



## Molecular ecology of denitrifiers

Laurent Philippot

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**ABSTRACTS**

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## DARWIN'S TREE OF LIFE

### THE GREAT TREE OF LIFE: FROM DARWIN TO BACTERIAL DIVERSITY

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The tree of life as a mythological concept is common to many cultures, ancient and modern. As a scientific metaphor for the evolutionary descent of organisms from common ancestors, it is relatively modern, although several instances of its use predate Darwin – most notably in Lamarck's *Philosophie Zoologique* (1809). Early on, phylogenetic thinking also permeated disciplines outside biology, including historical linguistics and textual criticism. Darwin left an iconic image of a phylogenetic tree in his B notebook from 1837–8. He included a phylogenetic tree as the only figure in *The Origin of Species*, where he also provides an eloquent description of 'the great Tree of Life, which fills with its dead and broken branches the crust of the earth, and covers the surface with its ever branching and beautiful ramifications'. Alfred Russel Wallace also used this metaphor in his essay from 1855 – 'a complicated branching of the lines of affinity, as intricate as the twigs of a gnarled oak' and is now buried under the trunk of a fossil tree. Darwin's followers, particularly German naturalist Ernst Haeckel, created more elaborate trees. However, rigour and a measure of objectivity in tree building arrived only in the late twentieth century, with the advent of cladistics and molecular phylogenetics. Molecular biology has provided support for a common origin and ancestry of all living organisms. In recent decades, the tree of life metaphor has simultaneously been uplifted by wide-ranging and evermore sophisticated surveys of biological diversity and downgraded by the discovery of rampant horizontal gene transfer in bacterial and archaeal genomes, twinned with doubts as to its applicability to the earliest stages of evolution.

### VIRUS EVOLUTION: SYMBIOSIS, MUTUALISM AND SYMBIOGENESIS

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Viruses are generally thought of as annoying pathogens, but that represents only a small proportion of their lifestyle choices. If we think of viruses as symbionts, where they clearly belong in the original definition of symbiosis, we see that they can be mutualists or antagonists, and are probably most often commensalists. Mutualistic viruses have only recently been studied, but they have dramatic effects on the survival of their hosts. We have found that viruses can be instrumental in the rapid adaptation of their hosts to extreme environmental shifts. This is because viruses may be a unique source of new genetic information. This process has clearly happened throughout the evolution of life and the footprints of these events can be seen in the evidence of past viral integration into the genomes of their hosts, a process of symbiogenesis. This presentation will discuss current discoveries of viral mutualistic symbiosis; evidence of the role of these events in the past; and where viruses belong on the tree of life.

### THE NTH EUKARYOTIC 'SUPERGROUP' AND THE EVOLUTIONARY AND ECOLOGICAL COMPLEXITY OF *CERCOZOA*

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Recently, developments in phylogenetic analyses have enabled significant advances towards resolving evolutionary relationships among protozoan lineages, which themselves represent a large proportion of the evolutionary history of eukaryotes including their earliest branches. These developments include methods analysing the evolution of gene sequences among taxa, the distribution of molecular characters such as gene fusions and insertions, and the simultaneous analysis of multiple genes culminating in the current emergence of large-scale 'phylogenomic' studies. Such approaches are simplifying the classification of eukaryotes into a small number of diverse 'supergroups', e.g. unikonts (=opisthokonts plus *Amoebozoa*), excavates, plants, haptophytes plus cryptophytes, and the SAR group (alveolates, stramenopiles, and *Rhizaria*). However, this process is very much in flux and the supergroup view of eukaryote life varies among research groups as new data appear and different analytical approaches are taken. I will discuss the evolutionary diversity and phylogeny of *Rhizaria*, one of the least well known of the supergroups. *Rhizaria* is very lineage-rich and morphologically/ecologically diverse, comprising the phyla *Cercozoa*, *Radiozoa*, and *Foraminifera*. However, recent results are questioning this trinity and may lead to the creation of new taxa in the near future. I will then focus on the relatively new phylum *Cercozoa*, its internal relationships and those with other *Rhizaria*, and will outline some of the studies currently underway on members of this highly diverse phylum.

### EVOLUTION OF THE MINIMALIST PLASTID GENOME OF DINOFLAGELLATES

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Dinoflagellates possess an extremely reduced plastid genome relative to those examined in plants and other algae. The organization of this genome is also radically different. Analyses have revealed the genome reduction is the result of widespread transfer of genes to the nucleus. The remaining genes mostly encode subunits of Photosystems I and II, the cytochrome *b<sub>6</sub>f* complex, and ATP synthase, as well as rRNAs and a few tRNAs. In typical plastid genomes all the genes are physically linked on a single molecule. However, in dinoflagellates the plastid genes are located on small plasmids, commonly referred to as 'minicircles'. In the majority of cases a single gene is present on each minicircle, but examples with up to four genes have been documented. A number of other unusual features are associated with the dinoflagellate plastid genome, such as the presence of a region of high sequence identity on each minicircle, unusual codon usage, polyuridylation of transcripts and the presence of 'empty' minicircles. We will discuss our current understanding of the dinoflagellate genome and possible reasons for its evolution.

### A NEW ROOT FOR THE TREE OF LIFE

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A rooted tree of life provides a framework to answer central questions about the evolution of life. Here we review progress on rooting the tree of life, and introduce a new root of life obtained through the analysis of indels, insertions and deletions, found within paralogous gene sets. Through the analysis of indels in 8 paralogous gene sets, the root is localized to the branch between the clade consisting of the *Actinobacteria* and the double membrane (Gram-negative) prokaryotes and one consisting of the archaeobacteria and the *Firmicutes*. This root provides a new perspective on the habitats of early life, including the evolution of methanogenesis, membranes, hyperthermophily; and the speciation of major prokaryotic taxa.

Our analyses exclude methanogenesis as a primitive metabolism, in contrast to previous findings. They parsimoniously imply that the ether archaeobacterial lipids are not primitive, and that the cenozoic prokaryotic population consisted of organisms enclosed by a single, ester-linked lipid membrane, covered by a peptidoglycan layer. These results explain the similarities previously noted by others between the lipid synthesis pathways in eubacteria and archaeobacteria. The new root also implies that the last common ancestor was not hyperthermophilic, although moderate thermophily cannot be excluded.

### GRAFTING MITOCHONDRIA ONTO THE TREE OF LIFE

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Eukaryotic life without mitochondria is unimaginable. It seems therefore quite amazing that these essential eukaryotic organelles are actually bacterial in origin. For years, the origin of the mitochondrial symbiont has been sought amongst the parasitic alphaproteobacteria such as *Rickettsia*. However, recent genomic and cell biological efforts aimed at understanding various protist lineages seem to underscore the notion that the bacterium that gave rise to mitochondria more likely resembled a metabolically more versatile free-living alphaproteobacterium. This realization has important implications for how this key endosymbiosis became established in the first place. As we currently know of no eukaryote that is primarily amitochondriate, theories containing amitochondriate eukaryotic hosts can no longer be maintained. Recent studies into anaerobic mitochondria such as hydrogenosomes and mitosomes have transformed the way we now see how this key event in eukaryotic evolution took place.

### THE EUKARYOTIC TREE OF LIFE: ENDOSYMBIOSIS TAKES ITS TOL

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It is difficult to overstate the impact of endosymbiosis on the evolution of eukaryotic life. Plastids, the photosynthetic organelles of modern-day plants and algae, evolved from once free-living cyanobacterial endosymbionts that became permanent fixtures inside a heterotrophic host eukaryote. Subsequent to the evolution of 'primary' plastids,

photosynthesis has spread across the eukaryotic tree by 'secondary endosymbiosis', a process in which a primary plastid-bearing eukaryote is assimilated by an unrelated non-photosynthetic eukaryote. Cryptophytes and chlorarachniophytes are two evolutionarily significant microalgal lineages that are unusual in that the nuclei of their algal endosymbionts persist in a highly reduced form—the 'nucleomorph'. At less than 1 Mbp in size, nucleomorph genomes are the most highly reduced nuclear genomes known, having lost or transferred most of their genes to the nuclear genomes of their secondary hosts. In this presentation I will review the current state of knowledge with respect to the origin and evolution of nucleomorph genomes across the known diversity of cryptophyte and chlorarachniophyte algae. I will also discuss ongoing efforts to sequence the host nuclear genomes of the cryptophyte *Guillardia theta* and the chlorarachniophyte *Bigelowiella natans*, and what they tell us about genome mosaicism in secondary plastid-containing algae.

