did not contain an active biofilm. Higher concentrations of Fe and Mn in bulk water resulted in increased deposition of Fe and Mn when biofilms were present. The Fe deposition rates reached 0.24 and 0.45 µg/ring/d on glass rings of the non-chlorinated reactors that received 0.05 and 0.3 mg Fe/L, respectively. In contrast the Fe deposition rates in the chlorinated reactors were only 0.03 and 0.04 µg/ring/d. The Mn deposition rates in the absence of the disinfectant were 0.22 and 0.87 µg/ring/d in reactors that received 0.02 and 0.4 mg Mn/L, respectively. In contrast, the deposition rates in the chlorinated reactors were 0.05 and 0.21 µg/ring/d.

Similar observations were made with KIWA biofilm monitors in a field study. Field experiments also demonstrated seasonal effects. During late summer and early autumn, adenosine triphosphate (ATP) concentrations in pipe wall biofilms were 8 times higher compared with other times of the year. The increase in ATP correlated positively with an increase in Fe and Mn deposition.

Based on the lab-scale investigations the detachment of pipe wall biofilms due to death or flow dynamics could dislodge the entrapped Fe and Mn into bulk water, triggering a discoloured water event. Prevention of biofilm formation and removal of bulk water Fe and Mn via treatment may effectively minimise discoloured water events.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.020 ISOLATION OF MIXED SPECIES BIOFILMS FROM AN OIL SANDS TAILINGS POND

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Extraction of bitumen from the oil sands of Northern Alberta, has resulted in the generation of millions of cubic meters of toxic waste that under strict legislation must be remediated. Tailings ponds are composed of a diverse mixture of water, sand, clay, residual bitumen, complex hydrocarbons and metal ions. We have hypothesized that bioremediation of the tailings pond can be optimized using mixed species biofilms that are endogenous to the tailings environment. Thus, using the Calgary Biofilm Device (CBD) as a microscale reactor, we considered if both aerobic and anaerobic mixed species biofilms could be cultivated directly from oil sands tailings pond sediments, demonstrating a cultivation technique that can be employed for subsequent bioremediation investigations.

We have succeeded in culturing both aerobic and anaerobic mixed species biofilms directly from an oil sands tailings pond under a variety of different culture conditions. Microbial diversity within biofilms were evaluated by denaturant gradient gel electrophoresis (DGGE) which showed very diverse mixed species biofilm communities could be generated using only the tailings pond sediments as an inoculum providing the organisms, carbon and energy sources. Furthermore, DGGE patterns showed that unique mixed species consortia were recovered using different incubation temperatures ranging from 4°C to 25°C, oxygen tensions (aerobic, microaerobic, and anaerobic) and varying growth medias such as minimal medium (R2B), complete medias (Trypticase Soy Broth, Luria-Bertani Broth) and a general anaerobic media.

In this study, we have effectively demonstrated that mixed species biofilms comprised of endogenous species from an oil sands tailings pond can be successfully isolated on the CBD under different laboratory conditions.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.021 GENE EXPRESSION DYNAMICS OF *PSEUDOMONAS PUTIDA* KT2440 BIOFILMS UNDER WATER DEPRIVATION

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In soil, bacteria can form colonies that are exposed to changing hydration conditions, exerting a stress to which the bacteria should adjust. Some of the phenotypes associated with water deprivation, such as the production extracellular polymeric substance (EPS) and the limitation of motility, have previously been observed. However, it is not known how these responses are regulated and temporally expressed. Here, we aimed to investigate the gene level responses by identifying the differentially expressed genes at -0.4 MPa water stress compared to non-stressed condition. We hypothesized that under water stress flagellar and EPS genes would be significantly differentially expressed, the former being down- and the latter being up-regulated.

The novel Pressurized Porous Surface Model (PPSM) was used to expose KT2440 colonies to -0.4 MPa water stress for 4, 24, and 72 hours. Agilent whole genome 1-color cDNA-microarray was used for gene expression profiling. The genes with log₂-fold change ≥ 1.5 and adjusted P-value ≤ 0.01 were identified as significantly differentially expressed.

Considerably more genes were expressed after 4-h compared to after 24 and 72-h. While significantly induced after 4-h, the alginate genes were not detected after 24 or 72-h. Surprisingly, flagellar genes of which number and expression level increased with the exposure time, were induced. The rest of the identified genes play roles in adaptation to atypical conditions, protein fate, DNA repair, energy metabolism, cell envelope, transport and binding proteins.

Early induction of alginate genes suggests that alginate production is an acute adaptive response to water stress, strengthening its role in creating hydrated environment, while flagellar gene induction appears to be rather a late response. Furthermore, the trends in both the number and expression levels of the genes from 4 to 72-h suggest a shift towards a non-stressed behavior.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.022 INFLUENCE OF TERMINAL OXIDASE *CBB*₃ IN *PSEUDOMONAS AERUGINOSA* BIOFILM FORMATION UNDER ANAEROBIC ENVIRONMENTS

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Pseudomonas aeruginosa is an ubiquitous gram-negative bacterium that grows under aerobic and anaerobic environments. As a part of adaptation strategies for different environments, *P. aeruginosa* possesses a highly branched aerobic respiratory chain, and it has been indicated that *P. aeruginosa* can conserve energy in different environmental conditions by controlling the level of multiple terminal oxidases. Still, it is little known about the physiological influence of these

terminal oxidases on growth of *P. aeruginosa*. In this study, we focused on *cbb₃* oxidase. Growth in planktonic shaking culture under aerobic or anaerobic conditions was compared between PAO1 strain and a *cbb₃* oxidases deletion mutant. Under anaerobic condition, the *cbb₃* mutant grew better than PAO1 strain. Moreover, we observed that the ratio of the dead cells to live cells decreased in the stationary phase in the *cbb₃* mutant which was observed by staining with SYTO9 and propidium iodide. Thus, it was suggested that *cbb₃* oxidases induced cell death under anaerobic environments. Interestingly, PAO1 strain formed many aggregates under anaerobic condition and *cbb₃* mutant did not. Staining of the aggregates with SYTO9 and propidium iodide suggested that the aggregate is a complex of live and dead cells and extracellular DNA. Compared with the culture of *cbb₃* mutant, that of PAO1 strain in the stationary phase contained a large amount of extracellular DNA. It is known that extracellular DNA is a component of biofilm matrix. Therefore, we next compared biofilm formation under anaerobic condition between PAO1 strain and *cbb₃* mutant by using a 96 well microtiter plate. *cbb₃* mutant formed biofilm poorly in comparison with PAO1 strain. Taken together, our results demonstrate that terminal oxidase *cbb₃* induce cell death and release extracellular DNA under anaerobic environments, which lead to promotion of biofilm formation.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.023 EVALUATION OF MICROBIAL COMMUNITIES GENETIC AND FUNCTIONAL DIVERSITY IN NATURAL BIOFILMS TO OPTIMISE GROWTH AND SAMPLING

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Numerous studies have shown that the majority of micro-organisms favour developed biofilms in natural conditions. Indeed, their functioning and their organisation are studied in various areas such as medical applications or industries as well as environmental applications. However, because of the diversity of potential biofilm surfaces, it is especially difficult to study natural biofilms.

In order to define an adapted growth and sampling method for natural biofilms, we first evaluated the influence of the nature of natural surfaces (stone, leave, bark, plant) on the genetic and functional diversity of biofilms' microbial communities in a natural river system. Then we tested the ability of artificial surfaces (glass, plexiglas, sterile wood) to grow biofilms with genetic and functional diversities similar to those on natural surfaces. The biofilms' genetic diversity was assessed using a molecular method; Denaturing Gel Gradient Electrophoresis (DGGE) of a gene fragment coding for either 16S rRNA or 18S rRNA, for bacterial and fungi respectively, while functional diversity was estimated with ECOLOG® plates.

Results showed the importance of varying natural biofilm surfaces in order to recover a representative microbial diversity. Biofilms that developed on the artificial surfaces had similar genetic patterns between themselves, as well as similarities with the natural water samples. However these biofilms differed from the natural biofilms collected in the same sampling spot. The combination of these two approaches, using genetic and functional microbial diversity as criteria, enabled us to optimize methods to sample and to culture biofilms. Moreover, we were

able to compare the evolution of biofilms in a natural environment and to estimate maximum diversity in river biofilms. Finally, this is one of the few studies that have evaluated fungal diversity in natural biofilms, although in the present case, it was estimated to be higher than for bacteria.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.024 DOES COLONY MORPHOLOGY PARTLY EXPLAIN THE VARIABILITY OF AUTOINDUCER INFLUENCE ON BIOFILM DEVELOPMENT? A MODELLING APPROACH

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A number of bacteria regulate biofilm development by autoinducers, a strategy usually called quorum sensing. Due to contradictory experimental results, the general interrelation between autoinducers and biofilms is still debated. Biofilms or, more basically, microcolonies can grow in different basic shapes - mats, ball-, tower- or mushroom like - dependent on e.g. species and environmental condition. Hithero, scientific focus has been laid on the question, how autoinducers influence biofilm development. The question has been largely neglected, how the colony morphology impacts autoinducer systems. Experimental investigations are difficult, because induction of different biofilm shapes may not only change the autoinducer regulation, but also a number of other traits, making a proof of a direct link difficult. We use a modelling approach in which the production, decay and diffusion of the signalling substance is described by a system of partial differential equations. Based on quantitative data of the autoinducer system in *Pseudomonas putida* IsoF, we consider ellipsoidal and specifically deformed ellipsoidal colony shapes and solve numerically the associated mathematical system for a stationary state. The numerical techniques utilize a double-domain pseudo-spectral collocation point method. The analysis of the numerical results indicated that the colony form has a significant impact on the induction properties, caused by differences in autoinducer accumulation. Flat mats and, even more, towers require a significant higher number of cells to be induced, or, in extreme, can be almost non-inducible. In contrast, in convex colony forms like hemispheres only a limited amount of cells is required. Thus, a part of the contradictory results may be explained by differences in biofilm/microcolony morphology. It would be interesting to investigate experimentally, whether bacteria use these differences for certain functional traits, or may rather be subject to them.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.025 EFFECT OF THE QUALITY AND QUANTITY OF DISSOLVED ORGANIC MATTER ON DEGRADATION OF THE PHENYLUREA HERBICIDE LINURON BY A MICROBIAL BIOFILM COMMUNITY

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Dissolved organic matter (DOM) is an important natural source of carbon, energy and nutrients for heterotrophic microbial communities in the environment. The quality and quantity of DOM are known to influence enzymatic activity, growth and composition of these communities. However, knowledge on the effects of DOM on specific functions like pesticide degradation is scarce. In this study, the effect of DOM on a bacterial consortium consisting of *Variovorax* sp. WDL1, *Hyphomicrobium sulfonivorans* WDL6 and *Comamonas testosteroni* WDL7 which synergistically mineralize the phenylurea herbicide linuron, was studied. The consortium was cultivated in continuously irrigated flow cells allowing biofilm formation. To study the effects of DOM on linuron degradation, consortium biofilms were grown in minimal medium with linuron as sole carbon and energy source until steady-state conditions were achieved. Then, the influent medium was changed to minimal medium with linuron and/or additional DOM at median surface water concentrations. After a new steady-state was reached, the system was irrigated again with minimal medium containing only linuron. Effluent concentrations of linuron and the metabolite 3,4-dichloroaniline were measured with HPLC. Specific fluorescent labelling of each strain allowed analysis of biofilm morphology with CLSM. Citrate added in addition to linuron was shown to inhibit linuron degradative capabilities of the community, but not at environmentally relevant DOM concentrations. Moreover, the community restored its degradation capacity when linuron was again the only carbon and energy source. This contrasts with previous results in which we showed when citrate was temporarily the only carbon and energy source, resilience of the linuron degradation capacity was affected, emphasizing the importance of selectivity. Also, the biofilm composition and architecture was only moderately affected by the intermittent mixed-substrate feed.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.026 THE SUPERINFECTIVE PROPHAGE IN PSEUDOMONAS AERUGINOSA BIOFILM DRIVES BACTERIAL ADAPTATION

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Prophage sequences have been identified in most sequenced bacterial genomes and thus are likely to contribute significantly to the genetic repertoire of bacteria. However, the effects of prophage and accessory genes on the host are yet to be fully elucidated. A recent study by Rice *et al.* has shown that a prophage of *Pseudomonas aeruginosa* plays a key role in maturation, cell death, dispersal and variant formation during biofilm development, an important mechanism for bacterial survival in the environment (ISME J. 2009; 3:271-282). Further, these effects are manifested in the biofilm when the phage becomes superinfective. Biofilm flow cells were

employed to determine the development of biofilm structures between *P. aeruginosa* variants that constitutively produce superinfective phage and the wild-type. Biofilms of the constitutively superinfective variants did not undergo microcolony formation and displayed early cell death during the biofilm developmental process. Sequence analysis of the superinfective phage indicated that super infection is linked to either single nucleotide polymorphism in a gene with homology to the repressor C gene of the Enterobacteria phage P2 or is the result of large deletions of the Pf4 phage genome. Given the pronounced effects of the prophage on the life cycle of *P. aeruginosa* biofilms, elucidation of the host-phage interaction may be key to understanding biofilm formation and adaptation in the environment.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.027 INVESTIGATION OF STARVATION-INDUCED DISPERSAL OF PSEUDOMONAS AERUGINOSA BIOFILMS

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Glucose starvation has been shown to induce dispersal of *Pseudomonas aeruginosa* PAO1 biofilms, however, the molecular mechanism by which this occurs has not been fully elucidated. This study aimed to identify the molecular signalling pathways that regulate glucose starvation-induced dispersal of *P. aeruginosa* biofilms. We have monitored glucose starvation-induced dispersion of PAO1 biofilms using an online biofilm monitoring system, which enables continuous determination of biofilm and dispersal biomass. The response to carbon starvation of PAO1 wild-type (WT) and mutant strains (*nirS*, *vfr*, *bdlA*, *rpoS*, *cyaA* and Δ Pf4) which have been implicated to be involved in dispersal or carbon starvation responses was compared. Glucose starvation was applied to biofilms grown in M9 medium by replacing the M9 medium with glucose-free medium. In addition, the biofilms were treated with carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), a proton ionophore that dissipates proton motive force, hence inhibiting ATP production. Serine hydroxamate was also used to induce the stringent response. Our findings indicate that the biofilms disperse when glucose starvation is induced. Mutant strains *nirS*, *vfr*, *bdlA*, *rpoS* and Δ Pf4 formed less biofilm biomass than the WT strain, and these biofilms dispersed when starved, suggesting that these genes are not associated with glucose starvation-induced dispersal. Biofilms of the *cyaA* mutant strain dispersed dramatically faster than the WT. Treatment with CCCP for 30 minutes prior to starvation inhibited dispersal. This result suggests that energy derived from oxidative phosphorylation is essential for the dispersal of the biofilm. Induction of the stringent response did not lead to dispersal of starved biofilms, indicating that translation is not necessary for glucose-induced dispersal. These results demonstrate that the biofilm lifecycle is intimately linked to central metabolism and suggests that biofilm formation is a core behaviour of bacteria.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.028 INFLUENCE OF CELL SURFACE PROTEINS IN *LACTOBACILLUS PLANTARUM* JCM1149 BIOFILM FORMATION

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High resistance of lactic acid bacteria (LAB) biofilms to environmental stresses cause various problems in industry and dentistry. In order to solve these problems, it is important to understand how LAB form biofilms. Recently, cell surface proteins of LAB were shown to be important in adherence to human colonic mucin. However, it is not known whether cell surface proteins are involved in lactobacilli biofilm formation. Here we investigated the influence of the cell surface proteins of *Lactobacillus plantarum* JCM1149 on the biofilm formation. *L. plantarum* JCM1149 biofilms were grown for 1 day with de Man, Rogosa, Shape (MRS) broth. The biofilms after washing with sterile water were treated with proteinase K. The amount of *L. plantarum* JCM1149 biofilms decreased by about 20% compared to the untreated biofilms. Several proteins of *L. plantarum* JCM1149 were localized on the cell surface below pH6 but released into the medium at alkaline pH, indicating that lactobacilli rapidly modify their surface properties in response to changes in pH. Furthermore, to investigate the role of the surface proteins on *L. plantarum* biofilms, we grew *L. plantarum* JCM1149 at pH4, 6, and 8 in flow cell chambers for three days. At pH 8, where several surface proteins release into the medium, *L. plantarum* JCM1149 did not form biofilms while they formed biofilms below pH6. These results suggest that the cell surface proteins of *L. plantarum* JCM1149 are involved in biofilm formation. Since lactobacilli don't have motility machineries, we propose that the rapid modification of their surface properties in response to changes in pH could provide a novel strategy for the bacteria to control biofilm formation.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.029 COMPETITIVE EXCLUSION IN SESSILE MICROBIAL COMMUNITY MODELS

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Though mathematical modeling has a long history in ecology, including microbial ecology, its application is less familiar in the area of sessile microbial communities (microbial mats and biofilms), in large part due to the complexities that arise in modeling spatially heterogeneous systems. Such models have been common in the engineering community however, with a more than 30 year history of application to subjects such as waste and ground water treatment. The aim here is thus to apply basic ecological questions to standard sessile microbial community models, particularly with regards to issues of competitive exclusion.

We analyze standard single spatial dimension (1D) continuum models of sessile microbial communities, using both analytical and computational methods.

We are able to show that commonly utilized 1D models exhibit a sort of competitive exclusion, namely that species ecology throughout the community is determined by competitive outcomes at the community substratum. We can show clear illustrations of this prediction through simple computational examples.

We can conclude that the predicted competitive exclusion principle is probably too restrictive and hence unrealistic. This suggests that a class of commonly used 1D models is inadequate. We are able to conclude that the needed model change requires at least some microbial mobility within the community.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.030 ANALYSIS OF BACTERIAL BIOFILM COMMUNITY IN PRODUCED WATER SYSTEMS WITH AND WITHOUT NITRATE TREATMENT

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The increase of hydrogen sulfide production by sulfate-reducing bacteria (SRB) in petroleum environments can result in negative consequences for the industry, such as reservoir souring and biocorrosion. Nitrate treatment has been introduced worldwide, including Brazil, as an alternative procedure to control SRB in oil industry. In order to better understand the impact of nitrate treatment, the aim of this study was to analyze the bacterial diversity in biofilms formed on carbon steel coupons in reactors containing produced water from a Brazilian oil platform. Two reactors were operated: a reactor treated with continuous nitrate flow (N reactor) and other used as a control (C reactor). Three set of experiments (E1, E2 and E3) were carried out using the same experimental conditions, and the reactors were maintained for 5, 45 and 80 days, respectively. PCR-DGGE approach using 16S rRNA gene was performed to evaluate the predominant bacterial groups involved in the process of biofilm formation in both reactors. In general, DGGE profiles did not show remarkable changes in the community structure during the experiments. However, in N reactor, the DGGE profile showed differences after nitrate injection in E2 and E3. Two bands extracted from the DGGE of E2 were identified as *Marinobacter* sp. and *Achromobacter xylosoxidans*, respectively. Most probable number counts of SRB showed a reduction after nitrate injection in N reactor during E3. Carbon steel coupons had a significant weight loss in N reactors in all experiments. When the surface of the different coupons was analyzed by scanning electron microscopy, an increase on corrosion was observed in those from N reactors, when compared with those of C reactors, in E2 and E3 experiments. In conclusion, although the viable SRB count has been reduced by nitrate treatment, an increase of biocorrosion was observed in the coupons from N reactor.

Abstract Category
29 Biofilms and Sociomicrobiology

PS.29.031 BIOFILM FORMATION AND ACID STRESS RESPONSE OF LACTIC ACID BACTERIA

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In nature, bacteria have developed various mechanisms of resistance to environmental stresses and one of which is formation of biofilms. Investigation of bacterial biofilm formation and the responses of biofilm bacterial cells to environmental stresses in a particular place contributes to our understanding of the ecology of bacteria in a particular environment, which leads to advances in microbial control. The aim of this study is to investigate the ecology of lactic acid bacteria (LAB), which cause spoilage, contamination or deterioration of many types of food. We have focused on the biofilm formation by spoilage LAB in raw materials, food products, and the production line.

Lactobacillus plantarum subsp. *plantarum* JCM1149, *Lactobacillus plantarum* M606 (an isolate), *Lactobacillus brevis* JCM 1059, and *Leuconostoc mesenteroides* NBRC3832 were used in the study. Planktonic LAB cells were cultured in a glass tube containing de Man, Rogosa, Sharpe (MRS) medium. Biofilms were formed on glass cover slips in the wells of a polystyrene microtiter dish containing MRS medium by static incubation at 30 or 35°C. The biofilm formation was observed by confocal laser scanning microscopy and monitored by staining with crystal violet. The responses of biofilm cells and planktonic cells of LAB to acetic acid were investigated by survival tests.

All the LAB species tested formed biofilms on glass cover slips and the biofilm bacterial cells showed more resistance than the planktonic bacterial cells in acetic acid. The biofilm formation was stimulated by fructose, which is contained in raw materials of food such as vegetables or fruit. The expression levels of a gene associated with production of extrapolymerized polysaccharide was increased by fructose. These findings suggested that spoilage LAB may form robust biofilms on food of plant origin and survive under environmental stresses.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.032 PYOVERDINE PRODUCTION IN *PSEUDOMONAS AERUGINOSA*: A COOPERATIVE BEHAVIOR THAT IS STABILIZED BY THE EXTREME DURABILITY OF THE PYOVERDINE MOLECULE

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In response to iron deficiency, the bacterium *Pseudomonas aeruginosa* releases pyoverdine, its primary siderophore, into the local environment to scavenge iron. Pyoverdine production can be understood as a cooperative behavior because pyoverdine molecules can be shared among bacteria in the local community. Such cooperative acts can be exploited by cheating mutants that are defective for pyoverdine production, whilst still being able to take up pyoverdine in complex with iron produced by others. Here, we ask whether the durability of pyoverdine - i.e. the time over which the same pyoverdine molecule can be recycled and repeatedly used to scavenge iron - stabilizes this cooperative system and reduces selection for cheating. We extracted pyoverdine from *P. aeruginosa* strains that produce either pyoverdine type I, II or III. We measured durability of pyoverdine using fluorescence spectroscopy and tested whether durability differs between pyoverdine types and environmental conditions. To assess the effect of pyoverdine durability on fitness, we conducted competition assays between cooperative and cheating strains under conditions where we experimentally manipulated durability. We find that pyoverdine molecules are generally very durable and remain functional over extended periods of time, although significant differences exist between pyoverdine types and environmental conditions. While extended durability strongly decreased selection for cheating, reduced durability resulted in cooperative strains being outcompeted by cheating strains. Our results suggest that extended durability of the pyoverdine molecule is an important factor that helps stabilizing this cooperative system. A proximate explanation for this is that cooperators can down-regulate pyoverdine production once enough pyoverdine has accumulated in the media, thereby saving metabolic costs and reducing the probability for cheats to invade.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.033 EFFECT OF POLY(3-HYDROXYBUTYRATE) ON BIOFILM FORMATION UNDER COLD CONDITIONS IN THE NOVEL ANTARCTIC SPECIES *PSEUDOMONAS EXTREMAUSTRALIS*

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P. extremaustralis is an Antarctic strain, capable of synthesizing polyhydroxyalkanoates (PHAs) in the form of polyhydroxybutyrate (PHB). Previous studies have shown that PHA is essential to grow under cold conditions and to cope with the oxidative stress. In this study we hypothesized that PHB producing strains can be found in cold environments in both planktonic and biofilms, while mutant strains unable to synthesize it can only withstand with oxidative stress derived from cold in the biofilm state. *P. extremaustralis* and its PHB minus (*phaC*) mutant were used to assess the effect of PHB accumulation on biofilm formation capability under low temperature (10°C). We also analyzed cold survival in a planktonic reducing environment (low oxygen content). Surface attachment of the strains was evaluated using a microtiter assay. Number of viable cells of wild type strain was similar in both biofilm and planktonic state. By contrast, the mutant was able to survive in the sessile form in cold. We performed liquid cultures under 3 different aeration conditions using Erlenmeyer flasks and hermetically sealed bottles in order to analyze the effect of the oxygen level on growth under cold conditions. Both strains showed similar growth at room temperature but at 10°C, while the wild type grew in all oxygen conditions, the

mutant only grew in microaerobiosis, showing that in reducing environments mutant strain was able to solve the PHB deficiency. Complementation of the mutant strain with *phaC* restored the wild-type phenotype in all the experiments. Under biofilm life style, cells are protected against many environmental stressors and our results suggest that in this state the PHB mutant could avoid the redox imbalance, alleviating the oxidative stress. PHB allowed the survival of the wild type strain in both planktonic and biofilm states increasing its capability to colonize different habitats. Other advantages related with biofilm life style could also be involved.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.034 THE EFFECT OF MICRO-FABRICATED SURFACES ON MARINE MICROBIAL COLONISATION AND BIOFILM FORMATION

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Surfaces immersed in marine waters are rapidly colonized by organisms with a sessile lifestyle - in a process known as biofouling. Colonisation of ship hulls and other man made surfaces carries major energy and economic costs for global maritime and coastal industries and traditional methods for dealing with this problem impact negatively on the environment. In this study, a novel antifouling approach using micro-fabricated polydimethylsiloxane surfaces with micro-scale surface topographies was assessed for activity against microbial attachment in marine waters. Microfabricated surfaces were first analysed quantitatively using confocal microscopy with live/dead stain to assess the extent of microbial colonization. Micro-scale topographies smaller than 4 μm were found to prevent micro-colony formation, the early stage of biofilm formation. Moreover, a significant reduction in microbial colonisation to both 4 μm and 10 μm topographies was observed during seven days of exposure in marine waters. Second, qualitative 16S rRNA gene denaturing gradient gel electrophoresis analysis revealed that the early succession (2 days) of bacterial colonization to microfabricated surfaces were mainly composed of γ -proteobacteria (83%), while α -proteobacteria (47%) dominated the late-succession (14 days) communities. In addition, we found that bacterial biofilms after 14 days in marine waters displayed surface-specific community composition at the genus level. It is proposed that the physicochemical characteristics, including surface wettability and surface hydrodynamic forces, of the different micro-scale topographies used in this study had a pronounced effect of the attachment, development and the composition of surface associated bacterial biofilms. Hence, the ecological impacts of microbial biofilm formation on manmade surfaces are now available for testing. Microfabrication technologies may greatly contribute to the development of novel antifouling control measures.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.035 NITRIC OXIDE AS A TOOL FOR BIOFILM REMOVAL: FROM LABORATORY SCALE TO INDUSTRIAL APPLICATION

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During the recent years, low levels (subnano molar) of nitric oxide (NO) were established as a signal in biofilm development. NO induced dispersal was demonstrated in a wide range of model microorganisms and in environmental biofilms. Prevention of biofilm formation was demonstrated in fewer species, but not in environmental samples. In contrast, it was found that higher levels of NO enhance biofilm formation. The mechanisms by which NO triggers biofilm dispersal, prevention or enhancement are poorly understood. In order to accurately determine concentrations of NO that trigger biofilm dispersal, we used a flow system with NO gas as a source for this reactive signal. The system was initially set to supply NO for 6 hours to a 24h old *Pseudomonas aeruginosa* biofilm. The surface coverage was measured with confocal laser scanning microscope. It was found that biofilm dispersal of *P. aeruginosa* occurred within the concentration range of 0.1 to 15nM. It was further found that at lower concentrations, NO triggered dispersal of environmental biofilm samples from sea water desalination plant. While 5 to 10 nM were ineffective, initial concentration of 0.1nM reduced both the biofilms average thickness and surface coverage. However, exposure of these samples to NO for longer periods resulted in a shift from biofilm dispersal to enhancement. In freshwater delivery systems, similar treatment of biofilm samples resulted in increased density and viability of bacteria. The use of controlled NO delivery to water facilities may reduce biofilm related expenses, for example, reducing biofilm formation on reverse osmosis membranes. NO might be a suitable tool for this and other purposes due to the low effective concentration of it and to the lack of safety problems in drinking water.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.036 CHARACTERIZATION OF MUTANTS FROM *RHIZOBIUM TROPICI* SEMIA4080.

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Exopolysaccharide is produced by many rhizobia species in free-living and in symbiotic condition. A number of genes of rhizobia involved in synthesis of EPS have been identified in an *exo* cluster of genes, which has been sequenced. In this work, we describe the construction and characterization of mutant strains obtained from *Rhizobium tropici* SEMIA4080 via insertional inactivation of the *exoZ* gene by chromosomal integration using a kanamycin resistant transposon cassette. Plasmid isolation, DNA ligation, restriction mapping, transformation, genomic DNA extraction, and plasmid profile were performed by standard methods. The construct was transferred to *R. tropici* by electrotransformation. Ninety six recombinants, selected by their mucoid characteristic, were grown on modified RDM agar plates supplemented

with 10% (wt/vol) sucrose and 100 mg liter⁻¹ Congo red. Replacement of the mutated gene was forced by selecting for the resistance of the transposon marker (Kan^r). EPS-producing strains had a mucoid phenotype that could easily be distinguished from mutants deficient in EPS synthesis. The *exoZ* gene of *R. tropici* SEMIA4080 is involved in, but not essential for, the production of acidic exopolysaccharide. The analysis of the nucleotide sequence of this DNA fragment revealed high similarity (90.0%) to the ExoZ protein of the *Rhizobium etli* CIAT652 and (87.0%) *Rhizobium* sp. strain NGR234. We suggest that gene disruption combined with electrotransformation is the major cause of genome rearrangements by illegitimate recombination, producing deletions not only on the target gene but also on adjacent domains, since when these mutants grown on plates, their colonies arise in small number. Wild-type *R. tropici* SEMIA4080 colonies on RDM medium were highly mucoid in appearance but some of the recovered mutants seemed to have an intermediate phenotype, while most recombinants showed mucoid colonies, suggesting that they produced EPS.
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Abstract Category
29 Biofilms and Sociomicrobiology

PS.29.037 QUORUM SENSING REGULATES CHITINASE PRODUCTION IN ACTIVATED SLUDGE

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Many bacteria use quorum sensing or cell-cell signalling to alter their behaviour in response to increases in population density. Cell-cell signalling has generated interest from the wastewater treatment industry. In this study, we investigated the local accumulation of N-acyl-L-homoserine lactones (AHLs), in activated sludge flocs, using a bacterial monitor strain derived from this environment. Additionally, 50 different activated sludge isolates were identified by sequencing SSU rRNA genes and tested for AHL production using CviR, TraR, LuxR and LasR based biosensors and for expression of six known AHL dependent phenotypes (surfactant, elastase, chitinase, lipase, cellulase and antimicrobial production). A surprising 77% of isolates produced AHLs and of these AHL producing isolates 75% expressed known AHL dependent phenotypes. Bacteria belonging to the Aeromonadaceae family were common amongst the AHL producers and many of them produced extracellular lipase and chitinase activity. Addition of N-oxohexanoyl-L-homoserine lactone (OHHL) to activated sludge stimulated chitinase production. These experiments provide insight into the role of AHL mediated gene expression in activated sludge and may ultimately create opportunities to improve sludge performance.

Abstract Category
29 Biofilms and Sociomicrobiology

PS.29.038 F- PHENOCOPIES INDUCE *ESCHERICHIA COLI* BIOFILM FORMATION

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The ecological role of plasmids within biofilms has been largely overlooked, and it has been reported that conjugative plasmids directly induce bacteria to form biofilms via an expression of conjugative pili. In this report, we described an adaptive behavior of bacterial conjugation during the biofilm formation of *Escherichia coli* harboring a natural IncF conjugative F plasmid (F+). We showed that cell-to-cell interactions via an unusual mating-pair formation of F pili among F+ x F+ cells (namely, F- phenocopy phenomenon) promote the early *E. coli* biofilm development stages. The presence of F+ x F+ population is resulted from heterogeneity inside biofilms leading to sessile bacteria grow at different rates in which the late-stationary phase growing cells acted as F- phenocopy cells. According to global transcriptional analysis and proteomic experiment, biofilm lifestyle shared similar gene expression pattern with F- phenocopies. In which, during F+ *E. coli* biofilm formation, F- phenocopy cells expressed specific sets of chromosomal genes (i.e., general stresses and two-component systems) that control the regulation regions of F transfer operon by blocking surface exclusion proteins and DNA transfer machineries. However mating-pair proteins were stabilized and consequently promoted F+ x F+ pili assembly, suggesting by loss-of-function experiments. Thus F- phenocopy phenomenon is an effective adaptive behavior of bacterial cells during biofilm formation.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.039 IN SITU MEASUREMENT OF BIOFILM ACTIVITY: IMPLICATIONS FOR DIAGNOSIS OF INFECTIVE ENDOCARDITIS

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Infective endocarditis is a microbial infection of the endocardium of the heart and is associated with significant morbidity and mortality. The treatment of choice for endocarditis is high-dosage antibiotic therapy. If the infection is not cured at an early stage, surgery and heart valve replacement are necessary. It is assumed that endocarditis is a biofilm infection; however, this view is based mainly on clinical observations, recalcitrance to antibiotic treatment, and conventional histology of the heart valves. In the present study we aimed to detect bacterial communities within human heart valves and to analyze their architecture and activity in situ. Heart valve biopsies from 110 suspected endocarditis patients were obtained during surgery and submitted to fluorescence in situ hybridization (FISH). To detect the activity of single bacterial cells more precisely we developed FISH probes for the 16S-23S internal transcribed spacer that is only present in actively transcribing cells. All 39 FISH positive samples showed only monospecies infections. In 22 cases we succeeded in visualizing rich, well structured, and highly organized bacterial communities. Strong FISH signals indicated high ribosome content, suggesting recent metabolic activity of bacteria, also in culture negative cases. Using the 16S-23S spacer FISH we also detected positive, actively transcribing cells in heart valves of patients under adequate therapy. In summary, biofilm features like spatial organization and limited growth in vitro play an important role in human endocarditis. These findings confirm the higher resistance of bacteria toward antibiotic treatment in biofilms in the clinical setting. They stress the point that our current diagnostic techniques regarding cultivation and antibiotic resistance

testing of planktonic cells in vitro are not satisfactory. In situ techniques might allow visualizing the effect of antimicrobial therapy on in vivo grown biofilms in situ.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.040 C-DI-GMP SIGNALING PATHWAY REGULATOR MORA ACTS VIA RELATED PROTEIN MORC IN CONTROLLING MOTILITY AND BIOFILM FORMATION IN PSEUDOMONAS

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We have previously described MorA as a negative regulator of motility that affects the timing of flagella expression and biofilm formation in *Pseudomonas* spp. *Pseudomonas putida* PNL-MK25 is an antibiotic-resistant derivative of the plant growth-promoting rhizobacterial strain. MorA possesses a transmembrane domain with central sensory domain of PAS-PAC motifs. It also contains C-terminal c-di-guanylate cyclase GGDEF domain and c-di-guanylate phosphodiesterase EAL domain. MorA knockout strain exhibits hypermotility and reduction in biofilm formation. Expression analyses revealed that transcript levels of the flagellar master regulators *fleQ* and *fliA* remained unchanged between wild type and *morA* mutant strain while flagellin encoding *fliC* expression was highly upregulated in the mutant strain. Therefore, the mechanism by which MorA regulates flagellin expression in the *P. putida* remains unknown. To identify signaling pathway members in the motility pathway, random mutagenesis was performed in the *morA* mutant strain followed by screening for mutants that have reverted to normal motility. A total of 76 second site revertants in the *morA* signalling pathway were obtained and 23 of these were identified by sequencing. One of these genes encodes MorC, a soluble protein containing PAS-PAC motifs and C-terminal GGDEF and EAL domains. In addition, MorC also have a nucleotide binding GAF domain. Markerless knockout of MorC reduces motility while biofilm formation is not affected. In combination with MorA knockout, both motility and biofilm formation is reduced. Therefore, we hypothesize that MorA acts via MorC in controlling the motility pathway but not biofilm formation. Studies on biochemical functions of MorC and its various domains, both in vitro and in vivo, are on-going. These studies will reveal the regulatory networks of cyclic-di-GMP signalling system.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.041 INDUCTION AND REGULATION OF ALGAL-FLOCCULATION, USING MARINE MICROBIAL ISOLATES AND HIGH PROTEIN EXTRACT

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Marine isolates from the IMO Culture Collection were screened for EPS. There were 29 EPS producing marine isolates, characterized and screened for flocculation of four microalgae species, *Chaetoceros muelleri*, *Isochrysis* sp. *Tetraselmis suecica* and *Nannochloropsis oculata*. Only 14 of the EPS producing isolates were found to induce flocculation. After the prior

screening, the marine isolates were identified using the API kits. Flocculation dynamics were also studied with the incorporation of starch and high protein extracts. Floc size and intensity were measured over a period of 21 days using Axio photomicroscopy. The microbial isolates that induced flocculation were identified as, *Saccharomyces cerevisiae*, *Candida krusei/inconspicua*, *Candida guellermondii*, *Bacillus cereus*, *Bacillus firmus* and *Staphylococcus* sp. Of the identified isolates, all were shown to induce flocculation of all four microalgae species studied. Floc diameter generally increased for 14 days after flocculation induction was carried out before declining. The diameter size of some isolates showed an increase, while others exhibited a slow decrease after more substrate was added on day 17. Floc intensity started to decrease after day 2 but stabilized after day 7. However, when more substrate was added on day 17, floc intensity exhibited an increase. Molecular studies were conducted to correlate the identity of microbial-algal components of the flocs. PCR-DGGE analysis showed that the DNA extracted from the flocs resembles the microbial and algal isolates used in the induction process. Induction of flocculation for the four microalgae species which are significant for the aquaculture industry, was successfully done using selected marine microbial isolates. Floc size and intensity were monitored thru time to determine inoculation-induction intervention. Individual isolates were characterized, identified and were confirmed to be present in the induced flocs.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.042 INHIBITION OF QUORUM SENSING SIGNAL PRODUCTION OF *PSEUDOMONAS AERUGINOSA* BY BICYCLIC COMPOUNDS

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Bacteria have a cell density-dependent gene regulation, which is referred to as Quorum sensing (QS). Various functions are controlled by QS, which is mediated via self-generated extracellular signals. *Pseudomonas aeruginosa* is a ubiquitous bacterium that can be found in various environments. It secretes a compound 2-heptyl-3-hydroxy-4-quinolone, referred to as *Pseudomonas* quinolone signal (PQS), which is known as one of QS signals in *P. aeruginosa*. PQS regulates the production of numerous virulence factors, which is toxic to other bacteria. Therefore, other bacteria may have a mechanism to inhibit PQS production to diminish *P. aeruginosa* virulence. Recently, it was reported that indole and 7-hydroxyindole (7-HI) diminish *P. aeruginosa* virulence (Lee *et al.*, MB, 2009). In this study, we explored compounds that inhibit PQS production. Bicyclic compounds such as indole derivatives and naphthalene derivatives inhibited PQS and PQS-controlled virulence factor production of *P. aeruginosa*. The addition of bicyclic compounds at the concentration of 500 μ M repressed the production of PQS. This concentration did not affect growth curve of *P. aeruginosa*. In addition, bicyclic compounds repressed the production of pyocyanin and membrane vesicles, which are regulated by PQS. While indole did not inhibit swarming motility of *P. aeruginosa* at the concentration of 500 μ M, 1-naphthol diminished swarming motility at the same concentration. In the transcriptional analysis, the addition of bicyclic compounds reduced the expression of PQS biosynthetic operon *pqsABCDE*. These results suggest that bicyclic compounds, which are secreted by many bacteria, inhibit PQS production in a transcriptional level. Since indole compounds are known as QS

signals in many bacteria, these bacteria may secrete them to diminish *P. aeruginosa* virulence. In addition, since 1-naphthol is synthesized by naphthalene-degrading bacteria, they may interact with *P. aeruginosa* through naphthalene derivatives.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.043 MYXOBACTERIA GROWTH IS DENSITY DEPENDANT UNDER PH STRESS

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Relatively simple fitness benefits of high population density such as predator avoidance or ease of mate location can promote group living and thereby increase the chance that complex social interactions may evolve. Thus, the study of density benefits may provide insights in the evolutionary roots of complex social behaviours. The bacterium *Myxococcus xanthus* exhibits several sophisticated social traits, including coordinated motility and fruiting body development upon starvation. We demonstrate a previously unknown fitness benefit of high cell density in *M. xanthus* — increased growth rate under pH stress — that is expected to promote group living under adverse environmental conditions. Density—dependent growth under pH stress was observed both in *Myxococcus* strains fully proficient at social motility and development and in mutants defective at these traits. We propose that density—dependent resistance to environmental stress may have facilitated for the evolution of complex social traits exhibited by *M. xanthus* and other myxobacteria species.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.044 GENOMIC VARIATION AT THE POPULATION AND SPECIES LEVEL IN THE SOCIAL BACTERIUM *MYXOCOCCUS XANTHUS*

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Cells of the highly social bacterial species *Myxococcus xanthus* cooperate to swarm through the soil, hunt other microbes and construct fruiting bodies upon starvation. We have begun to analyze patterns of variation among the genomes of several closely related *M. xanthus* strains that were isolated at the centimeter scale and exhibit divergent social phenotypes, including intense antagonism toward one another during co-development. By comparing the genomes of these sympatric clones we seek to analyze how *M. xanthus* genomes from the same population diversify over short evolutionary time scales and identify candidate polymorphisms that may play an important role in the social differentiation of these strains. We present preliminary results from comparison of three natural isolates and the well described reference strain DK1622.

Abstract Category

29 Biofilms and Sociomicrobiology

**PS.29.045 ROLE OF TYPA AND VIOLACEIN IN MICROBULBIFER SP. D250
BIOFILM FORMATION**

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Surface attached biofilms are a predominate mode of life for the vast majority of environmental bacteria, yet we are still only just beginning to understand how these complex structures are formed, the molecular mechanisms leading to their development and their ecological significance. We have recently demonstrated an increase in the production of the antipredatory molecule violacein in biofilms compared to planktonic cultures in a number of marine bacteria including the epibiotic strain *Microbulbifer* sp. D250 (Matz *et al.*, 2008). In order to further characterize the relationship between violacein production and biofilm formation, we have generated violacein-deficient transposon mutants in strain D250. Here, we show that two such mutants, designated 250-dV1 and 250-dV2, were disrupted in homologs of the genes *typA* and *vioB* respectively. TypA homologs have been previously shown to regulate virulence in a number of pathogenic bacteria and this is the first time such a regulator has been shown to control secondary metabolite (ie. violacein) production in an environmental, presumably non-pathogenic, organism. Further, both VioB and TypA mutants were significantly reduced in their ability to form both surface attached and "suspended" biofilms as compared to the wild type D250. While the exact mechanisms by which violacein enables biofilm formation in this strain is yet to be elucidated, this study suggests that violacein performs multiple functions in this organism. For example, in addition to the previously described antipredatory and antibacterial activities, violacein promotes biofilm formation, thus giving D250 a further ecological advantage by allowing it to rapidly colonize marine surfaces. Consequently, a putative model of TypA regulated violacein production, leading to biofilm formation by *Microbulbifer* sp. D250, is proposed.

Abstract Category

29 Biofilms and Sociomicrobiology

**PS.29.046 ECOGENOMIC ANALYSIS OF NAEGLERIA CONTAMINATED
WATER DISTRIBUTION PIPELINES**

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Naegleria spp. are thermophilic free-living amoebae that occur in soil, freshwater lakes and thermal springs. In addition to natural water bodies, man-made thermally polluted waters, e.g. poorly chlorinated swimming pools, power plant cooling ponds, and domestic water supplies, provide engineered environments favourable for both pathogenic, *N. fowleri*, and non-pathogenic *Naegleria* growth. Water distribution pipes are noted to support bacterial growth through biofilm formation on the inner surface of the pipe walls. These biofilms provide a food source for thermophilic amoebae as well as protection from disinfectants. In Western Australia, many regions have drinking water supply temperatures consistently above 30°C for >4 months annually, providing ideal conditions for *Naegleria* spp. colonization. The extended periods of elevated temperature further aid microbial growth, thus creating favourable conditions for *Naegleria* growth. While the ecological requirements of both temperature and bacteria are

recognized as contributing to the presence of all *Naegleria* spp, the role of the bacterial composition in contributing to the presence or absence of pathogenic or non-pathogenic *Naegleria* species is not well understood. In this study we profiled the biofilm composition at six different water distribution pipeline locations contaminated with either *N. fowleri* or non-pathogenic *Naegleria*. The ecogenomic results indicate a significant difference in the bacterial biofilm composition supporting pathogenic *N. fowleri* growth when compared to biofilms supporting non-pathogenic *Naegleria*. The results indicate that not only the presence of sufficient bacterial food source, but the composition of the bacterial food source, and the related physical-chemical conditions controlling the biofilm composition, may be important ecological factors regulating the presence of pathogenic *N. fowleri* in water distribution pipelines.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.047 BIOGEOGRAPHY OF MICROBIAL COMMUNITIES COLONIZING ARTIFICIAL MINERAL SUBSTRATES

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The nature of microbial communities that form biofilms on artificial mineral substrates remains little studied, with the notable exception of those involved in the degradation of historical monuments. Although biofilm formation is largely influenced by environmental conditions (temperature, humidity, solar irradiation), microbial communities that form on such substrates under similar environmental conditions offer ideal model cases for studying microbial biogeography. Here, we aimed at comparing the microbial community structure in similar composite mineral supports exposed to the open natural environments for several years in different locations along a west-east transect in the European temperate zone. We analyzed samples from Ireland, West and Central France, and from sites in Chernobyl area exposed to different radiation intensities. We characterized the microbial diversity by SSU rRNA-based methods using specific primers for archaea, bacteria and eukaryotes, as well as by using DGGE and optical and electron microscopy. Biofilm communities were complex and exclusively composed of bacteria and eukaryotes. Bacterial sequences affiliated to the Cyanobacteria, Alphaproteobacteria, Deinococcales, Actinobacteria, Acidobacteria, Bacteroidetes, Betaproteobacteria, Deltaproteobacteria and Planctomycetes. Members of the radiotolerant and polyextremophilic Deinococcales, typically adapted to desiccation, were found to be dominant in French samples. Surprisingly, deinococci were not abundant in the most irradiated Chernobyl samples, which were dominated instead by members of the Actinobacteria having as closest relative *Rubrobacter radiotolerans*. Cyanobacteria were very diverse and abundant with representatives of the Oscillatoriales, Pleurocapsales, Chroococcales and Nostocales. Most eukaryotic sequences corresponded to Ascomycota (fungi) and to Chlorophyta (green algae), some of which, e.g. *Klebsormidium* and *Trentepohlia* species, are involved in the formation of lichens.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.048 AUTOINDUCER-2 PROMOTES COMPETITIVE INTERACTIONS BETWEEN PIONEERING ORAL STREPTOCOCCI

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Streptococcus oralis and *Streptococcus gordonii* are pioneer colonizers of clean tooth surfaces and promote the development of multi-species dental plaque. Both species produce autoinducer-2 (AI-2): a proposed bacterial inter-species signal molecule that is indicated to mediate communication between bacteria. The aim of this work was to determine if AI-2 alters the competitive interactions between *S. gordonii* DL1 and *S. oralis* 34 under conditions relevant to the human oral cavity. While both species produce AI-2 in a growth-phase-dependent manner, the luxS mutant of each removed chemically synthesized AI-2 from solution, suggesting a dynamic production and degradation system for each species. *S. gordonii* DL1 formed confluent biofilms in saliva while *S. oralis* 34 formed scant biofilms. Compared to the wild-type, the isogenic luxS mutant of *S. gordonii* DL1 formed denser biofilms with finger-like projections into the lumen while the luxS mutant of *S. oralis* 34 formed biofilms that were identical to the wild-type. Addition of 1nM AI-2 to saliva restored *S. gordonii* DL1 luxS mutant biofilms to that of the wild-type phenotype. When grown together in dual-species biofilms in all possible wild-type and luxS mutant combinations, *S. oralis* 34 was only competitive and retained a biofilm niche when AI-2 was present in the local environment. Either species needed to produce AI-2 to allow *S. oralis* 34 to compete against *S. gordonii* DL1. In the absence of AI-2, *S. oralis* 34 cells were seldom present in the dual-species biofilms. The addition of 1nM of chemically synthesized AI-2 to dual-species luxS biofilms restored the competitiveness of *S. oralis* 34 luxS mutant. In conclusion, this work demonstrates that AI-2 alters the biofilm competitiveness of pioneering oral streptococci which, in nature, will alter the subsequent succession of species into oral biofilms and the overall ecology of dental plaque.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.049 BACTERIAL UTILISATION OF BIOPOLYMERS AT MICROGRAM-PER-LITER LEVELS IN TAP WATER UNDER STATIC AND DYNAMIC CONDITIONS

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Oligotrophic aquatic environments contain low concentrations of biopolymers that can promote growth of heterotrophic bacteria and biofilm formation. Biofilm formation occurs in natural aquatic environments, but also in drinking water distribution systems where it may cause growth of bacteria that impair water quality and safety. The aim of this study was to quantify bacterial growth and biofilm formation at µg C/l levels of biopolymers in oligotrophic water under static and dynamic conditions. Batch experiments were conducted to investigate the growth kinetics of recently isolated *Flavobacterium johnsoniae* strain A3 with biopolymers at 0 - 200 µg C/l in tap

water. Biofilm forming properties of tap water enriched with 10 µg C/l of either amylopectin, maltose or both were assessed by analyzing ATP concentration, heterotrophic plate count, total cell count and T-RFLP fingerprint of a biofilm developing under dynamic conditions during 35 days. Substrate affinity (K_s) values of strain A3 for maltose, laminarin, amylopectin, xyloglucan and gelatine ranged from 3.2 to 20.3 µg C/l, whereas v_{max} values varied from 0.210 to 0.328 1/h. Thus strain A3 can rapidly utilize biopolymers at the µg C/l level in water under static conditions. Under dynamic conditions, tap water enriched with 10 µg amylopectin-C/l did not enhance biofilm formation compared to the blank. In contrast, biofilm formation was promoted by tap water with 10 µg maltose-C/l. Biofilm formation with both maltose and amylopectin was initially synchronic to biofilm formation with only maltose, but biofilm formation continued with amylopectin when the maximum biomass concentration with maltose was reached. Biopolymers are utilised at concentrations of a few µg C/l ($\ll K_s$) in oligotrophic aquatic environments under static conditions, but only promote biofilm formation under dynamic conditions in certain situations, for example when an adapted biofilm is already present on the substratum.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.050 PHENOL BIODEGRADATION BY BACTERIAL BIOFILMS PREPARED FROM POLY(ETHYLENEOXIDE) CRYOGELS

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Xenobiotic degrading bacteria were immobilized on poly(ethylene oxide), PEO, cryogels as carriers. Bacterial cultures of *Pseudomonas rhodesiae* KCM R5 and *Bacillus subtilis* KCM RG5 were isolated from heavy metal and aromatics polluted industrial area. PEO cryogels were prepared by P. Petrov and Ch. Tsvetanov (Institute of polymers, BAS, Sofia) following an original method. The capability of both immobilized systems to degrade phenol as single source of carbon and energy was investigated in batch experiment. The PEO cryogels-bacterial biofilms thus obtained were treated consecutively with 300, 600 and 1000 mg L⁻¹ phenol for 28 days. The immobilization system PEO-KCM R5 removed phenol at concentration of 1000 mg L⁻¹/24 h while the system PEO KCM RG5 was less effective degrading phenol at concentration not exceeds 600 mg L⁻¹/24 h. Our experimental results showed a vast bacterial colonization. The optimal reproduction of the immobilized bacteria is connected with the high content of free-water in the cryogels. An initial adaptation of the immobilization systems with 100 mg L⁻¹/24 h was found to protect the bacterial cells from the phenol shock. The liquid debit through the biofilms at the end of the experiment was significantly lower than the starting one. The PEO cryogels obtained are non-toxic, demonstrate high biocompatibility with bacteria and possess good mechanical characteristics as polymer carriers. After the long and aggressive phenol treatment the PEO-biofilms remained compact, porous and elastic. The investigated new materials demonstrate potential for complete phenol degradation in the industrial wastewaters.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.051 EFFECT OF MIMICKING MICROGRAVITY ON THE MOTILITY OF PSEUDOMONAS AERUGINOSA ON AGAR MEDIUM

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Pseudomonas aeruginosa is a prevalent contaminant of clinical instruments and a well-known opportunistic pathogen. The persistence of this organism in the specific sites is attributed to the biofilm formation depending on the coordinated motility. The purpose of this study was to know about the motility behavior of *P. aeruginosa* under the microgravity environment formed in the spacecraft. To this end, we examined the swarming and twitching motility of *P. aeruginosa* strains, PAO1 and a type IV fimbriae-negative mutant of PAO1 (*pilA* mutant), by growing them on clinostat, a device creating an environment nearly equivalent to actual microgravity. The swarming and twitching motility was measured on Luria-Bertani medium containing 0.6 and 1.0 % (w/v) agar, respectively. Strain PAO1 showed a higher rate of spreading colony development on the agar medium under clinorotation than under normal static condition. In contrast, the twitching motility of strain PAO1 was fully inhibited on clinostat and the degree of inhibition was comparable to the result of the *pilA* mutant under the static control condition. This finding showed that the formation of type IV fimbriae was inhibited by clinorotation. To examine whether the clinorotation influences the regulation of the fimbrial genes at the transcription level or translation level, molecular genetic studies targeting the type IV fimbrial genes are now in progress.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.052 LINK BETWEEN QUORUM SENSING AND EXOPOLYSACCHARIDE PRODUCTION IN PSEUDOMONAS AERUGINOSA

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Pseudomonas aeruginosa is an opportunistic pathogen that regulates expression of hundreds of genes through quorum sensing. Previous chromatin immunoprecipitation-on-chip analysis and electrophoretic mobility shift assays have shown that the central regulator of quorum sensing, LasR, binds to the putative promoter region of *pslA*. *pslA* is the first gene of the *Psl* exopolysaccharide locus, one of the two key exopolysaccharide loci that contribute to biofilm formation in *Pseudomonas aeruginosa*. We are interested in further investigating the role of quorum sensing in exopolysaccharide production in *P. aeruginosa*.

We used colony phenotype assay to examine the effects of a *lasR* mutation on exopolysaccharide production in the clinical strain ZK2870. Wrinkled colony morphology is generally associated with enhanced exopolysaccharide production. We generated *pel* and *psl* deletion mutants in a *lasR* mutant background to identify the exopolysaccharide biosynthetic loci involved. We also investigated how cyclic-diguanylate levels affect the colony phenotype of the *lasR* mutant by

overexpressing a phosphodiesterase. We employed suppressor transposon mutagenesis to identify the genes capable of suppressing the wrinkled phenotype of a *lasR* mutant.

A *lasR* deletion mutant forms a wrinkled colony at 37°C indicating enhanced exopolysaccharide production as compared to the smooth wild type phenotype. Further genetic analysis suggests that LasR inhibits exopolysaccharide synthesis by affecting the *pel* rather than *psl*. Degradation of cyclic-diguanylate in a *lasR* mutant results in reduction of wrinkles indicating its involvement. None of the established pathways mediate QS-dependent colony morphology. Suppressor analysis implicated the *Pseudomonas* quinolone signal in this process.

Our data so far suggests that the *las* quorum sensing system repress *pel* exopolysaccharide production in *Pseudomonas aeruginosa* by a cyclic-diguanylate dependent process.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.053 USE OF MICROFLUIDIC DEVICE TECHNOLOGY FOR BIOFILM METABOLISM AND GROWTH MONITORING

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In nature, microorganisms often form surface-associated communities and aggregate that are referred to as biofilm. Because 90% of all microorganisms are estimated to exist in biofilms rather than in the planktonic state, the metabolic activity of these biofilms has a major role in global element cycles. To date, biofilm monitoring studies have been performed using relatively large scaled experimental devices such as flow cells, or continuous reactors. On the other hand, it has been demonstrated that microfluidic device systems can provide a powerful platform for biological assays. A microfluidic device is an "on-chip" liquid handling device that focuses on controlling tiny quantities of liquids to allow chemical assays to be integrated on a small chip. The microfluidic device is advantageous, because it reduces the consumption of reagents and sample solutions, has a short analysis time, is easily automated, and has a low cost. The main purpose of this study is to establish the utility of microfluidic device in biofilm studies. Here we demonstrated that the growth and the metabolic activity of a biofilm can be monitored on a microfluidic device. We first addressed that whether the biofilm growth in the microfluidic device could be visualized and quantified with a modified confocal reflection microscopy techniques (COCRM)⁽¹⁾⁽²⁾. Next, we integrated an electrochemical sensor for the monitoring of the metabolic activity of biofilm. The results showed that *Streptococcus mutans* biofilm growth could be monitored with COCRM, and the nitrification activity of activated sludge could be monitored with an ammonium selective electrode. According to these results, we propose that the microfluidic device can be a powerful tool for biofilm studies.

References

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Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.054 TRANSCRIPTOME ANALYSIS OF PHENANTHRENE-DEGRADING SPHINGOMONAS BIOFILMS EXPOSED TO ENVIRONMENTAL STRESSES

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Polycyclic aromatic hydrocarbons (PAHs) such as phenanthrene are widespread environmental contaminants. Bioremediation of these pollutants is often hindered by the loss of catalytic activity of endogenous or introduced pollutant-degrading bacteria due to various environmental stresses. This study aims at identifying abiotic stress factors which influence the survival and catabolic potency of phenanthrene-degrading bacteria to improve the treatment of environmental pollution with emphasis on PAH-degrading *Sphingomonas*. The phenanthrene-degrading strain *Sphingomonas* sp. LH128-Gfp was grown in phenanthrene containing flow chambers, continuously irrigated with a minimal medium for 11 days during which the biofilms were acutely (4 hours) or chronically (3 days) exposed to either solute stress (450 mM NaCl) or matric stress (10% w/v PEG6000). Biofilm morphology was monitored with confocal microscopy. To study differential gene expression, the genome of *Sphingomonas* sp. LH128-Gfp was sequenced, encoding about 7000 predicted genes. Based on these data a high density micro-array was designed for transcriptome analysis. While no clear morphological changes in the biofilm architecture were observed after imposing the stresses, micro-array analysis indicates that acute matric stress induces a transcriptional response during which 7 % of the genes were differentially expressed, while long exposure gene expression patterns were more similar to pre-stress conditions. Under short solute stress about 10 % of the genes were differentially expressed which was also the case during chronic stress exposure. Under all stress situations, genes involved in the catabolism of aromatics were down-regulated. These data suggest that although no structural biofilm changes are noticeable, there is a significant difference at the level of gene expression, and that, depending on the imposed stress, gene regulation may or may not be restored to the pre-stress situation.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.055 THE EFFECT OF MARINE PREDATORS ON THE COMMUNITY COMPOSITION IN A MIXED-SPECIES BIOFILM

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Protozoa are ubiquitous predators of marine microbial biofilms. Here, we investigate the impact of grazing by protozoans feeding on biofilms at different stages of development. A model marine mixed species biofilm consisting of *Microbacterium phyllosphaerae*, *Shewanella japonica*, *Dokdonia donghaensis* and *Acinetobacter lwoffii* was exposed to predation by *Rhynchomonas nasuta* (early biofilm feeder), *Tetrahymena* sp. (intermediate feeder) and *Acanthamoeba castellanii* (late biofilm feeder). Co-inoculation assays were performed in 24 well-microtiter plates. The impact of grazing was determined by assessing colony forming units of planktonic and biofilm communities, and ANOVA analysis. Results showed that the mixed species biofilms provided resistance to specific members when exposed to *R. nasuta* grazing, whereas single species biofilms were not resistant, at any stage of biofilm development. Selective grazing pressure exerted on *A. lwoffii* by *R. nasuta* in the early and intermediate stage mixed species biofilms induced a shift from attached to planktonic cells. *Tetrahymena* sp. preferentially grazed on *D. donghaensis* and *A. lwoffii* in early and intermediate biofilm development, while *A. castellanii* grazed primarily on *D. donghaensis* and *A. lwoffii* in early mixed species biofilms. In contrast, mature, rather than early and intermediate, mixed species biofilms were resistant to amoeba grazing. We propose that the community composition of mixed species biofilms is dependent on the stage of biofilm development, the type of predator and their feeding preferences.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.056 GENE EXPRESSION IN *PSEUDOMONAS AERUGINOSA* SWARMING MOTILITY

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Swarming motility of *Pseudomonas aeruginosa* is characterized by a fast and coordinated group movement over a semi-solid surface resulting from intercellular interactions and morphological differentiation. A striking feature of swarming motility is the complex fractal-like patterns displayed by migrating bacteria while they move away from their inoculation point. Understanding this group behaviour is important to gain further insight on bacterial structured communities. Using GeneChip® Affymetrix microarrays, we obtained the transcriptomic profiles of both bacterial populations located at the tip of migrating tendrils and swarm center of swarming colonies and compared these profiles to the one of a control population of bacteria grown on the same media but solidified to not allow swarming motility. Differentially expressed genes between the three conditions were selected using a threshold of 1.5 fold (\log_2), which gave a total of 378 selected genes (6.3% of the predicted open reading frames of strain PA14). Unexpectedly, microarrays expression data reveal that only a minority of genes are upregulated in tendril tip populations. Among them, we found energy metabolism, ribosomal protein and transport of small molecules related genes. Moreover, tendril tip cells display a general down-regulation of genes associated with virulence while swarm center cells appear to be under oxidative and copper stress responses. These results allow us to propose a model where tendril tip cells function as scouts whose main purpose is to rapidly spread on uncolonized surfaces while swarm center cells' function is to establish a permanent population.

Abstract Category
29 Biofilms and Sociomicrobiology

PS.29.057 STUDY OF THE SURVIVAL AND DYNAMICS OF *LEGIONELLA* AND OF ASSOCIATED MICROBIAL FLORA IN WATER AND BIOFILM AFTER DISINFECTION

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—Legionellosis, caused by *Legionella* bacteria kills 10% of patients. The management of *Legionella* risk is therefore a health issue of prime importance.

Legionella are mainly encountered in biofilm which is present in cooling towers and Hot Water Distribution Systems (HWDS). Various disinfection procedures are used to eliminate this bacteria but none of them can eradicate it totally. Moreover, no reproducible and comparative method has been developed to evaluate the intrinsic efficiency of treatments against *Legionella* in biofilm. For this purpose, a pilot-scale 1 was constructed in order to simulate a real HWDS. It consisted in two identical loops (control and treated) which each have a sampler of biofilm. This unit was contaminated with *Legionella* by river water. Three heat shocks (70°C 30 min) and three chemical treatments were tested. *Legionella* concentrations were followed in control and treated water and biofilm by culture on GVPC medium and by quantitative PCR (qPCR). The results showed a temporary reduction of cultivable *Legionella* and little effect on qPCR counts. For the first two thermal shocks, no cultivable *Legionella* has been detected within 48 h following treatment and return to initial levels was observed between 48 h and 72 h after the shock. The third heat treatment proved ineffective. Regarding the chemical treatment, a transient effect was also observed on the amount of cultivable *Legionella* but the initial concentrations were again reached between 72 h and one week after stopping treatment. Furthermore, the use of advanced molecular tools (sequencing, SSCP) has identified the diversity of *Legionella* (five species) in the biofilm of HWDS and characterized the microbial flora accompanies (proteobacteria, amoebae...). This work brings new elements to test the effectiveness of anti-*Legionella* treatments and opens up many possibilities in providing new information on the microbial consortium associated with *Legionella* in the biofilm.

Abstract Category
29 Biofilms and Sociomicrobiology

PS.29.058 INTERACTION BETWEEN MALE-SPECIFIC BACTERIOPHAGES AND *ESCHERICHIA COLI* BIOFILMS

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It has been demonstrated that a conjugative F plasmid induces *Escherichia coli* biofilm formation. F-pili mediate cell-cell interactions. F-pili tips also bind male-specific non-lytic filamentous bacteriophages, whereas lytic icosahedral bacteriophages, bind along the filament sides. Despite the increasing studies on bacterial biofilms and relatively large numbers of bacteriophage are found in many of the environments, few studies have addressed the effects of bacteriophages on *E. coli* biofilm formation. In this report, we observed that male-specific bacteriophages could eliminate the early bacterial attachment and microcolony formation, especially when infected with filamentous bacteriophages (i.e. fd, f1 or M13), suggesting that the tip of F-pili is most likely the specific receptor site for the formation of mating pairs, and the side of F-pili plays a non-obligatory role. Spatial localization of the bacteriophages within biofilm was performed using immuno-florescence labelling, indicating the unique phage propagated patterns during biofilm maturation. In addition, we found that the bacteriophages failed to enter into a biofilm matrix of the mature biofilms. We further demonstrated those specific bacteriophage-resistant mechanisms at transcriptional level, in which the mature biofilms regulated F-pili synthesis and increased curli production to prevent the bacteriophages infection. Thus, these indicated a close evolutionary relationship among the capacity to form a biofilm, the presence of a conjugative plasmid and the resistance to male-specific bacteriophages.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.059 LONG-CHAIN ACYLHOMOSERINE LACTONES INCREASE THE ANOXIC AMMONIUM OXIDATION RATE OF AN OXYGEN-LIMITED AUTOTROPHIC NITRIFICATION/DENITRIFICATION BIOFILM

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The oxygen-limited autotrophic nitrification/denitrification (OLAND) process comprises partial nitrification and anammox, catalyzed by aerobic and anoxic ammonium-oxidizing bacteria (AerAOB and AnAOB), respectively. Since AnAOB grow more slowly and have a lower specific ammonium-oxidizing activity than AerAOB, factors enhancing the AnAOB activity are of practical interest. Therefore, the goal of this study was to investigate whether quorum sensing influences the anoxic ammonium oxidation.

Specific ammonium oxidation rates were determined in batch activity tests. Biomass supernatant was obtained following mechanical homogenization and centrifugation. The presence acylhomoserine lactones (AHLs) was tested with reporter strain *Chromobacterium violaceum* CV026, and the AHL identity was tested with LC-MS/MS.

The specific anoxic ammonium oxidation rate of an OLAND biofilm significantly increased with a factor of 1.5 ± 0.2 at high biomass concentrations compared to low biomass concentrations. A

similar increase in activity was obtained when supernatant of a dense OLAND biofilm was added to low biomass concentrations. In this supernatant, AHL presence was shown by the reporter strain, and the AHL was identified as N-dodecanoyl homoserine lactone (C12-HSL). Furthermore, C12-HSL detection was negative in an AerAOB-enriched community, but positive in an AnAOB-enriched community. The addition of C12-HSL to low OLAND biofilm concentrations resulted in a significantly higher anoxic ammonium oxidation rate.

These results indicate that C12-HSL quorum sensing is related to the AnAOB in the OLAND biofilm. Anoxic ammonium oxidation could either be self-regulated by quorum sensing using C12-HSL or steered by heterotrophic bacteria associated with the AnAOB. This is the first study demonstrating that AHL-mediated quorum sensing plays a role in anoxic ammonium oxidation.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.060 BACTERIAL SWIMMING, SWARMING AND CHEMOTACTIC RESPONSE TO HEAVY METALS' PRESENCE: WHICH COULD BE THE INFLUENCE ON WASTEWATER BIOTREATMENT EFFICIENCY?

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In developing countries it is frequent to find high metal loads in surface waters as a consequence of poorly or not at all treated wastewater discharges. The development of easy to implement and rentable remediation alternatives, using indigenous bacteria, is essential to create options for the industry to diminish polluted discharges. Fixed-bed reactors are usually designed for this purpose, where the biofilm establishment and maintenance play the most important roles. Biofilm development strictly relies on different types of bacterial motility: swimming, swarming, twitching and chemotaxis, which can be altered by the microenvironment conditions. The aim of our work is to perform an integrated study on the different effects of Cu, Cd, Zn and Cr on swimming, swarming and chemotaxis of *Pseudomonas veronii* 2E, *Delftia acidovorans* AR and *Ralstonia taiwanensis* M2 to improve biofilm development for metal loaded wastewater biotreatment in fixed-bed bioreactors. These bacteria showed the ability to biosorb Cd(II), Cu(II) and Zn(II) and biotransform Cr(VI) either in planktonic or biofilm states. Swimming and swarming experiments were carried out at different metal concentrations as well as chemotactic response evaluation, using a modified chemical in plug assay, based on the characteristics of each strain. Clearly, the effects were independent, since a delay in swimming did not mean an inhibition of swarming or viceversa, nor a change in chemotaxis showed a correlation to swimming and swarming changes. *P.veronii* 2E motility was not affected by metal presence, being this strain optimal for fixed-bed reactor development. *D.acidovorans* AR swarming was inhibited by Cd and Zn. Although *R.taiwanensis* M2 showed high resistance to Cu, Cd, Cr and Zn, motility was definitively altered in all aspects. In spite of not being as useful as expected for biofilm reactors, further studies on *R.taiwanensis* M2 resistance mechanisms would be particularly interesting.

Abstract Category
29 Biofilms and Sociomicrobiology

PS.29.061 *Pseudomonas veronii* 2E BIOFILM DEVELOPMENT FOR A PROPER PACKED-BED BIOREACTOR DESIGN

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Biofilm-mediated wastewater treatments represent a proficient and safer alternative, especially when metal retention is involved. Extracellular materials, usually present in bacterial biofilm structures, enhance microbial metal immobilization mechanisms. Bacterial attachment or detachment to matrices may be strictly related to environmental conditions, being well described for *Pseudomonas aeruginosa*. *Pseudomonas veronii* 2E is an indigenous strain which was isolated from polluted sediments of Reconquista River. The aim of our work is to study the biofilm development of *P. veronii* 2E under different experimental conditions so as to improve the bioreactor performance for metal-loaded wastewater treatments. For such purpose, biofilm establishment and maintenance were explored over glass coverslips under different culture broth composition (M9-glucose, M9-citrate and PYG-Casein Peptone-Yeast Extract-Glucose), nutrient concentration, incubation time, temperature and Fe(II) presence. Adequate biofilm visualization was achieved following different techniques after crystal violet staining: 1. ethanol extraction and Absorbance 590nm measurement; 2. direct microscopic observation and 3. sample scanning with image analysis by MATLAB program. The best attachment was achieved up to 31hs 28°C incubation in M9-glucose when cell detachment began. Meanwhile, 55hs 32°C incubation with medium renewal was required to reach the highest bacterial adhesion in PYG. The maximal concentration of crystal violet obtained together with the image capturing supported these results. Biofilm structure developed with M9 appeared to be a monolayer, but in PYG numerous cell cluster and channels were appreciated when samples microscopically observed. For *P. veronii* 2E, the final biofilm structure was defined by temperature, medium composition and nutrient quality. These parameters directly influenced cellular differentiation, necessary for a proper biofilm establishment.

Abstract Category
29 Biofilms and Sociomicrobiology

PS.29.062 REDUCTION OF MARINE BIOFOULING BY COMPLEX COMMUNITIES OF MICRO-ORGANISMS ON ORGANIC POLYMERIC SURFACES OF MARINE ENVIRONMENTAL MICROSENSORS

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The oceans play a key role in the regulation of the Earth's climate. However, much of the oceans' biogeochemical processes remain undersampled, making future predictions on the Earth's

climate unreliable. Oceanographic surveys are limited to a relatively small scale due to the high costs of ship based water sampling (~£15k per ship, per day) or the opacity of deep seawater to electromagnetic radiation used for remote sensing. For this reason in situ sensors have been identified as a solution to providing large scale data on biogeochemical processes in the world's oceans.

Sensors that are deployed in the ocean for long periods are prone to biofouling, a process by which microorganisms colonise immersed surfaces, producing a slime or biofilm on the sensor equipment. These biofilms can have a detrimental effect on the functioning of sensors and so need to be limited to ensure accurate, long-term measurements by the sensors.

We are developing methods of biofilm remediation using a combination of biocompatible polymers and controlled release of chemical effectors of cell physiology. One such chemical that has been tested for its efficacy for reducing biofilm formation is nitric oxide.

Nitric oxide reduces biofilm formation by triggering an innate dispersal mechanism. In laboratory based, in vitro studies of complex microbial communities, nitric oxide has been shown to reduce marine biofilm formation by up to 75% on hydrophobic, abiotic surfaces.

Further experiments will study the effects of other chemical agents and their effects on biofilm community structure, using DNA based community analysis techniques.

While it is unlikely that any chemical will completely prevent biofilm formation, it is hoped that using a combination of techniques, such as modification of surface physico-chemistry, will keep biofilms at a level that is less detrimental to sensor function.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.063 EXTRACELLULAR METABOLOMICS OF PLANKTONIC AND BIOFILM SALMONELLA TYPHIMURIUM USING GAS CHROMATOGRAPHY MASS SPECTROMETRY

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Salmonella species are environmentally persistent pathogens capable of forming biofilms on many surfaces. This study compares the extracellular metabolite profiles between planktonic and biofilm Salmonella at different days of growth. We hypothesize that the exometabolome of planktonic Salmonella is different to the exometabolome of biofilms.

Salmonella enterica serovar Typhimurium ATCC 14028 biofilms were formed using the Calgary Biofilm Device. Supernatants of planktonic and biofilm cultures were taken at 1, 3, 5 and 7 days of growth, filtered and freeze dried. Samples were derivatised and analysed with an Agilent 6890 gas chromatograph system coupled with an Agilent 5973 mass selective detector using a 20-1 split injection. Data analysis was performed using SpectralWorks AnalyzerPro and principal component analysis (PCA) was performed on the normalised data using CAMO Unscrambler.

PCA score plot for 10% Luria Bertani broth with no salt (control) and planktonic and biofilm supernatant samples shows that the controls are separated from biofilm and planktonic

supernatants. In general, separate clusters were observed between planktonic and biofilm supernatants. There was also a downward shift along the second principal component (y-axis) with respect to increasing age of sample supernatants. The specific metabolites causing the variance between planktonic and biofilm supernatants is currently being determined.

Metabolite profiles of planktonic and biofilm *Salmonella Typhimurium* vary with age and the metabolites secreted by planktonic cultures are different to metabolites secreted from biofilms. In addition, the biochemical reasons for metabolome differences between planktonic and biofilm modes of growth should be investigated.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.064 COMPETITION AND SOCIALIZATION BETWEEN POLYCYCLIC AROMATIC HYDROCARBON (PAH)-DEGRADING BACTERIA ON SOLID SURFACES CONTAINING PAH CRYSTALS

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Polycyclic Aromatic Hydrocarbons (PAHs) are important soil pollutants. From PAH-contaminated soils, PAH-degrading bacteria belonging to different genera can be isolated. Those organisms apparently cohabit the same macro-environment. However, it remains unclear whether different PAH-degrading bacteria within a soil macro-environment occupy the same microniche, whether they socialize or compete in such a microniche and which environmental and bacterial characteristics define the niches. To assess microniche occupation, the interactions between PAH-degrading bacteria belonging to different bacterial genera were visualized during colonization of a PAH-containing glass surface through Confocal Laser Scanning Microscopy analysis. The main questions were (i) whether a particular PAH-degrading strain will visibly socialize, collaborate or compete with other PAH-degrading species when using a PAH as the sole source of carbon and energy in the system and (ii) whether or not the behavior of PAH-degrading bacteria when grown in single species systems is affected by the presence of other organisms in multispecies systems. The organisms used were the PAH-degrading bacterial strains *Sphingomonas* sp. LH128, *Pseudomonas putida* OUS82 and *Mycobacterium gilvum* VM552. The compartment and microniche occupation of each species showed to be highly dependent on the selected PAH source, the presence of other carbon sources, and the characteristics of the bacterial strain, like species-specific PAH bioavailability-promoting properties or (secondary) metabolite production. Compartment of particular strains in dual species systems was clearly affected by the presence of other strains. Also, different colonization patterns of the strains, showing both socializing and competing behavior, were observed in multispecies systems compared with their behavior in single species systems, emphasizing the complexity of interactions between PAH-degrading bacteria in environmental settings.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.065 BIOTRANSFORMATION OF URANIUM (VI) AND CHROMIUM (VI) BY DENITRIFYING MIXED MICROBIAL GRANULAR BIOFILMS

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Microbial reduction of radionuclides and toxic metals to less soluble forms represents a useful bioremediation strategy for eventual removal of redox-active elements from industrial effluents and aqueous environments. Aqueous effluents of nuclear industry origin contain high concentrations of nitrate along with various elements in smaller quantities. Removal and transformation of redox-active elements such as uranium (VI) and chromium (VI) by microbial granules was investigated in 100 ml serum bottles under anaerobic conditions. Mixed microbial granules (granular biofilms) were developed under anoxic conditions in a laboratory scale sequencing batch reactor (SBR) after inoculation with activated sludge. Transformation of flocculent sludge to microbial granules occurred within one month of SBR startup. Microbial granules exhibited stable and high rate denitrification suitable for the treatment of high strength nitrate bearing effluents of nuclear industry origin. These microbial granules exhibited efficient removal of U(VI) and Cr(VI) in serum bottles containing glucose as electron donor. Removal of U(VI) (0.5 mM) and Cr(VI) (0.2 mM) was almost complete within 2 days from media containing 1 g (wet weight) pre-grown granules. Results confirmed the role of cell metabolism in U(VI) or Cr(VI) removal by microbial granules. Spectroscopy analysis confirmed microbial transformation of U(VI) and Cr(VI). Denaturing gradient gel electrophoresis was used to determine community composition and dominance of functionally important bacteria. Our study shows that mixed microbial granule-based systems are suitable for treatment of high strength nitrate bearing effluents containing U(VI)/Cr(VI) as co-contaminants. This presentation covers development of mixed microbial granules, biotransformation of U(VI) and Cr(VI), changes in microstructure (determined using confocal microscopy) and bacterial community composition and characterisation of U(VI)/Cr(VI) reducing bacteria.

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.066 ADAPTIVE TRANSFORMATION OF *PSEUDOMONAS AERUGINOSA* BIOFILM STRUCTURE IN RESPONSE TO CHANGING RESPIRATION CONDITIONS

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Biofilms are surface-associated multi-cellular communities formed by microbial cells that often exhibit elaborate and complex three-dimensional structure. Because many microorganisms are observed to exist in biofilms, they do play a major role in global element cycles. The structures of biofilms have been indicated to correlate with metabolic efficiency. In animals and plants, a ubiquitous phenomenon is that environmental perturbations induce changes in the posture of

individuals, such as mammals curling their bodies to reduce heat radiation in the cold. Using novel methods [1,2], our study demonstrates that such adaptive responses can also be found in bacterial biofilms. Here we demonstrated that, biofilm could adaptively control their structure in response to environmental shift. When aerobic conditions were replaced by less-energetically efficient denitrifying conditions, mushroom-shaped aerobic *Pseudomonas aeruginosa* biofilm structures underwent a drastic transformation into three-dimensional mesh-like structures, concomitant with improvements in denitrifying efficiency. The mesh-like structure was more permeable to small molecules, and cells inside the biofilm began to express a critical denitrification gene along with the formation of the mesh-like structure. Based on these results, we conclude that the shift in the available electron acceptor would induced a transformation of biofilm structure that increased the fitness of the *P. aeruginosa* biofilms. These results reveal that bacterial biofilm would be a dynamic community that can adapt to environmental shifts by controlling its physical structure.

1. Y. Yawata, N. Nomura, H. Uchiyama, Appl. Environ. Microbiol. 74, 5429-5435 (2008).
2. Y. Yawata, H. Uchiyama, N. Nomura, Microbes Environ. doi:10.1264/jsme2.ME09169 (2010).

Abstract Category

29 Biofilms and Sociomicrobiology

PS.29.067 CHARACTERIZATION OF INDIGENOUS THERMOPHILIC MICROORGANISMS OF HOT SPRINGS IN LIMA AND HUARAZ

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Microbial diversity in extreme temperature environments is low, indigenous microorganisms have the adaptive physiological characteristics that allow survival in the ecosystem. To determine the morphological, physical and chemical properties of native microorganisms isolated from hot springs of Churin (Lima) and Chancos (Huaraz), to determine the presence of spores and mobility of these organisms.

We took 3 samples of each hot spring, were grown at 70°C for 40 days, one of the control samples was only thermal water, the second shows added 1% meat extract and the third was added 1% citrate-nitrate, the same procedure for the waters of the surrounding canals, swimming pools or wells which were grown at 50°C.

The sample had very little microbial growth, good microbial growth was found in the sample with beef extract at 70°C with Gram positive pleomorphic cytoplasmic granules similar to *Thiobacillus* sp and thick rods, mobile, *Desulfovibrio*. Slender rods with cytoplasmic granules similar to *Beggiatoa*. In the samples cultured at 50°C showed higher microbial diversity, fungi, yeasts and algae.

The cultures at 70°C with 1% beef extract has a higher microbial growth with nitrate-citrate, channel samples and 50°C and has a higher diversity of microorganisms. Definitive identification of microorganisms at 70°C requires molecular testing.

Abstract Category
29 Biofilms and Sociomicrobiology

PS.30.001 CYANOBACTERIA FROM A RADON-RICH HOT SPRING

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The Paralana hot spring in the Flinders Ranges, South Australia, is subjected to heat, ionising and ultraviolet radiation. It sustains a flourishing microbial community despite this combination of extreme conditions. Previously, we have investigated the microbial diversity of this ecosystem. 16S rDNA cloning and sequencing studies have allowed us to conclude that cyanobacteria are a significant component of the biota. We have now enriched and identified a number of these cyanobacteria as the first phase of an analysis of their contribution to the ecosystem. Samples were incubated on BG11 agar and putative cyanobacterial-positive cultures identified by microscopy. Following serial transfers, enrichments were analysed using morphological characteristics and 16S rDNA-based techniques. Microscopical observation of samples prior to culturing revealed a variety of cyanobacterial cell morphologies and sizes. BG11 enrichment resulted in the growth of filamentous and single-celled cyanobacteria and other microbes. Genera tentatively identified microscopically include *Oscillatoria*, *Fischerella* and *Synechococcus*. Three enrichment cultures analysed using molecular methods were dominated by cyanobacteria or green non-sulfur bacteria (*Chloroflexus aurantiacus*). Of the four major 16S rDNA cyanobacterial sequences identified, two displayed database similarity of >99% with previously cultured species (*Fischerella major* and *Oscillatoria amphigranulata*), whilst the others may represent novel taxa. Enrichment of members of the Paralana hot spring cyanobacterial community, and their eventual axenic isolation, represents an important initial step in ascertaining their specific role in Paralana, such as shielding of other species from high UV levels. It also will provide a useful resource for future studies on extremophiles simultaneously exposed to multiple stressors.

Abstract Category

30 Miscellaneous

PS.30.002 ESTIMATION OF ABUNDANCE OF BACTERIA ON ASIAN DUST BY QUANTITATIVE PCR

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Dust storms originating in the arid regions of Asia usually occur from late winter to early spring, and more than one million tons of dust per year is transported to distant locations, such as the Korean Peninsular and Japan, by the prevalent westerly winds (Asian dust events; Kosa). The major dust sources are considered to be arid regions in China. We supposed that these soil particles could be carriers of microbes. In order to clarify the dynamics of microbes moving with these dust events, bacterial cells attached to dust particles were detected by culture-independent approaches. Dust source soil samples were collected from Loess plateau, Taklamakan desert and Gobi desert and Asian dust particles were collected in Beijing, China and Osaka, Japan. In Osaka, Asian dust particles were directly collected at an altitude of 6000 feet by an airplane to avoid the contamination of soil particles from ground. The number of bacterial cell was

determined by quantitative real-time PCR with the eubacterial primer set. The average diameter of these particles collected in Osaka was 2 - 5 μm and smaller than that of Beijing and dust source region. The number of bacterial cells on Asian dust particles was estimated as $10^5 - 10^7$ cells g^{-1} . These findings show that bacterial cells can "travel" by atmospheric transport of soil and might affect the microbial ecosystem in Eastern Asian countries when severe Asian dust events occur. This study was supported by the Ministry of the Environment, Japan; the Global Environment Research Fund (B-0902).

Abstract Category
30 Miscellaneous

PS.30.003 APPLICATION OF QUANTITATIVE REAL-TIME PCR FOR ENUMERATION OF TOTAL BACTERIAL, ARCHAEAL, AND YEAST POPULATIONS IN KIMCHI

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Kimchi is a traditional fermented Korean food made of brined vegetables, with a variety of spices. Various microorganisms are associated with the kimchi fermentation process. This study was undertaken in order to evaluate the applicability of quantitative real-time polymerase chain reaction (PCR) targeting the 16S and 26S rRNA genes for investigation of the dynamics of bacterial, archaeal, and yeast communities during fermentation of various types of kimchi. Although the total bacterial and archaeal rRNA gene copy numbers increased during kimchi fermentation, the number of yeasts was not significantly altered. In 1 ng of bulk DNA, the mean number of rRNA gene copies for all strains of bacteria was 5.45×10^6 which was 360 and 50 times greater than the number of gene copies for archaea and yeast, respectively. The total gene copy number for these three domains differed among the different types of kimchi; however, the relative ratios among them were similar. A higher number of rRNA gene copies for bacteria in the various types of kimchi suggest that bacteria might be principal duties in the kimchi fermentation process.