## POLYPLOIDIZATION AND THE EVOLUTION OF GENE ORDER ALONG YEAST GENOMES

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Abstract not received

## WHERE ARE VIRUSES IN THE TREE OF LIFE? AND WHAT CAN THEY TELL US ABOUT THE TREE?

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The nature of viruses (living or not?) and their place in the tree of life (legitimate or not?) have been recently hotly debated (1–4). In the traditional view, viruses are merely by-products of cellular evolution and play a secondary role in the history of life, mainly by serving of vehicles for cellular genes and by interfering in cellular competition (3). I will argue that this view is biased by a misconception on the nature of viruses that denies the possible existence of true viral genes (born in the virosphere). In my opinion, viruses (defined as capsid encoding organisms, 5) and derived mobile elements are the major driving force in Darwinian evolution of the whole biosphere, both at the level of variation and selection. They have co-evolved with their hosts (i.e. with the tree of life) and form a tree of their own in the shadow of the cellular tree. In each of the three domains (*Archaea*, *Bacteria*, *Eukarya*), different families of viruses and related mobile elements thus co-evolved with their hosts (4). Although some very ancient families are present in all domains, viruses are very different from one domain to the other. In my opinion, the existence of three distinct viral domains is a strong argument against scenarios in which one domain originated from another one or from the two others (e.g. fusion scenarios between archaea and bacteria for the origin of eukaryotes, or rooting the tree of life in one of the three domains).

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## SEARCH FOR A TREE OF LIFE IN THE PHYLOGENETIC FOREST

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The wide spread of horizontal gene transfer (HGT) among prokaryotes is often considered to undermine the Tree of Life (TOL) concept. However, the possibility remains that the TOL could be salvaged as a statistical central trend in the phylogenetic 'Forest of Life' (FOL). We performed a comprehensive comparative analysis of 6901 phylogenetic trees for prokaryotic genes including a group of 102 nearly universal trees (NUTs). Despite the high topological inconsistency among the trees in the FOL, most likely, owing to extensive HGT, a distinct signal of vertical inheritance that was particularly strong among the NUTs was detectable. The HGT events among the NUTs seemed to be distributed randomly and did not obscure the vertical signal. The topology of the NUTs was highly similar to the topologies of numerous other trees in the FOL, so although the NUTs cannot represent the FOL completely, they might reflect a significant central trend. Thus, the original TOL concept

becomes obsolete but the idea of a 'weak' TOL as the most pronounced trend in the thicket of the FOL appears viable and worth further investigation.

## EVOLUTION OF MULTICELLULARITY: FROM STRESS SENSING TO DEVELOPMENTAL CONTROL

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Dictyostelids or social amoebas aggregate to form fruiting bodies consisting of stalk cells and spores in response to food depletion. To provide a framework for studying the evolution of multicellularity in the Dictyostelids, we constructed a molecular phylogeny of all known Dictyostelids. Plotting of morphological traits characters to the phylogeny showed that an evolutionary trend towards the formation of large unbranched fruiting structures with more differentiated cell types in the more derived species, as opposed to small, clustered and branched structures with fewer cell types in the more basal groups. The evolution of larger fruiting structures is accompanied by the use of cAMP as a chemoattractant to bring cells together in aggregates and the loss of encystation as an alternative survival strategy to sporulation in fruiting bodies.

We next identified and functionally analysed developmental signalling genes in species that span the phylogeny. In its more common intracellular role, cAMP triggers initiation of development and maturation of spore cells in fruiting bodies, where it also mediates inhibition of spore germination by high osmolality. We found that the intracellular role of cAMP in spore maturation and germination is evolutionary derived from a homologous role in encystation and excystation of solitary amoebas.

Apart from its role as chemoattractant, extracellular cAMP, detected by cell surface cAMP receptors (cARs), coordinates fruiting body morphogenesis and induces prespore cell differentiation in the derived species *D. discoideum*. cAR genes could be identified throughout the phylogeny. Others reported that disruption of cAR genes in *D. discoideum* blocks aggregation and further development. However, we found that in basal taxa, loss of cARs did not affect aggregation, but disrupted fruiting body morphogenesis. Moreover, the fruiting bodies had cysts instead of spores in the spore head. Combined, the data suggest that cAMP signaling in social amoebas evolved from cAMP-mediated encystation in solitary amoebas; cAMP secretion in aggregates prompted the starving cells to form spores and not cysts, and additionally organized fruiting body morphogenesis. cAMP-mediated aggregation was the most recent innovation.

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## ENVIRONMENTAL DNA COMBINED WITH FLUORESCENT *IN SITU* HYBRIDIZATION REVEALS A MISSING LINK IN THE FUNGAL TREE OF LIFE

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Chytridiomycete fungi are important microbial components of aquatic environments. However, due to a reliance on culture based analysis, the evolutionary complexity of the 'chytrids' has been largely underestimated. Environmental gene library analyses of aquatic environments has recently demonstrated a highly novel form of deep branching 'fungi', the environmental diversity and complexity of which indicate that these uncharacterized organisms are an important component of aquatic ecosystems. Here, we use a combination of environmental PCR and fluorescent *in situ* hybridization (FISH) to elucidate the complexity and evolutionary history of these novel 'fungi'. PCR amplification and DNA sequence analysis extending through the 18S, 5.8S and 28S rRNA encoding genes, allowed improved phylogenetic analysis using complex models. The development of FISH probes



reveals our target group to be a picoeukaryote of 4–5 µm in diameter and ubiquitous within local freshwater sites. Cells were morphologically similar and were often visualized in high numbers within structures we suggest to be sporangia. Interestingly, cell wall staining reveals our target organisms to be lacking in the characteristic fungal cell wall, unlike known 'chytrid' groups. This, in combination with our phylogenetic data, suggests this highly diverse group may be a missing link in the fungal tree of life.

#### FROM DARWIN TO FLEMING: ANCIENT AND MODERN PATTERNS OF FUNGAL EVOLUTION

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DNA sequences and metagenomics have uncovered a breathtaking spectrum of fungal biodiversity. Recent work on the base of the fungal tree has uncovered whole new phyla that were considered distinctly 'un-fungal', such as the intracellular microsporidia, and other as-yet undescribed clades representing a huge pool of diversity. Sampling the tips of the fungal tree, global sampling and reverse-taxonomy has enabled the discovery of cryptic species and the description of recent and rapid evolutionary processes that have great impacts on ours, and planetary, health. This talk will outline the functionality of sampling and describing this huge span of diversity; the evolutionary processes that create and maintain these fungal species, and their eventual fate.

#### PROTISTS – THE HIDDEN MAJORITY IN THE EUKARYOTIC TREE OF LIFE

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Protists are unicellular eukaryotes that have conquered almost every habitat on our planet. More than two billion years of evolutionary history gave rise to a diversity in protists that is probably unmatched by any other eukaryote kingdom. Therefore, it is not surprising that neither microscopy-based nor molecular-based surveys of protistan diversity conducted in a variety of environmental systems has sampled protistan diversity to completion. Instead, these studies indicated that the species richness of protistan communities may be so high as to border on being intangible, and even the largest diversity surveys appear to be no more than small samples from apparently endless lists of species present at any locale studied. The recent introduction of highly parallel tag sequencing strategies in microbial ecology and diversity research offers the possibility for an unprecedented scale of sampling at reasonable time and cost. We applied such a tag sequencing strategy (454 pyrosequencing) with the aim to sample the protistan diversity in water samples from two contrasting anoxic marine basins to saturation. Based on the data obtained, I will discuss the potential of this approach to access the hidden majority in the eukaryotic tree of life and to fill in the 'protistan gap' in this tree.