Abstract Category
30 Miscellaneous

PS.30.004 THERMOCOCCUS SPP. CAN GROW BY HYDROGENOGENIC FORMATE OXIDATION

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Formate and H_2 are in thermodynamic equilibrium ($\text{HCOO}^- + \text{H}_2\text{O} \rightarrow \text{HCO}_3^- + \text{H}_2$, $\Delta G^0 = +1.3 \text{ kJ/mol}$). Recently, growth on formate was reported for syntrophic associations of hydrogenogenic formate utilizers with methanogens (Dolfing et al., 2008, Appl. Environ. Microbiol. 74:6126). Then, growth of a single microorganism, *Thermococcus onnurineus*, at the

expense of H₂ production from formate was demonstrated (Y.J. Kim et al., manuscript in preparation). The aim of this work was to test the occurrence of this capacity in other thermophilic microorganisms.

For cultivation a marine medium supplemented with 2 g/l of sodium formate and 0.5 g/l of yeast extract was used. Microbial growth was monitored by direct cell count. H₂, CO₂ and formate were determined by GC and HPLC.

14 *Thermococcus* strains were tested. *Thermococcus gammatolerans* type strain, *T. barophilus* Ch5 (Mid-Atlantic Ridge isolate) and *Thermococcus* sp. DS-1 (East Pacific Rise) but not other thermococci tested were found to be able to grow on formate producing H₂ and CO₂. The results, in case of thermococci with sequenced genomes, were in agreement with the presence/absence of formate hydrogen lyase gene clusters that include formate transporter genes. From samples of hydrothermal chimneys of Lau Basin, eight formate consuming, hydrogen producing enrichments apparently dominated by thermococci were obtained in medium with pH 3.5 or 5.5 at 75 - 92°C, and strain *Thermococcus* 434-R3-1 was isolated. Thus, growth at the expense of hydrogenogenesis on formate proved to be a capacity spread among diverse thermococci inhabiting deep-sea hydrothermal environments all over the world.

Abstract Category
30 Miscellaneous

PS.30.005 ARSENIC AND COPPER HYPERTOLERANT MICROORGANISMS FROM MINE HEAP AND THEIR ROLE IN As PRECIPITATION

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Arsenic speciation is affected by the microbial metabolism that participates in the biogeochemical cycle of the As. Previous works demonstrated that acidophilic (pH <1) and moderate thermophilic (48°C) microorganisms isolated from samples taken in mine heaps (up to 66 m deep), can grow in a culture media with As(III) and Cu in concentration up to 11.7 mM (879 mg/L) and 37.32 mM (2,370 mg/L), respectively; the soluble As and Cu did not affect the live biomass suggesting ongoing activity of the surviving microorganisms. (Torres-Cázares *et al.* 2008, ISME-12). Our recent findings showed that such microorganisms growth under saturated conditions with 117 mM (8790 mg/L) of As and 373 mM (23,700 mg/L) Cu and may precipitate *circa* 57% of the soluble As(III). Thus, it is possible to biostabilize the soluble As(III) by means of As(III) biooxidation and A(V) precipitation as ferric arsenate, FeAsO₄. Thus, the survival of chemolithotrophic microorganisms under As exposure has practical applications in mine bioleaching and for bioremediation tasks. These As and Cu hypertolerant community determined by 16S rRNA gene sequence analysis, was mainly composed by the chemoorganotrophic bacterium *Acidobacterium* sp., the sulfuroxidizers *Sulfobacillus thermosulfidooxidans* and *Sulfobacillus thermotolerans*, the ferrooxidizer *Leptospirillum ferriphilum*, and *Leptospirillum* sp. It is well known that these microorganisms present the ars operon, whose genes are involved in arsenic tolerance. Remarkably, we identified other species in the same samples, which could

be exploited to implement other environmental solutions, e.g.: phosphorous removal by *Acidovorax temperans*; polyethylene degradation by *Brevibacillus borstelensis*; phenol degradation by *Ochromonas danica*; or dibenzo-p-dioxins degradation by *Sphingomonas* sp.

Abstract Category
30 Miscellaneous

PS.30.006 USING DILUTION AND MICROPIPETTE TECHNIQUE TO ISOLATE OIL PRODUCING ALGAE

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Oleaginous algae has a cellular lipids content of over 20% (w/w), making it potential feedstock for biodiesel production during the energy crisis this days. Taiwan is located in the subtropical hemisphere with long day light for photo-synthesis, which is an optimum environment for algal growth. This study investigates and isolates oleaginous algae from freshwater for biofuel production. Water samples (100ml) collected from a fountain were filtered with a membrane filter and enriched with BG11 and 3N-BBM media for 3 weeks. The algal enriched water samples were diluted and the micropipette technique was used to isolate single algal cells. The single algal cells were cultured. Seventeen isolates were successfully isolated from five water samples. From the Nile red staining results, ten isolates were preliminarily screened for high oil content algae. These isolates were identified according to their morphology as *Kirchneriella* sp., *Botryococcus* sp., *Monoraphidium* sp., *Chlorella* sp., *Scenedesmus* sp., *Coelastrum* sp. and *Golenkinia* sp. Among these isolates some species were reported as the oleaginous algae. The growth and oil production characteristics of these isolates will be investigated further.

Abstract Category
30 Miscellaneous

PS.30.007 INTRASPECIES PHYLOGENY OF ANTIBIOTIC-PRODUCING RHODOCOCCUS ERYTHROPOLIS STRAINS EVALUATED BY MULTILOCUS SEQUENCE ANALYSIS AND MALDI-MS ANALYSIS

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In the last decade or two, *Rhodococcus* strains, a member of *Actinobacteria*, has been studied mainly for their remarkable ability to degrade persistent chemicals such as PCBs and dioxins, and also their plant- or mammal-pathogenic aspects. In the past few years, antimicrobial activity of some *Rhodococcus* strains has been discovered and studied. These findings indicated the genus *Rhodococcus* is a good resource for new antibiotics and its biosynthetic genes. Our recent study demonstrated that *R. erythropolis* contained 15 antibiotic-producing strains and they were classified into three groups (Groups I, II, III) according to their antibiotic spectrum and growth compatibility. Antibiotic compounds of them were partially purified and it was found that each

of the group produces distinct antibiotics. Most interestingly, each group inhibited the other two groups' growth severely. However, the 16S rRNA gene sequences of these 15 strains were identical, so their taxonomic relationship could not be elucidated. A new characterization method is therefore needed to specify and discriminate these strains with different antibiotic activities.

In order to classify these close relatives, multilocus sequence analysis (MLSA) and ribosomal protein mass analysis by MALDI-MS were performed and compared its resolving power. Partial nucleotide sequence of five housekeeping genes, *gyrB*, *rpoB*, *recA*, *trpB*, and *atpD* were determined and used for MLSA, and 30 ribosomal protein masses obtained by MALDI-MS were selected for the classification.

Both analyses classified the 15 strains finely, and the result matched to the antibiotic grouping. The phylogenetic trees based on them showed highly similar topology and also showed comparable resolving power. These results indicated that *R. erythropolis* has wide intraspecies diversity in a small phylogenetic breadth and also indicated that MALDI-MS analysis can be powerful and reliable tool for identification and phylogenetic classification of bacteria.

Abstract Category
30 Miscellaneous

PS.30.008 STUDY ON FED-BATCH FERMENTATION KINETICS OF COMPOUND FLOCCULANTS-PRODUCING BACTERIA

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For optimizing the fermentation process of compound flocculants-producing bacteria F+, their growth characteristics were investigated and the fermentation kinetics was analyzed. The characteristics of the compound flocculants-producing bacteria F+ were studied and their influencing factors were investigated. Based on the equations of "Logistic equation" and "Luedeking-Piret equation", the kinetic models reflecting their fermentation features of strain growth, substrate consumption and product generation were constructed respectively. The batch fermentation kinetics model parameter was obtained. The good agreement of predicted values with the experimental results showed that the kinetic models were applicable for predicting the actual fermentation process.

Abstract Category
30 Miscellaneous

PS.30.009 INFLUENCE OF GROWTH ENVIRONMENT ON TOLERANCE TO UV-B RADIATION AND MICROENCAPSULATED OF THE FUNGUS BEAUVERIA BASSIANA

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The development of biopesticide as environmental friendly alternatives to chemical pesticides for pest control has generated a considerable amount of interest. In this study in the ultraviolet experiment, the artificial illumination of utilizing different time separately. Such as salicylates, cinnamates and benzophenones also have these properties. These kinds of ultraviolet absorbents which is used the industry of cosmetics are added in this study, shown that with 0.2% (w/v) have good performance of absorbing to UV-B, and same result by added the sunflower oil. Using such as materials, the cultivation state experimental result show after shining 2 hours, it is better performance to UV-B radiation.

In spray-drier experiments, various polymers such as HPMC, chitosan, dextrin, skimmed milk, and PVP K90 were examined as encapsulation matrices. Different inlet/outlet temperatures, compositions of the carrier systems, and conidia concentrations were examined with respect to spore viability. Moreover, the production characteristics such as particle size distribution, entrapment efficiency, and shape were investigated. The best encapsulated products were obtained by using a matrix composed of 10% dextrin, 10% skimmed milk, and 5% PVP K90 as the coating material. The conidia exhibited 80% viability even after storage for 6 months. The mean particle size was in the range of 2.5-10 μm . The microspheres resulted in a product yield of approximately 60-80%, while that with dextrin only as coating material was 80 ± 3 . This study, using low temperature spray drier to encapsulate fungus conidia, In order to prolong the *Beauveria bassiana* activity and improve their shelf life.

Abstract Category
30 Miscellaneous

PS.30.010 MICROBIAL PHOSPHATASE ACTIVITY INVOLVED IN SUBSURFACE URANIUM SEQUESTRATION

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Due to the widespread contamination of soils and groundwater, in situ sequestration of heavy metals and radionuclides has proven to be a cost-effective strategy for remediation. We are currently investigating a remediation approach that utilizes the phosphatase activity of bacterial strains extant within the contaminated soils of the DOE Field Research Center in Oak Ridge, TN, for in situ contaminant sequestration. To determine the microbial community response to exogenous organophosphate, soil slurry and flow-through reactor experiments were conducted at pH 5.5 and 7.0 with U(VI) and NO_3^- contaminated soils under oxic and anoxic growth conditions. Soil slurry and flow-through reactor experiments were conducted for up to 80 days at 25°C with 10 mM G2P and 15 mM NO_3^- as the sole C, P and N sources, respectively. Under oxic growth conditions, greater than 4 mM soluble PO_4^{3-} was measured at the end of the slurry incubations and NO_2^- was not detected while anoxic soil slurry incubations indicated an accumulation of greater than 1 mM PO_4^{3-} . Following triplicate incubations, 16S rDNA diversity of slurries were analyzed via high-density 16S oligonucleotide microarray (PhyloChip). Preliminary data suggests that under oxic conditions, the microbial community structure is enriched in proteobacterial taxa at low pH when compared to the diversity of unamended soils.

Analyses of slurries incubated under anoxic conditions are underway to identify bacterial taxa capable of organophosphate hydrolysis in both oxic and anoxic environments. Flow-through reactor studies of soils with an initial pH of 3.7 demonstrated robust microbial activities once a pore water pH of 5.5 was achieved. Both denitrification and organophosphate hydrolysis were detected within 2 days of pH adjustment. Our soil slurry and reactor studies demonstrate the potential efficacy of phosphate-mediated sequestration of U(VI) by the extant microbial community present in contaminated subsurface soils

Abstract Category
30 Miscellaneous

PS.30.011 TEMPERATURE, ORGANIC MATTER, AND THE CONTROL OF BACTERIOPLANKTON IN THE NEUSE RIVER AND PAMLICO SOUND ESTUARINE SYSTEM (NC, USA)

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Estuaries are some of the most fertile and ecologically significant aquatic ecosystems on Earth and yet are also among the most anthropogenically stressed, making an understanding of microbial processes and carbon cycling in these systems critically important in the face of regional and global change. A four-year, spatially extensive study of the Neuse River-Pamlico Sound estuarine system, North Carolina, was used to evaluate the temporal patterns and bottom-up controls of the resident bacterioplankton community. This meso- to eutrophic estuarine system had high concentrations of dissolved and particulate organic matter and supported an active and abundant phytoplankton community. Temporal analysis of bacterioplankton productivity (BP), measured by leucine uptake, revealed a strong seasonal pattern, similar to water temperature. Comparisons of BP and several environmental variables grouped by season and year showed little correspondence between bacteria and either river discharge or organic matter at either scale. Temperature explained about half of the variation in BP data, but without any obvious threshold in the semi-log temperature-productivity relationship, implying the absence of a shift to substrate limitation at higher temperatures. The temperature effect varied spatially and was lower at the freshwater station, suggesting differential substrate availability or the presence of a resident microbial community different from the brackish stations. Proxies for bacterial substrates, including DOC, PON, and chlorophyll *a*, improved the predictive power in multiple regression models, but at least one third of the variation in BP remained unexplained. The regression models differed by station group further highlighting the possible difference in biota and/or resources between the freshwater and brackish sites.

Abstract Category
30 Miscellaneous

PS.30.012 INFLUENCE OF HYDROLOGICAL CONDITIONS ON THE *ESCHERICHIA COLI* POPULATION STRUCTURE IN CREEK WATER ON A RURAL WATERSHED

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Escherichia coli is a commensal bacterium of the gastro-intestinal tract of human and vertebrate animals, used as a bacterial indicator of fecal contamination of water. The aim of this study was to investigate the effect of hydrologic conditions on the structure of *E. coli* population in creek water on a small rural watershed.

Structure of *E. coli* population was characterized by the distribution in four main phylo-groups A, B1, B2, D which is related to the host species, its diet or geographic origin. Samples were collected from the creek water in four contrasted hydrologic conditions (i.e. wet period, dry period, rain during dry period and wet period) in a well characterized watershed.

The results showed that both concentration and structure of the population of *E. coli* depended on the hydrologic conditions and the land use of the watershed. After a rainfall, the increase of fecal contamination related to an input of human and animal contamination, was accompanied by a modification of the distribution of phylo-groups within the *E. coli* population, with change in the ratio A to B1 phylo-groups. In this watershed, *E. coli* B1 strains were the main phylo-group isolated in water. Diversity of 112 *E. coli* B1 strains isolated in water was investigated based on *uidA* alleles (600 pb), presence of *hly* gene, O-type and antibiotic resistance. Among the forty epidemiological types (ETs) thus identified, five *E. coli* B1 ETs would be more abundant in slightly contaminated water.

This study showed that the structure of *E. coli* population in environmental water is not constant, but instead varies on the basis of hydrologic conditions, current land use of watershed, and fecal bacteria contamination level.

Abstract Category
30 Miscellaneous

PS.30.013 PAENIBACILLUS RIGUI SP. NOV., ISOLATED FROM WETLAND FRESHWATER

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A rod-shaped endospore-forming Gram-variable bacterium, strain WPCB173T, was isolated from fresh water sample collected from Woopo wetland, Korea. The isolate was identified as a member of the genus *Paenibacillus* on the basis of phenotypic characteristics and phylogenetic inference based on 16S rRNA gene sequence. The major polar lipids are phosphatidylethanolamine and unidentified phospholipids. The diamino acid found in the cell wall peptidoglycan is meso-diaminopimelic acid. The predominant menaquinone is MK-7. The major cellular fatty acid is anteiso-C15:0 (65.2 %). The DNA G+C content is 48.3 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain WPCB173T falls within the radiation of a cluster comprising the *Paenibacillus* species; its closest neighbours were *Paenibacillus chinjuensis* WN9T (96.7 %) and *Paenibacillus soli* DCY03T (96.4 %). Genomic DNA-DNA hybridization of strain WPCB173T with of *Paenibacillus soli* DCY03T and

Paenibacillus chinjuensis WN9T showed relatedness of only 10 and 19 %, respectively. On the basis of phenotypic and molecular genetic evidence, strain WPCB173T represents a novel species of the genus *Paenibacillus*, for which the name *Paenibacillus rigui* sp. nov., is proposed. The type strain of the new species is strain WPCB173T (= KCTC 13282T = JCM 16352T).

Abstract Category
30 Miscellaneous

PS.30.014 INHIBITORY EFFECTS OF CONDENSED TANNINS ON SULFATE-REDUCING BACTERIA POPULATIONS AND HYDROGEN SULFIDE PRODUCTION FROM SWINE MANURE

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Odoriferous compounds and emissions associated with consolidated storage of swine manure are produced as a result of anaerobic microbial digestion of materials present in the manure. Hydrogen sulfide (H₂S) is one such offensive and toxic odorant that can reach hazardous levels during manure storage and handling. Production of H₂S involves sulfate reduction largely by anaerobic sulfate-reducing bacteria (SRB). The objective of this study was to test the effects of applying condensed tannins to swine manure in an effort to reduce odors and emissions and target bacterial groups responsible for odor production. Fresh swine manure was diluted in anaerobic buffer to a 10 % final solution, then treated with condensed tannins ranging in concentration from 0.1-1% w/v and incubated anaerobically at room temperature. H₂S production was measured and aliquots of manure were removed periodically for enumeration of the bacterial populations. Treatment of swine manure with tannins resulted in a decrease in H₂S production, with the highest treatment dose of 1% w/v tannins inhibiting 99% of H₂S production, compared to the control. A quantitative PCR (qPCR) approach was used to target and quantify the SRB population in manure, in response to treatment with tannins. This assay targeted the conserved *dsrA* gene of SRB, which encodes a key enzyme involved in sulfate respiration and production of H₂S. Addition of tannins reduced the SRB population in stored swine manure by 95% after 7 days of treatment, and this reduction was maintained throughout the course of the study. Specific SRB groups were targeted for enumeration and data suggest that *Desulfobulbus*-like SRB were most significantly affected by condensed tannin treatment. Total bacteria, as quantified by 16S rDNA-targeted qPCR, were also significantly reduced by tannin treatment. This study demonstrates that condensed tannins are effective at reducing both H₂S production and bacterial populations in the manure.

Abstract Category
30 Miscellaneous

PS.30.015 PRODUCTION OF DEGRADABLE POTS FROM BACTERIAL CELLULOSE

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The production of degradable pots from bacterial cellulose for plant seedlings to replace the plastic pot which is a cause of global warming was investigated. Primarily, bacterial cellulose was produced as the main raw material for degradable pot production from *Acetobacter xylinum* in different media from low cost raw substrates such as whey, molasses and ripe banana. The optimum conditions for maximum yield of cellulose production from whey was pH 4.5, sugar 7 Brix; from molasses pH 4.0, sugar 7 Brix and from ripe banana pH 4.3, sugar 8 Brix incubated at room temperature for 20 days. It was found that *Acetobacter xylinum* in whey produced the thickest cellulose of 38 mm and 1,080 g wet weight. Cellulose from ripe banana was 25.3 mm thick with 1,324.8 g. wet weight and that from molasses was 8.6 mm thick with 335 g. wet weight. The degradable pots were prepared by mixing bacterial cellulose, starch, bee wax and benzoic acid before moulding the pot was water resistance, stability and resistance of mold. Degradable pots were investigated biodegradation by soil burial experiments for 2 months.

Abstract Category
30 Miscellaneous

PS.30.016 EFFECT OF DISSOLVED OXYGEN ON *DESULFOVIBRIO VULGARIS* BIOFILM AND PLANKTONIC CELLS

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D vulgaris Hildenborough, although an obligate anaerobe, is capable of surviving in environments that are exposed to mM levels of O₂. *D vulgaris* is capable of forming biofilms when grown anaerobically, and SRBs in natural environments such as subsurface sediments are more readily exposed to low concentrations of dissolved oxygen (DO) rather than air. In this study, lactate and pyruvate grown planktonic and biofilm cells were exposed to different concentrations of DO to elucidate different physiological responses. Experiments were carried out with lactate and pyruvate as substrates. Both planktonic and biofilm cells were washed anoxically to lower sulfide levels upon re-inoculation in the presence of DO, and sulfide levels normalized to protein were similar between planktonic and biofilm cells. Lactate-grown biofilm cells were non-viable when exposed to 1.5 ppm DO, but planktonic cells tolerated up to 1.9 ppm DO. Even at DO levels that inhibited growth, viability did not significantly decline until 1 week post-inoculation. Regardless of DO concentration, biofilm cells exhibited a lag phase when re-suspended in the planktonic phase. Pyruvate-grown cells displayed similar tolerance as lactate to DO when transferred from planktonic or biofilm mode, but had 2 to 3h lag in both physiological states. Both planktonic and biofilms cells had increased growth rates when DO was increased from 0.3 to 0.8 ppm DO in the presence of lactate but not pyruvate. The results indicated that pyruvate-grown planktonic and biofilm cells were more sensitive to DO exposure compared to lactate-grown cells.

Abstract Category
30 Miscellaneous

PS.30.017 *EX SITU* CONSERVATION AND MOLECULAR GENETIC TYPIFICATION OF WILD FILAMENTOUS FUNGI IN THE STATE OF MICHOACAN, MEXICO

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Vegetative mycelia of wild filamentous fungi from natural areas can be long-term conserved *ex situ* through different techniques without loss of viability. Collections of mycelia are a strategy for conservation of physiological and genetic diversity of filamentous fungi. Local and regional collections of wild fungi strains can be tools for developing biotechnological applications. The present work aims at isolating strains of wild filamentous fungi from the state of Michoacan, Mexico, evaluating the survival of strains preserved by *ex situ* conservation techniques, and making a molecular genetic characterization of the obtained mycelia. Samples were taken from different natural areas of Michoacan, inoculated in potato dextrose agar and incubated at 28 °C. 150 vegetative mycelia were isolated and the nuclear ribosomal unit ITS region were sequenced in 61 isolates. Identities resulting from BLAST alignments with sequences in the Genbank included: 10 strains at the species level including *Didymella bryoniae*, *Fusarium sambuanum*, *Fusarium incarnatum*, *Neurospora sitophila*, *Glonium pusillum*, *Pestalotiopsis cyptomerae*, *Umbelopsis ramaniana*, *Infundibulicybe gibba*, *Trametes versicolor* and *Pleurotus djamour*; 48 strains at the genus level, of which 48% were related with *Hypocrea* and its anamorphs within *Trichoderma*, 20% to *Fusarium* and *Gibberella*, and 10% with *Pestalotiopsis*, the remaining with *Bionectria*, *Diaporthe*, *Phomopsis*, *Lasiodiplodia*, *Mucor*, *Rhizomucor* and *Cerebella*, among other genera. Of the 150 isolates, survival after 33 months was observed in 82.9% of the strains preserved in distilled water at room temperature, and in 94.5% of strains after cryopreservation at -80 °C in 50% glycerol (v/v). The results show that our strain collection mainly contains wild mycelia of Ascomycota including some anamorphs, and only a few species of Zygomycota and Basidiomycota. The applied preservation methods ensure the long-term survival of most stored mycelia.

Abstract Category
30 Miscellaneous

PS.30.018 BIOLOGICAL CONTROL EFFECT ON ROOT-KNOT NEMATODE OF AMINO ACIDS EXTRACTED FROM CHICKEN FEATHER BY USING

***Chryseobacterium* sp. FBF-7**

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The amino acids such as arginine, glutamic acid and methionine have been approved for controlling the activities of juveniles and the hatching of eggs on root-knot nematode. In the present study, we investigated the characteristics of keratinolytic protease of chicken feather-degrading bacteria and control effect of keratinolytic amino acids (KAA) against *Meloidogyne incognita*. Thirty-one chicken feather-degrading bacteria were collected from chicken farm (waste feather, compost and wastewater). In the course of screening of keratinolytic protease

activity, *Chryseobacterium* sp. FBF-7 strain was showed 75% feather-degrading activity and 2.3×10^8 cells/ml of optimal growth on 20°C. The keratinolytic protease activity of *Chryseobacterium* sp. FBF-7 was 15~17 unit/ml/min and enzyme was stable at temperature range from 15 to 20°C. Therefore, enzyme was categorized as a psychrotrophic keratinolytic protease. For the control effect of root-knot nematodes, amino acid was extracted and analyzed. Total amino acid extracted from 2% of chicken feather meal by *Chryseobacterium* sp. FBF-7 was 1661.6 $\mu\text{mol/ml}$. Thirty-two percent of total amino acid was essential amino acids and major amino acids were valine, glutamic acid, aspartic acid, glycine and proline. Among the amino acids, which controlling the activities on root-knot nematodes were comprised arginine (41.5 $\mu\text{mol/ml}$), glutamic acid (182.2 $\mu\text{mol/ml}$) and methionine (16.7 $\mu\text{mol/ml}$). Effect of KAA on *Meloidogyne incognita* using 24well plate assay was examined, it showed 54-76% suppression of the egg hatching and 61-68% mortality of the juvenile activity. Based on these results, the KAA produced by *Chryseobacterium* sp. FBF-7 was expected prospective biological control products on root-knot nematode.

Abstract Category
30 Miscellaneous

**PS.30.019 BIOHYDROGEN PRODUCTION FROM CELLULOSE BY A
CLOSTRIDIUM CELLULOLYTICUM H10 AND *DESULFOVIBRIO VULGARIS*
HILDENBOROUGH CO-CULTURE**

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Hydrogen gas production via dark fermentation of cellulose has been investigated as a potential source of renewable energy. Some microorganisms, such as *Clostridium* species, are capable of both cellulose hydrolysis and H₂ production. However, both H₂ yield and cellulose degradation efficiency remains very low with *Clostridium* species. In this study, a co-culture of *Clostridium cellulolyticum* H10 and *Desulfovibrio vulgaris* Hildenborough was developed for efficient cellulose degradation and high-yield H₂ production from cellulose using a consolidated bio-processing strategy. The impacts of temperature, initial pH, cellulose concentration and yeast extract concentration on hydrogen gas production by the co-culture were studied. Under optimized conditions, cellulose degradation, H₂ production, volatile fatty acids and alcohols, and soluble sugars were monitored in the co-culture and both mono-cultures. The data showed that the co-culture performed better in terms of cellulose degradation and H₂ production (2.96 mol H₂ mol⁻¹ glucose) than the mono-culture (1.8 mol H₂ mol⁻¹ glucose) with 4 g cellulose L⁻¹ as the sole carbon source. Experiments were conducted with SEM and co-culture microarray analyses as well. SEM images suggest that the binding of cells to cellulose fibers is greater in the co-culture than in the *C. cellulolyticum* monoculture, suggesting that the addition of *D. vulgaris* may enhance contact or interactions between bacteria and cellulose and increase cellulose degradation rates. Microarray analysis also shows that *C. cellulolyticum* genes involved in cellulose degradation and hydrogen production were more highly expressed in co-culture than in monoculture. The results demonstrate that the developed co-culture system can efficiently degrade cellulose and produce H₂ at a high yield. Future work will focus on the further

understanding of mechanisms of cellulose degradation and H₂ production, and interactions within the co-culture.

Abstract Category

30 Miscellaneous

PS.31.001 ALIGNMENT OF TERMINAL RESTRICTION FRAGMENT LENGTH POLYMORPHISM (T-RFLP) PROFILES OF 16S RRNA USING A BAYESIAN MODEL-BASED METHOD

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Clustering is a popular method utilized in analyzing T-RFLP data that is used in studying the diversity of human microbial ecosystems (Zhou et al. 2007 for example). Clustering is used to identify microbial community kinds that share similar structure. Clustering T-RFLP data takes place in two steps: 1) similar operational taxonomic units (OTUs) between different samples are identified based on the similarity of associated fragment lengths (a step referred to as alignment or binning) and 2) different sample profiles are clustered based on the abundance of the identified OTUs in each sample. Both steps jointly affect the accuracy of the final clustering. In this poster I present a new, model based Bayesian alignment approach to inferring the microbial community composition of microbial ecosystems. I use this method with a model based clustering, T-BAPS (Tang et al. 2007) and compare it to a distance-based alignment and clustering method that we developed in early research work. I utilize simulated data in these comparisons. Preliminary results indicate that the model-based approach is superior to the distance-based one in recovering the true clustering structure of the data. This is due to the improved efficiency of the model-based approaches in utilizing the information available in the data.

Abstract Category

31 Methodological Developments

PS.31.002 NOVEL MICROFLUIDIC APPROACH FOR QUANTIFYING BACTERIAL CHEMOTACTIC TRANSPORT PARAMETERS IN WELL-DEFINED CHEMOATTRACTANT GRADIENTS

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Population-scale transport parameters (chemotactic sensitivity χ_0 and random motility μ) have been traditionally used to quantify bacterial chemotaxis and predict bacterial fate and transport in natural environment. Previously, transport parameters have been derived theoretically using probabilistic models incorporating microscale swimming behavior, yet the mechanistic foundations of this upscaling process have not been verified experimentally. Moreover, chemical gradients are poorly characterized in traditional chemotaxis assays which have been formerly used to quantify transport parameters.

We present novel microfluidic experiments to quantify bacterial transport parameters by designing a microfluidic device to generate and accurately measure gradients of chemoattractant (α -methylaspartate) while simultaneously capturing the swimming trajectories of individual *Escherichia coli* bacteria using videomicroscopy and cell tracking. By measuring swimming speed and bias in swimming direction of single cells for a range of chemoattractant

concentrations and concentration gradients, we directly computed the chemotactic velocity and the associated chemotactic sensitivity. We also used microfluidics to generate steady linear and non-linear steady chemoattractant gradients and probed the population-scale response of *E. coli* to quantify chemotactic transport parameters.

Measured values of both χ_0 [single-cell assay: $(12.4 \pm 2.0) \times 10^{-4} \text{ cm}^2 \text{ s}^{-1}$, population-scale assay: $(4.5 \pm 0.5) \times 10^{-4} \text{ cm}^2 \text{ s}^{-1}$] and μ [$(3.3-7.0) \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$] are comparable to literature results. The microscale approach to bacterial chemotaxis lends experimental support to theoretical derivations of population-scale transport parameters from single-cell behavior. The population-scale verification of bacterial response provides the first quantification of chemotaxis of free-swimming cells in steady nonlinear microfluidic gradients and opening the door to bacterial chemotaxis studies in gradients of arbitrary shape.

Abstract Category

31 Methodological Developments

PS.31.003 EFFECTS OF DNA EXTRACTION METHODS ON CULTURE INDEPENDENT MICROBIAL COMMUNITY ANALYSIS OF WASTE WATER TREATMENT PLANT SAMPLES

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One of the main drawbacks in microbial ecology is coming from the bias in the DNA isolation techniques. In this study, two different home-made DNA extraction methods (CTAB and phenol based Home-made-1 & lysozyme and ammonium acetate based Home-made-2) and three different commercial DNA extraction kits (C-1, C-2, C-3) were used to isolate total genomic DNA from anoxic, anaerobic and aerobic waste water treatment plant samples. The major goal was to determine the most effective isolation methodology which yields an adequate quality and quantity of DNA for downstream microbial community analysis methods. The DNAs obtained have been initially analyzed in terms of amount and purity. Home-made-1 was found as the method which yields the highest DNA concentration. Genomic DNAs served as a template for PCR-DGGE analysis of archaeal and bacterial communities for diversity analysis. DGGE profiles of the C-1 method housed up to 21 taxa for bacterial and 9 taxa for archaeal communities as the highest numbers reached. DGGE profiles were also subjected to the pairwise similarity comparisons via Neighborhood-based using Pearson correlation coefficient. In archaeal DGGE analysis, Home-made-2 and C-3 kit have shown similarities up to 95% with Home-made-1. However, in bacterial DGGE, Home-nade-1 and Home-made-2 have shown the highest similarity ratios among the other methods but this time, C-3 couldn't be as successful. As a result, C-2 has shown low similarities in all analyses and stayed behind the other extraction methods, in contrast, C-1 has been seen to be the most effective isolation method via its shown taxas from DGGE gel profiles. Obtaining different DNA profiles even from the same samples shows us that the DNA extraction methods used in the phylogenetic analysis are actually such important tools and can affect the results directly.

Abstract Category
31 Methodological Developments

PS.31.004 EVALUATION AND IMPLICATIONS OF PYRONOISE, A PROGRAM FOR ERROR CORRECTION IN 454 PYROSEQUENCE DATA

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2nd generation sequencing technologies such as 454 pyrosequencing offer the capacity for unprecedented depth of sampling of microbial communities. It is a challenge to maintain validated quality control standards in the face of a rapidly developing technology. Accumulative error in large datasets without the possibility for repeated measurement is particularly worrisome, as in the case of pyrosequencing of PCR amplicon libraries derived from environmental DNA. PyroNoise was recently reported (Quince et al. 2009 Nat. Meth.) as a novel strategy for correcting errors in pyrosequencing data, particularly errors in the length of homopolymeric runs and PCR point mutations.

We evaluated PyroNoise and its implications for biological interpretation using our own dataset.

Requirements for hardware and computing capabilities are likely to limit the adoption of PyroNoise. Comparison with other sequence processing methods was complicated by changes to accession numbers and because quality screening is based on raw flowgram signals rather than on derived quality scores. However, working from raw flowgrams is a strength of PyroNoise. A pyrosequencing + PCR error rate of 0.34% was implied based on the number of corrections made by PyroNoise. This is compatible with published estimates of pyrosequencing accuracy. However, the implied error rate was inversely related to the number of identical sequence reads; more corrections were made to rare sequence variants than to common variants. More base changes were more likely to occur at conserved than variable positions. Compared to standard methods, output from PyroNoise could be grouped into a comparable number of operational taxonomic units (OTUs) with a less stringent dissimilarity cutoff.

PyroNoise provides greater discriminatory power than the alternative approach of accounting for errors by means of overly broad grouping, and is a valuable quality control procedure for 454 pyrosequencing.

Abstract Category
31 Methodological Developments

PS.31.005 COMPARATIVE STUDY OF DNA EXTRACTION METHODS FROM EXTREME, LOW BIOMASS ENVIRONMENTS

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Microbial life in deep sediments and Earth's crust is now acknowledged by the scientific world. The deep subsurface biosphere contributes significantly to fundamental biogeochemical processes. However, despite great advances in geomicrobiological studies, deep terrestrial ecosystems are microbiologically poorly understood. A major obstacle for molecular approaches in such deep geological low biomass containing rocks has been DNA extractions. Therefore, the aim of this study was to compare DNA extraction methods and optimize a procedure adapted to extreme, low biomass environments.

Four Triassic rock samples have been collected at around 2000 meters in borehole drilled by French National Radioactive Waste Management Agency (ANDRA) in the eastern Paris basin. Each core was crushed in aseptic and anoxic conditions. A comparative study of direct DNA extraction methods, including commercially available procedures, was carried out. The quality of the environmental DNA extracts (crude and after dialysis) was verified by the ability to amplify a fragment of 16S rRNA and to obtain genetics fingerprints with DGGE (Denaturing Gel Gradient Electrophoresis) technique.

Although extracted DNA was undetectable, a polymerase chain reaction amplification using universal bacterial primers enabled positives results. Amplification results between crude and dialysed DNA extracts didn't show a significant efficiency difference for a same extraction method. However, in most cases, a high quality DGGE fingerprint was obtained with amplicons of dialysed DNA extracts. Similarities between fingerprints retrieved from the different DNA of the four Triassic rocks will be discussed.

This methodological study has never been carried out on deep rock samples and proved the necessity to optimize DNA extraction procedures for future investigations of extreme, low biomass ecosystems.

This work has been performed within the framework of the TAPSS2000 program and the GNR FORPRO II (CNRS-ANDRA).

Abstract Category

31 Methodological Developments

PS.31.006 THE RIBOSOMAL DATABASE PROJECT: TOOLS AND DATA FOR HIGH-THROUGHPUT rRNA ANALYSIS

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The ribosomal small subunit rRNA (16S) gene is used as the most important molecular marker for understanding the vast diversity of microorganisms. The Ribosomal Database Project (RDP; rdp.cme.msu.edu) offers aligned and annotated 16S rRNA sequence data and analysis services to the research community. These products are widely used in molecular phylogeny and evolutionary biology, microbial ecology, bacterial identification, characterizing microbial populations, and in understanding the diversity of life. Next Generation sequencing technologies such as 454 and Illumina are transforming the study of microbial diversity by offering greatly

enhanced sequencing breadth and depth. RDP's Pyrosequencing Pipeline provides an effective toolkit to automate the computationally-intensive processing and analysis of these large datasets. The Pipeline takes raw high-throughput reads through initial processing steps to hierarchical clustering into OTUs or taxonomic assignment using the RDP Classifier. Several common ecological metrics are calculated including Chao1, Shannon Index and rarefaction. Results are available in formats suitable for common statistical and ecological packages. Other RDP tools include the RDP Classifier, also available as an open-source package; Sequence Match for finding nearest neighbors; Library Compare for determining differentially represented taxa between two libraries; RDP Probe Match for determining taxonomic coverage of primers and probes; Tree Builder for rapid phylogenetic tree construction; and Browsers that provide entry to the public sequences. RDP is updated monthly and, as of March 2009, maintains 1.3M aligned and annotated quality-controlled rRNA sequences. There are, on average, 18,633 analysis sessions per month by 5,634 researchers using RDP. The myRDP features support over 2,500 active researchers to analyze over 4M pre-publication sequences.

Abstract Category

31 Methodological Developments

PS.31.007 THE RDP PIPELINE FOR GENE-TARGETED METAGENOMICS

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Gene-targeted metagenomics is an approach involving the use of PCR amplification of specific functional genes, followed by sequencing with high throughput technologies. Being gene-specific and deep in sequencing coverage, this approach offers not only extensive insight into microbial gene diversity, but holds promise to reveal functional, ecological and evolutionary patterns of the gene targets. RDP's Functional Gene Pipeline has been significantly enhanced to support gene-targeted metagenomics. This pipeline consists of a workflow of components to facilitate each step along the entire analysis process. Fungene Repository provides an updated database of gene homologs based on HMM/HMMER search of the GenBank nr database and includes both protein and DNA alignments. Integrated tools are provided for primer testing, phylogenetic analysis and model refinement. Several new tools have been developed to analyze high-throughput gene-targeted metagenomic data, including RDP FrameBot, which extends and implements an existing dynamic programming algorithm to detect and correct frameshift artifacts and filter out non-target reads, and RDP mcClust, which implements the memory-constrained hierarchical clustering algorithm for clustering large numbers of protein reads and includes a distributed computing option. This pipeline has been tested on several important functional genes including biphenyl dioxygenase (*bph*), important for bioremediation, nitrogenase reductase (*nifH*), a key component in nitrogen fixation, and butyryl-CoA transferase (*but*) and butyrate kinase (*buk*), important for production of butyrate, the main energy source of human colonic epithelial cells.

Abstract Category

31 Methodological Developments

PS.31.008 DEVELOPMENT OF GENETIC TOOLS IN *VERRUCOMICROBIUM SPINOSUM*

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Bacteria within the phylum *Verrucomicrobia* are phylogenetically and phenotypically diverse, and inhabit a wide variety of environments including soils, sediments, and marine habitats. Some members of *Verrucomicrobia* are found in association with eukaryotes that include both invertebrates (ciliates, nematodes, corals, sponges, tubeworms, termites, sea cucumbers) and vertebrates (rat, dugong, gorilla, human). Additionally, members of *Verrucomicrobia* have been shown to exhibit cellular compartmentalization previously found only within the bacterial phylum *Planctomycetes*. Despite their apparently broad distribution and intriguing cell biology, members of this phylum remain poorly characterized and their contributions to ecological and biogeochemical processes are largely unclear. Furthermore, the lack of genetic tools in culturable members of the phylum presents a significant hurdle. There are no published studies describing the transformation of exogenous DNA into any members of the *Verrucomicrobia*. Here we present a procedure for the transformation of a Tn5 transposon system into the type strain of *Verrucomicrobium spinosum* via electroporation. Additionally, we describe the first random-transposon mutant library in this organism. In this study we employed the EZ-Tn5 transposon system to isolate 101 transposon mutants. Mapping of several insertion sites has identified mutations in genes encoding a superoxide dismutase, a transcriptional regulator of the tetR family, and a Pkn10 serine/threonine kinase. To our knowledge this study represents the first description of the introduction of exogenous DNA into any species within the *Verrucomicrobia* and the first random mutant library in the species *V. spinosum*. Further work is needed to determine whether the method described here is more broadly applicable to other members of the *Verrucomicrobia*.

Abstract Category

31 Methodological Developments

PS.31.009 UCLUST: FAST CLUSTERING, SEARCH AND ALIGNMENT FOR HUGE SETS OF SEQUENCES

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Biological sequence data is accumulating faster than the computational resources needed to analyze them. High-through-put methods are required to process the exponentially growing datasets found in environmental sequencing, metagenomic projects, public sequence repositories and other contexts. Common steps in sequence analysis of large datasets include clustering, alignment and assignment to known databases in order to reduce redundancy, quantify diversity and obtain subsets tractable for more computationally expensive methods used in downstream analysis. UCLUST is a new algorithm that enables computational classification of huge sets of sequences. UCLUST enables generation of high-quality clusters and alignments at rates of hundreds of thousands of sequences per minute on a current laptop computer. A test dataset with one hundred million 454 reads of 16S rRNA genes was clustered and aligned in a few hours

using less than 50 Mb memory. UCLUST can classify 16S reads against a reference database with high accuracy at speeds more than 100x faster than MEGABLAST. Compared to CD-HIT, a widely-used high-throughput clustering program, UCLUST is substantially faster, uses less memory, produces clusters of higher quality, enables clustering at lower identities, and enables clustering of much larger datasets. UCLUST is freely available for academic use from the author's web site at <http://www.drive5.com/uclust>.

Abstract Category

31 Methodological Developments

PS.31.010 CLASSIFICATION OF EXTREMOPHILIC BACTERIA USING FOURIER TRANSFORM INFRARED SPECTROSCOPY- ATTENUATED TOTAL REFLECTANCE

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For the large-scale classification of microbial communities, Fourier Transform Infrared spectroscopy is more discriminative than morphology, and faster and less expensive than 16S RNA sequencing. In contrast to absorption infrared spectroscopy, attenuated total reflectance characterizes surface features and hence is less sensitive to differences in growth conditions. The aim of this research is to develop an attenuated total reflectance method for the classification of alkaliphilic bacteria in the Nebraska Sandhills lakes.

Spectra of axenic cultures of classified bacteria were collected over the mid-infrared region using a Bruker Equinox 55 and Pike single bounce attenuated total reflectance cell. We trained Bayesian and support vector machine classifiers and tested them in cross-validation experiments for classification accuracy.

Spectra from both nylon and PVDF filters were required to obtain sufficient spectral information. Storing these filters in a desiccant vial under a nitrogen blanket preserved the integrity of the sample for at least 13 days. Spectral differences induced by small differences in growth media were significantly less for our spectra than for spectra obtained by using absorption spectroscopy methods. At this time, the small number of spectra we have collected from isolates known to be members of separate Phyla support our aim. We are currently collecting the required spectra necessary to validate our method.

Preliminary results show that our method accurately classifies bacteria at the Phylum level. We expect that this method will be capable of classifying bacteria to species if not strain. Because spectra are less sensitive to sample preparation as compared to absorbance infrared methods, less error in classification will be introduced into a library of spectra collected between different laboratories or over long periods of time.