#### REPRODUCTIVE ISOLATION IN SACCHAROMYCES

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*Abstract not received*

#### FUNGAL BIODIVERSITY: DO YOU BELIEVE IN GENERA?

PEDRO W. CROUS

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Fungi represent a largely unexplored group of organisms of which only the most common examples are known at species level. A conservative estimate based on the number of unique fungi per plant species suggest that at least 1.5 M species of fungi should occur on plants, of which around 7% have been described to date. Many habitats, ecosystems and host plants have, for instance, never been investigated, and thus their microbial inhabitants remain unexplored, unknown, and underutilized. The implementation of molecular techniques provides a quick insight into microbial diversity, and a potential means of rapid identification. The current taxonomic system, however, is largely based on the fungal phenotype. Many fungal genera have been defined based on single characters judged to be informative at the generic level. There are numerous examples, however, of unrelated taxa placed in genera due to the fact that they shared apparently similar characters arising from adaptation to similar niches and due to convergent evolution. Merging structure with sequence will demand that a new taxonomic system is established. This will prove highly problematic, as in many cases genetic relatedness cannot be established based on the phenotype, or is expressed

in features not previously considered informative at the generic level. Furthermore, dual nomenclature for fungal species has no future once their DNA data are known, suggesting that a single nomenclature would be preferable for well defined clades, and that a new taxonomic system is called for based on true evolutionary relationships.

#### EVOLUTIONARY GENOMICS OF YEASTS

BERNARD DUJON & THE GÉNOLEVURES CONSORTIUM\*

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Yeasts have emerged from various phylogenetic branches of fungi, and share similar lifestyle despite considerable evolutionary distances. The *Saccharomycotina*, or hemiascomycetous yeasts, have been most extensively studied at the genomic level owing to the large number of species identified in this subphylum of *Ascomycota*, the importance of some of them in fermentation processes or pathogenicity, and the considerable role played by *Saccharomyces cerevisiae* as a reference and model organism. Today, the genomes of more than 30 species of *Saccharomycotina* have been sequenced, analysed and compared and population genomic studies have now been conducted on natural isolates of several species.

I will summarize genome characteristics of major families of *Saccharomycotina*, trying to replace them in an evolutionary perspective. Recent results on the genomes of protoploid *Saccharomycetaceae* will be discussed in relationship with the basic protein repertoire of this family of yeasts, the presence of tandem or dispersed paralogs, the evolution of non-coding RNAs and the presence of horizontally-acquired novel genes. Some unexpected features about genome organization and characteristics among *Saccharomycotina* will be discussed in an evolutionary perspective.

\*See [www.genolevures.org/](http://www.genolevures.org/) for a list of participants of the Génolevures Consortium (GDR2354, CNRS)

#### HYPHAL TIP STRUCTURE AND FUNGAL PHYLOGENY

W. ROBERT ROBERSON

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Hyphal growth and morphogenesis are complex processes that have allowed the fungi to successfully utilize a wide range of ecological habitats and develop multiple lifestyles. Bioimaging studies, and more recently molecular studies, of hyphal tip biology have placed great emphasis on the Spitzenkörper. The Spitzenkörper appears to have evolved only in filamentous fungi where it is present in all members of the Basidiomycota and Ascomycota studied thus far. Spitzenkörper have been verified only in a few members of the 'lower fungi.' Though progress is being made towards better understanding the cellular and molecular biology of the Spitzenkörper, important details remain unclear regarding its structural diversity and distribution among the filamentous fungi. In this presentation, light and electron microscopy are used to address Spitzenkörper structure, behavior, and distribution among diverse fungal taxa.

#### MUSHROOMS IN THE GREAT TREE OF LIFE

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Darwin's great tree of life grows unevenly. In every major branch, some forms come to dominate, while others make relatively small contributions to biodiversity. Prior attempts to understand the processes responsible for differential evolutionary 'success' have focused on two kinds of evolutionary processes. Key innovation tests have been used to explore hypotheses that certain organismal attributes promote diversification, while tests of bias in character-state transformations have been used to address whether there is directionality in evolution. We are investigating the correlates of evolutionary success in the Agaricomycetes, which contains the vast majority of mushroom-forming Fungi, using binary state speciation-extinction analysis implemented in the program BiSSE (by W. Maddison and colleagues). We hypothesize that pileate-stipitate fruiting body forms and ectomycorrhizal (ECM) symbioses are correlated with evolutionary success in Agaricomycetes. Comparative analyses of the Agaricales and Boletales suggest that there is a directional trend favoring the evolution of pileate-stipitate forms in both clades, but there is no consistent directional trend in the evolution of ECM forms. Models estimated with BiSSE suggest that pileate-stipitate lineages in the Agaricales and Boletales and ECM lineages in the Boletales have elevated rates of both speciation and extinction. However, the only evidence of a key innovation effect is in pileate-stipitate lineages of the Agaricales.

## PUTTING MICROBES TO WORK

### PROCESSES AND MICROBIOLOGY FOR MAXIMAL USE OF RESOURCES PRESENT IN USED WATER

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Recently we have entered the era of 'bio-economy'. All processes and products must be considered in relation to the use of renewable resources. In this respect we must reconsider also our current concepts about the treatment of 'waste'-water and opt for the maximum use of the resources it contains. Starting from this mindset: for *decentralized* systems there is a primary role for anaerobic digestion, with a polishing task for processes as OLAND and activated sludge. For the *centralized* systems, a total redesign in which up-concentration at the treatment plant is primordial, is proposed. In these centralized plants, emphasis should be shifted to total recovery. Nitrification should be aligned with IO-membrane recovery processes for nitrate. Denitrification should be avoided in the future. The effluents must be integrated in the production of 'NEWater'. All of this necessitates new focus for the microbial process engineers. Specifically, the concept of Microbial Resource Management (MRM) of activated sludge needs to be developed. One must benchmark the concept by using molecular tools and examine its operational validity and its cost/benefit for practitioners.

Moreover, one needs to better integrate quantitative microbial risk assessment (QMRA) in the water treatment. One should designate the potentials of seeding/bio-supplementation of sludge. Various new methods of retro-fitting plants with advanced removal technologies are warranted. Finally, methods to upgrade treatment plants with systems to generate bio-electrochemical recovery products or even products with value in the feed chain are becoming of interest.

### FUNGAL DETERIORATION OF PLASTICS

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Man-made polymeric materials are ubiquitous in our everyday lives and have an enormous range of applications from man-made textiles to plastics, coatings, paints and additives. As a consequence, a vast array of man-made polymers accumulates in the environment and landfill waste sites where they cause considerable water and land pollution problems. Over the last few decades, plastics and plasticizers due to their wide production and distribution have led to a large increased environmental burden. While some plastics are relatively recalcitrant in the environment, other plastics such as polyurethane, or plasticizers present in the plastic such as plasticized polyvinylchloride, are susceptible to microbial deterioration and degradation when exposed to the environment. Our studies have shown that fungi play a key role in the deterioration of these materials and using a combination of culture and molecular-based techniques we have studied the temporal colonization and community organization of the fungi on these surfaces. We have also investigated the influence of biocides on developing fungal communities that are commonly incorporated into these materials to prolong the life time of the plastic-based materials in the environment.

### BIOCATALYSTS FROM EXTREMOPHILES

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*Abstract not received*

### MICROBIAL FUEL CELLS

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The direct conversion of organic compounds to electricity in microbial fuel cells shows promise for a wide diversity of applications including: powering electronics in remote environments or the human body; promoting the bioremediation of contaminants; and the conversion of wastes to electricity. Optimizing these applications requires a better understanding of how micro-organisms transfer electrons to the anode surface. *Geobacter sulfurreducens* produces current densities that are among the highest known for pure cultures and it is closely related to the *Geobacter* species that naturally colonize the anodes of microbial fuel cells deployed in a variety of environments. *G. sulfurreducens* forms relatively

thick (50–75 µm) biofilms on graphite anodes and cells at substantial distances from the anode appear to significantly contribute to current production. Several lines of evidence will be presented that suggest that this long-range electron transfer is possible because *G. sulfurreducens* forms electrically conductive biofilms. The degree of biofilm conductivity is related to the abundance of PilA, the structural protein for pilin filaments previously reported to be conductive and referred to as microbial nanowires. OmcZ, an outer-surface c-type cytochrome, is also an important component in the electron transfer; apparently facilitating electron transfer at the anode surface. The implications of these and other findings for engineering microbial fuel cells with enhanced current production capabilities will be discussed.