Abstract Category

31 Methodological Developments

PS.31.011 SELECTIVE AND HIGHLY EFFICIENT ENRICHMENT OF UNCULTURED *NITROSPIRA*

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Nitrospira species are numerically dominant and carry out nitrite oxidation in both engineered system and natural environment. Nevertheless, the knowledge of *Nitrospira* species is limited because the genus *Nitrospira* contains only three described and cultured species. The cultivation and isolation of various phylotype are still required for more comprehensive understanding of physiological properties. In this study, a new approach to obtain highly enriched culture of *Nitrospira* was conducted, that is a combination of 1) cultivation of *Nitrospira* using continuous feeding reactor, and 2) separation of *Nitrospira* cells using the "cell sorting system". The *Nitrospira* species were enriched by continuous feeding of nitrite as substrate. The activated sludge in the sewage wastewater treatment plant was used as primary inoculum. Nonwoven fabric material was used as carrier for maintaining sufficient amount of *Nitrospira* cells in the reactor, while high flux rate of the feeding substrate (hydraulic retention time of 3h) was kept during the cultivation. During the enrichment procedure, the abundance of the *Nitrospira*-like bacteria gradually increased up to over 80% of the total bacterial population. *Nitrospira*-like bacteria in activated sludge are known to form relatively dense and strong microcolonies, whereas other microbes are easily dispersed by simple sonication to be planktonic cells. This indicates that *Nitrospira* microcolonies-containing enrichment samples can be separated from planktonic microbial cells (mainly heterotrophic bacteria) by using a flow cytometric cell sorting system because the size of the microcolony is 5-10 μm which is easily separated from the single cell by flow cytometry. As a result of applying to enriched sample described above, the ratio of *Nitrospira* to total bacteria was increased more than 99%. The above approaches to cultivate and separate *Nitrospira* can also be applied to other uncultured microorganisms.

Abstract Category

31 Methodological Developments

PS.31.012 CHAPERONIN GENES RECONSTRUCT THE ARCHAEL PHYLOGENY FROM FOUR NEUTRAL HOT SPRINGS

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Background and aims: Chaperonin (alpha and beta) subunits genes are known to confer thermotolerance and are also broadly distributed in *Archaea*. Thus this gene was selected as a phylogenetic marker to assess the archaeal diversity from filaments growing in four neutral hot springs at different temperatures. Methods: Filaments were collected from four Icelandic hot springs and its environmental DNA was extracted. 16S rRNA genes were amplified by PCR using primers specific for the *Archaea* domain and the PCR products were subsequently cloned and sequenced. Sequences were compared to available databases by use of Blast and aligned in ARB. For the amplification of the chaperonin genes a set of sequences from different archaeal species were aligned and two degenerate primers were designed. These primers amplified an 800

bp fragment which was cloned to construct a library and sequenced. Results: 16S rRNA gene diversity analyses identified members of the *Crenarchaeota* group which belonged to *Desulfurococcales*, *Thermoproteales*, the candidate division *Korarchaeota* or were closest related to sequences from uncultured *Archaea*. On the other hand, despite the paralogy observed in the chaperonin phylogeny, cultivated representatives from the main groups detected in the 16S rRNA gene analysis could be unequivocally assigned to either the alpha or beta subunit in its phylogenetic tree. Both markers revealed that the sequences retrieved from the different hot springs were grouped according to their taxonomic affiliation. Future experiments will be conducted in order to quantify the chaperonin gene content in each sample analyzed. Conclusion: The main taxonomic affiliations were observed with both the 16S rRNA and chaperonin phylogenetic analyses indicating that the chaperonin gene can be used as a novel genetic marker to detect these species in this extreme environment. Acknowledgments: This work was supported by the grant CGL2009-10756 from the MICINN (Spain).

Abstract Category

31 Methodological Developments

PS.31.013 SEQUENCE-SPECIFIC BACTERIAL GROWTH INHIBITION USING ANTISENSE PEPTIDE NUCLEIC ACID TARGETED TO RRNA

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Antisense peptide nucleic acid (PNA) oligomers, which targeted to the mRNA of essential genes, could inhibit cell growth with sequence specificity. rRNA is also one of the promising target molecules of antisense oligomers for bacterial growth inhibition. In previous studies PNA targeted to the functional domains of 23S rRNA can inhibit translation and cell growth. However, effective inhibition of translation and cell growth using 16S rRNA-targeted PNA has still not been achieved. Here, we report that PNA targeted to the functional site of 16S rRNA could inhibit both gene expressions in vitro and bacterial growth in pure culture with sequence specificity.

We designed 10- to 15-mer PNAs conjugated with a cell-penetrating peptide, which targeting several highly conserved regions of 16S rRNA, and then estimated whether these PNA oligomers actually have an inhibitory effect by using in vitro translation assay (in a cell-free translation system). In this assay, a peptide-PNA oligomer (PNA-mRBS) that targeted the mRNA binding site at the 3' end of 16S rRNA clearly showed an antiribosomal effect. Using 0.6 μ M of the PNA-mRBS, cell-free β -galactosidase production decreased by 50%, whereas PNA-mRBS with 1 or 2 mismatches to the target sequence showed much weaker inhibition effects. To determine the growth inhibition and bactericidal effects of the PNA-mRBS, we performed OD measurement and viable cell counting. We observed dose- and sequence-dependent inhibition of cell growth and bactericidal effects. These growth inhibitory effects are observed both in the Gram-negative bacterium of *Escherichia coli* and the Gram-positive bacteria *Bacillus subtilis* and *Corynebacterium efficiens*, although inhibitory concentrations were different for each bacterial species. These results present possibilities for 16S rRNA sequence-based specific bacterial growth inhibition using a peptide-PNA conjugate.

Abstract Category

31 Methodological Developments

PS.31.014 A QUEST FOR HIGH-THROUGHPUT DEREPLICATION OF UNIDENTIFIED MICROBIAL DIVERSITY

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Dereplication is a rather ambiguous term, originally referring to the grouping of isolates at strain level, although now generally used for the grouping of isolates at any taxonomic level in the first steps of an identification strategy. MALDI-TOF MS has high taxonomic resolution, high speed and simplicity, making it an interesting tool for dereplication. Here, the applicability of MALDI-TOF MS for dereplication of an unidentified microbial diversity was assessed through comparison with rep-PCR, widely applied for dereplication at subspecies-to-strain level.

Approximately 400 isolates were obtained from the rhizosphere of potato plants from the Central Andean Highlands. MALDI-TOF MS was performed on 4800 Plus MALDI TOF/TOF™ Analyzer (Applied Biosystems). Rep-PCR fingerprints were generated using the (GTG)₅-primers. Data generated was analyzed using BioNumerics 5.1 (Applied Maths), with Pearson correlation coefficient.

First, 18 isolates were selected arbitrarily for an optimization study of MALDI-TOF MS spectra. Incubation on 10% TSA for 48h at 28°C and cell extracts for sample preparation were chosen as standardized conditions. MALDI-TOF MS spectra from approximately 400 unidentified bacterial isolates were generated using the CHCA matrix. Also, rep-PCR fingerprints of alkaline lyses of all isolates were obtained. Reproducibility for both methods was determined by analyzing 10% of the isolates in triplicate. Closely related clusters were delineated using results for reproducibility, cophenetic correlation analysis and cluster cut-off analysis. All members of arbitrarily chosen clusters were identified through 16S rRNA gene sequence analysis for verification of cluster delineation.

This study is the first to evaluate the applicability of MALDI-TOF MS for dereplication of an unidentified microbial diversity, through comparison with rep-PCR. This work was carried out in the framework of the VALORAM funded under 7th FWP.

Abstract Category

31 Methodological Developments

PS.31.015 CHALLENGING "THE GREAT PLATE COUNT ANOMALY": ISOLATION OF METHANOTROPHIC BACTERIA

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Methanotrophic bacteria are ubiquitous and play a vital role in reducing emissions of the greenhouse gas methane. The isolation of these fastidious organisms into pure culture has proven difficult and laborious. The majority are so far not-yet-cultured. Therefore, next to traditional

agar-plating, non-conventional and/or miniaturized high throughput cultivation techniques have been developed recently. This study compares a miniaturized high-throughput dilution-to-extinction culturing with plating for the retrieval of novel methanotrophic bacteria.

Samples were taken from a landfill, compost soil, aerobic digester and wetland soil. Methane oxidation activity was measured with gas chromatography. Bacteria were enumerated with MPN technique; isolation was done by plating (gellan gum as solidifying agent) and miniaturized dilution-to-extinction culturing. Liquid non-visible growth was determined through ATP measurements. Strains were screened via microscopy and gene sequence analysis.

Initially, an activity-abundance sample screening was performed on four different environmental samples. One sample was chosen as inoculum for an elaborate isolation campaign using four variations of a diluted mineral medium (with ammonium or nitrate and different copper concentration). Plating, MPN and miniaturized dilution-to-extinction procedure were performed in parallel. The incubation was at 20°C with extended incubation times. Isolates thus obtained were purified and confirmed as methanotrophs through GC analysis and high-throughput DNA extraction and PCR screening. Phylogenetic analysis of 16S rRNA gene and *mmoX* and *pmoA* genes was used to compare culturable diversity from different media and culturing techniques. **CONCLUSIONS.** A miniaturized, high-throughput dilution-to-extinction protocol for methanotrophic bacteria was evaluated. This procedure can be readily applied to other interesting environmental samples, either anthropogenic or natural.

Abstract Category

31 Methodological Developments

PS.31.016 CPNDB: A CHAPERONIN SEQUENCE DATABASE

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Chaperonins are molecular chaperones present in virtually all prokaryotes and eukaryotes. Sequences encoding the 60 kDa chaperonin (CPN60, type I chaperonin, also known as GroEL or HSP60), are particularly useful for phylogenetic studies and as targets for detection and identification of organisms since a 549—567 bp segment of the *cpn60* gene can be amplified with universal PCR primers. Chaperonin sequences provide more discriminating power for closely related species than *16S rRNA* gene sequences. cpnDB (<http://cpndb.cbr.nrc.ca>) is a curated database of chaperonin sequences collected from public databases or generated by researchers exploiting the *cpn60* target in clinical, phylogenetic, and microbial ecology studies. Since its inception, cpnDB has grown to over 14,000 records representing bacterial reference strains, clinical isolates, field isolates and uncultured organisms detected in microbial ecology studies, as well as a wide range of archaea and eukaryotes. cpnDB includes coverage of the bacterial taxonomic hierarchy proposed by Garrity *et al.* (TOBA Release 7.7, March 2007, <http://www.taxonomicoutline.org/>) and employed by the Ribosomal Database Project. cpnDB also includes type II chaperonin sequences (Thermosome, TCP or CCT) found in eukaryotes and archaea. The taxonomic coverage and intense curation of cpnDB have made it a valuable

resource for researchers interested in exploiting chaperonin sequences in diagnostic methods or as targets for phylogenetic or metagenomic microbial ecology studies. Here we present a summary of the contents of cpnDB, an update on the cpnDB web interface and a description of user applications of cpnDB in diagnostic, metagenomic and phylogenetic investigations.

Abstract Category

31 Methodological Developments

PS.31.017 METARIBOSCRIPPTOMICS: A NEW APPROACH FOR POPULATION SURVEYS BY NEXT-GENERATION SEQUENCING

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Numerous studies have addressed microbial population phylogenies using rRNA gene-specific PCR, using both traditional cloning and newer next-generation (clone-free) methods. However, there are well-documented problems with ribosomal PCR primer design and specificity, and during the normal thirty to forty cycles of amplification, chimeras and other artifacts have been shown to arise. These inconsistencies are not removed by next-generation (NGS) sequencing technologies, although the volume of microbial population rRNA sequence information has grown exponentially after the advent of NGS.

I present a novel phylogenetic library construction method using the unique properties of transposomes (transposase/DNA complexes). Total RNA is primed with random 9-mers to make first strand cDNA, then is treated with RNAses and self-annealed. Then, Nextera™ Transposomes (Epicentre) are used to simultaneously fragment and tag double-stranded cDNA. Due to the highly folded nature of rRNA cDNAs and their abundance, they are preferred targets for tagging. The di-tagged cDNA fragments are amplified in 15 cycles of PCR and then sequenced. The annealing of first strand cDNA is largely intramolecular, reducing the potential for chimeras during PCR. Only sequences with double-stranded regions serve as transposome targets and the contiguous sequences between transposon tags will be amplified. In initial experiments the vast majority of tags map to ribosomal cDNA of both the large and small subunits. Ribosomal cDNAs from all taxa should be selected by this method because of their universal folding domains.

A Nextera library was constructed with spring water total RNAs from organisms retained on a 0.45 micron filter. The library yielded 16-18S and 23-26S rRNA sequences of two domains and ten phyla, with an average fragment length of 180 nt. The small survey yielded both bacterial and eukaryotic sequences including rRNAs of water fleas and diatoms. The sequences were contiguous with GenBank entries, and there was no indication of chimerism in the limited number of samples. Studies are under way to determine the accuracy and breadth of sequence data obtained with this method and its applicability to deep sequencing.

Abstract Category

31 Methodological Developments

PS.31.018 RAPID ENUMERATION OF RESPIRATORY ACTIVE MYCOBACTERIA WITH FLUORESCENT DOUBLE STAINING

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Clarifying the dynamics of nontuberculous mycobacteria (NTM) in aquatic environments is important to prevent their infection to human beings. For this purpose, methods for the detection of active mycobacterial cells are required. Culture methods are widely used for the detection of mycobacterial cells, but these methods are rather difficult to detect mycobacteria quantitatively and rapidly. Mycobacterial cells are quantifiable under a fluorescent microscope within several hours with acid-fast bacilli staining. In addition, several fluorochromes are available to determine the bacterial activities. In this study, we attempted to detect mycobacteria with physiological activity within 90 min by combining Auramine O acid-fast staining with 5-cyano-2, 3-ditoyl tetrazolium chloride (CTC) as an indicator of respiratory activity (Auramine O-CTC double staining). We firstly optimized the condition of the Auramine O-CTC double staining method to address the following problems; (i) CTC formazan dissolved in the decolorization step and (ii) fluorescent intensity of Auramine O was decreased by fluorescent resonant energy transfer (FRET). Specificity of this method was confirmed by staining mycobacterial cells (*Mycobacterium abscessus*, *M. avium*, *M. goodnae*, *M. intracellulare*, *M. smegmatis*) and non-targeted bacteria (*Bacillus cereus*, *Escherichia coli*, *Legionella pneumophila*, *Pseudomonas putida*, *Staphylococcus aureus*). Results can be obtained within 90 min by the optimized procedure, while more than one week is required for the culture-dependent approach. In conclusion, the Auramine O-CTC double staining method was useful for rapid detection of respiratory active mycobacteria. This method may contribute to identifying dynamics of NTM in aquatic environments. This work was supported by the Grant in Aid for Scientific Research (A) (20249007) promoted by the JSPS and the RIHN C-06 research project.

Abstract Category

31 Methodological Developments

PS.31.019 PCR PRIMERS FOR SEPARATE DETECTION OF INTRA-SPECIFIC GROUP VARIATIONS IN A BLOOM-FORMING CYANOBACTERIUM, *MICROCYSTIS AERUGINOSA*

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The cyanobacterial genus *Microcystis* commonly forms blooms in eutrophic water systems. Some members of the genus produce the cyclic heptapeptide hepatotoxins, microcystins, which cause hepatocellular carcinoma. Hence, *Microcystis* blooms are often responsible for the livestock mortality and cause serious problem in water management. The classification of *Microcystis* species had been unified into *Microcystis aeruginosa* based on the 16S rRNA gene sequences. The morphological features, however, are different not only among natural environments but also between natural environment and selective conditions, especially under unialgal and axenic culture. Here, we describe that new PCR primer sets and a new quantitative method using real-time PCR with the SYBR Green I assay. We considered 71 of 16S-23S rDNA

internal transcribed spacer (ITS) regions and designed three group-specific PCR primers, which are successfully selective for morphologically *M. wsenbergii*-like non-toxic group (Group-3) and *M. viridis*-like toxic group (Group-4) from *M. aeruginosa*-like one (Group-1) containing toxic and non-toxic *Microcystis*. This procedure was applied to water samples from field experimental pools and limnic environments. The Group-1 dominated during the development of the water bloom in the field experimental pool-A, while temporal shifts in the compositions of the group variations in *Microcystis* were observed in pool-B where smaller amount of *Microcystis* were inoculated. Compositions of *Microcystis* group were different between a reservoir and an irrigation pond. The Group-3 dominated in the reservoir, while the Group-1 dominated in the irrigation pond. The data presented show differences in the composition of ITS grouping in *Microcystis* among water blooms in nature. We showed the newly developed real-time PCR technique targeting 16S-23S rDNA ITS region is rapid and simple for the separate enumeration of the number of group variations in *Microcystis aeruginosa*

Abstract Category

31 Methodological Developments

PS.31.020 COMPARISON OF MICROBIAL POPULATIONS ISOLATED FROM A VARIETY OF SOILS USING DIFFERENT HOMOGENIZATION METHODS DURING DNA EXTRACTION

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Efficient DNA extraction is the key step in molecular analysis of soil for microbial ecology investigations. In fact, the choice of extraction method may have a significant effect on the data collected and mechanical lysis is generally considered best. Soils can vary greatly in their microbial diversity and load, which is influenced by factors including pH, organic content, moisture content, and texture of the soil. Consequently, careful study of the choices in mechanical lysis methods is important for obtaining reproducible data that accurately reflects the soil microbial assemblage. To assess bacterial and fungal diversity from different soils of varying characteristics, we used terminal restriction fragment length polymorphism (TRFLP) analysis, a reliable and well-established method for estimating microbial assemblage structure in environmental samples. DNA extraction of six different soil samples was evaluated using four different methods of mechanical disruption, varying the process (vortex vs. PowerLyzer™ and the matrix (glass beads vs. garnet). The use of the PowerLyzer™ generally resulted in higher DNA yields than the vortexer, as did the glass beads compared to garnet. As expected, soils containing higher sand content yielded much less DNA compared to soils rich in organic content. The effect of using the different methods on the microbial assemblage structure as assessed by TRFLP was strongly dependent on the soil type and the microbial assemblage (fungal or bacterial).

Abstract Category

31 Methodological Developments

PS.31.021 DEVELOPMENT AND EVALUATION OF A NEW PROTOCOL FOR DNA AND RNA ISOLATION FROM MICROBIAL MATS

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Microbial mats are complex biofilms consisting predominantly of bacteria and archaea arranged in layers. Mats typically develop in extreme environments becoming up to several centimeters thick as the microbial populations stratify and the matrix composed of extracellular polymeric substances expands. Over time, mats can become very rigid, making nucleic acid purification extremely difficult. As a result, treatment of mats to dissolve the EPS matrix is often necessary to enable efficient lysis of the microbial community. A protocol was developed that combined EPS treatment with cell lysis to facilitate removal of polysaccharides, humic substances, metals, salts, carbohydrates, and proteins from microbial mat samples. The resulting lysis buffer formulation, in combination with heating and either bead beating or vortex lysing was extremely efficient at releasing both DNA and RNA into the supernatant. After an inhibitor removal step, subsequent protocol variations were made depending on whether purified DNA or RNA was desired then validated through independent sources. DNA was easily obtained either with bead beating or vortex lysing while RNA recovery and quality was better with vortex lysing only. Depending on the mat, nucleic acid yields varied, but downstream applications such as PCR and RT-PCR were successful with every sample. These protocols provide a new approach to isolating DNA or RNA from microbial mats without the need for extensive and time consuming sample pretreatment.

Abstract Category

31 Methodological Developments

PS.31.022 QUANTITATIVE ASSESSMENT OF THE REMOVAL OF HUMIC ACID FROM PURIFIED DNA AND ENVIRONMENTAL SAMPLES USING INHIBITOR REMOVAL TECHNOLOGY

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Humic Substances (HSs) are a ubiquitous component of environmental samples such as soils, sediments, and biofilms. They are also a leading contributor of dissolved organic matter in water. It has been well documented that carry-over of HSs during DNA and RNA purification from environmental samples interferes with the function and efficiency of enzymes used in downstream applications. Inhibitor Removal Technology® (IRT) is a patented method for removal of humic substances as well as polyphenolics and polysaccharides. The method consists of two buffers that use changes in pH to first solubilize charged molecules then precipitate the insoluble large macromolecules, separating them from nucleic acids. The efficacy of IRT was evaluated by adding a series of humic acid (HA) dilutions to DNA purified from cultured microorganisms. End-point PCR was used to compare DNA containing humic acid to DNA that had been treated with an IRT based clean-up protocol. No products were generated in samples containing even low levels of humic acid. However, with the addition of IRT, PCR product was generated for all samples. Compost samples processed with and without IRT were also compared. Only the IRT treated samples showed a clean UV wavelength scan and were successful in PCR. The same samples without IRT demonstrated high 230 and 340 values and

complete PCR inhibition. For further validation, IRT was applied to protocols for water DNA and biofilm RNA isolation with samples obtained from sources high in humic substances, metals, and pesticide contamination. For water, DNA was isolated both with and without IRT then evaluated using quantitative PCR. When IRT was used Ct values improved by 7+ cycles. For biofilm, RNA was isolated with different quantities of IRT buffers. RT-PCR products were consistently generated when IRT buffers were increased. Whereas using less of the IRT buffers required a second IRT based clean-up protocol for a positive RT-PCR result. These applications provide direct evidence of the benefit of IRT in protocols for DNA and RNA isolation from environmental samples rich in humic substances.

Abstract Category

31 Methodological Developments

PS.31.023 INTRODUCING EzTaxon-e: 16S rRNA SEQUENCE DATABASE WITH NEW HIERARCHICAL CLASSIFICATION SYSTEM FOR BACTERIA

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For last few years, DNA sequencing-based community analysis has been widely propagated due to the invention of pyrosequencing. The major challenge in applying pyrosequencing to microbial community analysis lies on dealing with massive sequences and comparing community datasets. The existing 16S rRNA databases have provided taxonomic framework for the identification of bacteria, but inaccurate and insufficient taxonomy on "uncultured bacterium" have hampered correct identification. Here we present the EzTaxon-e, a new 16S rRNA database with complete hierarchical classification scheme for bacteria, which is an extension of EzTaxon database (<http://www.eztaxon.org>). In EzTaxon-e, we selected representative sequences from public domain to serve tentative type strains of yet uncultivated species using 3% sequence similarity for the species boundary. The classification for uncultured sequences was solely based on extensive phylogenetic analysis and sequence similarity values, resulting in complete and accurate hierarchical taxonomic structure for all species. The new system consists of 88 bacterial phyla and over 25,000 species including 132 candidatus species and >8400 species with valid names. Multiple metagenomic samples from diverse environments were examined to evaluate usefulness of our database. Bacterial community structures revealed using EzTaxon-e database were compared with those based on taxonomic schemes of the Ribosomal Database Project, NCBI and Greengenes databases. Even though community structures were generally similar at the phylum level, our system was shown to have more coverage in the identification of sequences at the family, genus and species levels, especially for as-yet uncultured taxa. We conclude that EzTaxon-e database should provide a sound taxonomic backbone in the identification of massive sequence data and can facilitate comparison of multiple bacterial communities. The database is available at <http://www.eztaxon-e.org>.

Abstract Category
31 Methodological Developments

PS.31.024 APPROACHES TO DEFINE THE PHYSIOLOGICAL STATE OF BACTERIA

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Upon environmental stress bacteria can enter different physiological stages including dormant states. In case of dormancies cultivation methods underestimate the amount of viable bacteria. Thus, new detection methods are needed to define the physiological state of bacteria. Therefore a number of different parameters were selected to study gene expression, membrane integrity and the cellular molecular pattern of stressed bacteria.

Using hygienically relevant bacteria the gene expression of the universal stress protein *usp* and other stress responsive targets were determined. The sigma factor *rpoS*, *pbp5* involved in peptidoglycan synthesis and *rcaA*, a regulator in biofilm synthesis in *Pseudomonas aeruginosa*, enterococci and *Escherichia coli* respectively were investigated. Gene induction and repression of the above mentioned genes became obvious during stress application.

PCR based population analysis in combination with propidium monoazide (PMA) treatment was applied to determine the efficiency of innovative disinfection measures and to distinguish between killed, injured or living cells. Depending on the applied treatment, the investigated natural populations using PCR and subsequent denaturing gradient gel electrophoresis (DGGE) represented the living part of the communities exhibiting intact membrane integrities. Mass profiling with whole cell approaches using matrix-assisted laser desorption/ionization (MALDI) mass spectrometry and cluster analyses applied on enterococci resulted in distinct grouping of bacteria exhibiting different physiological behaviors.

The presented methods form a powerful analytical platform for the characterization of the physiological states of bacteria applicable to both pure cultures and natural mixed populations.

Abstract Category
31 Methodological Developments

PS.31.025 TAKING SNAPSHOTS OF MICROBIAL COMMUNITIES—RNA-BASED ASSESSMENT OF SOIL MICROBIAL COMMUNITIES STORED IN A NOVEL BACTERIOSTATIC RNA PRESERVATION SOLUTION

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The development of next-gen sequencing technologies for molecular microbial ecology research have allowed more holistic views of microbial communities and detection of minute changes in metabolic patterns and community structure. Undoubtedly, the ability to effectively capture "snapshots" of microbial communities is essential to such studies. Given that on-location DNA/RNA extraction and/or deep-freezing of samples are difficult to achieve for field research, preserving total nucleic acids for later extraction is often the only option. Since temperature

conditions during transport and storage can have profound effects on microbial communities, stabilization of DNA/RNA and biostaticity are both critical for such preservation solutions. Reagents commonly used for such purposes are known to be inadequate for soil samples in many cases, yielding little or low quality RNA after extraction, thus indicating a need for alternative methods. We report the assessment of a novel reagent, the MO BIO LifeGuard™ Soil Preservation Solution, for its ability to stabilize total microbial RNA in soils stored up to 30 days at various temperatures. RNA yield and integrity of samples preserved with LifeGuard, RNALater, and GITC/Solution D (5M guanidine HCl) were examined after storage at temperatures from 25°C to -20°C over a four-week period. Only LifeGuard™ yielded high quality RNA at all time-points and temperatures. To assess the preservation of microbial community structure in LifeGuard-preserved samples, RT-tRFLP analysis was performed. The data indicated that storage at -20°C was optimal over the longest time period for both temperate soil and Antarctic Dry Valley soil, and that after two weeks at 4°C, the RT-tRFLP profile of Antarctic soil was >80% similar to that of the zero-time sample. These results suggest that LifeGuard is an effective nucleic acid stabilizer, and that LifeGuard-preserved DNA/RNA samples can be used to generate metagenomic/metatranscriptomic data resembling *in situ* microbial communities. Additionally, because LifeGuard is bacteriostatic rather than bactericidal, it may also be used for cultivation applications.

Abstract Category

31 Methodological Developments

PS.31.026 RAPID QUANTITATIVE DETECTION METHOD OF THE SULFATE REDUCING BACTERIA BY REAL-TIME PCR USING TAQMAN-MGB PROBE TECHNOLOGY

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A rapid detection method of SRBs was developed using the real-time PCR. The target gene used in this study was the conserved region of 16S rDNA sequence of SRBs. A TaqMan-MGB probe for amplification was designed for the SRBs detection. The detection limit of the assay for SRB DNA was 1 genome equivalent per reaction. All 14 samples obtained from Daqing Oilfield contained SRBs. The detection precision of this method was much higher than that of traditional method, and the whole process only need about 6h.

Abstract Category

31 Methodological Developments

PS.31.027 c16S: A HMM BASED ALGORITHM FOR ACCURATE TAXONOMIC CLASSIFICATION OF 16S RIBOSOMAL RNA GENE FRAGMENTS

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Existing 16S rRNA gene classification methods draw taxonomic inferences either by performing sequence alignments of given query 16S rRNA gene sequences with known 16S rDNA

sequences present in a database or by using word frequency based approaches. 16S rRNA gene sequences have stretches of conserved regions alternating with hyper-variable regions. It is observed that hyper-variable regions of different species belonging to a genus are more or less conserved. Based on this observation, we have devised a novel 16S rRNA gene classification algorithm (c16S), that uses genus-specific Hidden Markov Models (HMMs) for taxonomic classification of 16S rDNA sequences. In order to achieve high levels of accuracy, c16S incorporates several steps, which take into account the quality of the HMM alignment, prior to the final taxonomic assignment.

Multiple sequence alignments of 16S rDNA sequences from various species belonging to each individual genera were generated. These alignments were used for building genus-specific HMMs. Thresholds for determining the HMM alignment quality were also precomputed utilizing training data obtained with 16S rDNA sequences originating from diverse taxonomic clades. During classification step, query 16S rDNA sequences are first scored against precomputed HMMs. Based on the quality of the alignment, the query sequence is assigned at an appropriate taxonomic level. The performance of c16S algorithm was compared with that of Ribosomal Database Project (RDP) classifier.

Results obtained with c16S algorithm for various validation data sets indicate a high accuracy of classification of the input 16S rRNA gene sequences, even in scenarios where the input sequences originate from hitherto unknown organisms. In addition, the accuracy of c16S algorithm is 15-20% higher compared to the RDP classifier.

Given its high accuracy, the c16S algorithm is expected to have immense utility for microbial ecologists.

Abstract Category

31 Methodological Developments

PS.31.028 A NOVEL METHOD FOR DISCRIMINATION, SORTING, AND MULTIPLE DISPLACEMENT AMPLIFICATION OF SINGLE-INFECTED CELLS OF *EMILIANA HUXLEYI*.

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Viral infection of bacteria and phytoplankton plays a major role in gene transfer, biogeochemistry, and community dynamics. Often, viruses are host-specific at the strain level. Understanding the factors that determine when and how a particular virus can and cannot infect an algal host is of great interest due to the various ecological implications. A crucial step towards understanding these factors is to be able to discriminate single-infected from healthy cells. In the current study we took advantage of known physiological responses to viral infection in *E. huxleyi*, specifically oxidative stress, increased levels of DNA, and membrane patchiness to develop a method for discriminating infected cells prior to fluorescence-activated sorting. Then we optimized the conditions for whole genome amplification using the non-PCR based technique Multiple Displacement Amplification to obtain sufficient material for molecular analysis. For discrimination of healthy and infected cells we tested three flow cytometric assays: 1) CM-

H₂DCFDA dye for detection of intracellular Reactive Oxygen Species; 2) DNA dye SYBR Green I; 3) FM 1-43 lipid dye for detection of membrane blebbing. FM 1-43 gave the most unequivocal separation of healthy and infected cells and allowed discrimination from 6 h post-infection. With the other two dyes we could only discriminate infected cells from 24 h post-infection, therefore, missing early stages of infection. PCR amplification of Multiple Displacement Amplification products using genetic primers specific to the Major Capsid Protein gene of *E. huxleyi*-viruses revealed that all the sorted cells were infected. We were also able to amplify *E. huxleyi*'s 18S rDNA and calcium binding protein gene fragments from the same multiple displacement amplicons. This novel method to reliably discriminate infected from healthy cells will allow answering numerous questions regarding the mechanisms of viral infection of *E. huxleyi* and most likely other phytoplankton groups.

Abstract Category

31 Methodological Developments

PS.31.029 DIFFERENT APPROACHES OF CALCULATING CORRELATIONS FOR RANDOM MATRIX THEORY NETWORK CONSTRUCTION

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The increasing availability of high-throughput technologies, such as microarrays and pyrosequencing, has provided biologists an unprecedented chance to access biological systems. The construction of gene networks with these genetic data is considered a useful way to predict and verify gene interactions in complex biological systems, and further provide clues to unknown gene functions or construct systematic biological models to illuminate the mechanism of cellular processes from a novel perspective. One of the most used network construction methods is relevance network. Calculation of the correlation between nodes can greatly affect the efficiency and accuracy of the resulting network. Here, we build networks using random matrix theory (RMT) methods and several different correlation measurements to determine which one is more robust for RMT-based network construction. The correlation methods used were Pearson correlation, Biweight correlation, Spearman rank correlation, reliability scores, and different combinations of these. We constructed networks through *Escherichia coli* microarray datasets (M3D, Faith JJ et al, 2007) and our results show that all of these correlation methods are able to reveal some gene interactions in different aspects. However, different measurements show different network topological characters, such as small-world, scale-free, modularity, and have different impact upon the efficiency and accuracy of the RMT network construction methods.

Abstract Category

31 Methodological Developments

PS.31.030 RAMAN MICROSPECTROMETRY FOR THE SINGLE CELL IN SITU AND IN VIVO DETECTION OF THAUMARCHAEOL-CONTAINING AMMONIA-OXIDIZING ARCHAEA

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Thaumarchaeol (formerly known as crenarchaeol, Spang *et al.*) is a cell membrane lipid, which has been found only in thaumarchaeal ammonia oxidizers. This diglycerol tetraether lipid contains cyclopentane and cyclohexane rings (Damst *et al.*, 2002). These cyclic structures usually show strong and distinct peaks in Raman spectra at wave numbers between 400 cm⁻¹ and 1100 cm⁻¹. Thus, by using single-cell Raman microspectroscopy, thaumarchaeol could allow the quick detection of thaumarchaeotal cells in environmental samples. To test this approach, we did comparative Raman analyses of the thaumarchaeal ammonia oxidizer *Nitrosopumilus maritimus*, a non-nitrifying crenarchaeon (*Sulfolobus* sp.), and two bacteria (*Escherichia coli*, *Streptococcus* sp.). As expected, the Raman spectra of *N. maritimus* cells showed a distinct peak pattern clearly different from the spectra of the other organisms. Four possible diagnostic peaks for *N. maritimus* were identified approximately at wave numbers 485 cm⁻¹, 700 cm⁻¹, 780 cm⁻¹ and 1055 cm⁻¹. Based on these peaks, *N. maritimus* cells were identified in defined mixtures of bacterial and archaeal cells. These results were verified by concomitant FISH with rRNA-targeted probes. Subsequently, Raman microspectroscopy was used to detect thaumarchaeol-containing archaea in environmental samples. Follow-up experiments using purified thaumarchaeol and other representatives of the ammonia-oxidizing archaea should be performed to confirm the diagnostic peaks for the unique membrane lipids of these organisms. Spang A. *et al.* Archaeal evolution revisited: A unique set of information processing genes in the novel phylum *Thaumarchaeota*. Manuscript under review.

Damst J.S. *et al.* 2002. Crenarchaeol: the characteristic core glycerol dibiphytanyl glycerol tetraether membrane lipid of cosmopolitan pelagic crenarchaeota. *J Lipid Res.* 43:1641-1651.

Abstract Category

31 Methodological Developments

PS.31.031 BIODIVERSITY, EVOLUTION AND NICHE ADAPTATION OF *LACTOCOCCUS LACTIS* AS REVEALED BY GENOMOTYPING

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Lactococcus lactis is a lactic acid producing bacterium, which is widely used in the manufacturing of many fermented dairy products. However, the species also occurs in many non-dairy niches, such as fermented plant material. Recently these strains have gained increasing interest, as they possess potential flavor-forming activities and have diverse metabolic properties. An extensive genotypic diversity analysis was now performed on 39 *L. lactis* strains, isolated from dairy and plant niches. Pangenome comparative genome hybridization arrays were used to assess presence or absence of genes and gene clusters in all strains, relative to all known *L. lactis* sequences in public databases. Known chromosomal and plasmid-encoded genes were analyzed separately. A large strain variability was found in gene clusters which function in growth on plant substrates, such as arabinose, xylan, α -galactosides and galacturonate. Further niche-

specific differences were found in genes for exopolysaccharides biosynthesis, stress response (iron transport, osmotolerance) and defense mechanisms (nisin biosynthesis). Strain variability of functions encoded on known plasmids included proteolysis, lactose fermentation, citrate uptake, metal ion resistance and exopolysaccharides biosynthesis. These results can now be used to further investigate *L. lactis* diversity, which can help in optimizing bacterial communities for better and safe fermentation procedures.

Abstract Category

31 Methodological Developments

PS.31.032 DOUBLE LABELING OF OLIGONUCLEOTIDE PROBES FOR FLUORESCENCE IN SITU HYBRIDIZATION IMPROVES SIGNAL INTENSITY AND INCREASES rRNA ACCESSIBILITY

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Fluorescence *in situ* hybridization (FISH) with singly labeled rRNA-targeted oligonucleotide probes is widely applied for direct identification of microbes in the environment or in clinical specimens. Recently, it has been demonstrated that the detection limits of conventional FISH with a Cy3-labeled probe EUB338-I are 370 +/- 45 16S rRNA molecules per cell for *Escherichia coli* hybridized on glass microscope slides and 1,400 +/- 170 16S rRNA copies per *E. coli* cell in activated sludge [1]. One of the most frequently reported FISH problems, however, is a low signal intensity of the detected microbes as a consequence of falling below these limits. Here we show that a replacement of singly labeled oligonucleotide probes with 5-, 3-doubly labeled probes at least doubles FISH signal intensity without causing specificity problems. Furthermore, Cy3-doubly labeled probes strongly increase *in situ* accessibility of rRNA target sites and thus provide more flexibility for probe design [2].

1. Hoshino, T., et al., *Quantification of target molecules needed to detect microorganisms by fluorescence in situ hybridization (FISH) and catalyzed reporter deposition-FISH*. Appl Environ Microbiol, 2008. 74(16): p. 5068-77. 2. Stoecker, K., et al., *Double labeling of oligonucleotide probes for fluorescence in situ hybridization (DOPE-FISH) improves signal intensity and increases rRNA accessibility*. Appl Environ Microbiol, 2010. 76(3): p. 922-6.

Abstract Category

31 Methodological Developments

PS.31.033 BACTERIAL COMMUNITY COMPARISONS BY TAXONOMY-SUPERVISED ANALYSIS BYPASSING SEQUENCE ALIGNMENT AND CLUSTERING

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High throughput sequencing of the 16S rRNA gene is rapidly increasing our understanding of microbial community structure, but is also making the analysis of more samples and much larger data sets more challenging. We compared the bacterial community-by-OTU matrix that

characterizes bacterial communities by the abundance of clustering-determined OTUs (taxonomy-unsupervised analysis), and the bacterial community-by-'taxonomy-bin' matrix that is based on existing bacterial taxonomy (taxonomy-supervised analysis) on 1.3 million 16S rRNA V4-region gene sequences from 211 different bacterial communities. Beta-diversity measures calculated by using these two types of matrices were significantly correlated to each other, as were the ordination configurations, indicating that similar ecological conclusion would be drawn from both methodologies. The taxonomy-supervised analysis is more tolerant to sequencing errors, allows comparisons when sequences are from different regions of the 16S rRNA gene, and is not limited by the exhaustive computation required for the alignment and clustering required by the taxonomy-unsupervised analysis. However, it does not resolve as well were the current taxonomy is limited, but should perform better than the taxonomy-unsupervised analysis when the taxonomy is improved. With the tremendous expansion in 16S rRNA data acquisition underway, the taxonomy-supervised approach offers the potential to provide for more rapid and extensive community comparisons among habitats.

Abstract Category

31 Methodological Developments

PS.31.034 SIP GOES PROTEOMICS - PROTEIN-SIP REVEALS METABOLIC ACTIVE SPECIES IN FUNCTIONAL METAPROTEOME APPROACHES

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Background and aims For the detection of early stages of metabolisation and to cover also species with low incorporation of ¹³C from substrates techniques with high sensitivity for incorporation are needed. For unravelling food webs in addition to high sensitivity also an accurate determination of the ¹³C incorporation is crucial. The detailed information on incorporation of ¹³C will provide a measure to detect the metabolisation of an isotopically labelled substrate by a microbial species and to track the carbon flux in microbial communities. **Methods** In a mixed culture containing one species degrading toluene under anoxic conditions (*Aromatoleum aromaticum* strain EbN1) and a gluconate consuming enrichment culture (UFZ-1) the metabolisation of ¹³C labelled toluene and ¹²C gluconate was detected in terms of incorporation into proteins. In a LC-MS approach (Orbitrap-MS) the determination of incorporation was conducted on the basis of the half decimal place rule. **Results** In the mixed culture only proteins from the toluene degrading species EBN1 showed incorporation of ¹³C. The level of incorporation was about 93% and rather homogenous over all 38 analysed proteins (Jehmlich et al. 2008). The LC-MS based approach yielded identifications of peptides on the basis of the solely ¹²C containing peptides and the incorporation into ¹³C containing peptides with about 100 peptides showed a deviation about 15% in comparison to the hypothetical ¹³C content (Jehmlich et al., 2010). **Conclusions** Protein-SIP features high sensitivity for the

detection of incorporation and the accuracy of species specific incorporation allows tracking the carbon flux throughout microbial communities. Jehmlich et al., 2008, ISME Journal, 2,11,1122-33. Jehmlich et al., 2010, Mol Cell Proteomics, Jan 11, Epub.

Abstract Category

31 Methodological Developments

PS.31.035 DEVELOPMENT OF A VIROCHIP TO ASSES THE VIRAL DIVERSITY IN MICROBIAL COMMUNITIES

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Functional gene arrays constructed with key genes involved in various biological and geochemical processes have been widely used to analyze the bacterial, archaeal or eukaryotic microorganisms in microbial communities present in a diverse range of environments. However, less attention has been paid to the viral components of these communities. Studies have shown that viruses can outnumber prokaryotic organisms in the ocean by an order of magnitude and play important roles in these environments, such as the release of organic carbon back into the environment through cell lysis and the horizontal transfer of genes between host organisms. Here a ViroChip has been developed to assess the bacteriophage and algal viral community in environmental samples and give a more complete picture of microbial community dynamics. To accomplish this, specific gene sequences were retrieved by keyword searches on publicly available databases, and retrieved sequences were verified with experimentally confirmed sequences using HMMER alignment (<http://hmmer.wustl.edu/>). Second, sixty two genes (forty two for bacteriophages and twenty for algal viruses), covering ~9400 unique sequences that provide functional and structural components necessary for viral propagation were selected for probe design. These included genes for host recognition (69 tail fiber proteins sequences), genome integration (171 integrase sequences), genome replication, genome packing (216 terminase sequences), and host lysis (1083 holin and endolysin sequences), and their probes were designed using in-house probe design software to be sequence-specific and group-specific (both exclusive and inclusive) with different specificities in order to increase gene coverage. The developed ViroChip is currently being synthesized and further experiments are underway to examine viral diversity in soil. ViroChip is expected to be able to specifically assess the viral communities from different environments.