### ENGINEERING MICROBIAL METABOLISM FOR PRODUCTION OF ADVANCED BIOFUELS

JAY D. KEASLING

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Today, carbon-rich fossil fuels, primarily oil, coal and natural gas, provide 85% of the energy consumed in the United States. As world demand increases, oil reserves may become rapidly depleted. Fossil fuel use increases CO<sub>2</sub> emissions and raises the risk of global warming. The high energy content of liquid hydrocarbon fuels makes them the preferred energy source for all modes of transportation. In the US alone, transportation consumes around 13.8 million barrels of oil per day and generates over 0.5 gigatons of carbon per year. This release of greenhouse gases has spurred research into alternative, non-fossil energy sources. Among the options (nuclear, concentrated solar thermal, geothermal, hydroelectric, wind, solar and biomass), only biomass has the potential to provide a high-energy-content transportation fuel. Biomass is a renewable resource that can be converted into carbon-neutral transportation fuels.

Currently, biofuels such as ethanol are produced largely from grains, but there is a large, untapped resource (estimated at more than a billion tons per year) of plant biomass that could be utilized as a renewable, domestic source of liquid fuels. Well-established processes convert the starch content of the grain into sugars that can be fermented to ethanol. The energy efficiency of starch-based biofuels is however not optimal, while plant cell walls (lignocellulose) represents a huge untapped source of energy. Plant-derived biomass contains cellulose, which is more difficult to convert to sugars, hemicellulose, which contains a diversity of carbohydrates that have to be efficiently degraded by microorganisms to fuels, and lignin, which is recalcitrant to degradation and prevents cost-effective fermentation. The development of cost-effective and energy-efficient processes to transform lignocellulosic biomass into fuels is hampered by significant roadblocks, including the lack of specifically developed energy crops, the difficulty in separating biomass components, low activity of enzymes used to deconstruct biomass, and the inhibitory effect of fuels and processing byproducts on organisms responsible for producing fuels from biomass monomers.

We are engineering the metabolism of platform hosts (*Escherichia coli* and *Saccharomyces cerevisiae*) for production of advanced biofuels. Unlike ethanol, these biofuels will have the full fuel value of petroleum-based biofuels, will be transportable using existing infrastructure, and can be used in existing automobiles and airplanes. These biofuels will be produced from natural biosynthetic pathways that exist in plants and a variety of microorganisms. Large-scale production of these fuels will reduce our dependence on petroleum and reduce the amount of carbon dioxide released into the atmosphere, while allowing us to take advantage of our current transportation infrastructure.

### A MICRO SOLUTION TO A MEGA PROBLEM – CAN MICROBES BE USED TO CLEAN UP DIRTY OIL?

[RICHARD JOHNSON](#)<sup>1</sup>, BEN SMITH<sup>2</sup>, STEVE ROWLAND<sup>3</sup> & CORINNE WHITBY<sup>1</sup>

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Naphthenic acids (NAs) are a complex mixture of carboxylic acids found principally in weathered crude oil. NAs cause environmental problems as they are recalcitrant, corrosive, and toxic. Aromatic compounds make up a small but unexplored fraction of this mix. This project aims to characterize aromatic NA-degrading micro-organisms in relation to NA biodegradation rates, metabolite production and NA structure. Enrichment cultures were set up, using environmental consortia from a coal tar-contaminated site, on four aromatic NAs



that differed in the methyl branching of the alkyl side chain. GC-MS analysis demonstrated complete metabolism of the least branched compound [4-(4'-n-butylphenyl)butanoic acid] through  $\beta$ -oxidation within 14 days, with complete mineralization in 49 days. NA biodegradation using pure cultures also followed a similar  $\beta$ -oxidation degradation pathway. NAs with increased alkyl branching were more recalcitrant and resulted in a persistent metabolite, which remained after 125 days. Analysis of 16S rRNA gene clone libraries demonstrated a shift in community composition during NA degradation. Our findings suggest that the presence of specific micro-organisms and NA structure dictates the degree of biodegradation. This novel research will facilitate studies on NA biodegradation pathways and allow the development of more effective bioremediation approaches for removing NAs in the environment.

#### BACILLUS SUBTILIS SPORES TO DISPLAY FUNCTIONAL ENZYMES

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Display of polypeptides like feed enzymes at the surface of viable bacterial spore systems has a great potential use for animal feed. To deliver their full potential in the gut, feed enzymes must survive the harsh conditions of the feed preparation and of the gastrointestinal tract. Resistance to unkind environment allow spores of probiotic strains to be a potentially valuable tool to improve formulation of bioactive molecules, and thus to become an innovative nanostructure for commercial purposes. Some successful examples have been reported, in which abundant structural proteins of the *Bacillus subtilis* spore coat were used as carriers for the display of recombinant proteins. However, a very limited number of examples resulted in the display of functional enzymes. In our study, we show that the spore coat structural protein CotG can expose an endogenous phytase, commonly used feed enzyme for monogastric animals. Moreover, we explore the use of a coat-associated enzyme as an original anchoring motif. We give evidence that the endogenous phytase can be fused to *B. subtilis* OxdD, a spore coat associated oxalate decarboxylase. We also demonstrate the display of a bioactive heterologous passenger; the multimeric beta-glucuronidase enzyme from *Escherichia coli*, fused to OxdD.

#### MYCORRHIZAL BIOREMEDIATION OF HEAVY METAL CONTAMINATED SITES

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Most plants adapted to terrestrial ecosystems are obligate or facultative mycorrhizal symbionts. Their survival on heavily polluted places depends on selection of resistant strains of mycorrhizal fungi. Some of these fungi influence the uptake of potentially toxic elements by the plants. Plant-based technologies in industrial areas are mostly focused on phytostabilization, involving the use of plants to stabilize the contaminated tailing material, in order to minimize leaching and wind or water erosion. The mycorrhizal status of plants commercially available, that were seeded on the industrial wastes, and those that were transferred from wet or dry places was studied. Field and laboratory experiments were carried out to evaluate whether introduction of plants assisted by well selected microbiota could help to increase above- and under-ground diversity. Selected plants were analysed by Total Reflection X-ray fluorescence method. Commercially available grasses are often devoid of well established mycorrhiza and their populations dramatically decrease with time. Much more stable vegetation communities arise from plants that appear on the wastes spontaneously. The plants need appropriate below-ground ecosystems, especially at difficult sites. Recent experiments concerning the use of AMF in phytoextraction of metals such as Ni and radio-Cs pointed out even broader spectrum of fungal application.

#### BIOMINING: AN ESTABLISHED AND DYNAMIC BIOTECHNOLOGY

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'Biomining' is generic term to describe the application of living organisms to extract and recover metals from mineral ores and waste materials. Since its inception as a crude technology ('dump leaching') for treating 'run of mine' rocks and boulders that contained too little copper to be processed by conventional processing, engineering options used in biomining have become increasingly refined and diverse. Currently, microbiological

processing is used to extract both base metals (copper, nickel and zinc) and precious metals (gold and silver) from ores and mineral concentrates in heaps and stirred-tank bioreactors, as well as in dumps. All biomining operations necessarily operate as open and non-sterile systems. However, because of the prevailing conditions, only living organisms that are able to survive in environments that are both highly acidic and that contain greatly elevated concentrations of toxic metals and other dissolved solutes are active in biomining operations, which restricts indigenous life forms almost exclusively to some 'extremophilic' bacteria and archaea. Microbial consortia, comprising iron- and sulfur-oxidizing chemoautotrophs and organic carbon-degrading mixotrophic and heterotrophic acidophiles, establish in all commercial biomining operations. How these may be 'fine-tuned' to optimize metal recoveries from different types of ores and mineral concentrates is one of the current challenges for microbiologists.

#### EVOLUTION OF MUTUALISM AS A 'SOLUTION' TO IRON SUPPLY IN THE SEA

DAVID GREEN<sup>1</sup>, SHADY AMIN<sup>2</sup>, MARK HART<sup>1</sup>, WILLIAM SUNDA<sup>3</sup>, FRITHJOF KÜPPER<sup>1</sup> & CARL CARRANO<sup>2</sup>

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Marine dinoflagellates and coccolithophores are fundamental components of the marine ecosystem, but are possibly better known for their toxic blooms and the White Cliffs of Dover. As a part of studies to understand the roles that bacteria that associate with these algae may have, we observed one genus of bacteria to be ubiquitous. This algal-bacterial specificity suggested the existence of a symbiotic relationship. Abundant siderophore production was observed and viewed as one possible connection between the two biota—as iron is often very scarce in the sea. Structural analysis identified the siderophore to be vibrioferrin (VF), and that the VF-Fe complex was exquisitely sensitive to light, producing biologically-available Fe(II) as the product of photolysis. Using bacterial-free dinoflagellate cultures supplied with VF-Fe chelates, light exposure significantly enhanced algal Fe uptake. Genome analysis of one of our VF-producing symbionts revealed an unusually high incidence of eukaryotic-like proteins and the secretion systems, I, II, IV and VI. This coupled with metabolic data, suggests that this bacterium is tuned to the metabolism of algal metabolites, and has the genetic apparatus to intimately associate with eukaryotes. We propose that this model represents the evolution of a mutualistic relationship to solve the perennial problem of algal iron supply in exchange for a place to live.

#### MICROBIAL BIOSENSORS- PUTTING MICRO-ORGANISMS TO WORK

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The revolution of microbial biosensors in environmental analysis has not yet been realized. In part this is because regulators remain more comfortable with traditional chemical analysis, in part because of the lack of credible field-scale trials. Here I will report on the coupling of chemical and biosensor approaches to better understand the modes of action of chemicals during environmental exposure. In the first instance biosensors have enabled us to understand the fate and impact of metals in the environment and this has aided us in hazard assessment models. Examples of genuine environmental sites will be considered. Furthermore, when coupled to clever non exhaustive extraction techniques we are able to relate biosensor responses to issues such as biodegradation which allows significant developments in the evolution of remediation techniques and pollutant mitigation. Remedios have coupled such approaches to decision support tools to underpin sustainable land remediation at the site scale.

#### GENOME WIDE INSIGHTS INTO BIOCONTROL BY TRICHODERMA

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The genus *Trichoderma/Hypocrea*, contains some of the most abundant fungi in soil, many of which are potent mycoparasites, active colonizers of the plant rhizosphere, and also frequently found as endophytes. The combination of these parameters led to their use as biocontrol agents against various plant pathogenic fungi. The biochemical and physiological mechanisms responsible for these abilities are not clearly understood, however. The recent

## PUTTING MICROBES TO WORK

(continued)

genome sequence analysis of three *Trichoderma* genomes, two mycoparasitic (*T. atroviride*, *T. virens*) and one not (*T. reesei*) has enabled a comparative analysis of the genome features characteristic for mycoparasites. Among them are a large and diverse set of chitinolytic enzymes and chitin binding domains, genes required for hydrolysis and metabolism of hemicelluloses encountered in the plant rhizosphere, an expanded family of cell surface-bound proteins and a large array of enzymes synthesizing polyketides and non-ribosomal peptides. Genome sequence comparison was also complemented by transcriptome analysis and metabolic network construction. Genes overexpressed during the onset of mycoparasitism in *T. atroviride* revealed that the fungus reacts with a strong stress response, sensing of nitrogen shortage and increased lipid catabolism. The availability of these genome sequences and the transcriptome data will enable a new and more comprehensive interpretation of the physiology of mycoparasitism, and aid in the selection of traits for breeding of biocontrol strains by recombinant techniques.

## MICROBIAL POLYSACCHARIDES

## BACTERIAL POLYSACCHARIDES EXPORT AND ASSEMBLY

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Bacteria are capable of expressing a diverse range of cell surface polysaccharide capsules. The capsule coats the outside of the bacterial cell and as a consequence plays an intimate role in mediating interactions between the bacterium and its immediate environment. In the case of bacterial pathogens, as well as acting to protect the bacterial cell from local insults there is emerging evidence that capsular polysaccharides may act as signalling molecules to modify the host response and adapt the local environment.

The expression of a polysaccharide capsule presents a number of serious challenges to the bacterium. It needs to synthesize component sugars, polymerize the individual sugars into high molecular weight polysaccharides and then finally transport the high molecular weight polysaccharide onto the cell surface. In this talk I will highlight with examples the diverse strategies employed by bacterial pathogens to address these questions and how an understanding of these processes is important in the prevention and treatment of disease.

## BACTERIAL POLYSACCHARIDE DIVERSITY, GENETICS AND EVOLUTION

PETER REEVES

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Most bacteria have surface polysaccharides that vary among isolates as shown by serology and confirmed by chemical analysis.

We will focus on O antigens of *Salmonella* and *Yersinia pseudotuberculosis*, and capsules of *Streptococcus pneumoniae*, with excellent coverage for the serological diversity, and good to complete coverage for the structures that comprise the variation, and of the gene clusters that encode them.

88 of the 90 named capsule type of *S. pneumoniae* are synthesized by the ABC transporter pathway, and the gene clusters have distinctive conserved and variable regions. The 50+ O antigens of *Salmonella* are mostly synthesized by the Wzx/Wzy pathway and map to their own distinct same locus, but fall into 2 groups, those with N-acetylglucosamine as initiating sugar and those with galactose. These groups differ in several other ways, and the latter group may have entered the species after divergence from *E. coli*, which has a very high proportion in the N-acetylglucosamine initiated group. *Y. pseudotuberculosis* has only about 20 O-antigen types, almost all resemble the *Salmonella* Galactose-initiated group in structure, but have N-acetylglucosamine as initiating sugar.

There are clear patterns in the diversity and we will look at some examples in detail and discuss their evolutionary origins.

## EPIDEMIOLOGY OF SEROTYPE DIVERSITY AND ASSOCIATION WITH DISEASE

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Abstract not received

## MODULATION OF LIPID A IN GRAM-NEGATIVE BACTERIA: TURNING FOE INTO FRIEND

DAVID A. SIX<sup>1</sup>, QINGKE KONG<sup>2</sup>, LILLIAN GU<sup>1</sup>, ROY CURTISS III<sup>2</sup> & CHRISTIAN R.H. RAETZ<sup>1</sup><sup>1</sup>Dept of Biochemistry, Duke University Medical Center, Durham, North Carolina 27710, USA;<sup>2</sup>Center for Infectious Diseases & Vaccinology, Biodesign Institute and School of Life Sciences, Arizona State University, Tempe, AZ 85287-5401, USA

The development of a safe live, attenuated *Salmonella* vaccine may be facilitated by the detoxification of its lipopolysaccharide (LPS). The recent characterization of the lipid A 1-phosphatase, LpxE, from *Francisella tularensis* has allowed the construction of recombinant, plasmid-free strains of *Salmonella* that produce predominantly 1-dephosphorylated lipid A in the LPS. Nearly complete lipid A 1-dephosphorylation by LpxE was seen only after selection of the optimal promoter and chromosomal locus in addition to codon-optimization. Complete lipid A 1-dephosphorylation was confirmed in standard culture conditions as well as low pH, low Mg<sup>2+</sup> culture conditions that induce a variety of lipid A modifications that might have interfered with 1-dephosphorylation. Recombinant *Salmonella* strains expressing LpxE had LD<sub>50</sub>s in mice up to 10,000-fold higher than the wild-type *Salmonella typhimurium*. Moreover, mice inoculated with these LpxE strains gained robust protective immunity to infection with the wild-type strain. Candidate *Salmonella* vaccine strains expressing an antigen

from the pneumococcal surface protein A (PspA) were also confirmed to possess nearly complete lipid A 1-dephosphorylation by LpxE. After inoculation by the LpxE/PspA strains, mice produced robust levels of anti-PspA antibodies and survived challenge by lethal doses of wild-type *Streptococcus pneumoniae*, validating *Salmonella* as an antigen delivery system.

## IN SEARCH OF PEPTIDE IMMUNOLOGICAL MIMICS OF POLYSACCHARIDES

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Bacterial polysaccharides are major targets of protective immune response against bacterial pathogens. Unfortunately polysaccharides are, in general, T-independent antigens, giving rise to poor immune responses lacking immunological memory, and thus they are often not easily suitable for the development of an effective vaccine. Several research groups have demonstrated that, by using phage-display technology, it is possible to identify peptides mimicking the protective carbohydrate serotype-specific epitopes. These peptides specifically bind to the anti-carbohydrate antibodies used for their selection, and therefore represent antigenic mimics (*mimotopes*) of the sugar epitopes. Immunogenicity studies of the mimotopes nevertheless show that the induced anti-polysaccharide antibody titre, when present, is generally low. Comparison of the structure of the protective carbohydrate epitopes and of the selected mimotopes (free and combined to the antibody) would contribute to define the molecular basis of the mimicry, and would therefore be a powerful strategy to improve mimotope sequences. This approach might represent a viable strategy to achieve identification of peptide leads, allowing development of mimotope-based anti-carbohydrate vaccines.