Abstract Category

31 Methodological Developments

PS.31.036 SOURCES OF VARIABILITY IN ESTIMATES OF MICROBIAL ABUNDANCE USING QUANTITATIVE PCR

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Quantitative PCR (qPCR) provides useful estimates of the relative abundance of microbial taxa; however, interpretation of these results may be hampered by variability due to methodological sources rather than real variability in taxon abundance. We used qPCR to estimate the abundance

of ammonia-oxidizing bacteria in salt marsh sediments. We explicitly tested whether some of the variation in our estimates was due to differences in: (1) the number of freeze-thaw cycles of template DNA, (2) DNA extraction efficiency among samples and (3) PCR inhibition among samples. The same 40 samples were used in multiple qPCR reactions, and each estimate was an average of three replicates from separate qPCR reactions. To estimate differences in extraction efficiency among samples, we added a known amount of green fluorescent protein (GFP)-labeled *Pseudomonas aeruginosa* cells to the samples before extraction. To estimate the variability of PCR inhibitors among samples, we then added an equal number of pCMV-SPORT6-PLAC8 to each extracted sample. Finally, in some of the reactions, we varied the number of freeze-thaw cycles (1 to 3) of the template. Differences in the number of template freeze-thaw cycles did not add variability to our estimates of AOB abundance. Similarly, differences in PCR inhibitors did not add variability (the number of PLAC8 copies detected did not differ), although template dilution increased estimated abundance by up to 8 fold. In contrast, extraction efficiency appeared to vary among samples (the number of GFP copies detected differed). These results suggest that studies using qPCR to estimate microbial abundance might be improved by investigating, and when possible, controlling or accounting for, methodological sources of variability.

Abstract Category

31 Methodological Developments

PS.31.037 CAPTURING GREATER 16S rDNA SEQUENCE DIVERSITY WITHIN THE BACTERIAL DOMAIN

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Since the molecular revolution in 1987, the number of Bacterial Divisions listed under the NCBI databank has increased from 12 to at least 92. However, many Divisions are severely underrepresented due to a lack of sequence homology to 'universal' bacterial PCR primers such as 27F/1492R, designed originally to target better-known groups such as *Proteobacteria* and *Firmicutes*. Currently, many Divisions that have no cultured representatives, such as Candidate Division TM7, are defined exclusively by 16S rDNA gene sequence data. Thus, the phylogeny, distribution and abundance of these Divisions are poorly characterised. Our aim was to develop a bacterial PCR primer set that covers a significantly broader range of the Bacterial domain. Forward and reverse primers (338F/1050R) were designed using alignments generated from the Ribosomal Database Project (RDP). RDP probe match was utilised to test potential primer sequences. The final primer set, spanning 710 bp, was compared against 27F/1492R using soils samples derived from Antarctic, sub-Antarctic and Australian coastal environments using next generation amplicon sequencing, obtaining approximately 10,000 reads per sample. In silico evaluation via the RDP server of 338F/1050R, resulted in 85% coverage of known 16S rDNA sequences, and displayed strong homology to Candidate Divisions OD1, SR1, WS3, TM7. There was a significant increase in the 16S rDNA sequence diversity of Bacterial Divisions obtained in each soil environment using this primer set when compared to 27F/1492R. Improved coverage of the bacterial domain with our 'universal' primer set highlights the potential for future studies to reveal even greater bacterial diversity, whilst altering our concepts of community structure in

certain environments. This primer set will now be used for the assessment of microbial diversity in Antarctic soil and offers a powerful alternative for expanding phylogenetic information currently available on the Bacterial domain

Abstract Category

31 Methodological Developments

PS.31.038 REVEALING THE HIDDEN ENVIRONMENTS OF AN ACIDIC GEOTHERMAL LAKE USING SONAR BATHYMETRY, HIGH-RESOLUTION GPS MAPPING, AND TIME-LAPSE PHOTOGRAPHY

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Boiling Springs Lake (BSL) is a 1.8 ha pool of pH 2.2, 52°C water in California's Lassen Volcanic National Park (LVNP). As a Microbial Observatory site focused on understanding the interactions among the entire microbial community, it requires delineation of all habitats. However, the lake's depth and bathymetry is obscured by suspended sediments that limit light penetration to <10 cm. The shoreline contains many highly variable features such as mud pots, fumaroles, and seasonal pools, and the lake undergoes seasonal changes due to winter snow, when access is problematic.

A student-designed radio-controlled vehicle (ROV) was used to characterize bathymetry, and sediment mineral composition was determined by X-ray diffraction. High-resolution GPS mapping around the lake's perimeter and shore was used to create a database of specific features, and time-lapse photography was used to study lake convection over hourly, daily, and seasonal scales. A tethered buoy measured hourly temperatures at the surface and depth over winter months.

The basin consists of soft, unconsolidated clay sediments that slope steeply to a central plateau at 9 m, interrupted by several gas vents originating from a series of flooded fumaroles. BSL water contains only 69 mg L⁻¹ suspended solids, but these consist of finely dispersed kaolinite and mixed layer clays that effectively limit light penetration, and gas upwelling over min-hour time scales keeps the lake convectively mixed. BSL undergoes a seasonal winter T depression to ca. 48 C in January, with storm-associated dips to 44 C from snow input, and flooding of the shore environment.

The characterization of BSL's physical environment indicates a diverse suite of habitats and seasonal variability. This physical context will guide studies of biological diversity, including virus and cell counts, sediment, water, and near-shore clone libraries, metagenomics, and cultivation studies.

Abstract Category
31 Methodological Developments

PS.31.039 SIZING ERROR IN THE T-RFLP ANALYSIS OF 16S RIBOSOMAL RNA GENE IS DEPENDENT ON THE BACTERIAL SPECIES AND THE FRAGMENT SIZE

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Background. Terminal restriction fragment length polymorphism (T-RFLP) analysis has become a widely used molecular tool for assessment of differences in the composition of bacterial communities. However, identification of terminal restriction fragment (TRF) origins is prevented by discrepancy between true TRF length predicted from sequence information and observed TRF length. Methods. In this study, we prepared various lengths of fluorescently-labeled DNA oligomers extended from 8F primer (5'- AGA GTT TGA TYM TGG CTC AG -3') by using dye-primer chemistry. Sizing error of each fragment was determined for 16S rRNA gene of 31 oral bacterial species. Results. Sizing errors of the 35-700 bases DNA fragments were in the range of 13 bases (-9 to +4 bases). On the other hand, size variations of the DNA fragments with the same length were within 5 bases among the 31 species of 15 bacterial genera. The variation was almost within 2 bases only in the genus of Streptococcus (11 species). Conclusions. These results suggested that sizing errors in the T-RFLP analysis are dependent on the bacterial species and the fragment size. Bacterial assignment software adopted the different size error settings for each bacterial genera may enable to predict the composition of human commensal microbiota from T-RFLP profiles with higher accuracy.

Abstract Category
31 Methodological Developments

PS.31.040 INTRODUCING MATHFISH: A WEBSITE THAT RUNS MATHEMATICAL MODELS OF FLUORESCENT IN SITU HYBRIDIZATION FOR THERMODYNAMICS-BASED PROBE DESIGN

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The traditional design of oligonucleotide probes used in Fluorescent in situ Hybridization (FISH) is a largely experimental practice. Probe sensitivity is tested by hybridization with target organisms, while specificity is addressed using non-target organisms with one or two mismatches to find the optimum denaturant (formamide) concentration for the adequate discrimination of mismatches. This approach is limited by the availability of target and non-target organisms or clones of targeted molecules (e.g. 16S rRNA). Furthermore, sequence databases often reveal a long list of non-target organisms which cannot be exhaustively evaluated. Therefore, predictive tools that rationalize probe design and allow systematic evaluations are desired. To this end, mathematical models have been developed based on the thermodynamics of nucleic acid interactions taking place in FISH. These models have been used for the demonstration of the global accessibility of Escherichia coli 16S rRNA to FISH probes, simulation of formamide denaturation profiles, and systematic evaluation of mismatch

discrimination potential. To facilitate the use of these models, a web-based tool named mathFISH has been developed as a user friendly environment for the mathematical simulation of FISH experiments. The website is encrypted in Java and runs using MATLAB modules. The main user inputs are probe and target sequences. A key output is the overall free energy of hybridization, which for perfectly matching probes is recommended to be in the range -13 to -15 kcal/mol. In addition, formamide profiles and free energy calculations are provided for a quantitative evaluation of mismatch discrimination ability, where, a mismatch destabilization free energy of less than 4 kcal/mol generally indicates difficulty in mismatch discrimination. In total, eight tools for different types and levels of thermodynamics-based probe analysis are available. The function of each tool and recommended usage will be presented.

Abstract Category

31 Methodological Developments

PS.31.041 FIXATION-FREE FLUORESCENCE *IN SITU* HYBRIDIZATION FOR TARGETED ENRICHMENT OF MICROBIAL POPULATIONS

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Fluorescence *in situ* hybridization (FISH) is a powerful method for visualizing microbial populations in a community setting and can be used as the basis for separating targeted populations from a multi-species background through fluorescence activated cell sorting (FACS). Cell fixation is an accepted and reportedly integral part of the FISH protocol that serves to stabilize cell integrity and facilitate probe permeability for imaging. For microorganisms, this is most commonly achieved with paraformaldehyde (PFA) which crosslinks proteins and nucleic acids. However, for genomic or proteomic applications, unfixed biomass is desirable to minimize possible sequencing biases introduced by crosslinking and also to avoid possible cell lysis difficulties. In this study, we made a simple modification to the standard ribosomal RNA-targeted FISH protocol by removing the PFA-fixation steps to allow recovery of unmodified nucleic acids. Surprisingly this resulted in no appreciable effect on the quality of FISH images from two bioreactor sludges and termite hindgut samples using epifluorescence microscopy. We then targeted one bacterial and one archaeal population in the sludge samples with group-specific oligonucleotide probes using in solution fixation-free FISH and sorted hybridized populations using fluorescence activated cell sorting (FACS). We could show that sorted populations were highly enriched for the target organisms based on 16S rRNA gene sequencing, thus confirming probe specificity using the modified FISH protocol. This approach should facilitate subsequent genomic sequencing and analysis of targeted populations since DNA is not compromised by cross-linking during fixation.

Abstract Category

31 Methodological Developments

PS.31.042 MICROCALORIMETRIC ASSESSMENT TO EVALUATE QUALITY OF LONG-TERM SHRIMP AQUACULTURE SOILS IN HAINAN ISLAND OF

SOUTHERN CHINA

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The study of shrimp aquaculture ecotoxicity and microbial rapid response in soil has recently received much attention, thus analysis their relationships were very necessary. In this study, we monitored soil quality, microbial activities and diversity in soil with high salinity by using microcalorimetry, soil enzyme activities and PCR-denaturing gradient gel electrophoresis (DGGE) technology. Soil samples were collected from two upland pond sites, eastern and western coast of Hainan island of southern China, respectively, where shrimp aquaculture have been carried out for more than seven years. The very long values of peak time obtained in all samples, indicated the lower microbial metabolism in this high salinity soils. A higher dissipation of heat per cell unit indicated that a lower microbial metabolism in soil was observed in upland pond soils in comparison with native soils. The less population of culturable microbes, the lower activities of dehydrogenase, urease and acid phosphatase, the less microbial diversity presenting by Shannon-Weaver index from DGGE, were also observed in upland pond soils. In conclusion, the soils were degraded after long-term shrimp aquaculture from both microbial activities and microbial diversity respects. In addition, our results also claimed that microcalorimetric method is a useful tool to detect microbial activity even at high salinity soils.

Abstract Category

31 Methodological Developments

PS.32.001 BACTERIAL DIVERSITY, PIGMENT AND NITROGEN FIXATION OF BIOLOGICAL DESERT CRUSTS FROM THE SULTANATE OF OMAN

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Biological desert crusts are relatively common in the arid deserts of the Sultanate of Oman, however little is known about their microbial community composition and role in soil fertilization. We compared three crusts from geographically different locations for their soil texture, bacterial community structure, pigment composition and nitrogenase activity. The crusts were growing on alkaline (pH 7.6 to 8.7) loamy sand and silty loam soils. Microscopically, *Microcoleus vaginatus* was the most abundant cyanobacterium but Nostoc and Scytonema types dominated in cultures. The 16S rRNA sequences showed close similarities in the crusts' bacterial composition with 77-81% of the total clones belonging to cyanobacteria and the rest distributed among *Alpha*- and *Deltaproteobacteria*, *Bacterioidetes*, *Gemmatimonas* and *Planctomycetes*. 37% of the cyanobacterial clones were affiliated with heterocystous types like Nostoc, Scytonema, Brasilonema and Petalonema. Chlorophyll a concentrations suggest a similar abundance of phototrophs in all crusts. High levels of the UVA sunscreen scytonemin were detected in the exposed crusts. The three crusts exhibited comparable acetylene reduction rates in light and in the dark with a maximum rate of 58.5 ± 2.6 $\mu\text{moles reduced C}_2\text{H}_2 \text{ m}^{-2} \text{ h}^{-1}$. We conclude that the crusts, regardless of their geographical location, were rich in heterocystous cyanobacteria that can fix nitrogen and could possibly improve soil stability and productivity.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.002 THE DIVERSITY OF BACTERIAL AND FUNGAL COMMUNITIES IN TOURIST ACCESSIBLE CAVES AT UNESCO WORLD HERITAGE LISTED NARACOORTE, SOUTH AUSTRALIA.

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The impact of tourism and environmental factors on microbial diversity was investigated in caves at Naracoorte Caves National Park (a World Heritage Site) using culture-dependent and independent techniques in order to assist in cave management protocol development. The two caves used in this study (Stick Tomato and Alexandra Caves) were selected based on tourist numbers (>20,000 annual visits) and configurations. Stick Tomato Cave had two large openings (no doors) with storm water access while Alexandra Cave was shielded from the environment by lockable openings. The control cave was Strawhaven Cave with no tourist access. 16S rDNA and Intragenic Spacer Region-based DGGE analysis showed differences in the bacterial and fungal community profiles between the caves' disturbed and non disturbed areas with *acidobacteria* and *alphaproteobacteria*-like sequence unique to disturbed areas. Bacterial survey of these caves also showed *Proteobacteria* (culture-independent) dominance while fungal survey showed *Ascomycota*-like sequences (both culture dependent and independent techniques) dominance

irrespective of sampling area. The predominance of *Microascales*-like *Ascomycota* (*Trichurus*, *Doratomyces* and *Wardomyces*) in tourist accessible and control caves suggest they have significant ecological roles in these caves and were not strictly related to tourism. Generally, the bacterial and fungal community in tourist (disturbed) accessible areas of these caves showed a higher Shannon Diversity Values than in non tourist accessible (non disturbed) areas. Although differences in microbial community in disturbed and non disturbed areas were observed in this study, these were not due to tourism alone. Spatial heterogeneity of soil samples, access to storm waters and air movement within the caves were also thought to affect microbial distribution. Therefore, the cave ecosystem of Naracoorte is a complex one in which microbial distribution was often affected by a combination of factors.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.003 MICROBIAL DIVERSITY IN LAKE UVS, MONGOLIA

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Background: Lake Uvs is located at the boundary of dessert and tundra in Mongolia. Temperature ranges from -58°C to 47°C while salinity remain very high throughout a year. Bacteria which inhabit in the unique environment are expected to have unique characters. However, information on bacterial flora is limited until today.

Objectives: To understand diversity and characteristics of bacteria in Lake Uvs. Methods: Environmental parameters, including water temperature, pH and salinity, were measured in situ. Bacterial strains were isolated on nutrient agar and R2A media at 20°C and screened for enzyme activities (cellulase, amylase, protease and lipase). Optimal growth environment for each strain was assessed based on optical density (600 nm) in the temperature range 4-42°C and the salinity range 0-4%, for 48 hours. The structure of the bacterial community was analyzed by denaturing gradient gel electrophoresis (DGGE) and isolates were identified based on their 16S rRNA gene sequences.

Results: Based on presence of enzyme activities, we selected 15 strains. Six strains that isolated from surface water had high activities only for protease and lipase. Other strains had high activities of all enzymes. According to the growth rates of the isolates in the temperature range of 0-42°C, all strains showed the best growth at 20°C. All bacteria were tolerant to the salinity range of 0-4%. DGGE banding patterns were similar at all depths. The isolates were classified as a *Gammaproteobacterium*, *Pseudomonas* sp., *Vibrio vulnificus*, *Stappia aggregata*, *Pseudomonas mendocina*, *Dickeya dadantii*, or *Rheinheimera pacifica*.

Conclusion: Bacteria in Lake Uvs were tolerant to various environmental fluctuations, and bacteria diversity was significant.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.004 CHARACTERIZATION OF PHOSPHATE SOLUBILIZING MICROORGANISMS FROM TROPICAL SOIL AND THEIR ABILITY TO ENHANCE

NUTRIENT UPTAKE IN RICE

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Uplands constitute 60 % of the rice-growing environment in Ghana. The traditional upland rice-based cropping system that relies on a period of fallow to restore soil fertility is unable to maintain organic matter, nitrogen and phosphorus in soil. As a result of that the yield of rice keeps on declining. The aim of the study is to characterize by molecular methods phosphate solubilizing microorganisms to know their phylogeny and also amplify the *nifH* and *pqq* genes and know their copy numbers. The second aim is to develop cheaper and available local P fertilizer that will enhance the yield of upland rice. Fifty-two isolates, mostly bacteria, fungi and actinomycetes have been obtained. They are mostly Gram negative and can solubilize rock phosphates in cultural solution. One of such isolates produced 169 mg/l phosphate in culture solution within 2 weeks. Initial pot experiment showed that rice yield, N and P uptake were significantly ($p < 0.05$) influenced when rice was grown in soil of pH 4.5-5.7 with some of the isolates. Molecular characterization of isolates is ongoing to further screen for isolates with high nitrogen fixing and phosphate solubilizing ability.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.005 DIFFERENCES IN METABOLISM AND COMMUNITY STRUCTURE ACROSS A NATURALLY REDUCED GRADIENT IN A URANIUM CONTAMINATED AQUIFER

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Although many studies on the bioremediation of uranium-contaminated groundwater have focused on microbial processes associated with artificially stimulated U(VI) reduction, less is known about uranium immobilization within subsurface sediments that are naturally anoxic. A U(VI)-contaminated groundwater site located in Rifle, Colorado, contains distinct zones of highly reduced subsurface sediments that, in comparison with nearby sediments, have less microbially reducible Fe(III) and are enriched with solid-phase uranium. In order to determine whether these geochemical differences could be attributed to differences in microbial metabolism, sediments and associated groundwater were incubated under anaerobic conditions to replicate the in situ environment. Measurements of steady-state hydrogen concentrations indicated that Fe(III) reduction was the predominant terminal electron-accepting process (TEAP) in the less reduced sediments whereas sulfate reduction was the TEAP in the highly reduced sediments. Turnover rates for the acetate pool, determined with [2-¹⁴C]-acetate tracer studies, were similar in both sediments, but acetate concentrations were more than 4-fold higher in the highly reduced sediments, resulting in correspondingly higher estimated rates of acetate metabolism. Analysis of 16S rRNA gene sequences in subsurface sediment core samples revealed higher percentages of Firmicutes and *Geobacter* in the highly reduced zones in comparison with the less reduced zones, even though in some instances these zones were no more than 1-2 meters apart. The sediments with the highest Firmicutes percentages also had the

highest concentrations of organic matter. These results suggest that increased abundance of particulate organic matter in discrete pockets within the subsurface stimulate microbial fermentation and associated anaerobic oxidation of acetate and other fermentation products, which support enhanced reduction of U(VI) and associated uranium immobilization.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.006 OTUBASE: A PLATFORM FOR OPERATIONAL TAXONOMIC UNIT BASED ANALYSIS IN R.

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High throughput pyrosequencing of 16s rRNA is an important tool for identifying the microbes present in uncultured samples. Several tools are available to process and analyse the data collected by pyrosequencing. Programs such as Mothur cluster sequences into operational taxonomic units (OTUs) based on sequence similarity. These OTUs can then be used to estimate various ecological parameters such as species richness and diversity.

The tools currently available to analyse OTU data are very good. However, they do not provide the means to easily implement new analysis techniques. The casual researcher is constrained by the functionality provided by the software developer. It was with this in mind that we developed OTUbase, an R package that provides structure and functions to store and manipulate OTU data. OTUbase provides functions to read in OTU data and store it within R. There are already many packages available within R that provide the capability to analyze communities and calculate various ecological parameters. OTUbase allows these tools to be applied to OTU data. In addition, the rich plotting features of R are available to visualize the data.

In conclusion, OTUbase is a new R package that provides the structure and functions needed to store, manipulate and analyze OTU data. Among its strengths are the ability to quickly implement new analyses and the easy availability of the rich analysis and visualization capabilities of R.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.007 BACTERIAL DOMINANCE IN SUBSEAFLOOR SEDIMENTS CHARACTERIZED BY GAS HYDRATES

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Subseafloor sediments are recognized as austere environments for microbial survival based on diminished availability of electron acceptors. The Andaman Sea is a back arc basin that contains sediment composed of marine derived carbonaceous ooze with 1% organic carbon where oxidants are likely to become limiting with depth. Mixed thermogenic/biogenic methane is also present as hydrate between the depths of 300 to 600 meters below seafloor (mbsf). Geologic horizons demarcate this gas hydrate occurrence zone (GHOZ) as evidenced by increasing

porosity from 50% to 63% at 300 mbsf and a seismically observed bottom-simulating reflector where the methane is believed to exist as a free gas at 600 mbsf. Our objective was to determine the microbial abundance and diversity in sediments that transition the GHZO. Sediment cores were obtained from 43 depth horizons ranging between 30 and 700 mbsf in the Andaman Sea. Microscopic cell enumeration and domain-specific quantitative PCR (qPCR) revealed that most sediment layers harbored relatively low concentrations of Bacteria (below detection limit (10^2 cell cm^{-3}) to 10^4 cells cm^{-3}); however, sediment at the top and bottom of the GHZO contained greater cell numbers (10^5 cells cm^{-3}). In addition, DNA extraction followed by multiple displacement amplification and PCR amplification was attempted using primers specific for archaeal and bacterial 16S rDNA. Clone library analysis and terminal restriction fragment length polymorphism was performed on the amplified DNA from sediment within the GHZO and found that members of Firmicutes, such as *Bacillus* spp., dominated these sediment layers. Consistent with the low abundance of Archaea predicted by qPCR, archaeal 16S rDNA were never detected. Compared to other seafloor settings, this Bacteria-dominated deep seafloor habitat has unique phylogenetic diversity that may be stimulated at geologic horizons related to the GHZO.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.008 MICROBIAL DIVERSITY ON THE GEOTHERMICAL BELT OF "LOS AZUFRES", MICHOACÁN, MEXICO

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The "Los Azufres Geo" thermal Belt is a volcanic system located in the northern portion of the Mexican Volcanic Axis, in the state of Michoacán. Although it is relatively well studied geochemically, no microbiological studies have been carried out on the region till now. To mitigate this deficiency, we are investigating its microbial diversity, by applying both T-RFLP technique and clone libraries, in order to access the whole bacterial and archaeal communities (e.g. Liu et al., 1997; Marsh, 1999). These techniques are powerful to obtain the fingerprint of the microbial life because they can detect even the uncultivable microorganisms. We probed three samples, extracted from geysers and mud in the area of the "Spa Los Azufres" (19:46:51.7 N; 100:39:23.6 W; and 2,875 m height), comprehending the following characteristics: biomats, biofilms and mud (Bm, Bf and Mu, respectively). The total DNA, obtained by traditional methods (Tsay et al., 1991), was used to amplify the 16S rRNA (by PCR) using universal primers to bacteria (8F and 907R). For the TRFLP analysis we used the 8F primer fluorescently targeted, HaeIII and HinfI as the restriction enzymes, and a sequencer ABI Prism to measure the length of fragments. In order to obtain the cloning library, we used commercial kits (Fermentas). All the three sampling sites showed anaerobic (dissolved oxygen below of limit detection) and highly acid (pH between 2 and 3) physical characteristics. The highest temperature (above 85 C) was observed at Mu site, while for the Bf and Bm the measured temperatures were, respectively, 27 and 35 C. Despite the proximity of the sampling sites,

different predominant bacterial populations were found, changing also between the wet and dry season. Moreover, we probed the best supports for the formation of biofilms. We verified microbial growth on the carbon sources: sucrose, glucose, malt extract, meat, lactate, and tioglicolate; and with the specific media to RSB and Thiobacillus.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.009 FUNGI BIODIVERSITY IN AN EXTREME CONTAMINATED BY HEAVY METALS AND CARBONATES

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The fungi are omnipresent on most of the terrestrial ecosystems, with some species known by their pathogenic effect or industrial (pharmaceutical, chemical and food) use, and more recently by their potential for environmental bioremediation. However, most of the techniques for accomplishing the biodiversity of microorganisms, especially those that use methods independent of culture, have been used for studying bacterial communities and relatively little information has been gathered for the fungi. In this work we used a technique independent of culture, namely the ARISA (Amplified Ribosomal Intergenic Spacer), for investigating the fungi biodiversity in an anthropologically extreme site, with high concentrations of metals, pH above 12 and a salinity of 100 mg.L⁻¹. The site is located in the city of León, Guanajuato (Mexico). In order to avoid the interference of metals and salts in the process of obtaining the total DNA, we probed many pre-treatments (Brito et al., in preparation), and found the best results with a density-separation solution at low-speed centrifugation (Hopkins et al, 1991). After that, the cell fraction was submitted to the traditional DNA extraction technique (Tsay et al, 1991). The Intergenic Transcription Spacer was amplified using primers described on the literature (ITS1 and ITS4 from White et al., 1990). For searching the best PCR amplification conditions, we used the DNA of the laboratory strain *Aspergillus* sp. The amplified DNA was submitted to capillary electrophoresis (ABI prism), and the fragment lengths were analyzed using PCA methods. In general the observed fungi diversity was small, slightly higher on the solid waste than on the lixiviated channels sediment. ACKNOWLEDGEMENTS: This work was supported by FONCICyT-CONACyT, ECOS-NORD-SEP-CONACyT-ANUIES (M07A01). Piñón-Castillo received a fellowship from CONACyT, Mexico. Thanks are given to Cruz E. Martinez-Palacios for technical assistance.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.010 COMMUNITY STRUCTURE IN TWO GEOCHEMICALLY DISTINCT HYDROTHERMAL POOLS IN UZON CALDERA, KAMCHATKA, FAR EAST RUSSIA

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Contemporary hydrothermal systems are of interest as microecological systems. Two thermal pools in the Uzon Caldera, Arkashin Schurf (Arkashin) and Zavarzin Spring (Zavarzin), were sampled to characterize geochemical conditions and bacterial and archaeal community structure, including elemental composition, lipid composition, 16S rRNA sequencing, and C, N and S stable isotope ratios. Sediments in Arkashin relative to Zavarzin had higher concentrations of elements associated with magmatic source water. The archaeal glycerol dialkyl glycerol tetraether (GDGT) profiles from Arkashin were dominated by GDGT-4. Up to 350 $\mu\text{g g}^{-1}$ of phospholipid fatty acids (PLFA) were recovered from Arkashin and included biomarkers for *Aquificales*. In Arkashin, 206 Bacterial 16S sequences were assigned to 19 operational taxonomic units (OTUs). The most abundant were related to the "*Sphingobacteria*", *Hydrogenobaculum* (*Aquificales*) and *Variovorax* (β -*Proteobacteria*). In Zavarzin, relative abundance among archaeal GDGTs was more evenly distributed than in Arkashin. Ninety-one archaeal 16S rRNA sequences were assigned to 31 OTUs representing the *Crenarcheota* and "*Korarchaeota*". Approximately one quarter of the Zavarzin archaeal sequences appeared to represent unique clades of *Archaea*. Biomarkers in up to 455 $\mu\text{g g}^{-1}$ of PLFA recovered from Zavarzin included those for *Chloroflexi* as well as for heterotrophic bacteria. The 300 Bacterial 16S sequences analyzed from Zavarzin were assigned to 59 OTUs. The most abundant sequences were related to *Rosiflexus*, followed by γ -*Proteobacteria* and clostridia. These data, coupled with stable isotope ratios, indicate that *Hydrogenobaculum* is a primary producer in Arkashin and allochthonous inputs may be an important C source in Zavarzin. Thus, the distinct geochemical conditions give rise to unique community structures in each pool.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.011 DIVERSITY OF MICROBIAL COMMUNITIES IN BLACK CARBON AND AMAZONIAN ANTHROSOLS ASSESSED BY T-RFLP AND DGGE FINGERPRINTS

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The existence of anthropogenic dark soils (ADE) proves that infertile Ferralsols and Ultisols can be transformed into permanently fertile and productive soils. ADE soils exhibit approximately three times more soil organic matter, nitrogen and phosphorus and 70 times more black carbon (BC) content, when compared to their adjacent or background soils (ADJ). This study aimed at analyzing the structure of Archaeal communities in ADE, BC and ADJ from "Hatahara" and "Balbina" sites located at Central Amazonia, by denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP). Evaluation of T-RFs was performed using Peak Scanner 1.0, CANOCO 4.5 and Primer 5 and DGGE fingerprints were analyzed by Bionumerics software 6.01. The analysis in Archaeal community structure by T-RFLP defined three distinct groups, showing higher distance between BC and ADJ and lower distance between ADE and BC. The principal component analysis (PCA) based on T-RFs

indicated that the community structures were distinctly separated along the first and second axes from the graphic, which described 60.1% and 39.1% of the total variation, respectively. The ADJ soils showed a higher estimated richness of T-RFs when compared with ADE and BC. The hierarchical cluster analysis of DGGE fingerprints showed similarity between the Archaeal community in ADE soils and BC in both sites. ANOSIM analysis revealed statistically significant differences between "Balbina" (secondary forest) and "Hatahara" (abandoned crop) sites, which confirm the hierarchical clustering obtained by DGGE. PCA generated from T-RFLP also confirmed data expressed by hierarchical clustering of the microbial community profiles revealed by DGGE. Even considering the microbial complexity in highly fertile soils, comparisons using Venn Diagram indicated that black carbon may be a niche for unique microbial communities, confirmed by a complete sequencing analyses. FAPESP, CNPq

Abstract Category

32 Biodiversity and Community Analysis

**PS.32.012 DISTRIBUTION PATTERNS OF THE MEMBERS OF PHYLUM
*ACIDOBACTERIA***

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The distribution pattern of the phylum Acidobacteria, a previously uncultured bacterial group, was investigated by molecular ecological analysis. Acidobacterial 16S rDNAs were observed in almost all soil samples, and members of acidobacterial primer group A were detected in all samples that harbored the phylum Acidobacteria. Other primer groups, Y, G, and O, showed limited distribution patterns. We further divided the primer groups into acidobacterial subdivisions (class-level). Subdivisional distribution patterns were determined by comparing the observed T-RFs with theoretical T-RFs predicted by *in silico* digestion of acidobacterial 16S rDNAs. Consistent with the PCR results obtained with subgroup-specific primers, T-RFLP analyses showed that acidobacterial subdivision 1 belonging to primer group A was present in the majority of the soil samples. At the subdivisional level, acidobacterial subdivision 1 might be the most widely distributed group in this phylum, indicating that members of subdivision 1 might be adapted to various soil environments, and members belonging to other subdivisions might be restricted to certain geographic regions or habitats.

Abstract Category

32 Biodiversity and Community Analysis

**PS.32.013 SELECTION FOR BACTERIA, ARCHAEA, AND FUNGI IN CATTLE
AFFECTED SOILS - EVALUATION BY COMPARATIVE COMMUNITY PROFILING
OF TOTAL AND EXTRACELLULAR DNA**

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Cattle overwintering management applied to upland pasture soil affects the composition and activity of microbial communities in several ways. We performed comparative extracellular DNA (eDNA) and total DNA (tDNA) approaches to estimate the contribution of eDNA originated from soil or excrements, to total DNA- derived community fingerprints in soils under different cattle overwintering management (severe - SI, severe in past, now regenerating - SR, medium - MI, control - CO). eDNA can originate from genomic DNA by active extrusion (prokaryotes) or passive mechanisms (release after cell lysis) and therefore can represent an important portion of the soil metagenome. Molecular profiles were used to characterize the impact of cattle overwintering on microbial domains (Bacteria, Archaea) or kingdoms (Fungi). We challenged to assess the information about soil microbial communities stored in the extracellular fraction of the total soil DNA pool. We found the eDNA fraction, extracted from soils and excrements, to be a quanti-qualitative important portion of the soil metagenome. Community fingerprinting of bacteria, archaea and fungi of cattle impacted soils also revealed the presence of bands presumably originating from cattle excrements; in fact, these bands were not present in the control soil. For all target microbial community fingerprints, excrements showed distinctly different microbial community profiles with respect to the soil samples. Both severely impacted sites clustered together apart from non-impacted site, confirming the selective effect of cattle on soil microbial communities. eDNA- derived profiles of archaea and bacteria were congruent with tDNA- derived ones, and showed similar phylotype richness and community structure. The lower congruency was found for eDNA profiles of fungal origin, showing few bands and poor community profiles in comparison to tDNA generated ones. Our results suggest that eDNA was mainly of bacterial and archaeal origin.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.014 YELLOWSTONE LAKE CONTAINS RICH BACTERIAL DIVERSITY AND HIGH ENERGY GEOCHEMICAL INPUTS

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Yellowstone Lake is central to the balanced functioning of the Yellowstone ecosystem. Decades of studies have led to the conclusion that the lake is a "simple ecosystem." However, discovery of hydrothermal vents on the lake floor suggested this may be an overly simplistic view. The goal of this study was to determine how vent inputs might affect the bacterial communities found in different parts of the lake.

A remotely operated vehicle allowed sampling of surface zone waters and lake floor geothermal vent fluids. Extensive geochemical profiling was coupled with a survey of bacterial diversity using both Sanger sequencing and 454-pyrosequencing of bacterial 16S rRNA genes.

Vent emissions contained substantial H₂S (~600 μM), CH₄ (40-50 μM), CO₂ (1-10 mM) and H₂ (up to 120 nM), which could fuel bacterial communities distinct from solar energy. Both the pyrosequenced 16S amplicons and Sanger sequenced near full-length clones demonstrated that this lake is rich in bacterial diversity. The lake is well mixed as suggested by the fact that 90% of the pyrosequencing reads were in OTUs found in all four locations in the lake. However, the vents had localized impacts with 41% of the observed OTUs found only in vent waters. *Actinobacteria*, *Bacteroidetes*, and *α*- and *β*-*Proteobacteria* were the principal phyla, and surface waters were dominated by a novel *α*-*Proteobacteria* clade most closely (but distantly) related to the SAR11 group which are a significant component of marine microbial communities. Thermophile signatures were found exclusively or highly enriched in vent water samples.

Overall, results from this study suggest this lake is not simple in that primary productivity could derive from both phototrophy and chemolithotrophy. Furthermore, the bacterial diversity observed in this lake rivals that encountered in soil, and challenges the current view that soils are the most significant reservoirs of microbial diversity in the biosphere.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.015 TRAITS ANALYSIS OF MICROBIAL COMMUNITIES ADAPTED TO FLUCTUATING REDOX POTENTIAL IN TROPICAL FOREST SOILS

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How changing environmental conditions modify microbial community structure and how changing community structure affects community function, and thus ecosystem function, are central questions of microbial ecology. Complex microbial communities encompass a range of functions with varying relationships to phylogeny; there seems to be phylogenetic conservation of some functions (ie., methanotrophy) and little phylogenetic fidelity of others (ie., denitrification). A prevailing hypothesis is that there is a continuum over which functions vary in their phylogenetic restriction. To test this theory we used the results from a study of soil response to various redox potential patterns. Soil cores from a subtropical forest in Luquillo, Puerto Rico were incubated for 32 days under either static oxic, static anoxic, or fluctuating redox regimes with a 4-day oxic and 4-day anoxic cycle. Rates of CO₂ production, denitrification, iron oxidation and reduction, and methanogenesis were quantified and related to redox patterns. Using the high-density 16S rRNA gene microarray, PhyloChip, we analyzed standing (DNA) and active (RNA) microbial communities. Having previously shown that these communities are highly adapted to fluctuating redox potential, we used these data to identify taxa whose activity correlated strongly with the rates of specific functions. Examination of phylogenetic relatedness and traits analysis reveal that guilds associated with denitrification are the most phylogenetically dispersed, followed by iron reduction, then methanogenesis. However, measures of general heterotrophic activity (such as high correlation to CO₂ production or high activity based on RNA/DNA ratios) do not fit this model. This work seems to support the proposal that there is a

continuum of phylogenetic relatedness to function. Our approach may provide a useful analytical procedure for identifying functional guilds of microbes in complex natural environments.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.016 PHYLOCHIP ASSAY FINDS DEEPWATER OIL PLUME ENRICHMENT OF PSYCHROPHILIC OIL-DEGRADING BACTERIA

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The blowout of oil in the Gulf of Mexico resulting from the explosion of the Deepwater Horizon drilling rig is the deepest and one of the largest oil leaks in history. In an effort to mitigate the environmental impact, a commercial surfactant been injected directly at the wellhead resulting in a dispersed oil plume at 1099-1219 m depth (average temperature 4-6°C). The PhyloChip assay, revealed a decrease in microbial richness coupled with an upsurge in the abundance of multiple deep-ocean psychrophilic Gammaproteobacteria within the plume compared to non-contaminated waters at corresponding depths. Sixteen distinct taxa were significantly stimulated including those of known petroleum-degraders previously identified in cold ocean waters, sea ice and circum-polar habitats. Compared to non-plume samples, the cell densities doubled as observed by direct microscopic counts. Sequencing of 16S rRNA genes confirmed both the decrease in richness and the population increase of Gammaproteobacteria within the plume. These results suggest that *in situ* bioremediation potential of the oil plume exists in the deep-sea, and the psychrophilic oil-degrading microbial communities could play a significant role in degradation of deep-sea hydrocarbons in the Gulf.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.017 THE BACTERIAL SOIL COMMUNITY IN A BRAZILIAN SUGARCANE FIELD

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The assessment of bacterial communities in soils gives insight into the behavior of these under different environmental conditions. In this context, we assessed the composition of soil bacterial diversity in a Brazilian sugarcane experimental field. The experimental design encompassed common sugarcane (variety SP80-1842) and its transgenic form (IMI-1 - imazapyr herbicide resistant). Plants were grown in field plots in a completely randomized design with three

treatments, which addressed the factors transgenic and imazapyr herbicide application. Soil samples were taken at three developmental stages during plant growth and analyzed using 16S ribosomal RNA (rRNA)-based PCR-denaturing gradient gel electrophoresis (PCR-DGGE) and clone libraries. PCR-DGGE fingerprints obtained for the total bacterial community and specific bacterial groups - *Actinobacteria*, *Alphaproteobacteria* and *Betaproteobacteria* - revealed that the structure of these assemblages did not differ over time and among treatments. Nevertheless, slight differences among 16S rRNA gene clone libraries constructed from each treatment could be observed at particular cut-off levels. Altogether, the libraries encompassed a total of eleven bacterial phyla and the candidate divisions TM7 and OP10. Clone sequences affiliated with the *Proteobacteria*, *Actinobacteria*, *Firmicutes* and *Acidobacteria* were, in this order, most abundant. Accurate phylogenetic analyses were performed for the phyla *Acidobacteria* and *Verrucomicrobia*, revealing the structures of these groups, which are still poorly exploited in their importance for soil functioning and sustainability under agricultural practices.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.018 MOLECULAR AND CULTIVATION-BASED ANALYSIS OF MICROBIAL COMMUNITIES IN OIL FACILITIES ON THE NORTH SLOPE OF ALASKA

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The energy industry is becoming increasingly aware that microorganisms present in oil reservoirs can degrade hydrocarbons and influence the corrosion of metal structures. However, much remains to be understood about the microbial populations responsible for these activities and the ecology of these high temperature anaerobic ecosystems. Molecular and cultivation-based surveys of microbial communities were conducted from various points within a major oil production facility on the North Slope of Alaska. Samples from processing/separator facilities, injection wells, seawater inlet lines, and a pipeline undergoing physical corrosion mitigation using a Pipeline Inspection Gauge (PIG) were included in a molecular survey of the microbial community using pyrosequencing. The PIG sample, which represents an enrichment of material associated with the inner surfaces of the pipeline, was also used for cultivation studies. With the exception of the seawater inlet line, the microbial communities were largely similar in all samples. These communities were dominated by OTUs identified as members of the genus *Thermovirga* and contained other microorganisms associated with high temperature anaerobic environments exposed to hydrocarbons. The relative abundance of some of these taxa did shift noticeably in the PIG sample, which was instead dominated by members of the genus *Thermoacetogenium*. Therefore, members of the genus *Thermoacetogenium* may be preferentially associated with the pipeline surface. Additionally, members of the genus *Bacteroides* that were obtained as cultivated isolates were also present preferentially in the PIG sample. The research reported here implicates members of the genera *Thermovirga*, *Thermoacetogenium*, and *Bacteroides* as important microorganisms associated with oil degradation and microbially influenced corrosion. These core taxa will therefore be targeted in

future work directed at monitoring and mitigating microbial processes deleterious to oil production.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.019 ENUMERATION AND IDENTIFICATION OF MICROORGANISMS IN HYPER-SALINE SOILS OF ATACAMA DESERT

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Soils of the Atacama Desert, northern Chile, have elevated chloride, nitrate and sulphate levels. The local climate endures very low humidity and wide day/night temperature variation and is thus considered one of the most arid and extreme environments on earth. This study entailed the search for, and identification of microorganisms inhabiting sediment materials at this place. Samples were obtained at various depths at three points in the Salar Grande area, located 90 km south east of the city of Iquique, North Chile 620 m above sea level. At the first point a sample pool was taken from the open pit salt mine "Tenardita", the second sample pool was obtained 3 km south west of the mine and the third sample pool was collected from 5 km north west of the mine. Both second and third samples had a high sulphate and nitrate salt content, forming a hard crust called "Panqueque". The microorganisms present were enumerated using DAPI counting and microbial population was identified via gene clone libraries of the 16s rRNA gene segment. Counts were low (0 to 2E+05 cells/g) in the first point samples and attempts to identify the microbial community were unsuccessful. In the second sample point counts ranged from 0 to 2E+06 cells/g and sequences related to heterotrophic archaea of the *Halorubrum* genus and bacterial strains, most of them closely related to *Vibrio* spp. and *Bacillus* spp. were obtained. At point three, counts ranged from 0 to 4E+06 cells/g, and sequences related to genus *Acidianus*, associated with sulphur metabolism, were found. Despite high salinity and extreme dryness encountered in the Atacama Desert, we detected archaeal and bacterial microorganisms from the surface to 2 m depth. We identified 16s rRNA sequences related to archaea and bacteria. Bacterial sequences were previously reported found in Atacama soil samples (Lester *et al.*, 2007; Drees *et al.*, 2006) but no previous reports of archaea were published.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.020 DYNAMICS AND ECOPHYSIOLOGY OF FRESHWATER BACTERIA DURING A PHYTOPLANKTON SPRING BLOOM