## MOLECULAR MECHANISMS OF FUNGAL CELL WALL ASSEMBLY

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The fungal cell wall is a dynamic and multi-layered structure, containing a core of crosslinked chitin and glucan. Despite decades of work, there are huge gaps in our knowledge of the enzymes responsible for cell wall synthesis, modification and crosslinking. This is particularly pressing given the significant rise in fatal fungal infections of immunocompromised patients, and thus the need for novel, properly genetically/chemically validated, drug targets. I will present data from a multidisciplinary approach (covering genetics, biochemistry, structural biology, high throughput screening, synthetic chemistry and cell biology) that furthers our understanding of the function, biochemistry, inhibition and genetic/chemical validation of *A. fumigatus*/*C. albicans* enzymes involved in fungal cell wall biogenesis. Aspects of cell wall synthesis, sugar nucleotide biosynthesis, cell wall modification/hydrolysis and crosslinking/transglycosylation will be covered.

## CAMPYLOBACTER JEJUNI – A HYPERGLYCAEMIC BUG

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*Campylobacter jejuni* is the most common bacterial cause of human gastroenteritis world-wide and the most frequent type of infection to lead to Guillain-Barré syndrome, a disorder of the peripheral nervous system thought to be triggered when surface-exposed carbohydrate structures on an infectious agent mimic host antigens.

Our lack of knowledge of the pathogenesis of *C. jejuni* coupled with its socioeconomic importance was the driving force for the completion of the *C. jejuni* strain NCTC11168 genome project in 2000. The genome sequence revealed many previously unidentified determinants including a capsule polysaccharide. Also identified were loci for independent O- and N-linked glycosylation pathways, the first bacterium to be identified with two such systems.

The O-linked system consists of a 50-gene locus in strain NCTC11168 and encodes a variety of glycans that decorate the flagellin. This locus is highly variable between different strains. By contrast the N-linked general glycosylation system, which modifies over 40 proteins, is invariant. Both the O and N-linked glycosylation systems from *C. jejuni* have been characterized and represent useful models to understand protein glycosylation in more complex eukaryotic systems. Additionally, the cloning of the N-linked glycosylation system in *E. coli* enabled the production of recombinant glycoproteins. This spin off from basic research shows great promise in glycoengineering, the subject of the Tuesday workshop.

# MICROBIAL POLYSACCHARIDES

(continued)

## LPS AND CAPSULE BIOSYNTHESIS IN *BACTEROIDES FRAGILIS*

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*Bacteroides fragilis* is a normal resident of the human gastrointestinal tract and an opportunistic pathogen. It is the Gram negative obligately anaerobic bacterium most frequently isolated from opportunistic infections such as peritonitis, serious gynaecological sepsis, soft tissue abscess and bacteraemia. The latter has an estimated mortality of up to 19%. An individual strain can produce an extensive array of extracellular polysaccharides (EPS), including at least eight antigenically distinct micro-capsules, as well as phase variable large and small capsules. Complete genome sequencing has revealed multiple gene clusters potentially involved in biosynthesis of EPS. In *B. fragilis* NCTC9343 eight of these gene clusters are implicated in production of the antigenically variable micro-capsule; seven are switched ON and OFF by the site-specific inversion of promoter sequences, where recombination can be mediated by members of the serine family of invertases. Deletion of a putative *wbaP* homologue prevents expression of the phase variable large capsule and deletion of the single putative *wzz* homologue prevents production of antigenically variable high molecular mass polysaccharides (HMMPS) which form the micro-capsule. These findings shed some light on the controversy that has surrounded the nature of LPS and capsules in *B. fragilis*.

## POLYSACCHARIDES AND GLYCOSYLATION IN *BURKHOLDERIA*

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*Burkholderia pseudomallei* and *Burkholderia mallei* can cause melioidosis and glanders respectively in humans. Disease caused by *B. pseudomallei* is a significant problem in NE Thailand where up to 40% of people admitted to hospital with septicaemia have melioidosis. Glanders is primarily a disease of solipeds but when humans are infected it is frequently fatal. Pathogenic *Burkholderia* spp are reported to produce a variety of polysaccharides including, lipopolysaccharide, capsular polysaccharide or glycans attached to proteins. There is increasing evidence that some of these polysaccharides are protective antigens. Glycosylation of proteins has not been reported in *B. pseudomallei* or *B. mallei* and we have set out to identify proteins which are glycosylated in *B. pseudomallei*. Bacterial proteins were separated by 2-D gel electrophoresis and stained for carbohydrates. Reactive spots were analysed by mass spectrometry and proteins identified. The characterization of the glycan moiety is currently underway.

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## OLIGOSACCHARIDE ENGINEERING

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The synthesis of bacterial polysaccharides and lipopolysaccharides relies on the presence of nucleotide-sugars in the bacterial cytoplasm. In certain conditions, the pool of NDP-sugars can be diverted to the synthesis of intracellular oligosaccharides. Among those conditions are the simultaneous presence of endocellular (recombinant) glycosyltransferases AND acceptable substrates to achieve sequential glycosylation reactions. In this context, colanic acid is an interesting polysaccharide that contains specific monosaccharide units such as fucose and glucuronic acid.

The talk will focus on the diversion of the precursors of colanic acid to the production of fucosylated and glucuronylated oligosaccharides in an engineered *Escherichia* K12 strain.

Different strategies based on the over-expression of the *cps* operon or on the cloning of separated genes have been developed in order to provide NDP-sugars for heterologous glycosylation. The efficiency of the different strategies will be discussed in relation with the endogenous regulation pathway of polysaccharide synthesis.

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## IMMUNE RECOGNITION OF FUNGAL CELL WALL POLYSACCHARIDES

NEIL A.R. GOW

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The core components of the cell wall are shared by most fungal species and consequently the immune system has evolved to try to detect these molecules to induce protective responses. In the human pathogen *Candida albicans* the cell wall and septal cross-walls are composed of a robust chitin-glucan inner skeleton to which an outer shield of a matrix of highly glycosylated mannoproteins are attached. Emerging evidence has shown that all of the main components of the *C. albicans* cell wall skeleton and matrix may be recognized, both singly and in combination, by the immune system. However, while some components stimulate immune recognition others attenuate or block it. The composition of the cell wall is also far from fixed, and is modulated during cellular morphogenesis and in response to environmental changes. Therefore the dynamic nature of the cell wall makes it a moving target for the immune surveillance. This presentation will summarize how the fusion of fungal molecular genetics and immunology has led to the systematic dissection of the mechanisms by which cells of the innate immune system grapple and destroy fungal invaders and reciprocally how pathogenic fungi have adapted their cell wall structure create a defensive shield and camouflage jacket to protect themselves from the immunological sentinels of the body.

## BIOCHEMISTRY OF CELL WALL POLYSACCHARIDE BIOSYNTHESIS IN OOMYCETES

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Oomycetes have long been considered as a separate class within the kingdom Fungi. However, based on sequence comparisons and phenotypic analyses, they are currently classified in the Stramenopile kingdom together with heterokont algae and water molds. The oomycete phylum comprises saprophytes beneficial to natural ecosystems as well as pathogens responsible for severe environmental damage and economic loss. Typical examples are the fish pathogen *Saprolegnia parasitica* and *Phytophthora* species that infect trees and a range of agriculturally important plants such as potato, tomato and soybean. An important characteristic that distinguishes oomycetes from fungi is their cell wall composition, which consists essentially of cellulose, (1→3)- $\beta$ -D-glucans and (1→6)- $\beta$ -D-glucans. As opposed to fungi, minute amounts of chitin occur in the walls of some oomycetes. Despite the importance of cell wall polysaccharide biosynthesis in the morphogenesis and growth of oomycetes, the molecular mechanisms involved in this process remain poorly understood. This presentation will review our latest results on the biochemistry of cellulose, (1→3)- $\beta$ -D-glucan and chitin biosynthesis in oomycetes. The specific properties of oomycete carbohydrate synthase genes will be described, and a model for tip growth integrating the concept that oomycete carbohydrate synthases are located in plasma membrane microdomains similar to lipid rafts will be discussed.

## BACTERIAL POLYSACCHARIDE VACCINE PRODUCTION FROM PLANTS

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Polysaccharides play a vital role in bacterial pathogenicity and immunogenic capsular polysaccharides are key components of many bacterial vaccines. The polysaccharide encapsulated bacterium, *Streptococcus pneumoniae* (the pneumococcus) is a major respiratory pathogen. Its extreme variability, with over 90 known serotypes, poses a significant problem for vaccine development and current and prospective vaccines are composed of a limited number of polysaccharides. Although protein antigens have been synthesized in plants there are currently no reports of synthesis of bacterial polysaccharide antigens. Here we report that pneumococcal capsular polysaccharide can be synthesized *in planta* and that immunization with extracts of the transgenic plants protects mice against pneumococcal disease.

We infer that the plant cells redirect UDP-sugar precursors involved in cell wall synthesis to the manufacture of bacterial polysaccharide. This represents a frontier area in metabolic engineering, which with the expression of additional pneumococcal genes could lead to the production of vaccines offering complete serotype coverage.

#### SYNTHESIS OF THE CELL WALL AND CAPSULE OF *CRYPTOCOCCUS NEOFORMANS*

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*Cryptococcus neoformans* is a serious opportunistic pathogen. The main virulence factor of this fungus is a prominent capsule composed of two major polysaccharides: GXM (glucuronoxylomannan) and GXMGal (glucuronoxylomannogalactan), named after their components. These polymers are associated with the cell wall, another intricate structure composed mainly of polysaccharides. We are addressing some of the fascinating questions of biosynthesis of these extracellular polymers, including how and where they are made and the interactions between them. Our studies indicate that GXM components are generated within the cell and travel to the surface via the secretory pathway. Once there, this material associates with the cell wall via alpha-glucan, in a protein dependent manner. Interestingly, changes in capsule size are mediated at the polymer level, before secretion, rather than by alterations in capsule assembly after exit. Because cells lacking UDP-xylose produce stunted capsule fibers and are avirulent in animal models we are investigating cryptococcal xylosyltransferases. We have purified a beta-1,2-xylosyltransferase with specificity appropriate for incorporation of xylose into capsule polysaccharides. Intriguingly, the corresponding gene has multiple homologs in fungi, but none in other kingdoms. Gene deletion yields cells that produce GXM with reduced beta-1,2-xylose content and GXMGal which is almost devoid of this linkage. Xylose is also completely absent from cryptococcal glycoinositolphosphoceramides, indicating the enzyme functions in synthesis of three distinct glycoconjugates. Significantly, the mutant shows reduced growth in a short-term mouse model of infection. *C. neoformans* expresses a second xylosyltransferase with similar in vitro function but a less prominent role in capsule synthesis. A third transferase has the novel activity of adding xylose-phosphate to mannose residues, most likely as part of cryptococcal O-glycan synthesis. These processes offer a glimpse of the fascinating glycobiology of *Cryptococcus neoformans*.

## MICROBIAL FACTORIES

## MICROBIAL PHYSIOLOGY UNDERPINNING THE PRODUCTION OF DIFFICULT RECOMBINANT PROTEINS

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The Holy Grail of recombinant protein production remains the availability of generic protocols and hosts for the production of even the most difficult target products. First an explanation will be given why the shock imposed upon bacteria using a standard recombinant protein induction protocol not only arrests growth, but also decreases the number of colony-forming units by several orders of magnitude. Approaches commonly used to prevent deposition of recombinant protein in cytoplasmic inclusion bodies will be summarized. A generic approach to process design will be proposed that minimizes stress on the production host. It is based upon the observation that when a fluorescent protein tag is fused in-frame to the carboxy terminus of the target protein, fermentation optimization experiments provide a good prediction of what will happen if the same conditions are used to produce the protein without the tag. A C-terminal GFP fusion to a model target protein, *Escherichia coli* CheY, was exploited both as a reporter of the accumulation of soluble recombinant protein, and to develop a generic approach to optimize yields. The optimized protocol is suitable for both process scale-up and to the accumulation of an untagged heterologous protein that requires both secretion and extensive post-translational modification.

## ENGINEERING PROTEIN FITNESS AND FUNCTION USING THE TWIN-ARGININE TRANSLOCATION SYSTEM

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All living organisms, including bacteria, localize a fraction of their proteins partially or completely across hydrophobic lipid membranes. The remarkable feat of delivering proteins across tightly sealed membranes is achieved by complex protein machines such as the general secretory (Sec) and twin-arginine translocation (Tat) export systems. During the export of proteins by both Sec and Tat systems, a variety of fitness checkpoints ensure that only those proteins passing a stringent selection process are allowed to enter the transport cycle thereby preventing any harmful effects that might be caused by the deployment of export-incompetent proteins. We view these quality control (QC) mechanisms as a toolbox that can be exploited to effectively increase the diversity of functional gene expression and as potential sites for reprogramming cellular function. For instance, we have developed a simple genetic selection strategy for evaluating protein folding and solubility in *E. coli* that is based on the Tat pathway and its intrinsic QC mechanism. Since Tat pathway QC typically prevents export of misfolded proteins in *E. coli*, only correctly folded proteins reach the periplasm and confer cell survival. Our results for nearly 50 different protein sequences of prokaryotic and eukaryotic origin highlight the ability of Tat selection to assess the fitness of proteins and protein domains without the need for structural or functional information about the target protein. We have employed this genetic selection to evolve protein fitness by preparing libraries of random point mutations or gene fragments and selecting those that express in a soluble form in *E. coli*. This has resulted in the isolation of soluble variants of the aggregation-prone Alzheimer's A 42 peptide and poorly folded scFv antibodies. Finally, we are currently using the folding quality control in conjunction with Tat-mediated 'hitchhiker' export to engineer potent new protein therapeutics such as antibodies with properties (e.g. stability, binding affinity) that are far superior to those of their natural counterparts. Collectively, these studies highlight our ability to manipulate the fitness and function of target proteins using the unique mechanistic features of the bacterial Tat pathway.

## THE CAULOBACTER S-LAYER AS A PROTEIN PRODUCTION PLATFORM

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Secretion of recombinant proteins is desirable because of lower downstream processing costs and fewer problems if refolding is necessary. However many proteins of interest in biotechnology cannot be secreted via the universal Sec-dependent pathway. Type I secretion offers an alternative; it is essentially an ATP driven pump for partially folded proteins. Display of therapeutic or infection-blocking agents on bacteria is considered as a means of producing low cost agents for disease control. But high-density surface display is often not possible.

*Caulobacter crescentus* assembles a two-dimensional crystalline array on its outer surface; an

'S-layer' composed of a single highly expressed protein exported by Type I secretion. We have adapted the export system to enable secretion of a wide variety of proteins (using the C-terminal secretion signal) or for dense display of proteins on the cell surface, using the entire S-layer gene. As examples, a complete Protein G immunoglobulin binding capability and a number of antibodies, cytokines and receptors analogues have been displayed. Applications of the S-layer display in development include immune cell targeted anti-cancer vaccines and an HIV microbicide for distribution in 3<sup>rd</sup> world countries. The system uses small, high copy number plasmids that readily shuttle from *E. coli* and is available to the research and commercial community, formulated as protein secretion and protein display kits.

HETEROLOGOUS PROTEIN SECRETION FROM *BACILLUS SUBTILIS*: NOW YOU SEE IT, NOW YOU DON'T

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The use of secretion systems for the production of heterologous proteins has a number of advantages over intracellular systems, including reduced downstream processing costs. *Bacillus subtilis* is an attractive secretion host since it has the potential to secrete proteins at yields in excess of 20 g/l. However, such concentrations are rarely obtained during the production of heterologous proteins.

In *B. subtilis*, the majority of secreted proteins are transported via the Sec-dependent translocase in an essentially unfolded form. Consequently, they must fold in an environment that is dominated by a high density of immobilized negative charge. Recent progress has identified post-translocational folding in the extracytoplasmic environment as the main bottleneck to achieving high yields of heterologous proteins. While native *Bacillus* proteins have intrinsic characteristics that facilitate their rapid folding (assisted by a variety of extrinsic folding factors, including chaperone-like enzymes, peptides and metal ions), heterologous proteins do not. Heterologous proteins are therefore sensitive to membrane- and cell wall-associated proteases that act to clear misfolded or otherwise aberrant proteins from the translocase and/or the cell wall. This talk will review the developments of strains designed to by-pass the undesirable effects of these proteases, the operation of the secretion stress systems and the potential use of novel components from other secretion pathways.