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Algal biomass in the prealpine Lake Zurich (Switzerland) strongly increases during spring, concomitant with the onset of thermal stratification. A large proportion of algal derived carbon is channelled through the microbial food web. Heterotrophic bacteria from distinct phylogenetic groups might consume different fractions of this organic carbon and show more or less resistance to food web related mortality factors (predation, viral lysis). They, therefore, might exhibit contrasting growth patterns during the phytoplankton spring bloom. We followed the temporal dynamics of numerically important bacteria using fluorescent in situ hybridisation and we assessed their uptake of Leucine, Thymidine and N-Acetyl-D-Glucosamine via micro-autoradiography. Bacteria affiliated with Cytophaga-Flavobacteria (CF), Betaproteobacteria (BET) and Actinobacteria (ACT) all seemed to profit from the spring phytoplankton bloom. CF and BET showed similar overall growth patterns; they were first to be stimulated by increasing concentrations of dissolved organic carbon (DOC), and they appeared to be strongly affected by viral lysis and protistan grazing. By contrast, their substrate incorporation patterns clearly differed. Moreover, while most BET were related to a single genus (*Limnohabitans* sp.) throughout the study period, there was a distinct shift within the CF community. ACT were mainly affiliated with the freshwater Ac1 lineage. They showed high incorporation of all substrates and thrived best during the period of high predation on Beta and CF. Our results hint at contrasting roles of the studied bacterial taxa during periods of sudden input of algal-derived DOC into freshwaters.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.021 EUKARYOTIC MICROBIAL DIVERSITY IN A HYPERSALINE LAKE AND ITS VARIABILITY WITH PROKARYOTIC COMMUNITY SHIFTS AND ENVIRONMENTAL FACTORS

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In face of short water supply in arid regions worldwide, many soil and freshwater environments are becoming increasingly saline. In order to understand these environments and the microbial processes taking place therein, we have undertaken a biological assessment of a hypersaline lake ecosystem. The prokaryotic, eukaryotic and viral community of Lake Tyrrell in Victoria, Australia have been analyzed in regards to seasonal and temporal changes. This study focuses on variations in the eukaryotic microbial community over a three and a half year timescale. A microscopic survey of the eukaryotic microbial diversity has been conducted at sites of different salinities around the lake. Major protistan community members and their abundances will be presented. We have also collected and analyzed genomic and biochemical samples during the summer and winter seasons of 3.5 consecutive years at Lake Tyrrell. Microscopic and phylogenetic (>1,000 near full length 18S rRNA sequences) surveys agree and show very low protistan diversity. Dominance of the photosynthetic microalga, *Dunaliella salina* (Chlorophyta), and the small predatory flagellate *Colpodella edax* (acomplexan Alveolate), suggest that protists play an important role in the microbial food web and in structuring the community. Changes in community composition based on phylogenetic analysis are compared with changes in

temperature, salinity, nutrient availability, and prokaryotic community shifts. Lower abundances and diversity were found at higher salinity sites. We conclude that the eukaryotic microbial community is an integral part of the microbial diversity of the overall ecosystem. The results provide insight to the full functioning of an ecosystem with all its biogeochemical and biological entities.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.022 BIODIVERSITY OF ALKALIPHILIC HALOARCHAEA ISOLATED FROM SODA LAKES OF THE WADI AN NATRUN, EGYPT

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Soda Lakes, with alkalinity and high salinity, are extreme but often highly productive environments. In general, the basic ecology of the alkali environments is poorly understood, partially due to the lack of cultivability of prokaryotes from those environments. We employed culture-dependent techniques to study alkaliphilic haloarchaeal diversity in sediments and water samples of four large soda lakes in Wadi An Natrun, Egypt. Eight sediment samples and five water samples were obtained for physico-chemical and microbiological analyses. Physico-chemical analysis showed that the samples contain sufficient ions and high pH to support alkaliphilic haloarchaea. Archaeal isolation was performed by using ten different complex media with different carbon sources (glycerol, starch, yeast extract), salinity (15% - 25% NaCl) and pH (9-10.5). Samples of sediments and water from different lakes were used as inoculants and enrichments were incubated at 37°C for 3-4 weeks. Water samples of different lakes contained 10² to 3.5x10⁴ cfu/ml and sediments of different lakes contained 10² to 10³ cfu/g. Diversity and frequency of the genera were also occurred. A total of thirty-one morphologically different alkaliphilic haloarchaeal isolates were selected for phenotypic and genotypic characterization. Cells were chemo-organotrophic. They are strict aerobes, motile and extremely pleomorphic cells except strains WN3 and WN31 which are non-motile and coccoid-form. Also, most of the isolates have the ability to grow on wide range of salinities of 10-35% (w/v) NaCl, pH 8.5 to 11 and temperature range 25-65°C. However, the optimal growth obtained on 25% (w/v) NaCl with pH 9.5 at 37°C. Phylogenetic analysis based on 16s rRNA gene sequencing showed that the isolates belonged to the genera Natronococcus, Natronomonas, Natronorubrum and Natrialba.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.023 COMPARATIVE DIVERSITY ANALYSES OF FOUR THROMBOLITIC MAT TYPES FROM HIGHBORNE CAY, BAHAMAS USING HIGH-THROUGHPUT PYROSEQUENCING

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Thrombolites are clotted organosedimentary structures that form as a result of the metabolic activities of microbial mats. In this study we investigated the eubacterial, cyanobacterial, and archaeal lineages of four spatially distinct thrombolitic mats (black, beige, pink, button) of Highborne Cay, Bahamas using pyrosequencing of 16S rRNA genes. MOTHUR was used to process 16S rRNA reads from the amplicon libraries into operational taxonomic units (OTUs) at a 3% distance and generate diversity indices. OTUs were characterized phylogenetically using Megablast and TaxCollector (www.microgator.org). In all mat types, Alphaproteobacteria dominated the eubacterial libraries with sequences similar to phototrophic purple non-sulfur bacteria and methanotrophs, followed by Gammaproteobacteria with many sequences similar to known sulfide oxidizing bacteria. The most prominent differences between the four communities were within the cyanobacterial and archaeal populations. The beige and button mat types were rich in filamentous mat-building Oscillatoriales including *Leptolyngbya* spp., which are also present in the adjacent stromatolites. The button mats, however, were unique in that many of the recovered sequences were similar to filamentous *Diclothrix* spp., which are known to be associated with calcified filaments in thrombolites. Archaeal diversity in all mat types was low, representing less than 10% of the total microbial diversity. Sequences in the black and beige mat types were dominated by *Nitrosopumilus* spp., which are known ammonia-oxidizers. The button mat type, however, contained the highest archaeal diversity with most sequences similar to heterotrophic *Aciduliprofundum* spp. and methanogenic *Methanobacteria* spp.. Together, this study represents the first in-depth diversity analysis of thrombolitic mat communities using high-throughput pyrosequencing and provides a foundation for understanding the interactions and metabolisms of these complex microbial communities.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.024 MICROBIAL DIVERSITY ASSOCIATED WITH THE BASALTIC CO₂ INJECTION PILOT SITE AT THE HELLISHEIDI GEOTHERMAL POWER PLANT, ICELAND

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One of the options for reducing the anthropogenic CO₂ emissions in the atmosphere and the associated environmental consequences is to capture the gas emitted by industrial activities and to inject it into the deep subsurface. However, a diverse and abundant biosphere might be present at the storage sites that may have a positive impact as to promote the conversion of CO₂ into solid carbonates, or negative ones as the production of CH₄ or the clogging of the injection pipes. In order to assess and monitor the reactions taking place between this deep biota and the injected aqueous CO₂, we investigate the evolution of the microbial diversity associated with the Icelandic pilot site at the Hellisheidi geothermal power plant. This site is designed to study the feasibility of storing permanently CO₂ in basaltic rocks. Prior to the injection, the microbiological initial state was characterized through a regular sampling at various seasons. DNA was extracted and amplified for the observatory wells after filtration of 10 to 30 l of

groundwaters collected in the depth interval 400-980 m with a sampling protocol aiming at reducing contamination risks. An inventory of living indigenous bacteria and archaea was then performed using molecular methods based on the amplification of small subunit ribosomal RNA genes. After injection, the evolution of these microbial communities will be monitored using the Denaturing Gradient Gel Electrophoresis technique. The stratigraphic levels targeted to store the injected CO₂ harbor a large diversity of bacteria from the Delta-, Beta-, Gamma-proteobacteria, Firmicutes and Deinococcus-Thermus phyla. Euryarchaeota and Crenarchaeota close to archaea detected in subsurface or hydrothermal environments were also highlighted. Moreover, a large fraction of the identified microorganisms are very distantly related to any described species or environmental sequences analyzed so far and might represent new lineages of subsurface associated microorganisms.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.025 MOLECULAR ANALYSIS OF THE INDOOR AIR MICROBIAL DIVERSITY

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Like what we eat or drink, the air we breathe is essential to our survival. One person inspires every day not less than 14 m³ of air and so, is continuously subjected to many microbial contacts. By reading this page, you will breathe more microorganisms than you will see in a lifetime through pictures. Each day, most people spend 80 % of time indoors, where it is known that airborne microorganisms are common microbial contaminants, making microbial quality of air a particularly relevant matter. Indeed, airborne microorganisms are involved in some respiratory symptoms, allergies or infections among occupants. This work aims to use molecular tools in order to consider airborne microbial diversity and load. Such study could provide a better description of indoor airborne microbiology.

Air samples were obtained using an experimental high volume bioaerosol collector located in two different places (a museum and an open space office). Airborne microbial diversity and counts were assessed using molecular tools.

The first results show a load of fungi comprised between $2,9 \cdot 10^3$ and $8,6 \cdot 10^4$ GE/m³ of air and a load of bacteria comprised between $2,5 \cdot 10^5$ and $4,6 \cdot 10^5$ GE/m³ of air in the museum. In the office bacterial concentration reaches $4,9 \cdot 10^6$ GE/m³ due to high confinement while fungal concentrations observed match with those found in the museum. The Single Strand Conformation Polymorphism (SSCP) analysis shows a high diversity in indoor air and a relative stability of the bacterial community structure in a same place during a short time (few hours). On the contrary, outdoor studies show daily variations in bacterial community. As for eukaryotes, disparities exist in the community structure over a short period of time.

Pictures of the microbial diversity in enclosed spaces highlight an unsuspected diversity of the microbial communities. Monitoring microbial communities variations could bring some elements about spatial and temporal diversity.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.026 SOIL MICROBIAL COMMUNITIES AND N PERCOLATION IN SOIL COLUMNS AMENDED WITH UNTREATED OR FERMENTED CATTLE EXCRETA

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The expansion of farm-scale biogas plants has increased the landspreading of anaerobically digested slurry, replacing manure. We investigated if this change would alter the soil microbial community. We also tested if the anaerobic treatment would reduce the release of pathogens and increase the leaching of inorganic N to the environment. We applied 80 kg N ha⁻¹ of cattle excreta or anaerobic slurry to γ - and non-irradiated soils. Soil DNA was extracted after 0, 1 and 3 months of incubation at 20°C. Fungal and bacterial community structure was examined by DGGE. *E. coli*, *Listeria* spp. and *Salmonella* spp. were cultivated. A 100 d percolation experiment was performed and leached NH₄⁺-N and NO₃⁻-N measured.

Cluster analyses of fungal and bacterial banding patterns gave similar results. Two clades separated sterile from non-sterile treatments. Non-sterile soils were grouped according to incubation times, whereas sterile soils were mostly clustered based on amendment type. This suggests that allochthonous microbes were able to thrive in the soil, but not in the presence of autochthonous competitors. *Salmonella* and *E. coli* could not be isolated from the slurry or slurry amended soils, while they were present in soils amended with manure. *Listeria* was the most abundant in the excreta (15.4×10⁴ CFU g⁻¹), persistent in the digester and in the soil (1.7×10³ CFU g⁻¹). After 3 months, potential pathogens in amended soils were not more abundant than in the control. More CFUs were found in γ -irradiated and amended soils than in their non-sterilised counterparts indicating pathogen control by the autochthonous soil microbiota. Ammonia in the slurry was four-fold of that in the manure, while nitrate contents were similar. However, vertical losses of ammonia were comparable in all treatments, whereas those of nitrate were significantly higher in slurry amended soils. Nitrate leakage due to slurry application needs testing at various times and including cultivation of plants

Abstract Category

32 Biodiversity and Community Analysis

PS.32.027 ANALYSES OF THE MICROBIAL COMMUNITY IN FIBER AND EPITHELIAL FRACTIONS OF THE CROP OF CHICK AND ADULT HOATZINS USING 16S PYROTAGS

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The hoatzin (*Opisthocomus hoazin*) is a South American folivorous bird unique among known avian species due to its fermentative and enlarged crop that harbors an impressive array of microorganisms. The aim of this study was to profile the microbiota in the fiber and epithelial fractions of the crop from chick and adult hoatzins, previous to select samples for metagenomic analyses based on community stability (reproducibility) and presence of potential fiber degrading species. Genomic DNA was extracted from crop fiber and epithelial material of 7 chicks, 1 juvenile and 7 adult birds, with ~ 3 biological replicates per sample. The V8 region of the 16S rRNA gene was amplified and pyrosequenced and reads were binned and classified with Pyrotagger using a 10% quality filter and 97% sequence identity. Our results demonstrate that the chick samples were highly non-reproducible compared to the adult ones and that on average the adult crop is significantly richer than that of the chicks with no differences between the fractions. Among the non-bacterial biota, *Methanomicrobia* (archaea), *Apicomplexa* (protozoa) and *Dikaryotes* (fungi) dominated the chicks' crop while the juvenile and adults were dominated by *Methanobacteria* (archaea) and *Dasytricha* (ciliates). The adult epithelial and fiber fractions were dominated by *Methanomicrobia* and *Methanobacteria* respectively. The dominant bacteria were *Bacteroidetes* and *Firmicutes*, with lesser representation of 35 other phyla and the lineages that discriminate between fiber and epithelial fractions include *Prevotella* and other *Bacteroidales*, which were more abundant in the fiber fraction. These results confirm the presence of some of rare phyla (as detected by the PhyloChip) and show that *Prevotellaceae* is the most abundant and ubiquitous crop bacterial family, consistent with hemicellulolysis being a major fiber-degrading function. Our data suggests that the adult fiber fraction samples are the most interesting for metagenomic studies.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.028 MICROBIAL COMMUNITY STRUCTURE IN VOLCANIC DEPOSITS WITH DIFFERENT SUCCESSION STAGES OF VEGETATION RECOVERY IN MIYAKE ISLAND, JAPAN

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Early ecosystem development on newly deposited volcanic substrates has been focused from the aspect of soil genesis. The purpose of this study was to analyze the microbial community in volcanic deposits with different stages of vegetation development after the volcanic eruption. Our study sites (IG7 to IG9) were established on a 2000 volcanic ash deposit in the island of Miyake, Japan. The 2000 eruption at O-yama, an active volcano in the island, destroyed 60% of forest area in the island. In this study, the 2000 volcanic ash deposits (C1, upper layer; C2, lower layer) and soils (2A layer) buried under the volcanic deposit were sampled at three sites covered with little herbaceous vegetation (IG7), herbaceous vegetation (IG8), and herbaceous and ligneous vegetation (IG9). Samples were analyzed by culture-based methods (plate counts and Biolog Ecolplates) and 16S rRNA gene-targeted terminal restriction fragment length polymorphism (T-RFLP) profile. The bacterial population densities of the C1 and C2 layer deposits were higher in the samples IG8 and IG9 than in those of the sample IG7, suggesting that

the bacterial population density in the volcanic deposit was dependent on the vegetation cover level. The C1 layer T-RFLP profile contained 27, 36, and 32 of detectable terminal restriction fragments (T-RFs) for sites IG7, IG8 and IG9, respectively. The major T-RFs of the C1 layer was found at 574 base for IG7, at 193 and 219 base for IG8, and at 428 and 486 base for IG9, suggesting that the development of bacterial community in the C1 layer would be dependent on the above-ground vegetation development. The T-RFLP profiles of C2 layers were similar to those of 2A layers and composed of 57-116 T-RFs. Further analysis of volcanic deposit and soil bacterial communities using 16S rRNA gene cloning library is now in process to know the more detailed bacterial succession during the ecosystem development.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.029 MONITORING THE MICROBIAL COMMUNITY DYNAMICS DURING ACETIC ACID FERMENTATION OF ZHENJIANG VINEGAR BY DGGE AND RT-PCR

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Molecular approaches based on microbial small subunit rRNA gene were applied to investigate the diversity and population of bacteria and fungi during vinegar fermentation of Zhenjiang Vinegar, which is the most famous seasoning producer of vinegar in China. The specific primers P2/P3 and NS3/YM951r were used to amplify bacterial 16S rRNA gene and fungal 18S rRNA gene during the fermentation process, respectively. The DGGE profile indicated that the bands were well separated and the microbial diversity has a disciplinary series change in whole fermentation. Clone libraries (consisting of 1,143-bp inserts of bacterial 16S rRNA gene fragments or 398-bp of fungal 18S rRNA gene fragments that were amplified) were constructed. A total of 100 clones were sequenced. The results showed that bacteria present in the process of acetic acid fermentation were genus *Lactobacillus*, *Acetobacter*, *Gluconacetobacter*, *Staphylococcus*, *Enterobacter*, *Pseudomonas*, *Flavobacterium*, *Sinorhizobium*, etc., and the fungi present were genus *Saccharomyces*. The similarities were in a range of 96.84% to 100%. The results of quantitative RT-PCR suggested that the biomass of bacteria and fungi showed decreasing trend within the acetic acid fermentation. From the results we deduced that the aerobic solid-fermentation technique of Zhenjiang Vinegar may be different from traditional solid-fermentation, and the environment may be microaerobic or even partially anaerobic. This may be one of the primary reasons why there are many species of *Lactobacillus* and *Saccharomyces* during the acetic acid fermentation period. And our results also revealed that DGGE combined with sequence analysis is an effective technique for monitoring and analyzing the microbial community succession during the acetic acid fermentation of Zhenjiang Vinegar.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.030 EFFECTS OF MERCURY POLLUTION ON MICROORGANISMS ISOLATED FROM SOIL SAMPLES, WINDOWS AND LEAVES

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Background and aims: The aim of this study was to demonstrate the effect of long term mercury pollution in the selection of mercury resistance bacteria by comparison of the microflora of two air polluted mercury sites with one unpolluted control site.

Methods: The work was done in Lima city, Peru. Comparisons were made of soils, windows sediments and leaf surface microflora of (i) east Lima area polluted with mercury in the air, (ii) north Lima area polluted with mercury in the air, and (iii) clean Lima area without levels of mercury in air. Total viable counts of bacteria and determination levels of mercury resistances were made in these areas. Also, the mercury content in soil was determined by using EPA method 7470 A. A Probit analysis to obtain LC50 was applied to these results.

Results: The mean soil mercury levels were 3090, 4100 and 3680 mg Hg /Kg for clean area, east Lima and North Lima, respectively. LC50 were 13.82 and 13.67 in sediments and soil in clean area. LC 50 for sediments of mercury polluted areas were 17.55 and 17.45 while LC50 for mercury polluted soil were 34.53 and 36.34. There were a significative diference of mercury resistant microorganisms from soils and sediments between polluted and unpolluted samples. Results from leaf microorganisms also have shown that it is possible to isolate higher mercury resistance levels bacteria in mercury polluted zones.

Conclusions: It was demonstrated that microorganisms growing in mercury disturbed sites are adapted to persist in these environments by developing mercury resistant populations

Abstract Category

32 Biodiversity and Community Analysis

PS.32.031 IMPACT OF BIOGEOGRAPHY AND MICROBIAL COMMUNITY STRUCTURE AS DETERMINED BY PYROSEQUENCING ON THE SURVIVAL OF ESCHERICHIA COLI O157:H7

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Biogeographic patterns in microbial communities are traditionally explained by two factors, the environmental heterogeneity and historical events. In this study we investigated how biogeography of 15 eastern China soils affects the survival of E. coli O157:H7. Methods: Bacterial, archaea, and fungi community structures from these soils were investigated using 454 pyrosequencing technique. Survival of E. coli O157:H7 in the 15 soils collected from different geographic zones, coupled with the determinations of physical, chemical and biological properties of each soil by standard methods were done.

Based on pyrosequencing data, Actinobacteria, Proteobacteria, and Firmicutes were the dominant phyla from the 15 soils with the percentage of Firmicutes increasing from the cooler latitude (north) to the warmer latitude (south). Crenarchaeota and Euryarchaeota were the

dominant archaea phyla, whereas Ascomycota, Chytridiomycota and Neocallimastigomycota were the dominant fungi phyla. Bacterial community structures were grouped by two clusters consisting of seven and eight soils based on latitude. *E. coli* O157:H7 survival times in days (td) were between 1.4-26 days. The longer survival of *E. coli* O157:H7 was observed in northeastern Chinese soils, while in southeastern Chinese soils *E. coli* O157:H7 survived significantly shorter. Stepwise multiple regression analysis of td values showed that the survival of *E. coli* O157 was strongly correlated with soil pH, assimilable organic carbon (AOC), and microbial biomass carbon (MBC) and microbial community structure.

Pyrosequencing data of the 15 soils revealed two to three clusters representing north south direction of eastern China soils which differ significantly with the survival of *E. coli* O157:H7 decline.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.032 SOIL MICROBIAL COMMUNITIES IN APPLE ORCHARDS UNDER DIFFERENT MANAGEMENT PRACTICES

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Soil is a highly complex environment that is nutrient poor and microbial communities exist in discrete microhabitats which are influenced by the heterogeneity of soil. Soil communities are essential in the geochemical cycles and the productivity of terrestrial ecosystems. Agricultural management practices involve the enhancement of organic matter in soil in order to increase nutrient availability to plants. It is thus important to understand the distribution of microbial communities in soil and how they respond to soil treatments. The study evaluated the effects of different management practices on the microbial community structure and diversity in soils from an apple orchard over two years. Microbial communities were profiled using Automated Ribosomal Intergenic Spacer Analysis (ARISA). The fragment lengths of the highly variable internal spacer regions (ITS) were amplified using fungal and bacterial specific primers and converted into corresponding operational taxonomic units (OTU). Shannon (H') diversity indices were calculated and evaluated in Repeated measures ANOVA. Whittaker similarity (SW) indices were converted to similarity matrices and analysed using non-parametric multivariate analysis of similarity (ANOSIM). Microbial communities in apple orchards were found to be heterogeneous and bacterial diversity was consistently higher than fungal diversity in all samples. Mulch treatments showed an increase in bacterial diversity compared to other treatments. Differences in community structure were observed between all treatments with significant differences ($p \leq 0.05$) for fungal community structure during the growing season. This study showed that although different management practices may not have a significant effect on the diversity of microbial communities, the structure of the communities was significantly influenced.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.033 LARGE-SCALE BACTERIAL COMMUNITY STRUCTURE ANALYSIS OF UK SOILS

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Soil bacteria are an important group of microorganisms critical to many biogeochemical cycles and contribute to overall soil health. However, very few large-scale surveys have examined the biogeography of these microbes. In addition, there is uncertainty whether soil bacterial community structure, diversity and population distributions are governed by similar ecological principles to that of macro organisms. Understanding the factors that influence soil bacterial community structure is important to assess how these microbes may respond to future environmental change, and to help create a basic ecological framework for these organisms upon which future studies can be based. The primary aim of the study was to examine bacterial community structure and composition in over 1000 UK soils, sampled as part of the UK Countryside Survey 2007, using terminal restriction fragment length polymorphism (tRFLP) analysis, and cloning and sequencing. A variety of soil types were sampled from a wide range of habitat classifications, with concurrent measures of edaphic conditions, climatic variables and dominant plant species, enabling identification of the key drivers of soil bacterial diversity at a country-wide scale. We present an interpolated map representing broad scale changes in bacterial community structure across the UK. We show a significant increase in phylotype diversity with increasing pH, broadly consistent with emerging hypotheses. In addition, significant effects of all measured environmental variables were found, and bacterial communities responded to environmental gradients in a similar manner to plant communities. In addition spatial relationships were observed in the residual variance, suggesting possible biogeographic patterns. We therefore conclude that there is no evidence to suggest that soil bacterial communities follow different ecological laws to those of larger terrestrial organisms, where spatial and ecological criteria are key factors.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.034 SHIFTS IN MICROBIAL COMMUNITY COMPOSITION CORRESPOND WITH INCREASING URBANIZATION OF LAKE SHORELINES

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Urbanization has wide-ranging impacts on the organisms and functional processes in lake ecosystems. Bacteria play an important role in lake food webs and mediate essential ecosystem processes, therefore, understanding their community structure offers potential insight into ecosystem function. We examined bacterial community composition and abundance from 21 lakes along an urban gradient in the Pacific Northwest, U.S.A., which span a range of anthropogenic phosphorus (P) loads and productivity levels. A primary goal of this work is to understand how patterns in the abundance, richness and composition of the bacterial community correspond with changes in the productivity and biogeochemistry in lakes. Measured

environmental variables explained at least 60% of variation among lake characteristics; the majority of the variance among lakes was explained by a combination of productivity measures: total P, chlorophyll a, total nitrogen (N) and secchi depth. Bacterial abundance differed significantly among lakes and was most closely related to total P concentration. We used Automated Ribosomal Intergenic Spacer Analysis (ARISA) to examine bacterial community composition among and within lakes. Observed richness and bacterial community composition differed significantly among lakes and among stratified layers within lakes. The highest richness was observed in the hypolimnion, but community composition in the epilimnion was most distinct from the meta- and hypolimnetic communities. Total N concentration appeared to explain the most variation in community composition among lakes. Our results suggest that bacterial community structure is strongly related to patterns in urbanization and eutrophication of lakes and may play a role in how aquatic systems respond to increasing anthropogenic impacts.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.035 MICROBIAL PHYLOGENETIC DIVERSITY OF PHOTOTROPHIC MATS ASSOCIATED WITH EXTREMELY HALOPHILIC LAKE IN NORTHCENTRAL, WASHINGTON

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Autotroph-heterotroph microbial associations formed the foundation of the biosphere nearly 3 billion years ago with oxygenic photosynthetic prokaryotes and their associated heterotrophic partners colonizing shallow ocean zones. These associations are common planet-wide, representing metabolically interactive, self-sustaining communities that can assemble into highly organized microbial mats in extreme environments. Hot Lake is a highly saline epsomitic (MgSO₄) shallow meromictic lake near Oroville, Washington that contains a benthic microbial mat. The pH of the lake water was 8.5 and the Mg and sulfate concentrations at the time of sample collection were 5.2-9.8 g/L and 45-68 g/L, respectively. Pyrosequencing revealed many taxonomic units consistent with phototrophy, chemheterotrophy, sulfur cycling, and halotolerance. The most abundant phototrophs, based on nearest-neighbor phylogeny, included the filamentous cyanobacterium *Tychonema* and the phototrophic sulfur bacteria, *Halochromatium*. *Thiohalocapsula*, a purple sulfur bacterium, was also present as were purple non-sulfur bacteria affiliated with *Roseovarius* and *Rhodobaca*. Also present were many phyla representative of halotolerant, sulfur-respiring bacteria including *Halothiobacillus* and sulfate-reducing bacteria including *Desulfonatronum* and *Desulfotignum*. Laboratory microcosms were initiated from mat subsamples diluted into lake water resulting in a stratified community with green filamentous cyanobacteria at the surface with purple and brown underlying layers. Fritted glass squares were deployed vertically in the microcosm to allow the consortia to grow into the porous matrix and maintain stratification when removed for subsequent spatially-resolved analyses. Future research will include flow cytometry and cryosectioning in combination with the tools of systems biology to further characterize the functions and interactions among members of this mat community as a function of vertical position.

Abstract Category
32 Biodiversity and Community Analysis

PS.32.036 PARTICLE ATTACHMENT AS A HABITAT FILTER FOR LAKE FRESHWATER BACTERIAL ASSEMBLAGES

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In aquatic systems, particle-associated and free-living bacterial assemblages are generally distinct and respond differently to ecological drivers. Composition and dynamics of algae, in particular, may have a stronger influence on attached bacteria through access to algal resources. To better understand forces that influence bacterial assemblages with free-living and attached lifestyles, this study characterized and compared particle-associated and free-living bacteria in a suite of lakes over time. Particle-associated (PA, > 5 µm) and free-living (FL, < 5 µm) bacteria were collected from five humic lakes three times from July through August 2008 and from three of the lakes in July 2009 and characterized using automatic ribosomal intergenic spacer analysis. There were significant differences in community composition between fractions (ANOSIM R=0.556, p<0.001) and among bacterial communities from different lakes (ANOSIM R=0.520, p<0.001). Despite lake-specific variation in bacterial communities, particle-associated and free-living bacterial assemblages form distinct groups in ordination space, suggesting that attachment acts as a habitat filter structuring microbial communities in these lakes. Similarity in algal taxa and inputs of Sphagnum-derived organic matter into each lake may account for similarities in particle-associated bacteria. Within a lake, correspondence analysis demonstrated that particle-associated assemblages were more variable than free-living assemblages over time (mean centroid distance: PA=0.57, FL=0.20), potentially indicating that attached organisms utilize a more temporally variable resource base. rRNA sequence analysis was used to identify bacteria that were enriched in the particle associated fraction. These observations support the idea that attached bacteria are more strongly influenced by the temporally variable algal resource base and suggest that particles, inclusive of algal cells, play a pronounced role in community assembly.

Abstract Category
32 Biodiversity and Community Analysis

PS.32.037 AN ANALYSIS OF THE DIVERSITY AND PREVALENCE OF ENVIRONMENTAL MYCOBACTERIA (EM) WITH VARYING AGRO-ECOLOGICAL ZONES

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Environmental mycobacteria are ubiquitous species commonly detected in a variety of sources including soil, water and dust. The group are separate from the more pathogenic *M.tuberculosis* complex and hold clinical significance as they are capable of causing opportunistic disease especially in those that are immunocompromised. In addition, EM are hypothesised to be

responsible for the reduced BCG efficacy in lower latitude tropical countries. It is becoming increasingly important to determine the ecological variables which affect prevalence and diversity of EM species in the environment. For this study soils were chosen in a sampling location with a wide range of agro-ecological zones and varying climate region. Soil and water samples were collected from a total of 42 domestic locations in 9 different climatic regions. To determine EM composition, DNA was extracted and microbial tag-encoded Titanium amplicon pyrosequencing employed to target the 16S rDNA operon specific for the Mycobacterium genus and selected slow growing mycobacteria. Using multiphasic and bioinformatic techniques, an assessment of EM communities was achieved. This included the construction of rarefaction curves to assess sampling effort and phylogenetic analysis between communities to determine the effect of environmental variables. The study highlighted a wide diversity of EM species and differences based on the nature of the source. Community analysis demonstrated significant differences in EM profiles in different climatic and agro-ecological zones. The outcome of the study may have implications for the clinical relevance of EM, leading to a heightened interest into the correlation of human immune responses to EM exposure in these locations.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.038 INSIGHTS INTO THE INTERACTION BETWEEN *MICROCYSTIS AERUGINOSA* KW AND HETEROTROPHIC BACTERIA THROUGH THE COMMUNITY-SEQUENCING APPROACH

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Several heterotrophic bacteria have been suggested to be associated with *Microcystis aeruginosa* which cause toxic algal-blooms in eutrophic lakes or ponds. However, little is known about ecological relationships and physiological interactions between heterotrophic bacteria and *M. aeruginosa*. In this study, whole genomes in the culture solution of *M. aeruginosa* KW, which was maintained as uni-algal and non-axenic culture after it was sampled from Uiwang reservoir in Korea, were determined by two rounds of pyrosequencing, first on the total DNA in the solution and then on the phi29-amplified KW DNA. 16S rRNA sequences that belong to those of *Alphaproteobacteria*, *Actinobacteria*, *Bacteroidetes*, and *M. aeruginosa* were found in the assembled sequences through a homology search to the SILVA database. Most of the potential cyanobacterial genomic sequences could be aligned to the genomes of other completely sequenced *M. aeruginosa* strains, and many of the other sequences had significant similarities to those of *Rhizobiales* and *Actinomycetales*. Whole metagenomic analysis of the non-axenic culture of *M. aeruginosa* thus provides important clues to understanding the nutritional relationships such as the exchange of carbon and nitrogen sources among cyanobacteria, nitrogen-fixing *Alphaproteobacteria*, and other heterotrophic bacteria.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.039 ISOLATION AND IDENTIFICATION OF THERMOPHILIC BACTERIA SPECIES FROM HOT SPRINGS IN TURKEY

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Thermophilic bacilli grow best at temperatures between 45°C and 70°C, and therefore, it is possible to isolate them from different environments such as permanently cold habitats, deep ocean-basin cores, shallow marine hot springs, petroleum reservoirs, deep-sea hydrothermal vents and the leachate of a waste pile from a canning factory. The aim of this study was to identify and characterize thermophilic bacteria isolated from Yoncali, Ilica, Eynel, Göbel, Ömerli and Hudai hot springs in TURKEY. In this study, thermophilic bacteria were isolated from Yoncali, Ilica, Eynel, Göbel, Ömerli and Hudai hot springs in TURKEY. The water temperature and pH of hot springs was around 55-65°C and 8-9.5 respectively. The water samples which are collected were immediately transferred to laboratory and inoculated in nutrient broth (NB) and incubated at 55-70°C. Firstly, cultures obtained were purified and grouped based on their Gram reaction. Isolates were identified according to colony morphology, microscopic characteristics, nutritional characteristics, and cellular fatty acid profiles. The selected bacteria isolate was transferred on BUG medium and prepared suspension was inoculated in Biolog plates as per the standard protocol provided. All the media and Biolog plates were incubated for 24 to 48 h at 60°C temperature. Results were recorded and interpreted for identification using Biolog software. And then, the growth capacity of these isolates was investigated at the different salt concentration. Moreover, their enzyme activity was determined using API ZYM kit (BioMerieux). As a result of these identification tests, these isolates were belong to *Geobacillus*, *Bacillus*, *Microbacterium*, *Jonesia*, *Carnobacterium*, *Brevundimonas*, *Brevibacterium*, *Actinobacillus*, *Corynebacterium*, *Curtobacterium*, *Sanguibacter*, *Paenibacillus*, *Tsukamurella*.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.040 VARIATIONS IN MICROBIAL ABUNDANCE AND COMMUNITY STRUCTURE DURING *Microcystis* BLOOM IN AN EXPERIMENTAL POND

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Cyanobacterial blooms mainly composed by *Microcystis aeruginosa* are a serious symptom of lake eutrophication. Since the biomass of *M. aeruginosa* usually reaches to a high level in a bloom, the abundance and composition of heterotrophs dependent on the cyanobacterial primary production should be largely affected. However, variations in microbial community structure during the blooms are not well understood. To understand the changes in abundance and composition of microbial community during a *M. aeruginosa* bloom, we followed variations in ciliate, amoebae, heterotrophic nanoflagellate and bacterial abundance and community structure in outdoor experimental ponds. Lake Biwa plankton condensed using a 5 µm plankton net were respectively inoculated to three experimental ponds, pond A, B and C at a proportion of 5: 1: 0. Inorganic nutrients were supplemented to all ponds every two weeks. After 17 days, we found high abundance of amoebae and fluctuations of ciliates in pond A. After 35 days, *Microcystis*

bloom was detected in pond A, though no bloom was found in ponds B and C. Bacterial abundances rapidly increased after the bloom, while no significant increase in bacterial abundance was detected during the bloom. Catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH) showed the proportion of α -, β -, γ -proteobacteria and *Cytophaga-Flavobacterium* cluster (CF) before the bloom as 9.6 %, 40.0%, 6.2% and 13.8%, respectively. However, during the bloom, the detection of these phylogenetic groups decreased to less than 10% of total bacterial abundance. At the decline of the bloom, the proportion of each group was 16.6 %, 5.2%, 0.6% and 0.5%, respectively. Thus, bacterial community structure changed markedly during the wax and wane of *Microcystis* bloom in the present study, and we discussed the changes in bacterial community in relation to some biological and physico-chemical parameters.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.041 BACTERIAL COMMUNITY IN CRUDE OIL FROM TWO BRAZILIAN PETROLEUM FIELDS

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Bacterial community in crude oil samples with different Basic Sediments and Water (BSW) values from two offshore platforms (PA and PB) in Campos Basin, Brazil, were investigated by 16S rRNA gene clone library. Samples containing high BSW were named as PAH and PBH, while low BSW oil samples were named PAL and PBL. DNA extraction and PCR amplification are usually challenging for this type of sample. However, in this study, DNA was effectively extracted and 16S rRNA gene was successfully amplified after thoroughly washing the oil samples with Winogradsky Buffer and with incubations intervals at 40 °C for 30 min. BlastN identification and RDP classification tools were used to analyze a total of 157 clones obtained with pJET vector and the sequences showed that Gammaproteobacteria was related to ca. 76.4% of total clones, mainly affiliated with the genus *Pseudomonas*. In addition, clones affiliated with Betaproteobacteria (8.9%) and Firmicutes (1.9%) were also identified in all libraries. Principal component analysis (PCA) showed that the origin of oil samples influenced the bacterial diversity. Nevertheless, high BSW concentration of oil samples reduced the bacterial richness (Shannon index; LOW BSW: PAL=1.226694; PBL=0.923567; HIGH BSW: PAH=0.587501; PBH=0.818924). Most of the bacteria affiliated with the sequences of both platforms belonged to similar phylogenetic groups. The genera *Janthinobacterium*, *Comamonas*, *Acinetobacter*, *Bacillus* and *Stenotrophomonas* were usually observed. In this study, the bacterial groups that were detected are a potential risk to biodegradation of petroleum.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.042 COMMUNITY COMPOSITION OF MICROORGANISMS ATTACHED TO OIL IN A MESOTHERMIC OIL FIELD

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Oil is frequently produced by water injection. Wells in many mature, on-land reservoirs typically produce 5-10% oil and 90-95% water, with the water cut increasing with time. Microbially-enhanced oil recovery is one of the technologies helping increase the fraction of the recovered oil. Successful application of this technology requires thorough knowledge of the resident microbial community. Here we report the microbial community of the Enermark field, near Medicine Hat, Alberta that is attached to oil. Microorganisms attached to oil will be present in core matrix pores, where oil is trapped in micro-droplets. These microorganisms may have a greater oil recovery potential than those in the aqueous phase.

Produced water samples that contained oil were separated into oil and water phases in a separatory funnel. The water fraction was drained off and replenished by synthetic brine. Following shaking and phase separation, the synthetic brine was removed and the process was repeated at least twice to remove remnants of the produced water. Oil-associated biomass was then dislodged from the oil by adding synthetic brine, shaking and centrifuging at 25,000xg for 20 min. The principle of the separation is that whereas cells, which have a higher density than water, can bind to oil and go up into the oil phase at 1xg, they cannot do so at 25,000xg: instead, they will form a pellet. DNA was isolated from the cells obtained with this procedure and subjected to amplification via Polymerase Chain Reaction with bacterial and archaeal 16S primers. Amplicons were analyzed through Denaturing Gradient Gel Electrophoresis.

Significant differences were seen between oil- and water-associated fractions. The former were less diverse than the latter and included representatives of the Bacteroidetes, Desulfuromonadaceae, Spirochaetes, Pelobacteraceae (Bacteria), and Methanosarcinaceae (Archaea).

Abstract Category

32 Biodiversity and Community Analysis

PS.32.043 MICROBIAL STRUCTURE IN DIFFERENT LAND USES IN SOUTHWEST AMAZON BY T-RFLP

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The Southwest Amazon is a region with highest dynamic agriculture expansion worldwide in nowadays. Tropical forests and Cerrado (Brazilian savanna) are converted rapidly to pasture and agriculture. Following deforestation, soil C and N can be lost to atmosphere in form of greenhouse gases (CO₂, CH₄ and N₂O) and several chemical changes occur in soil due to fertilization and agricultural management. Changes are also expected in the microbial structures of the different land uses. The objective of this work was to compare microbial structures in Southwest Amazon Cerrado and Forest soils following land use changes to agriculture (soybean

— *Glycine max* L. Merrill) and pasture (*Brachiaria brizantha* A. Rich.) by Terminal Restriction Fragment Length Polymorphism (T-RFLP). Soil samples were taken from the three different land uses (native vegetation, soybean field and pasture), in two cities: Sinop (Tropical Forest - S 120553.3 W 552846.0) and Campo Verde (Cerrado - S 151588.8 W 550700.0); with pasture and soybean fields established more than 20 years ago. In each area, three replicate sites were chosen from each land use, and five plots in each site were selected and individual soil samples were taken from 0-15 cm soil depth. Soil chemical analysis and T-RFLP for 16S rRNA gene were performed and data analyzed by a Principal Component Analysis (PCA). Chemical changes were evident in the different land uses, with increase of pH, Ca, Mg, P, K, and N in the agricultural soils. Microbial structure was also affected by land use change, with Cerrado and Forest more close related with each other than Agriculture, and Pasture was intermediate among them. The average number of T-RFs was, at Sinop: Forest / 18, Pasture /19, Soybean / 23; and at Campo Verde: Cerrado / 19, Pasture /15, Soybean / 31. All the land uses showed distinct microbial structures, been agriculture the richest in T-RFs.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.044 VARIATIONS IN BACTERIAL COMMUNITY STRUCTURE ON TEN DIFFERENT SPELEOTHEMS IN KARTCHNER CAVERNS, ARIZONA

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Kartchner Caverns is a 3.9 km long wet living carbonate cave in southwestern USA near Benson, Arizona. The cave represents an oligotrophic environment with high humidity (average 99.4%) and elevated CO₂ [1]. Because of its unique geology Kartchner Caverns contains minerals from six different chemical classes: carbonates, sulfates, oxides, nitrates, silicates and phosphates, and is considered as one of the top ten caves in the world in terms of mineral diversity [2]. Furthermore Kartchner is also characterized by its variety of speleothems (secondary mineral deposits). In 2006, the cave was added to the National Science Foundation's Microbial Observatory Program. One goal of our studies in Kartchner is to characterize the heterogeneity of bacterial communities on speleothems. The objective of this study was to explore both, intra- and inter-speleothem variability in the bacterial community structure. Ten different speleothems located in a single cave room within an area of 10 m (length) x 2 m (width) were examined. A chemical element profile of a surface sample scraped from each speleothem was performed using ICP-MS analysis. The analysis revealed differences in the elemental content of the ten formations. Bacterial DNA community fingerprints were generated from each speleothem using DGGE analysis of PCR amplified 16S rRNA gene fragments. The intra-speleothem analysis revealed that the community profiles from the same formation are more similar to each other than to profiles from different speleothems. For the inter-speleothem analysis, bacterial community clusters were observed which appear to be influenced by the spatial location of the formation in the room.

[1] Buecher (1999) *J. Cave and Karst Stud.* 61, 108-120.

[2] Hill & Forti (1997) 2nd *Edition of cave minerals of the world.* 350-354.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.045 PATTERNS IN BACTERIAL COMMUNITY STRUCTURE ACROSS GROUNDWATER ARSENIC GRADIENTS

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Arsenic toxicity in drinking water is a global human health concern impacting tens of millions of people. The problem is especially acute in the Ganges- Meghna- Brahmaputra Delta (GMBD) region of Bangladesh where geogenic groundwater arsenic concentrations can be more than 10 times the World Health Organization's limit. There is evidence that microorganisms facilitate groundwater arsenic mobilization in the GMBD through cascading reduction and oxidation reactions involving labile organic carbon, humic substances, iron and arsenic in the aquifer sediments. However, our preliminary sequence-based data suggest that cultured representatives that are known to mediate these redox reactions are not abundant in the GMBD groundwater environment. Thus, the goal of this research was to investigate patterns in bacterial community composition across geochemical gradients in a groundwater aquifer in Araihasar, Bangladesh to generate hypotheses about the types of organisms that may be involved in arsenic cycling. We characterized the bacterial community by pyrosequencing 16S rRNA genes from aquifer sediment samples collected three locations along the groundwater flowpath, at a range of depths between 5 and 50 meters. Our analysis of the 31,517 16S rRNA sequences indicated that bacterial communities at our study site are diverse; 11,564 OTU's and 52 different bacterial divisions are represented in the dataset. Also, we performed a principal coordinates analysis and found that bacterial communities at deeper depths, and thus higher dissolved iron and arsenic concentrations, are more similar to one another than to communities at shallower depths from the same location. This research represents the first comprehensive characterization of the composition bacterial communities across geochemical gradients in the arsenic-rich GMBD groundwater environment.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.046 EFFECTS OF PCBs on MICROBIAL COMMUNITY IN ANAEROBIC ESTUARINE SEDIMENTS

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The coplanar PCBs, such as 3,4,4',5-tetrachlorobiphenyl (345-4 CB), 3,3',4,4',5-pentachlorobiphenyl (345-34 CB), and 3,3',4,4',5,5'-hexachlorobiphenyl (345-345 CB) have four or more chlorine atoms at both para and meta positions. These congeners display similar toxicity

like dioxin for organisms are named dioxin-like PCBs.

The toxicity of three coplanar PCBs on microbial communities of an estuarine sediment were assessed. Sediment slurries were amended with 2, 10 and 50 mg/L of 345-4 CB, 345-34 CB and 345-345 CB, respectively under anaerobic conditions. The fate and effects of these coplanar PCBs were studied over 250 days. Bacterial communities in sediment slurries were described by dehydrogenase activity and by bacterial populations deduced from the clone libraries. Dechlorination of 345-4 CB and 345-34 CB occurred at least after 100 days of incubation, but dechlorination of 345-345 CB was not observed over the entire incubation period. However, time profiles of dehydrogenase activity were similar in sediment slurries amended with 345-4 CB, 345-34 CB or 345-345 CB. After normalization of the effect of acetone we found that dehydrogenase activity was increased in sediment slurries amended with 50 mg/L, but were inhibited in those amended with 2 and 10 mg/L of coplanar PCBs. Extra addition of electron donors plus sulfate or sulfate could increase dehydrogenase activity significantly. The major microbial population in the sediment slurries incubated with 2, 10, and 50 mg/L of 345-4 CB were δ -Proteobacteria, Chloroflexi, and ϵ -Proteobacteria, respectively. This study shows that (1) dechlorination of coplanar PCBs did not change their effects on microbial metabolic activities, (2) concentration of coplanar PCBs had effects on microbial metabolic activities and community composition.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.047 CHANGES IN BACTERIAL RICHNESS IN LAKES IN DEPENDENCE OF THE LAKES' POSITION WITHIN A LANDSCAPE

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The acknowledgement that community assembly is affected by both local and regional dynamics is one of the major achievements in community ecology within the last 20 years. Moreover, there is a growing awareness that local and regional (metacommunity) processes influence biodiversity and thus ecosystem dynamics and stability. The aim of this study was to investigate the spatial pattern of bacterioplankton diversity in lakes with respect to their position within the landscape, i.e. to analyse whether lake position within a landscape is of importance for the lake's bacterioplankton richness and community composition. We hypothesised that species richness differs with lake order, e.g. lakes lower down in the landscape hold a greater species richness compared to lakes higher up in the landscape, due to changes in 1) local environmental properties or 2) regional processes, such as dispersal. Fifteen Swedish lakes, making up two converging lake-chains, were included in the sampling scheme. Bacterioplankton species richness was analysed employing 454 pyrosequencing of the hypervariable regions V3 and V4 of the 16S rRNA gene. A pilot study revealed that ~25'000 sequences per sample are sufficient to give a meaningful estimate of species richness. On average, 30'000 sequences per sample were obtained. Richness and diversity were rather similar among lakes. Neighbouring lakes generally depicted higher similarities. Moreover, lakes within one lake-chain were more similar to each other than to the lakes of the other lake-chain. However, in contrast to our hypothesis, lakes lower down in the landscape were not always more species rich in comparison to the ones higher

up. The pattern of species richness observed throughout the study lakes appears to be influenced by both spatial and local factors.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.048 DIAZOTROPHIC AND DENITRIFYING COMMUNITY OF TWO CONTRASTING SOILS IN THE CUATRO CIENEGAS BASIN

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Nitrogen is a limiting element for life on earth and this is more so in arid and semi-arid environments, and more importantly, this cycle is well understood molecularly and is regulated mostly by microorganisms. As arid environments expand due to global change, the assessment of the role of desiccation in changing microbial diversity is important to understand possible changes in biogeochemical cycles. We analyzed the community diversity of two soils with contrasting levels of humidity in the Cuatro Cienegas basin of Coahuila, Mexico. We constructed 16s rRNA clone libraries for general taxonomic and community descriptions and analyzed *nifH*, *nirK* and *nirS* clone libraries for functional group assessment. Biogeochemical analyses were performed for each type of soil at different times during one year. Differences in taxonomical composition were observed among soils. The more humid soil showed greater bacterial diversity and nutrient concentration than the drier soil. The greatest functional differences were observed within the denitrifying bacterial guild, while nitrogen fixers shared more taxonomic groups between sites. Our results suggest that differences in bacterial community composition and diversity are produced by nutrient availability, that in turn is caused by water availability. This supports the idea of moisture-limited taxonomic and functional diversity, remarking the relevance of the decrease moisture in the soils.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.049 THE MICROBIAL COMMUNITY OF A DISPERSED, DEEP-SEA OIL PLUME

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The Deepwater Horizon oil spill in the Gulf of Mexico is the largest spill in US history. The unremitting flow of oil from the wellhead puts this spill on the trajectory of becoming the largest in international history. In an effort to mitigate the environmental consequences of the spill, a dispersant has, nearly without pause, been injected directly into the wellhead. The consequence

of the application of this dispersant appears to be a dispersed oil plume at depth (1099-1219 m) in the deep-sea. To determine the probable fate of this deep-sea plume we used molecular methods to characterize the microbial communities within the plume and in an uncontaminated, deep-sea sample. Cloning and sequencing revealed that deep-sea plume samples were dominated by the order Oceanospirillales in the γ -proteobacteria. In fact, 93% of all sequences in both plume samples belonged to a single operational taxonomic unit (OTU) that is most closely related to *Oceanospirillales*. In a control sample this same OTU represented only 5% of all sequences analyzed. The dominant OTU in plume samples was closely related to known psychrophilic, hydrocarbon degrading γ -proteobacteria. Interestingly, the uncontaminated deep-sea control was dominated by a single OTU that was most closely related to a known hydrocarbon degrading Bacteria. The data suggests that the natural micro-flora in the deep waters of the Gulf of Mexico are hydrocarbon degraders. In addition, a relatively rare OTU in the uncontaminated deep-sea sample was detected as the dominant OTU in the plume. This suggests that the input of large volumes of oil, in conjunction with the injection of dispersants at depth, was sufficient to alter the microbial communities in the deep-sea. Ultimately, this microbial community will determine the fate of this unprecedented deep-sea oil plume.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.050 MICROBIAL BIOMASS AND ACTIVITY OF RUMEN COMPOST, OF ITS CONVERSION TO VERMICASTS, AND THEIR SUBSEQUENT COMPOST TEAS

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Physico-chemical and microbiological investigations were carried out on 9-month-old composted rumen waste, fresh vermicasts (obtained after passing through the guts of three earthworms species: *Eisenia fetida*, *Lumbricus rubellus* and *Perionyx excavates*) and their respective compost teas brewed for up to 48 hours. Although the nutrient levels were reduced in vermicasts compared to rumen compost, increased concentrations of soluble nutrients and humic acids were found in vermi liquor brew compared to rumen compost tea. Significant increases in microbial activities and populations as assessed by FDA hydrolysis and plate count methods were also observed in vermi liquor brew compared to vermi liquor. Community level physiological profiles of the solid and liquid phases of composted rumen waste and vermicasts revealed substantial differences in the functional response of microbial communities with vermi liquor brew showing higher diversities both for bacteria and fungi. Principal component analysis of this data also revealed differences in functional response of bacterial communities in rumen compost and vermicasts as well as their respective teas, nevertheless, rumen compost tea and vermi liquor brew showed proximate relatedness in the pattern of fungal communities in the two processes. The 16S rDNA and Internal Transcribed Spacer region based Denaturing Gradient Gel Electrophoresis profile analyses were also carried out to assess the effect of vermicomposting on bacterial and fungal diversity. While a significant increase in bacterial community diversity was observed in vermi liquor brew compared with those of rumen compost,

vermicast and rumen compost tea, no significant difference in fungal communities of rumen compost tea compared with those of composted rumen waste and vermicasts. In conclusion, transformation of rumen compost into vermicasts and its subsequent extraction and brewing of leachate can produce improved liquid biological fertiliser for agricultural use.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.051 BACTERIAL COMMUNITY ANALYSIS OF NATURAL URANIFEROUS SOILS

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Background and aims: A number of studies show that uranium is carcinogenic. This metal is a significant environmental pollutant, and it is of prime importance to better understand factors governing its bioavailability in soils and in particular, the potential role of bacteria in this process. In France, the uranium-rich area of Bessines (Limousin) was one of the most important uranium deposits and has been operated from 1949 to 1994. Nowadays mines are closed and the sites are rehabilitated. The aim of this project is to analyse the bacterial response to uranium.

Methods: Soil samples exhibiting contrasted uranium levels were collected near Bessines and their physicochemical characteristics were measured. The structure of bacterial communities was estimated using a genetic fingerprinting method that allows a comparative profiling of the samples (DGGE). We used both universal and group-specific PCR primers, coupled to a statistical analysis of the results. This molecular approach was coupled to the study of culturable bacteria in order to study bacteria-uranium interactions.

Results - Conclusion: The comparative analysis of bacterial community DGGE profiles on soils of Bessines, sampled at different time-periods, evidenced a large diversity in both uranium-rich and control soils. The community structure is remarkably more stable in the uranium-rich soils than in the control ones, indicating that uranium exerts a high selection pressure. A library of 230 culturable isolates, recovered from the soils, was challenged with uranium and resistant species were selected. Scanning electron microscopy (SEM) coupled to chemical analyses (EDX) revealed that a phylogenetically diverse set of uranium-resistant species were able to chelate uranium at the cell surface. Three *Microbacterium* related isolates, exhibiting contrasted uranium resistance levels, have been chosen as models to analyze the molecular mechanisms underlying the response to uranium.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.052 POPULATION DYNAMICS OF ANOXYGENIC PHOTOTROPHIC SULFUR BACTERIA IN THE MEROMICTIC LAKE SUIGETSU IN JAPAN

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The diversity and abundance of anoxygenic phototrophic sulfur bacteria (APSB) were investigated in Lake Suigetsu, a meromictic lake in Japan characterized by a permanent oxycline at a depth between 3 and 8 m separating the aerobic freshwater epilimnion from the anaerobic, saline, sulfidogenic hypolimnion. Water samples were collected at the central basin of Lake Suigetsu between May 2008 and March 2009. A PCR primer set targeting the gene coding for reverse dissimilatory sulfite reductase (*dsrA*) was designed to survey the diversity and abundance of APSB. APSB were found in the anoxic waters ranging from 10³ to 10⁶ cells ml⁻¹ by the real-time PCR assuming that all APSB have a single copy of *dsrA*. The densities of APSB in oxic-anoxic interface were generally higher than in deeper layer. APSB community composition was assessed by PCR amplification, cloning and sequencing of part of *dsrA*. The majority of the *dsrA* sequences were associated with members of the green sulfur bacteria of the genera *Chlorobium*, *Pelodictyon*, and *Prosthecochloris*. Clones related to purple sulfur bacteria within the γ -*Proteobacteria* were also detected from anoxic water layers of Lake Suigetsu. These APSB appear to be important in the sulfur and carbon cycles in the anoxic water column of Lake Suigetsu. Although there were no significant differences in the APSB community structures among the water samples, a minor change in the composition of *dsrA* operational taxonomy units between water depths and/or seasons was recognized. The relationships between environmental factors and APSB community composition were examined by canonical correspondence analysis (CCA). The CCA suggested that photon, sulfide concentration, and dissolved inorganic carbon content may be abiotic environmental factors associated with the minor changes in APSB community compositions in the water column of Lake Suigetsu

Abstract Category

32 Biodiversity and Community Analysis

PS.32.053 VARIABILITY IN MICROBIAL COMMUNITY STRUCTURE ALONG A GEOGRAPHICALLY DISTRIBUTED THERMAL GRADIENT AT QUEEN'S LAUNDRY, YELLOWSTONE HOT SPRINGS

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Yellowstone hot springs is well known for its unique and diverse microbial communities. At Queen's Laundry Hot Spring, we studied the changing microbial community structure along a thermal gradient as water flows out of the source pool at 92.5°C and cools downstream, ultimately reaching 21.0°C along our transect. In addition, this transect exhibits a pH gradient ranging from near neutral (7.2) at the source pool to more alkaline (9.2) at the downstream terminus. Along this transect we collected and analyzed 22 microbial mat samples using

Terminal-Restriction Fragment Length Polymorphism (T-RFLP) targeting both bacterial and archaeal small subunit ribosomal RNA (SSU rRNA) genes. Cluster analysis of these samples showed significant clustering along the cooling gradient indicating geographically discrete community types. The thermal gradient also correlated with a diversity distribution where the average bacterial Terminal-Restriction Fragment (T-RF) numbers increased as the sampling moved from hot (ave T-RF=6.4), to mid-temperature (ave T-RF=13.8), to cooler (ave T-RF=16.1) sites, showing that the bacterial communities get more complex as the transect becomes cooler. Some of the low temperature T-RFs have been putatively identified as *Cyanobacteria* spp. by performing *in silico* digests of SSU rRNA sequences from GenBank. Q-PCR was also used to assess the ratio of archaea to bacteria across this thermal gradient. We found the source pool to support the highest level of archaea at 48.7% with the bacteria were at 51.3% (i.e., nearly equal in proportion). The mid to low temperature sites were each at nearly ~100% bacteria. The archaeal community detected in the hotter source pool also exhibited exceptional diversity in terms of richness. Work is now in progress to further dissect the archaeal community from this source pool as well as detect and identify the major phylotypes from the distinct community types along our transect.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.054 TRANSITIONS IN BACTERIAL DIVERSITY ALONG A PRECIPITATION GRADIENT THROUGH THE HYPERARID REGION OF THE ATACAMA DESERT, CHILE

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The Atacama Desert of northern Chile has been described as the driest desert on earth. The hyperarid core of this desert contains large expanses that are devoid of vegetation and have mean annual precipitation values < 1 mm. This central core provides an excellent laboratory for the investigation of bacterial community structure and diversity at the hyperarid threshold of microbial life. Our recent analysis of noncultured microbial communities (DGGE profiles of 16S rRNA genes) along an east-west elevational transect (23.75 to 24.70°S) through the hyperarid core region of the Atacama Desert revealed that bacterial communities clustered into two separate groups. The first group included samples taken from 700 - 1931 m in the core region of the desert, while the second cluster included samples from the western slopes of the Domeyko Mountains (2500 m) to the slopes of Volcán de Lullailaco in the Andes (4500 m). Precipitation records are sporadic, but available records and vegetation data suggest that the two clusters reflect different precipitation exposures. The goal of the current study is to use 16S rRNA gene community surveys to characterize the differences in bacterial diversity of these two clusters. A combination of tools including pyrosequencing analysis (V6 region), Sanger-sequenced clone libraries (nearly full-length gene) and the sequencing of excised DGGE bands (V7/V8 region)

were employed to characterize the species richness and bacterial diversity of seven sites along the elevational transect. The results demonstrate an increase in species richness and phylogenetic diversity with precipitation. In addition, the pyrosequencing- and Sanger sequencing-based data sets were compared for the 2547 m sample to determine the effect of sequencing strategy on the apparent phylogenetic structure of the community and to identify rare, but potentially significant members of this hyperarid, oligotrophic ecosystem that are present in low abundance.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.055 IS EVERYTHING EVERYWHERE? THE EFFECTS OF GEOGRAPHICAL DISTANCE ON MICROBIAL COMMUNITY SUCCESSION

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One explanation of microbial biogeography asserts that "everything is everywhere but the environment selects". We put this theory to the test by examining patterns of microbial community succession in jars containing heterotrophic media separated by either short (~1m) or long (>1km) distances. We hypothesized that neighboring jars would show a geographic signal early in succession, but that over successional time that signal would erode. Sterile nutrient broth was transferred to autoclaved jars covered with 2 mm netting to exclude larger insects and plant material. We used a nested experimental design in which 9 pairs of flasks were placed across a ~13 km transect near Punta Allen, Mexico; temperature, shading and precipitation were relatively constant. Samples were taken 8 times over 99 hours. At each time point, subsamples were analyzed for optical density and then the remainder of the sample was frozen for further analysis. Samples were returned to Colorado, United States, and DNA was extracted. QPCR analysis of the bacterial to archaeal ratios was then performed. We found that growth rates between closer samples were more similar, particularly at the beginning of the experiment. Bacterial to archaeal ratios were orders of magnitude greater than those observed in other environmental samples. However, as microbial biomass increased, the bacterial to archaeal ratio decreased. As predicted, the bacterial to archaeal ratios were more similar at 1m vs 1km distances at the beginning of the experiment, but the effect of geography decreased over time. Samples have also been submitted for pyrosequencing to uncover the effects of geography on bacterial community composition. Our study has important implications for microbial dispersal and succession that may apply in other, more complex environments.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.056 PYROSEQUENCING REVEALS THE DIVERSITY AND VARIABILITY OF BACTERIAL COMMUNITIES ON SPELEOTHEM SURFACES IN KARTCHNER CAVERNS

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Kartchner Caverns, a carbonate cave in southern Arizona, USA, is characterized by oligotrophic conditions, high humidity and elevated carbon dioxide levels, characteristics defining a unique environment with potential for harboring unknown microbial life. In 2006, Kartchner was designated a National Science Foundation Microbial Observatory for which a major goal is to determine fundamental information about the structure and diversity of cave bacterial communities and the variation that can be expected on different formations. For this purpose swab samples were taken from nine speleothems in two separate untoured rooms of the cave. Two samples were taken in a portion of the cave which serves as a nursery roost for hundreds of bats during the summer. The other seven samples were taken in a region of the cave which is not impacted by bats. In addition, samples from a cave rock wall and from soil above the cave were taken to explore the uniqueness of speleothem bacterial communities. Genomic DNA was extracted from each sample and the V6 variable region of the bacterial 16S rRNA gene was amplified and sequenced using 454 pyrosequencing. A clone library of the nearly full length bacterial 16S rRNA gene sequences from one of the speleothem samples was constructed and used as a reference library for pyrosequencing results. The relative bacterial diversity and structure of each of the sample sites was characterized in terms of operational taxonomic unit richness and diversity. The phylogenetic distribution of each sample was determined and compared using the Ribosomal Database Project II's classifier. Preliminary results show that there is an average of 5,000 operational taxonomic units (97% similarity) per speleothem sample and that bacterial communities are more similar within the same room than in different rooms. This study provides the first extensive characterization of bacterial community diversity on speleothem surfaces in a carbonate cave.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.057 USING MICROBIAL MICROCOSMS TO INVESTIGATE BIOGEOGRAPHY IN FRESHWATER ENVIRONMENTS IN SCOTLAND

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Biogeography explains spatial patterns of species diversity. In the past, work in this area has focussed on plants and animals, but with the advancement of molecular techniques, many biogeographical studies have focussed on microbes. Recent studies have shown that environmental conditions can determine the distribution of microbial species. Additionally, increased geographic distance can isolate populations, leading to allopatric speciation. However, the study of microbial biogeography is still largely in its infancy, and there is still a debate as to whether it even exists.

We investigated whether microbial populations sampled from different environments produced similar communities when subjected to the same environmental growth conditions. We sampled from 11 lochs and one pond in Scotland that had different chemical compositions, pH, and altitudes and were at various geographic distances. Sediment and water were sampled and used to inoculate sterilised microcosms that consisted of pond sediment and water from Blackford pond in Edinburgh. They were sealed and incubated in a light incubator to allow microbes from the lochs to proliferate. After 16 weeks, the columns were destructively sampled and the microbial community compositions were determined using Denaturing Gradient Gel Electrophoresis (DGGE). We found that the initial source microbial communities were different to each other. This then influenced the microbial communities that developed in the microcosms, since microcosms from different sources were different. However, there was a clear convergence of microbial communities within each set of replicate microcosms. We can therefore conclude that different microbial source communities, when exposed to the same environmental conditions, can produce different stable microcosm communities. Further investigation will reveal how this depends on the geographic location and/or the environmental conditions of the lochs from which the source community was taken.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.058 DYNAMICS OF BACTERIAL CONSORTIA AND SULPHATE-REDUCING MICROORGANISMS ASSOCIATED TO ALWC PRODUCTS IN MARINE PORT STRUCTURES

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European port authorities are commonly concerned by Accelerated Low Water Corrosion (ALWC) occurring in low water zone of steel port structures. Chemical process of ALWC has been already investigated, however understanding of the role of microorganisms that might be involved in this process remains not clear. The goal of the MICSIFE project (Microbiologically Induced Corrosion of Steel structures In Port Environment) is to develop risk assessment methods to predict and assess ALWC for industrial end users. In this context, we studied the diversity, abundance and activity of bacteria and particularly sulphate-reducing microorganisms (SRM) in four ALWC products and one control (no ALWC process) from a European port. Abundances of total bacteria and SRM as their metabolically active parts were determined in all sample layers by qPCR and RT-qPCR targeting the 16S rDNA, *dsrB* gene and their transcripts respectively. Bacterial community structure from all sample layers were compared using CE-SSCP based on 16S rDNA. Total bacteria enumeration indicates high cell density in all corrosion product layers (10⁸ to 10¹⁰ cell/g-1 of corrosion product) which increases from the steel to the seawater. Important number of metabolically active bacteria is also detected in most of ALWC

products (2.108 to 3.1012 16S rRNA copies/g-1). Concerning SRM, they represent 35% to 98% of total bacterial community within inner layers of ALWC samples, while they reach only 25% within outer layers and the control sample. Moreover, particular abundance of metabolically active SRM is observed in inner layer of ALWC product while none could be detected in control sample. In this way, diversity analysis shows divergent bacterial community structures between inner and outer layers of ALWC products that seem to be related to this SRM distribution. Considering their abundance and activity, SRM represent the main population of the first layers of biofilm, and play certainly an important role in ALWC.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.059 TEMPORAL ANALYSIS OF LANDFILL LEACHATE-IMPACTED GROUNDWATER TO CHARACTERIZE THE FUNCTIONAL GENE STRUCTURE OF MICROBIAL COMMUNITIES

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A closed and unlined municipal landfill in Norman, OK releases leachate to a shallow aquifer resulting in an expanding subsurface plume of complex organic matter. Despite the increasing areal extent of contamination, previous studies suggest that spatially and temporally defined geochemical gradients remain relatively stable. We investigated the changes in functional genes diversity associated with the aquifer microflora within the leachate flowpath as a function of time. Groundwater samples were collected from one multi-level sampling well (MLS 35) in June 2008 and 2009, while a nearby MLS, well 37, was obtained in Feb. and Oct. 2009.

Microorganisms were collected on filters from which DNA was extracted and analyzed with a comprehensive functional gene array (GeoChip 3.1). Detrended correspondence analysis suggested that the community functional gene structure of MLS 35 changed with depth, but was stable over time. The number of unique genes increased with depth from 22.3% at 2 m to 35.1% at 11 m for MLS 35, but did not change appreciably over a year at the shallow depth. A similar relative stability in the functional gene structure was also detected over time in MLS 37. Positive genes within categories for C-, N-, P- and S-cycling, as well as those attributed to metal resistance and organic remediation showed little variation in numbers as a function of time. The *mcrA* genes varied temporally with measured methane values in both wells, most likely reflecting the turnover of organic carbon in the anaerobic aquifer. This hypothesis is supported by the identification of genes for contaminant remediation and the breakdown of complex cellulosic material. These results suggest that the functional gene structure of the microbial community is relatively stable despite the chronic exposure to landfill leachate.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.060 TITANIUM-PYROSEQUENCING DETECTION OF MULTIPLE-ANTIBIOTIC RESISTANT BACTERIA FROM A MUNICIPAL WASTEWATER TREATMENT PLANT IN SEOUL

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The aquarance and increase of antimicrobial resistance among microorganisms present in environmental water bodies raises concerns on risk to public health. Especially multiple antibiotic resistance would be serious concern to public health because it is difficult to treat infections by microbes exhibiting resistance against multiple antimicrobial agents. Despite, little is known about microbial communities exhibiting multiple antibiotic resistance. Municipal wastewater acts as a source of antibiotic resistant microbes, which is released into stream water after treated by biological wastewater treatment processes such as activated sludge. In this study, we explored bacterial communities exhibiting multiple antibiotic resistance from a municipal wastewater treatment plant (WWTP) in Seoul. For this work, we selectively cultured (LB medium with ampicillin, kanamycin, tetracycline) multiple antibiotic resistant microbial communities, which had been collected from the influent, recycled active sludge and effluent of the WWTP, and then their 16S rRNA amplicons were high throughput-analyzed using titanium pyrosequencing. Although the bacterial communities are polymicrobial with no single dominated microbial phylum, the most ubiquitous and predominat phylum among the 3 samples are *proteobacteria*, *acidobacteria*, *bacteriods* and *actinobacteria*. In the multiple antibiotic resistant communities from the effluent, *Haliscomenobacter*, *Klebsiella*, *Leptothrix*, *Propionivibrio*, *Prostheco bacter*, *Pseudomonas*, *Shigella* and *Victivallis* were found to dominate. *Haliscomenobacter*, *Prostheco bacter* and *Victivallis* are also present in the resistomes from the influent and activated sludge samples. The pyrosequencing results show which multiple antibiotic resistant bacteria were present in WWTP effluent, which is directly released into stream, and which multiple antibiotic resistant bacteria came from the source, influent wastewater.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.061 DIVERSITY OF LITHIFYING MICROBIAL COMMUNITIES IN THE GREAT SALT LAKE, UTAH.

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The earliest evidence of life on Earth comes from fossils of lithifying microbial communities dated to approximately 3.5-4 billion years ago. From that time until now, lithifying communities have played an important role in regulating global carbon cycling. The study of modern analogs of ancient communities can provide insight to the development of microbial metabolic pathways and community interaction that can help shed light on the evolution of biogeochemical cycles. In this study we compare the community composition of microbialites collected from the hypersaline South Arm (15% salt) and salt-saturated brines of the North Arm of the Great Salt Lake, Utah, USA. Phenotypically, South Arm microbialites are dominated by algae and

cyanobacteria while North Arm microbialites are contain a thick layer of pink color typically associated with halophilic bacteria and archaea. Phylogenetic microarray analysis suggests that the microbial communities are quite similar at the higher taxonomic class level, but differ greatly at the family level. The role of this observed difference on the biological functions associated with these microbialites is being investigated. Comparison of initial high-resolution, high-mass measurement accurate mass spectrometry profiles of peptide features suggests differences in the microbialite proteomes.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.062 MICROBIAL DIVERSITY OF A MODERN STROMATOLITE ANALOG IN YELLOWSTONE NATIONAL PARK, WYOMING: SEARCHING FOR THE DEPOSITIONAL COMMUNITY

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Stromatolites are commonly interpreted as evidence for early life and studies of modern analogs can shed light on the biological significance of ancient forms. Stromatolite analogs in a Yellowstone National Park, Wyoming hot spring exhibit fine laminations and domical morphology as seen in ancient stromatolites. The stromatolites are comprised of silica tubes thought to be the lithified remains of filamentous cyanobacterial sheaths. According to the developed growth model, microbial life in stromatolite interiors is the surviving, entombed constituents of the once surface community. Three morphologically dissimilar surface cyanobacterial mats were observed on the stromatolites, but it is not clear all three are responsible for constructing the stromatolites. Here, sampling was focused on determining which surface mat is the likely stromatolite builder. Core samples of one stromatolite taken at 2 mm intervals as well as surface community samples were analyzed by 16S rRNA gene content. A total of 15,528 total sequences of at least 225 nucleotides were obtained via pyrosequencing for an average of 1,530 sequences per sample. The predominant cyanobacterial phylotype in each mat type falls into a unique operational taxonomic unit at an identity threshold of 97%. Using BLAST against the SILVA102 non-redundant, small-subunit rRNA database, sequences from the predominant cyanobacterial operational taxonomic unit in each of the three surface mats are related to *Fischerella*, *Chlorogloeopsis* and an uncultured cyanobacterium, respectively. Clustering of 16S libraries using Unifrac distance, a measure of the similarity between communities, shows the surface mat with cyanobacteria related to an uncultured phylotype most resembles the interior communities. Also, Unifrac distance between the depositional mat and the interior samples increases with depth suggesting the stromatolites grow by external addition supporting the growth model.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.063 EFFECT OF DIFFERENT STRESS TREATMENTS ON MICROBIAL CATABOLIC DIVERSITY OF CHRONICALLY HYDROCARBON CONTAMINATED-SOIL IN BUENOS AIRES, ARGENTINA

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Understanding indigenous microbial function in contaminated soil is crucial to the successful development and use of bioremediation technologies. Due to the different petrochemical areas, Buenos Aires is a good example of soil exposed to a large wide of hydrocarbons. The soils exposed to chronic pollution may present extreme challenges to maintenance of diverse structural and functional microbial communities. There are different opinions about whether hydrocarbons pollution reduces the microbial diversity, and therefore produces less resilience of soil microbial communities. We investigated this behaviour in three different places from a Buenos Aires petrochemical area, including a pristine soil. Three stress treatments were imposed to each soil sample in low and high levels. They were declines in pH; increases in electric conductivity or increases in soil Cd (II) concentrations. Each treatment was carried out on three replicates. The soils treatments were incubated at 26±2°C for two weeks after the stresses, giving to the systems a period to recover or not from the stresses. After that, measurements of different enzymatic activities were carried out, alkaline phosphatase, acid phosphatase, dehydrogenase, urease and lipase activities. The hydrocarbons polluted soils showed high dehydrogenase activity and low urease and alkaline phosphatase activity. The acid stress provoked the decline of dehydrogenase and a stimulation of acid phosphatase activity in polluted soils. The stress by Cd (II) was significant in polluted soil, while the stress by salinity did not provoke any changes in these soils. The pristine soil remained unchanged by the Cd (II) addition; instead an increase of electric conductivity increased the urease and lipase activity. We also determined the community level physiological profiling (Biolog EcoPlate) and no significant changes due to stresses were detected from any soils. The chronic pollution of soils conditioned the response to different stresses.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.064 DETERMINANTS OF THE DISTRIBUTION OF NITROGEN-CYCLING MICROBIAL COMMUNITIES AT THE LANDSCAPE-SCALE

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Little information is available regarding the landscape-scale distribution of microbial communities and its environmental determinants. However, a landscape perspective is needed to understand the relative importance of local and regional factors and land management for the microbial communities. In this manuscript, we investigated the distribution of functional microbial communities involved in N-cycling and of the total bacterial and crenarchaeal

communities over 107 sites using a grid with a 16 km lag distance within Burgundy, a 31 500 km² region in France. After quantifying the abundances of the total bacterial, crenarchaeal, nitrate-reducing, denitrifying and ammonia-oxidizing communities and measuring 42 soil physico-chemical properties at each sampling site, we used by canonical variation partitioning to determine the relative contributions of land use, spatial distance, climatic conditions, time and soil physico-chemical properties to the distribution of the different communities. Although many environmental variables were significant predictors, only a few accounted to a large amount of the total variance in the distribution of the studied microbial guilds. Altogether we could explain up to 85 % of the spatial variation in community abundances with soil chemical properties as the main driver. We found spatial correlation up to 140 km and geostatistical modelling was used to generate predictive maps of the distribution of microbial communities across the 31 500 km² Burgundy region. The present study, which is the most comprehensive spatially explicit analysis of patterns of microbial communities to date, highlights the potential of such approach for microbial ecology to identify the overarching factors driving the spatial heterogeneity of microbial communities even at the landscape scale. It also provides the first maps of the distribution of microbial guilds at a scale which is of relevance for policy makers and stakeholders for ecosystem management.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.065 INDOOR FUNGI — A MOLECULAR VIEW

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Indoor microbial contamination due to excess moisture plays an important role in human health in both residential and occupational settings. Meanwhile, definitive information of the organisms present in indoor environments remains limited for the restrictions of traditional identification techniques.

Using large scale sequencing of ribosomal DNA together with quantitative PCR (qPCR), culture and ergosterol analyses we described the total mycoflora in indoor environment. The effect of moisture damage was assessed by comparing fungi in dust samples from damaged, renovated and healthy buildings, as well as species composition on building material samples and in dust. The total fungal communities as observed by sequencing were significantly more diverse compared to viable communities. QPCR indicated very low viability for most target organisms. Culture-dependent and independent methods agreed for the dominant species, but sequencing revealed the presence of additional, predominant groups from various fungal classes. The identified fungi include e.g. decomposing, plant associated, saprophytic and lichenous species, and thus occupy diverse natural ecological roles. Novel, verified phylotypes represented at least 10% of all diversity. Potentially toxigenic, allergenic and opportunistic fungi as well as members of human skin normal flora occurred regularly. Seasonal trends in incidences of different fungal groups were described. Investigation of the fungal diversity in contaminated building materials supported past literature at generic level, but provided more detailed species identifications. The effect of moisture damage renovation was seen as decreasing fungal diversity in dust.

We conclude that indoor fungal flora is diverse and while the use of universal DNA-based

method is indispensable for describing the true diversity of fungi in indoor samples, targeted methods like qPCR are more efficient for detecting building-related microbial problems.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.066 DISTRIBUTION OF BACTERIA IN AQUATIC SOURCES ASSOCIATED TO VOLCANIC SYSTEMS AT THE MOUNTAIN RANGES OF COLOMBIAN ANDES

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Biological diversity and geographical pattern distribution of bacterial communities in the Earth is of great importance because their abundance and its role as biogeochemical engines; they are essential as they mediate environmental processes that sustain life on the planet. However, the knowledge in Colombia about this group of microorganisms is inversely proportional to their diversity and importance at global scale. The aim of this study was to describe bacterial biogeographical patterns in order to test hypothesis about differential effects of historical and environmental factors on the structure of this communities.

Lakes and hot springs associated with two volcanic systems were sampled. Those systems located on mountain ranges of Colombia are separated more than 400 Km. Physicochemical parameters (pH, T°, conductivity and redox potential) were measured and integrated water samples were collected, filtered and processed to obtain community DNA. 16S rRNA gene was amplified and the product was analyzed by DGGE. Sites with similar community profiles were clustered using NMDS taking into account both environmental and biological matrices to test the hypotheses.

We found similarities between composition of bacterial communities associated to systems from different geographical localities of Colombian Andes: *Nudo de los Pastos*, Volcán *Nevado del Ruiz*, Thermal springs of Eastern mountain range. Measured physicochemical factors allow to distinguish groups of aquatic systems with similar properties, however distribution and structure of bacterial communities does not correspond to this clustering. Then structural and compositional differences in bacterial communities respond also to other ecological variables, for example, biotic - abiotic interactions and resources availability.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.067 MICROBIAL COMMUNITY OF IRON REDUCTION ZONE IN GROUNDWATERS FROM RIVERINE ALLUCIAL AQUIFER

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Anaerobic iron reduction process plays a key role in purification of metal and organic pollutants in aquifer. The bacterial community in iron reduction zone of ground waters of riverine aquifer of Mankgyeong River was investigated in three sites (B1, B3, and B6). In this study, the depth of iron reduction as the main anaerobic process was selected below redox boundary with high ferric iron concentration. Among known iron reducing bacteria, clones related *Rhodoferax ferrireducens* were one of the major clones (9.9%) in B3. Considering versatility of electron acceptors for *R. ferrireducens* and environmental conditions of B3, presence of *R. ferrireducens* does not correlate with iron reduction. At B6 site, clones related to *Geobacter* spp, the well-known iron (III) reducing bacteria, were observed as the main candidate of dominant iron-reducers. Although major clones of B1 were related to those found in iron-manganese nodules, there were no obvious candidates of iron-reducers. In addition to known candidates of iron-reducers, clones related to other anaerobic respiration processes such as sulfate- and nitrate-reduction were also observed. These results were supported by real-time PCR quantification iron-reducers. Abundance of *Geothrix* spp. was very low (<0.01%) and *Shewanella* spp was not detected in all samples. *R. ferrireducens* and *Geobacter* spp. were the major candidates of iron-reducers in B3 (15%) and B6 (15%) sites, respectively. Even though the sampling sites close each other, microbial communities (iron reducers) in iron reduction zones were quite different. For anticipation and exploitation of iron-reduction potential in bioremediation in riverine aquifer environments, in addition to the molecular analysis of microbial community, investigations on the other factors such as availability of other electron acceptors are required.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.068 SPATIAL RELATIONSHIPS BETWEEN SOIL MICROORGANISMS AND EARTHWORMS COMMUNITIES AT A MACROSCALE: THE CASE OF GRASSLANDS IN HAUTE NORMANDIE, FRANCE

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Soil is probably one of the more species-rich habitats in terrestrial ecosystems. This biodiversity is all the more important as values of soil organisms refer to the direct or indirect in the delivery of ecosystem services. Among soil organisms, microorganisms are considered as one of the important component performing over 90% of the transformation of substrates in soils. However their activities depend on a number of abiotic and biotic factors that adjust their effect on ecosystem functioning at different scales of time and space. In particular, earthworms by their activities can interact and influence the structure and composition of soil microbial communities at diverse scale. The important role of space in population, community and ecosystem processes has been largely recognized in soil ecology. Therefore, we evaluate relationships between the both communities at a macroscale. Spatially explicit samplings were carried out in grasslands in Normandie. Earthworms were sampled in 104 points to allow mappings of species densities. In the same way, a sampling design for the microorganisms was conducted on 24 points superposed to the first grid. Soil microbial structure and diversity were described by using ergosterol, PLFAs profiles and BIOLOG Ecoplate. Geostatistics were used to describe the spatial structure and co-

variation in patterns of both communities. We observed differences in the structure and functional diversity of microorganisms between the three grasslands. However, some of earthworm species presented a typical patchy distribution and these patches seemed to be independent from the structure of microbial communities. This study provides an example of the importance of considering biotic interactions while assessing the functioning of managed ecosystems. Ecosystem engineers such as earthworms represent an important group of soil invertebrates and their interactions an essential factor in the structuring of other soil organisms at different scales.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.069 SPATIAL VARIABILITY IN AIRBORNE BACTERIAL COMMUNITIES ACROSS COLORADO AND THEIR RELATIONSHIP TO THE COMMUNITIES OF POTENTIAL SOURCE ENVIRONMENTS

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Airborne bacteria are thought to be a large component of near surface atmospheric aerosol loads, however, the study of airborne microbiology has been largely restricted to fungal spores, pollens and dust mite allergens. With this work, we describe how land-use types influence bacterial communities in the near surface atmosphere with the goal of connecting airborne bacterial community members to their terrestrial source environments.

To address these knowledge gaps, we collected air samples from six sites within each of three distinct land use types across the Colorado Front Range as well as at a pristine location located 3300 m above sea level in the Colorado Rockies. Total bacterial abundances were assessed via flow cytometry, and bacterial communities were characterized using a high-throughput barcoded pyrosequencing approach. In addition, concentrations of biological ice nuclei in the collected samples were determined using a droplet freezing assay.

Microbial abundances and community composition were relatively stable across time and space. The airborne bacterial community composition across the three distinct land uses and above the high elevation research site were statistically distinct from one another, however, the airborne bacterial communities as a whole do appear to have a unique signature when compared to a large dataset of potential source environments such as the leaf surface and soil environment.

The near surface airborne bacterial community appears to be a rather large fraction of the coarse atmospheric aerosol load between ~ 2 and 10 μm , and the community composition seems to be unique from possible source environments. Therefore, there may be a ubiquitous population of bacteria resident in the near surface atmosphere. This potentially transient airborne bacterial community deserves more attention as they may have important effects on human health and atmospheric dynamics.

Abstract Category
32 Biodiversity and Community Analysis

PS.32.070 MICROBIAL COMMUNITY EXAMINATION BY FOURIER TRANSFORM INFRARED SPECTROSCOPY

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We examine a new method of characterizing function of the meta-metabolome by Fourier Infrared - Attenuated Total Reflectance spectroscopy. Fourier Transform Infrared spectroscopy is fast, samples can be stabilized in the field, and spectra are information rich. Key will be the ability to deconvolute the complex spectral patterns into meaningful biological information. The aim of this research is to compare the Fourier Transform spectra to 16S rRNA and fatty acid methyl ester profiles obtained from freshwater and extreme alkaline-saline lakes. We collected water samples from Salt Lake, Lincoln, Nebraska (November 2009). Water was pre-filtered through several pore sizes, then captured onto 0.2 micron filters, dried, and examined by mid-IR spectroscopy. Denaturing gradient gel electrophoresis, fatty acid methyl ester profiles, and scanning electron microscopy determined community composition. The results indicate Fourier Transform Infrared Spectroscopy can be used to examine a lake planktonic microbial community. Pre-filter pore size is important. Too large a pore-size can lead to too many eukaryotes on the filter - as indicated by scanning electron micrographs. Too small a pore size can lead to bio-fouling of the pre-filter and too little a signal. A pre-filter pore size of 8 micron is optimal. Larger volumes of water must be concentrated to analyze the microbial community by denaturing gradient gel electrophoresis and lipid analysis than infrared spectroscopy. Microbial communities from lake water can be quickly gathered onto filters, dried, and distinguished from one another by Fourier transform infrared spectroscopy. It is a highly sensitive method in that less biological material is needed to produce a significant signal. Further analysis of infrared spectra, microbial community deep sequencing, and lipid analysis, likely, will lead to increased biological information from the spectra.

Abstract Category
32 Biodiversity and Community Analysis

PS.32.071 BIOGEOGRAPHY OF EPSILONPROTEOBACTERIA IN SULFIDIC CAVES AND SPRINGS BASED ON THE 23S rRNA GENE

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The *Epsilonproteobacteria*, a diverse class within the *Proteobacteria*, are widely distributed in marine, freshwater, and terrestrial environments. Although well studied in habitats such as hydrothermal vents, *Epsilonproteobacteria* that live in sulfidic terrestrial environments are poorly understood. Based on previous work, we hypothesize that communities of *Epsilonproteobacteria* consist of both cosmopolitan and endemic members, driven by

environmental variables. This study used specific PCR primers to compare epsilonproteobacterial communities in sulfidic caves and springs at six sites in the United States and three in Slovenia. All sites featured high-sulfide and low-oxygen concentrations, and were chosen to cover a range of geographic distances (<1 m to 8511 km at maximum separation).

Because 16S rRNA genes from previous work provided limited genetic variability to evaluate biogeography within the *Epsilonproteobacteria*, a fragment of the 23S rRNA gene was amplified with newly designed epsilonproteobacterial-specific primers. Resulting community profiles of 23S rRNA gene PCR amplicons for each site were grouped by clustering methods and canonical correspondence analysis (CCA) to reveal relationships among community structure and selected environmental parameters.

Epsilonproteobacterial community similarity strongly correlated to minimum geographic distances, as well as temperature, sulfide, bicarbonate, and oxygen concentration. Higher resolution studies of community structure are currently underway via clone library analyses of the ITS region from the same community samples.

In contrast to 16S rRNA genes, community profiling with 23S rRNA genes indicates that few cosmopolitan groups dominate the terrestrial *Epsilonproteobacteria* and suggests that substantial uncharacterized diversity exists, depending on geographic proximity of sites to each other and environmental conditions.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.072 POPULATION TURNOVER IN A *MICROCYSTIS* BLOOM RESULTS IN PREDOMINANTLY NON-TOXIGENIC VARIANTS LATE IN THE SEASON

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Toxic freshwater cyanobacterial blooms are a health and recreation concern throughout the world. *Microcystis*, a toxigenic genus, is of particular concern to Northwestern lakes and reservoirs. Phycological analysis has shown *Microcystis* to be present in Copco Reservoir on the Klamath River since 2004. *Microcystis* populations typically include both toxigenic and non-toxigenic variants that are indistinguishable through microscopy. Our study looked at the population structure across the bloom season through multi-locus genetic methods. Surface samples of the 2007 bloom in Copco Reservoir on the Klamath River in Northern California were analyzed through PCR amplification of three loci: ITS, *cpcBA* and *mcyA*. Samples were taken between June and October, during which two cell-count peaks occurred, in mid-July and early September. From this analysis the sequences represented in clone libraries of the ITS and *cpcBA* loci could be classified into 4 or 5 allele groups, providing a convenient framework for temporal comparison of *Microcystis* sub-populations. Each group was dominated by a single, highly represented sequence. Other members of each group varied at 1 to 3 nucleotide positions, while groups were separated by up to 30 nucleotide differences. From this analysis a population

turnover occurring after the initial cell-count peak was indicated by changes in the allele composition of both the ITS and *cpcBA* loci. Different ITS and *cpcBA* genotypes are predicted to have been dominant at the two population peaks. Toxicity, reflected by microcystin per cell and *mcyB* copy number, was lower during the second peak and *mcyB* copy number fell further as the bloom declined. The relatively simple population structure represented by distinct allele groups offers opportunities for economical monitoring of genetic changes at the subpopulation level.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.073 HIGHER RATES OF N FERTILIZATION DECREASE SOIL ENZYME ACTIVITIES, MICROBIAL FUNCTIONAL DIVERSITY, AND DIVERSITY OF AMMONIA-OXIDIZING BACTERIA

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Little is known about effects of high nitrogen (N) fertilization on soil microbial community and diversity. Soil enzyme activities, microbial functional diversity, and the abundance and composition of ammonia-oxidizing bacteria (AOB) involving in soil N cycling were evaluated under conventional N rate (270 or 300 kg N ha⁻¹ season⁻¹, N4), 80% (N3), 60% (N2), 40% (N1) and 0% (N0) of N4 during a 2-year cucumber/tomato rotation. Soil activities of dehydrogenase, urase and neutral phosphatase, nitrification capacity, and microbial functional diversity were significantly decreased under the higher N3 and N4 rate, but in-between under the N2 rate. Microbial diversity indices were significantly lower under the highest N4 rate in the same vegetable season, but varied with seasons among N rates. Both principal component analysis and C substrate utilization patterns displayed significant separation of soil microbial communities between the higher N4 or N3 and other lower N rates. The ammonia-oxidizing bacteria (AOB) population size was significantly higher in the N2 rate than in either the non-N N0 or the highest N4 rate. Denaturing gradient gel electrophoresis (DGGE) profiles showed that N fertilization generally increased the diversity of AOB community under the N1, N2, N3 and N4 rate than under the N0 rate. However, the number of DGGE bands was more diverse under the N1 and N2 rate than under the N3 and N4 rate, with the greatest numbers of DGGE bands under the N2 rate. Phylogenetic analyses of the *amoA* gene fragments showed that all AOB sequences from different N fertilization rates were affiliated with Nitrosospira species and grouped into cluster 2, 3, 8 and 12. Our results indicated that a 40% reduction of the conventional N fertilization rate was practical to reduce excess N input while maintaining the sustainability of such greenhouse-based intensive vegetable systems in China's Yangtze River Delta.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.074 MICROBIAL COMMUNITY AND ECOTOXICITY ASSAY OF BIOREMEDIATED WEATHERED HYDROCARBON CONTAMINATED SOILS

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Disposal of bioremediated soil is usually carried after the soils have met legislated guidelines defined by chemical and ecotoxicity tests. Unfortunately, in some countries in Australia/Oceania, Asia and Africa ecotoxicity tests are not yet part of the mandatory tests required for the environmental safety assessment of remediated hydrocarbon contaminated soils. This study investigates the effects of bioremediation strategies such as biostimulation and mycoaugmentation in reducing the toxicity of soils contaminated with weathered hydrocarbon and evaluates the safety of such soils using ecotoxicity tests. Bioremediation strategies (in 12-week soil microcosms) resulted in up to 40% reduction in total petroleum hydrocarbon (5503 ppm) content of soils which resulted in these soils meeting the Australian guidelines for safe disposal requirements in terms of aliphatics (≤ 5800 ppm), benzopyrene (≤ 1 ppm) and metals content. Microbial community analysis using 16S rDNA (universal and AlkB) and Intragenic Spacer Regions-based Denaturing Gradient Gel Electrophoresis showed stable and adapted bacterial and fungal communities which were largely unaffected by biotreatment. However, ecotoxicology assays showed 100% earthworm (*Eisenia foetida*) mortality in potting soil mix containing $\geq 50\%$ (≥ 2751 ppm) of this treated soil over a 14 day period at concentrations deemed to be safe after in situ bioremediation. Substantial impairment of radish seed (*Raphanus sativus*) germination (up to 70% reduction) occurred in potting soils containing $\geq 10\%$ of contaminated soil (≥ 550 ppm); concentrations deemed to be safe for disposal of treated soils into residential areas after ex situ bioremediation. This showed that these soils were still toxic to the soil biota despite meeting the safety limits set by legislation and reinforces the need to include ecotoxicity assays as part of the mandatory guidelines for contaminant toxicity assessment of remediated soil in Australia.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.075 MICROBIAL DIVERSITY AND COMMUNITY STRUCTURE IN ENDANGERED SAND FYNBOS SOILS OF THE WESTERN CAPE, SOUTH AFRICA

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The Fynbos biome in the Western Cape, South Africa is famous for its high plant biodiversity. Highly endangered vegetation types such as the Sand Fynbos is extensively fragmented which makes it an important focus for conservation efforts. This study intended to understand the extent of microbial diversity in the unique ecosystem of the Western Cape's fynbos biome, focusing on the Sand Fynbos region. One of the main questions was to examine the extent to which the above-ground diversity is reflected in the below-ground microbial communities. The soil community composition of both fungal and bacterial groups in Sand Fynbos was characterized using Automated Ribosomal Intergenic Spacer Analysis (ARISA). The 16S-23S intergenic spacer region from the bacterial rRNA operon was amplified for bacterial communities (B-ARISA) from total soil community DNA. Correspondingly, the internal transcribed spacers, ITS1, ITS2 and the 5.8S rRNA gene from the fungal rRNA operon were amplified when examining fungal communities (F-ARISA). Samples from four different fragments were

analysed to determine the diversity on a local scale (alpha diversity) as well as a landscape scale (beta diversity). The microbial community structure from the different sites displayed strong spatial patterns which stayed consistent over four seasons of sampling. The above-ground floral community data from the Sand Fynbos were compared and closely linked with changes in the microbial community structure and diversity. From the study it was evident that the microbial communities of these areas are as sensitive to fragmentation as are the plant communities. We are grateful to Cape Nature for allowing access to the Riverlands Nature Reserve.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.076 MICROBIAL COMMUNITY STRUCTURES OF RIPARIAN ECOTONES INVADED BY NON-INDIGENOUS ACACIAS

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Riparian ecotones are situated at the interface between terrestrial and the aquatic environments and play an important role in ecosystem processes such as nutrient cycling. Many soil processes associated with riparian zones are mediated by soil micro-organisms. These processes play a role in maintaining water quality by controlling the flow of water to the aquatic environment and are involved in nutrient immobilization. This process is significantly influenced by the invasion of non-indigenous nitrogen fixing *Acacia* species into riparian zones. It is further known that soil biodiversity is often negatively affected by invasion of these transformer plant species. This study investigated the microbial community structure of two pristine fynbos sites in the South African, Western Cape region of which one was recently burnt, as well as a site invaded by *Acacia saligna*. A molecular approach based on the isolation of total soil DNA, was used to determine soil microbial community structure. Automated Ribosomal Intergenic Spacer Analysis (ARISA) was used to profile total soil microbial communities. The diversity as well as the community structure of the three riparian zones as well as the adjacent terrestrial zones were considered. The microbial diversity did not differ significantly between plots, however the community composition of the pristine terrestrial riparian zones and invaded riparian zones were shown to be radically different implying that the non-indigenous *Acacias* have a significant impact on the soil microbial communities of these sensitive areas. In addition, microbial communities appear to be highly heterogeneous within these zones and strong influenced by the dynamic fluvial processes as well as hill-slope hydrology. The Water Research Council, South Africa is acknowledged for their financial support, while Cape Nature is thanked for allowing access to these areas.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.077 ARID SOILS MICROBIAL ENZYMATIC ACTIVITY PROFILE AS AFFECTED BY GEOGRAPHICAL LOCATION AND SOIL DEGRADATION STATUS

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Evaluating soil health is critical for any successful remediation effort. Arid lands, with their minimal carbon and water contents, low nutritional status and therefore restricted and seasonal microbial activity pose specific challenges soil health restoration and by extension restoration of ecosystem representative plant populations. Thus it is hypothesized that microbial metabolic profiles in arid soil are sensitive to relatively minor climatic variations and also to changes in soils' physico-chemical parameters induced by human activities. We evaluated the total-soil enzymatic activity profiles for two distinct arid zone locations, one in the Northern Chihuahuan (NC) desert and a second one on tablelands in the Arizona/New Mexico Plateau (ANMP). At the latter site we targeted undisturbed areas as well as heavy metal contaminated lands remediated following surface coal mining. Enzymatic activity profiles were estimated by carbon substrate utilization via total soil respiration assays using the MicroResp® system. Results indicate that in general the NC samples exhibited more complex enzymatic activity profiles than the non-disturbed ANMP samples. Comparison of rhizosphere and non-rhizosphere soils indicated that the divergence in the enzymatic activity profiles was more obvious for the remediated areas than for the native non-disturbed areas. This suggests that the gradients in enzymatic activity profiles from rhizosphere to non-rhizosphere samples may be possibly used to assess the rate and status of remediation of such lands. Thus, for arid lands, the enzymatic activity profiling may be used to evaluate 1) the significance of local climatic and edaphic conditions on the metabolic range of the soil microbial activity and 2) the health status of degraded or remediated arid region soils.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.078 EXPLORING COMMUNITY DYNAMICS OF DENITRIFYING AND ANAEROBIC AMMONIA OXIDIZING BACTERIA IN A LARGE, UNREGULATED RIVER IN ALABAMA, USA

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The Cahaba River flows approximately 200 river miles from the valley ridge physiographic province through the coastal plain physiographic province to its confluence with the Alabama River. An unregulated river, the Cahaba is considered a hotspot of diversity as it supports rare species of mollusks, fish, and plants. Previously collected data show an approximately three-fold drop in water column nitrate concentrations from where the Cahaba flows through the city of Birmingham (population 1.2 million) into the coastal plains. Potential sinks for this lost nitrate are denitrification and anaerobic ammonium oxidation (anammox), which permanently remove nitrate by converting it to dinitrogen gas. My research focuses on understanding how two important factors known to control rates of denitrification, labile organic carbon and dissolved oxygen, influence the denitrifying and anammox Bacterial communities as the Cahaba changes from gravel and cobble dominated substrate to macrophyte beds on bedrock shoals to fine, sandy substrate. Denitrifying and anammox Bacteria can be identified by genes encoding key enzymes;

nitrite reductase for denitrifiers and hydrazine oxidoreductase for anammox. By studying the diversity, quantity and timing of *nir* and *hzo* genes, we will identify environmental factors that influence denitrifier and anammox community structure and respiration rates in a large river ecosystem. Currently, we are focused on Terminal-Restriction Fragment Length Polymorphism (T-RFLP) to determine changes in microbial community composition over time and space. Denitrification potential measurements show a drop in winter denitrifier activity with the highest activity in the bedrock shoals of the fall line year-round; these and other measurements such as dissolved organic carbon, sediment characterization, and nitrate levels will provide a compliment to gene data to determine the environmental factors that control denitrifier and anammox activity and distribution.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.079 ANOXYGENIC PHOTOTROPHIC BACTERIA COMMUNITIES OF HIGH ALTITUDE SALT LAKES IN NORTHERN CHILE

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The Chilean highland harbors a set of extreme habitats characterized by high UV-radiation, partly extreme saline conditions and high daily temperature variations. These unexpectedly quite productive habitats are dominated by microbial life, and phototrophic bacteria are assumed to have a major impact. However, little is known about the diversity and composition of the phototrophic prokaryotic communities. We compared the anoxygenic phototrophic bacteria (APB) communities of three athallassohaline lakes located at different altitudes in northern Chile: Salar de Atacama (2300 m), Salar de Aguas Calientes 3 (3950 m) and Salar de Tara (4400 m) using molecular genetic analyses. Specific primers for the functional genes *fmoA* (*Chlorobiaceae*) and *pufLM* (purple bacteria and *Chloroflexi*) were used. Terminal fragment length polymorphism (TRFLP) analysis of *pufLM* was used to compare the APB communities and cloning experiments and phylogenetic analysis of the functional genes was conducted. The lakes harbor distinct purple sulfur bacteria communities. Salar de Tara was dominated by a highly diverse set of phototrophic *Alpha*- and *Betaproteobacteria*. In contrast, the hypersaline Salar de Atacama was dominated by a so far unknown cluster of *Gammaproteobacteria* and a number of halophilic purple sulfur bacteria of the genera *Halochromatium*, *Thiohalocapsa*, *Ectothiorhodospira* and *Halorhodospira*. A cluster of unknown phototrophic *Proteobacteria*, which might be unique to these habitats, was identified. Further, aerobic phototrophic *Gammaproteobacteria* previously found in Tibetan salt lakes and potentially adapted to high altitudes were present in the Chilean lakes. The great number of yet uncultured and unidentified *pufLM* phylotypes emphasizes the uniqueness of the studied area as well as the need of further studies on phototrophic bacteria and the high potential for the isolation of new species and genera.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.080 FINGERPRINT ANALYSIS OF MICROBIAL COMMUNITIES IN GROUNDWATER SYSTEMS IN YUCATAN PENINSULA, MEXICO

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The rampant population growth and the resulting inadequate wastewater disposal options in Yucatan Peninsula have increased the threat of water pollution in groundwater systems. The saline groundwater layer is often used for wastewater effluent disposal in the region, possibly endangering the overlying freshwater aquifers. Although native microbial communities have always been degrading the organic material that ends up in the groundwater, these communities will likely change as a response to excess carbon load from different (anthropogenic) sources. A relatively fast way to track changes in these diverse microbial communities is community fingerprinting. Although this method does not allow identification of the microbes present, it can show the shifts in community diversity and composition. The purpose of this study is to test the effectiveness of four methods in tracking the changes in the native microbial communities: (1) random amplification of polymorphic DNA (RAPD) analysis; (2) ribosomal intergenic spacer analysis (RISA); (3) phospholipids fatty acid analysis (PLFA); and (4) repetitive DNA amplification (rep-PCR). These four methods were applied to samples taken during wet and dry seasons in one year from karst sinkholes (cenotes) with different amounts of carbon input as well as different distances from anthropogenic pollution sources. The results from RISA have revealed a sufficient variability between the different microbial communities, as well as variation within community composition seasonally. Preliminary results from the RAPD analysis show a similar pattern; however, there was a decreased variability in the RAPD results that may result from primer bias rather than the actual diversity.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.081 LONG-TERM AGRICULTURAL EXPERIMENTS REVEAL N INFLUENCE ON AMMONIA OXIDIZERS AND THE PREDOMINANCE OF CA. NITROSOSPHAERA

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Three long-term land use experiments, Broadbalk at Rothamsted Research in England and the Long Term Ecological Research Network sites in Michigan (Kellogg Biological Station) and Minnesota (Cedar Creek), were chosen to determine the effect of N fertilizer on the abundance of ammonia oxidizers in soil relative to other prokaryotes. At each site, N levels did not influence the abundance of ammonia-oxidizing Archaea (AOA) but did influence the abundance of bacterial oxidizers at two of the three locations, Rothamsted and Cedar Creek. The abundance of AOA was particularly high in one of the Broadbalk plots collected in May 2008. A metagenomic

analysis was done with DNA from this plot and revealed that less than 1% of the over 260 million reads could be mapped to known, sequenced prokaryotes. The most abundant archaeon *Crenarchaeota* in this metagenome was *Candidatus Nitrososphaera gargensis*. *Ca. Nitrososphaera* was confirmed to be the most abundant archaeon in all Broadbalk plots tested and was in the Florida Everglades agricultural soil, where it represented nearly 10% of all 16S rRNA sequences. These results suggested that at the high levels applied in agriculture, N is not a driver of archaeal abundance in soil, and that *Ca. Nitrososphaera* may be a dominant organism even in temperate soils.

Abstract Category
32 Biodiversity and Community Analysis

**PS.32.082 DEPTH DISTRIBUTION OF THERMOPHILIC BACTERIA IN
SEDIMENTARY GEOLOGICAL LAYERS IN THE NORTHERN CHIHUAHUAN
DESERT**

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Thermophiles are organisms that grow and thrive at temperatures above 45°C. They are widespread and are being commonly identified in non-thermophilic environments (e.g. places that are not heated by subsurface geothermal processes such as hot springs). We understand little about their possible role in desert soil ecosystems. As a starting point we decided to assess the distribution of recoverable thermophilic bacteria in an alluvionary deposit across a depth gradient extending from the surface soil crust to depths of over 3 m representing a time span of about 10,000 yrs. To achieve this we sampled two soil profiles in the Northern Chihuahua desert located on the site of the USDA ARS Jornada Experimental Range. It should be noted that, at the test sites, the climatic conditions are associated with very low seasonal precipitation with very little of it penetrating beyond the top 20-30 cm. A biogenic calcium carbonate layer found within the top 0.6 m also restricts any significant root penetration to depth. Soil sample extracts were plated on nutrient agar and incubated for 24 to 48 hrs at 50°C. Colonies recovered at this step were isolated, streaked on new plates and re-incubated under the same conditions. DNA was extracted from the isolates using various cell-lysing methods including culture extraction kit, freeze-thaw cycles, or boiling and followed by DNA ethanol precipitation. A PCR-DGGE amplification using the primers *rpoB* 1698Fgc and *rpoB* 2041R was employed to verify the diversity of the putative thermophiles across the sampled environment. This allowed us to verify the diversity of the recovered isolates and develop similarity trees. Phylogenetic similarity trees are also developed using data obtained from DNA sequencing and comparison with the publicly available DNA sequence libraries. Results are used to develop further hypotheses regarding the functional role of these organisms in the context of the metabolic potential of the sampled soil layers.

Abstract Category
32 Biodiversity and Community Analysis

PS.32.083 CATTLE OVERWINTERING AFFECTS MICROBIAL PROCESSES AND COMMUNITY COMPOSITION IN GRASSLAND SOILS

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Cattle overwintering changes physico-chemical and biological properties of grassland soils. High deposit of animal excrements and destruction of soil surface due to animal trading increase soil pH and contents of P, Ca, Mg, K, N, C. In summer, when cattle is not present, impacted soils undergo regeneration. The changes in soil properties are reflected in the composition of microbial community. The aim of this study was to determine to which extent cattle overwintering affects soil microbial processes of organic matter transformation, measured as activities of extracellular enzymes (α - and β -glucosidase, cellobiohydrolase, β -xylosidase, N-acetylglucosaminidase, phosphomonoesterase, phosphodiesterase, arylsulfatase and aminopeptidases). The biomass and microbial community structure was characterized using PLFA, ergosterol, and group specific qPCR. Study sites were located in Borová farm, Czech Republic. Sites with severe and limited impact were compared to control grassland soil with no access for cattle in spring (following overwintering), summer, and autumn (before cattle arrival). In spring, activities of all enzymes except β -xylosidase and N-acetylglucosaminidase differed significantly among affected and nonaffected sites. Control site showed lower microbial biomass. Microbial community composition differed between all three sites. More fungal biomass was recorded in spring compared to summer and autumn. The recovery phase in severely affected site was characterized by a decrease of pH, amount of P, Ca, Mg, K, N, C and increase of most of enzyme activities in autumn. Microbial community composition in sites affected by cattle overwintering in autumn still differed from control. Our results show that the practice of cattle overwintering strongly affects microbial processes and microbial community structure in grassland soils. During regeneration, differences in soil properties between affected sites and control decrease, but complete recovery is not achieved.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.084 METHANOGEN-INFLUENCED CORROSION IN PIPELINES TRANSPORTING WATER FROM THE SUBSURFACE TO A SAGD SITE

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Oil is produced from the Alberta oil sands by either surface mining or steam-assisted gravity drainage (SAGD). In SAGD high temperature and pressure steam is injected into the bitumen-containing subsurface to produce a mixture of water and oil. In view of limitations of using surface water, waters drawn from the subsurface are typically used. Use of the latter appears correlated with enhanced corrosion problems, prompting a more detailed investigation. Samples of water from the Grand Rapids (E1) and McMurray (E2) formation, as well as of combined water (E3) were obtained from an Industrial sponsor. Sections of cut-out, corroded pipe were also provided and were separated into pipe-associated water (PAW) and pipe-associated solids (PAS). The activities of relevant microbiological groups, including the sulfate-

reducing bacteria (SRB) were assessed. Corrosion rates were determined by the weight loss method. Microbial community composition was determined by DNA isolation, amplification of 16S genes and pyrosequencing.

Activities of microbial groups, including SRB, generally followed the order E2>E3>E1.

Corrosion rates for metal coupons, suspended in these waters were also generally higher for E2 and E3 than for E1. Analysis of microbial community composition indicated a high fraction of methanogenic Archaea in E2 and E3 waters. This fraction was even higher in samples of PAS, obtained from pipeline cutouts (>50%). These observations prompted us to investigate whether methanogens, rather than SRB, contributed to corrosion. Incubation of metal coupons in E1, E2 or E3 waters with no other added electron donor did indeed give methane formation in the order E2>E3>>E1.

Methanogenic archaea are the main community component in waters withdrawn from the subsurface for oil production by SAGD. They colonize the pipeline surface effectively and appear to contribute to corrosion of the pipelines used for water transport.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.085 MICROBIAL DIVERSITY AND ACTIVITY IN FRACTURING FLUIDS FROM A WYOMING GAS FIELD

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The production of natural gas from subsurface formations requires fracture technologies in which a fracturing fluid, consisting of sand suspended in the guar gum solution, is forced into the fracture to "prop" it open. The fracturing fluid is then flushed back to the surface by produced water, leaving behind the permeable channel of sand for outflow of gas. The guar gum serves to increase viscosity of the fracturing fluid, to reduce fluid loss and to decrease fluid friction. However, it also serves as a carbon and energy source for microbial growth in production lines, gas-fluid separators, storage tanks and other facilities. In some operations disposal of large amount of produced water, choking of filtration facilities, hydrogen sulfide production and microbially influenced corrosion are frequently encountered problems.

To assess microbial diversity and activity, samples were collected and inoculated into culture medium, containing guar gum as sole carbon and energy source in the absence (fermentative conditions) or presence of sulfate or nitrate as electron acceptors. Flow back collection pit samples had high activity of fermentative bacteria with a high rate of guar gum utilization and hydrogen gas production at 30, 60 and 80 °C. High activity of heterotrophic nitrate-reducing bacteria (hNRB) was also observed immediately at 37 and 60 °C, whereas activity of sulfate-reducing bacteria (SRB) was not observed until 20 days of incubation. This may be because, different from hNRB, SRB use only carbohydrate fermentation products as electron donor. Similar observations were made for a sample from a produced water holding tank. Hence, SRB can be responsible for high hydrogen sulfide levels if produced fluids are stored for prolonged periods before disposal. DNA isolation and 454 pyro-sequencing results showed the presence of Firmicutes, Bacteroidetes, Proteobacteria and Thermotogae as the major divisions present. Other divisions, including Synergistetes and Spirochaetes.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.086 A COMPREHENSIVE APPROACH IN THE SEARCH OF MICROBIAL INDICATORS ASSOCIATED WITH DIFFERENT AGRICULTURAL PRACTICES UNDER NO-TILL MANAGEMENT

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We present the first results of an interdisciplinary project (BIOSPAS) aimed at understanding how the microbial structure in soil under no-till farming are related to agricultural practices and soil properties. Three treatments were defined according to land use: 1) Sustainable agricultural management, subjected to intensive crop rotation and nutrient amendment, 2) Non-sustainable agricultural management with high mono-cropping without nutrient reposition, 3) Natural environment. Blocks of treatments were replicated 4 times in agricultural fields that had documented history of no-till management, at sites located across a west-east transect in Argentina's Pampas. Each sample was collected in triplicate, as a composite of 16 soil subsamples from 0-10 cm depth collected at 50 m intervals. The relative abundance of major taxonomic groups of bacteria was estimated using primer-specific quantitative PCR and PCR-DGGE. Pseudomonads were evaluated by quantifying the culturable fraction and by molecular markers *oprF* and *gacA*. Fatty acids profile, phosphatase and Glomaline Related Soil Proteins (GRSP) were also surveyed along with key soil variables (pH, moisture, organic carbon content, total nitrogen and P). Multivariate analysis was used to analyze microbial soil biology data. Differences in physicochemical and biochemical parameters were more consistently related to agricultural practices than bacterial community data. Principal component analysis separated soil management independently of site. Patterns of significant differences between treatments were obtained at the broad level of taxonomic resolution examined, but for most taxonomic groups the differences were site-specific. The results indicate that no-till systems support distinctive bacterial community composition and function according to land management, although the significance of these differences as indicators of soil quality requires further exploration.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.087 THE SYNCHRONISM OF VEGETATION, SOIL PROPERTIES AND THE SOIL MICROBIAL COMMUNITY IN NATURAL AND HUMAN-ASSISTED WETLAND RESTORATION

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Although the performance of vegetation has been widely used as a measure of each succession stage, the diversity and activity of the microbial community are crucial to the functions of

wetland biogeocenose. To investigate ecological interactions involved in wetland restoration, we examined the indicators of vegetation, soil properties and the microbial community in three human-assisted plots and 15 natural restoration plots that represent five succession stages in the modern Yellow River Delta. The microbial community level physiological profiles were determined with Biolog EcoPlate™, and the bacterial community structure was profiled using denaturing gradient gel electrophoresis. The relationships between all parameters were tested and correlations found using the Spearman rank correlations analysis combined with regression and multivariate analyses tested by Monte Carlo permutation. The ordination bio-plots demonstrated a significant increasing trend in vegetation diversity and evenness, soil microbial community activities, and metabolic and genetic diversity, synchronized with the fertility and desalination of soil, which may be plant-driven in a natural succession at the community scale. Meanwhile, obvious improvements in vegetation and microbial conditions were found in the human-assisted restoration project region, which can be ascribed to the significantly decreased salinity of soil by artificially increasing freshwater. In conclusion, the tight synchronization between trends within the vegetation-soil-microbe system indicates that interactions of their roles may be important in natural successional changes of the wetland ecosystem. Also human-guided restoration should be focused in future on interlinking the vegetation-soil-microbe elements to establish adaptive monitoring management. The results may be helpful for wetland restoration projects performed in similar regions.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.088 NON-RANDOM ASSEMBLY OF HOT SPRINGS BACTERIAL COMMUNITIES

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Understanding how communities are assembled is central to the field of ecology. However, this issue has generated much debate. Do species come together randomly, or are there rules that determine if certain combinations of species can coexist? Previous studies have determined that many communities are non-randomly structured, and that patterns of non-random assembly are similar among all three domains. However, it is not known if there are differences in assembly patterns among different bacterial divisions. The aim of this study is to evaluate assembly patterns for bacterial divisions. rRNA barcoded pyrosequencing data from a survey of microbial diversity along two thermal gradients in Yellowstone National Park, USA were used to create incidence matrices for eight bacterial divisions. Four of the divisions exhibited less co-occurrence than expected by chance: the Cyanobacteria, Chloroflexi, CFB, and Acidobacteria. We propose that both temperature-driven, niche-based processes and competition among organisms, respectively, contribute to the observed non-random community assembly.

Abstract Category

32 Biodiversity and Community Analysis

**PS.32.089 RESPONSES OF BACTERIAL AND ARCHAEAL AMMONIA
OXIDIZERS TO SOIL ORGANIC MATTER UNDER LONG-TERM MANAGEMENT**

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Ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA) co-exist in soil, but their relative distribution may vary depending on the environmental conditions. Effects of changes in soil organic matter and nutrient content on the AOB and AOA are poorly understood. Our aim was to compare effects of long-term soil organic matter depletion and amendments with labile and recalcitrant organic matter, with and without easily plant-available nitrogen, on the activities, abundances and community structures of AOB and AOA. Soil was sampled from a long-term field site in Sweden that was established in 1956. The potential ammonia oxidation rates, the AOB and AOA *amoA* gene abundances and the community structures of both groups based on T-RFLP of *amoA* genes were determined. Type and amount of organic matter had an impact on the AOB and AOA community structures and the AOA abundance. Our findings confirmed that AOA are abundant in soil, but showed that under certain conditions the AOB dominate, suggesting niche differentiation between the two groups at the field site. The large differences in potential rates between treatments correlated to the AOA community size, indicating that they were functionally more important in the nitrification process than the AOB. The AOA abundance was positively related to addition of labile organic carbon, which supports the idea that AOA could have alternative growth strategies using organic carbon. The AOB community size varied little in contrast to that of the AOA. This indicates that the bacterial ammonia oxidizers as a group have a greater ecophysiological diversity and potentially cover a broader range of habitats.

Abstract Category

32 Biodiversity and Community Analysis

**PS.32.090 ACTIVITY, ABUNDANCE AND COMPOSITION OF THE AMMONIA
OXIDIZING BACTERIAL AND ARCHAEAL COMMUNITIES IN PEAT SOIL**

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Boreal and subarctic peat soils cover nearly 3% of the Earth's land surface and when drained these soils become major sources of the greenhouse gas N₂O. We know little about ammonia oxidizers in these types of soils. Our aim was therefore to determine temporal and spatial differences in the abundance and community composition of the ammonia oxidizing bacteria (AOB) and archaea (AOA) at different depths in the upper 0.5 m of a drained and forested peat soil and relate this to emissions of N₂O and ammonia oxidation activity. We sampled soil and measured N₂O emissions at five replicated locations over a period of nine months in May, August, November and March. Further, we measured the substrate-induced ammonia oxidation (SAO) rates and estimated community composition and abundance by targeting the *amoA* genes for the AOB and AOA using T-RFLP analysis and quantitative real-time PCR. In contrast to what has been found in most other terrestrial environments, the AOB dominated over the AOA in abundance. We did not find any depth-related effects on the abundance, composition or

activity of the ammonia oxidizers present in the peat, probably explained by the rather homogenous soil profile with respect to soil parameters. However, both the AOB and AOA communities showed temporal variations in their composition, although temporal changes in abundance were only observed for the AOB with a decrease in numbers from May to March. Such differences were not reflected by the ammonia oxidation rates. The N₂O emissions were increasing over the sampling period to a maximum in March, but since these did not correlate to either the AOB or AOA abundances, changes in community composition or SAO activity, other organisms like denitrifiers rather than ammonia-oxidizers were likely involved.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.091 AMOEBOFLAGELLATES AND KINETOPLASTIDS DOMINATE THE PROTIST GRAZING COMMUNITY OF AN ACIDIC GEOTHERMAL LAKE

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Boiling Springs Lake (BSL) is a flooded fumarole that contains a 1.8 ha pool of pH 2.2, 52°C water in California's Lassen Volcanic Park. It is a Microbial Observatory site focused on understanding the interactions among the entire microbial community. Little is known of food webs in such environments, but we hypothesized that low community diversity would reduce the complexity of trophic interactions. Here we present the diversity and biogeographical distribution of the grazing protists in and around this feature.

We used a culture-based genetic approach to estimate the function of the physical environment on diversity. We sampled lake water and sediments along transects from the lake to the surrounding forest. We used denaturing gradient gel electrophoresis (DGGE) to explore the diversity of organisms present, using SSU rRNA (V8 region). We also enriched and isolated several organisms and developed group-specific ITS primers.

The near-lake environment is dominated by the heterolobose amoeboflagellate *Tetramitus thermoacidophilus*, genetically similar but distinct to that found in Kapchatka and Italy (Baumgartner et al., 2009). This organism grows optimally at 40-50 C, pH 2, and appears to favor ascomycete fungi as its food source. In contrast, the major bacterivore is a flagellate ubiquitous in several low-T acidic side pools, Bodo saltans. Samples further from the lake largely yielded the common soil lobose amoebae *Acanthamoeba* and *Hartmannella* spp. *Acanthamoeba* was isolated and detected from near-lake samples as well but did not grow under those conditions.

Biogeographical distribution of BSL's microeukaryotic grazing community, as assessed by genetic screens, suggests a unique, endemic community in the lake and an increasing gradient of diversity out to the surrounding forest. These patterns are discussed in the context of island biogeographical theory.

Abstract Category
32 Biodiversity and Community Analysis

PS.32.092 STUDYING THE DIVERSITY OF SPORE-FORMING MICROORGANISMS IN NATURAL COMMUNITIES

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The study of bacterial endospores has been generally restricted to the domains of medical research and food technology (well-known examples of this are *Bacillus anthracis* and *Clostridium botulinum*). By contrast, the diversity and function of spore-forming microorganisms in the environment is not yet well understood. New species of spore-forming bacteria (i.e. *Carboxydotherrnus hydrogenoformans* Z-2901) and new metabolic functions connected to bacterial spore-formers (e.g. metal reduction) have been reported recently. This suggests that both the metabolic and ecological diversity of spore-forming bacteria is far from being comprehensively assessed. One of the main difficulties when studying spore-forming microorganisms is the inability to specifically target this group within a complex microbial community. Therefore, the aim of this study is to develop a method to separate microbial spores from a complex mix of other cells and environmental matrix. So far, a combination of physical and chemical treatments is needed in order to achieve the best results in separation experiments with known cultivable spore-forming bacteria (*Bacillus subtilis*, *B. megaterium* and *B. alvei*). In addition, the potential to develop specific molecular markers to study the diversity of spore-forming bacteria *in situ* without cultivation is also investigated. A total of 57 genome sequences of spore-forming bacteria are retrieved from genome databases (Comprehensive Microbial Resource and Integrated Microbial Genomes) to identify a core of genes involved in sporulation. So far, six genes common to all genomes analyzed were identified. Phylogenies based on the common genes for sporulation show few differences with phylogenies based on the 16S rRNA gene. The diversity of spore-forming microorganisms is therefore assessed by two approaches: 1) *in situ* targeting with molecular markers and 2) separation, extraction and sequencing of spores directly from environmental samples.

Abstract Category
32 Biodiversity and Community Analysis

PS.32.093 ELUCIDATING NICHE DIVERSIFICATION IN AMMONIA OXIDIZING ARCHAEA AND BACTERIA IN COLUMBIA RIVER FRESHWATER SEDIMENTS

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Recent studies on ammonia oxidizing archaea (AOA) suggest that they are actively involved in global carbon and nitrogen cycles. While these organisms are ubiquitous across diverse ecosystems, their metabolic properties are not well understood. In previous work, we used combined microanalytical and molecular methods to analyze the geochemistry and microbial

populations in Columbia River sediments. We observed composition changes in putative AOA populations across steep O₂ and Eh gradients, although there were no significant differences in the archaeal ammonia monooxygenase subunit A (amoA) gene abundance. To better correlate changes in AOA populations with geochemistry, in this study we focused on finding sites at which collected sediment cores revealed changes in both AOA composition and number with depth. We also characterized additional geochemical gradients at fine vertical resolution. By linking molecular and geochemical data, we aim to discover the strategies utilized by AOA for niche diversification and to try to understand their coexistence with ammonia oxidizing bacteria (AOB). Sediment cores were collected from different sites along the Columbia River. O₂ and Eh profiles were generated at the mm scale onsite, which usually produced steep gradients. Nitrate and metals (Mn and Fe) were also measured. The cores were subsequently fractionated into 2 mm subsections and total DNA was isolated for molecular characterization. Abundance of putative AOA and AOB was quantified by qPCR using the amoA gene from each group as biomarker. The abundance of 16S rRNA genes was also quantified by qPCR to estimate contributions of AOA to total nonthermophilic Crenarchaeota populations. AOA and AOB community composition was analyzed in different sediment fractions by PCR-SSCP (single-strand conformation polymorphism) analysis. Combined results from these molecular and microanalytical measurements provide information about the metabolic strategies of AOA in freshwater sediments.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.094 ABUNDANCE AND GENETIC DIVERSITY OF E. COLI IN A TROPICAL WATERSHED

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E. coli is known to survive and even grow in tropical soil. Since soil-sourced *E. coli* may complicate water quality monitoring efforts, it is important to better understand the abundance and genetic diversity of *E. coli* in tropical soil and determine how it affects *E. coli* in water.

A ten-month monitoring was conducted in a tropical watershed that contains a rainforest and a densely populated urban community. Water and soil samples were collected from sites in both the urban and forest reaches of the watershed. The numbers of *E. coli* in soil and water samples were determined by plate counting. *E. coli* isolates were obtained from each sample, and then subjected to rep-PCR genomic DNA fingerprinting. The DNA fingerprints of the *E. coli* isolates were used to determine the genetic diversity of *E. coli* in the watersheds. In addition, the genetic relatedness between *E. coli* isolates from water and soil samples at same sampling sites were compared.

1. Higher *E. coli* concentrations in water are often associated with urban land use, while stream water in the forest section of the watershed usually has much lower concentrations of *E. coli*.
2. Tropical soil can harbor a very high number of *E. coli*.
3. Enormous genetic diversity of *E. coli* exists in the tropical soil.
4. No significant exchange between soil and water *E. coli* was observed.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.095 MICROBIAL DIVERSITY IN MEDICINAL CHILCA GAPS _PERÜ

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It is recognized by the community, various therapeutic properties of sludge Chilca saline, the mixture of microorganisms and their bearing minerals such as silicon sludge, instrumental in fostering the development of collagen in skin firmness make these lakes were visited by Tourists from different parts of the country in search of improvement to skin diseases, rheumatism, arthritis and so on. To determine the presence of contagious infectious organisms common in our area and isolate aerobic bacteria, anaerobic fungi own environment and saline lagoons Chilca.

We worked with samples of water and sludge lagoons miraculous, delighted and mellicera. To isolate infectious microorganisms and use traditional means to isolate environmental microorganism used in the preparation of culture media the same water of the lakes, with pH and temperature ecosystem.

In the traditional culture media for Enterobacteriaceae, Estaphylococcus aureus, Candida and dermatophyte cultures were negative in culture media prepared with water lagoons frequently highlights the aerobic growth of Thiobacillus sp, B. subtilis, Acuapirillum, Sarcina. In anaerobic media genres found Rhodospirillum, Rhodovibrium salivarum, spore-forming Gram-negative cocci and others not identified.

The absence of infectious organisms infect crops, confirms their sensitivity to these ecosystems, preventing infection among visitors. It is necessary to continue work to identify molecularly the organisms and ecosystems native to the sludge metabolic medicine.

Abstract Category

32 Biodiversity and Community Analysis

PS.32.096 LONG-TERM GRAZING INTENSITIES AFFECT SOIL MICROBIAL COMMUNITY AND DIVERSITY IN AN INNER MONGOLIAN STEPPE

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Ungulate grazing is known to play a crucial role in regulating nutrient cycling and controlling plant community structure and productivity in grassland ecosystems. However, little is known about the effects of grazing intensities on soil microbial community structure and diversity. In this study, we measured plant community biomass and diversity, soil characteristics and microbial community structure and diversity in a 16-year field experiment that had four grazing intensities (control site, CK; low-intensity grazing (LG), moderate-intensity grazing (MG) and

high-intensity grazing (HG) in relation to local grazing pressure) in an Inner Mongolia typical grassland. The CK, LG, MG and HG represent 0.00, 1.33, 4.00 and 6.67 sheep ha⁻¹, respectively. Bacterial community structure and diversity under grazing intensities were assessed with PCR amplification of DNAs extracted from soils and denaturing gradient gel electrophoresis (DGGE) separation. The results showed that the CK had higher soil moisture, organic C, NH₄⁺-N and NO₃-N concentrations in comparison to different grazing intensities sites. Among grazing intensities sites, the HG had the lowest plant aboveground biomass and diversity. Principal components analysis of DGGE patterns showed that the LG and MG sites were significantly different from the CK and HG sites. In addition, soil bacterial diversities in the LG and MG sites were significantly higher than the other two sites. Redundancy analysis of the relationship between environmental factors and microbial community patterns showed that soil moisture and organic C are more indicative of the changes of soil microbial community among all sites. With overall evaluation of factors above, our results indicated that low and moderate grazing intensities would be helpful to harbor greater soil microbial community structure and diversity and to provide grassland productivity in the Inner Mongolia steppe.

Abstract Category

32 Biodiversity and Community Analysis