SYSTEMS BIOLOGY APPROACHES IN *SACCHAROMYCES CEREVISIAE* AND ITS BIOTECHNOLOGICAL APPLICATIONS

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*Abstract not received*GENOME SCALE ENGINEERING OF PROTEIN PRODUCTION IN *PICHIA PASTORIS*

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Yeasts like *Pichia pastoris* have been proven to be valuable hosts for heterologous protein production. However, protein folding and secretion often limit productivity. Unfortunately there is a lack of state of the art analytical tools and of detailed physiological knowledge on *P. pastoris*, so that developments mainly depend on conclusions drawn from analogies to *Saccharomyces cerevisiae*.

In order to establish a genome scale approach to analysis and engineering of *P. pastoris*, we have sequenced the genome, developed a post genomics platform and employed it for the analysis of cellular reactions to intrinsic stress and extrinsic conditions of bioprocesses like temperature, oxygen supply or osmolarity. Combined transcriptomic, proteomic and metabolomic data indicate why such environmental conditions have a strong impact on heterologous protein production. The genome sequence highlights the major feature of *P. pastoris* as a protein production host, including the low level of secreted host cell protein leading to pure heterologous proteins, when applying glucose based expression systems, and the growth behaviour on glucose and glycerol.



Additionally we have applied DNA microarrays for the direct screening of genes encoding factors which support protein secretion. Combining transcriptomics with rational *in silico* selection of candidate genes enabled the rapid selection of factors enhancing protein productivity several fold. The results provide a pattern of subcellular processes which represent bottlenecks of heterologous protein secretion.

Taken together, these genome scale approaches lead to a detailed understanding of host cell physiology and to decipher additional physiological bottlenecks impeding heterologous protein production in *P. pastoris*.

## MODELLING FUNGAL METABOLOMICS

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*Abstract not received*

## SOLID-STATE FERMENTATION

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Cultivation of fungi on moist solid substrate (solid-state fermentation or SSF) can have advantages over cultivation in submerged cultures: It can give better product quantity or quality, can reduce equipment costs or reduce product recovery costs, depending upon the micro-organism and substrate used. It also has disadvantages compared to submerged cultures: Temperature control is much more critical and some degree of heterogeneity is inevitable in SSF.

In recent years our understanding of SSF has improved to the extent that design and control options can be predicted with similar accuracy as they can for submerged fermentation. What remains is the heterogeneity inherent to the presence of substrate particles: intra-particle gradients are needed for transport. These gradients may cause observed differences in product spectrum and they have been proven to affect the production rate.

In this presentation, I will discuss current research in our lab. One project aims at cultivation of oleaginous fungi for production of bio-diesel precursors from solid wastes or agro-industrial by-products. SSF is attractive here because of its low equipment costs. The second project aims at cultivation of entomopathogenic fungi for the control of malaria mosquitoes. SSF is attractive here because of the higher quantity and better quality of conidiospores. In both cases intra-particle gradients are very important for the product yield and product formation rate.

## SUBMERGED CULTIVATION AND MORPHOLOGY OF FILAMENTOUS FUNGI

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In biotechnological production processes performed with filamentous fungi the monitoring and control of morphological development is difficult to obtain due to the highly complex relation between morphology and productivity. Product formation by filamentous fungi, like *Aspergillus niger* is closely linked to their morphology. The morphological characteristics can vary between freely dispersed mycelia and distinct pellets of aggregated biomass with strong impact on rheology, mass transfer, mass transport and biochemical reaction. Mycelial growth has procedural disadvantages, for instance a high viscosity of the cultivation broth and therefore a low nutrient supply due to insufficient mixing. In comparison, cultivation broths

with distinct pellets show Newtonian flow behaviour, but disadvantages related to a limited nutrient availability within the inner part of the biopellets. Hence, in every biotechnological process, the optimal morphology varies due to specific product properties and cannot be generalized. The morphogenesis of *A. niger* cultivation can be controlled effectively by adjusting the pH-value and the volumetric power input.

In the early phase of cultivation the aggregation of *A. niger* conidia is dominantly affected by the pH-value, while the morphology of fungal pellets is mainly influenced by volumetric power input. The comparison of the volumetric power input caused by agitation and aeration revealed that the aeration has a higher impact to counteract the aggregation process, which leads to pellets with smaller diameter and an open structure, and also to a higher pellet concentration. Due to higher agitation induced power input denser pellets occur with a compact pellet surface.

Based on intracellular reactions up to physico-chemical and fluid dynamic phenomena at a macroscopic level, which determine the fungal morphology, the morphogenesis of mycelial growth and pellet formation via distinct aggregation steps ought to be completely covered by population balancing and verified by different particle size analysis techniques. The methods of population balancing will help to model and characterize morphologic developments and to connect fluid dynamic dependencies, the transport gradients, intracellular biological reactions and productivity in a comprehensive model for rational, target-oriented design of cultivation processes with filamentous fungi.

## CELLULASE PRODUCTION IN *TRICHODERMA REESEI*

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*Abstract not received*

## A SYSTEMS BIOLOGY APPROACH TOWARDS IMPROVEMENT OF ITACONIC ACID PRODUCTION IN *ASPERGILLUS* SP.

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Filamentous fungi are widely used for enzyme and metabolite production for a wide variety of uses – for example food, feed, textile, paper and pulp, fuels and chemicals, detergents – due to the development of extremely productive strains and production processes. With the advent of molecular genetic tools, in the last two decades very successful strain improvement programs for protein and secondary metabolites have been developed. However, for primary metabolites, such as organic acids, which are still by volume the largest fungal bioproducts, real breakthroughs have not been made in recent years. In our research we have addressed the production of one of the commercially interesting building-block organic acids, itaconic acid. To unambiguously identify the itaconic acid biosynthetic pathway several parallel approaches were taken using *Aspergillus terreus* as parental host strain. Using a combination of controlled fermentation design, reversed genetics and transcriptomics approaches the pathway specific *cis*-aconitate decarboxylase (CAD) encoding gene was identified. This gene encodes the enzyme required to make itaconic acid from general TCA cycle intermediates. In addition to this gene several other target genes for improvement of itaconic acid production were identified.

A highly relevant issue for the bioproduction of bulk-products like organic acids is also the cost of production. As production costs rely for 30–60% on the cost of feedstock, in frame with our itaconic acid project we also evaluated performance and feedstock versatility of several potential microbial production hosts on lignocellulosic substrates. From this analysis *Aspergillus niger* was selected as preferred host.

## ALTERNATIVE MODELS TO STUDY MAMMALIAN PATHOGENS

### AMOEBAE: NATURAL HOSTS AND VERSATILE MODELS TO STUDY THE VIRULENCE OF *LEGIONELLA PNEUMOPHILA*

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Environmental bacteria adopt different strategies to resist protozoa and other environmental predators. Bacterial survival strategies include biofilm formation, the production of toxins and intracellular replication. Amoebae are primordial phagocytes that share many features with metazoan phagocytes such as macrophages. Consequently, upon inhalation amoebae-resistant bacteria avoid degradation by alveolar macrophages, and thus may cause pneumonia.

*Legionella pneumophila* is an aquatic bacterium that colonizes biofilms and resists killing by free-living protozoa. *L. pneumophila* evolved the capacity to replicate intracellularly within a unique membrane-bound host compartment, the 'Legionella-containing vacuole' (LCV). The formation of LCVs in amoebae as well as in macrophages requires the bacterial Icm/Dot type IV secretion system and more than 100 secreted 'effector' proteins, which are believed to subvert conserved host cell vesicle trafficking and signal transduction pathways.

Thus, amoebae are valid and versatile models to study not only ecological aspects and transmission, but also virulence traits of the opportunistic pathogen *L. pneumophila*. In particular, the genetically tractable social amoebae *Dictyostelium discoideum* is a powerful model to analyse on a biochemical and cell biological level LCV formation and intracellular replication of the bacteria.

### HIGH-THROUGHPUT ANALYSIS OF BACTERIAL PATHOGENS USING NON-MAMMALIAN HOSTS

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*Caenorhabditis elegans* is a nematode that lives in the soil where it feeds on bacteria. This genetically tractable model organism is widely used in neurosciences and developmental biology. Since the pioneering work of Fred Ausubel, many laboratories have adopted *C. elegans* as an alternative model host for deciphering pathogen virulence. Then, the characterization of several bacterial infections in *C. elegans* allowed the identification of the nematode innate immunity.

This presentation will describe how this nematode can be used as an alternative model to study mammalian pathogens by performing candidate gene approaches, high-throughput forward genetic screens for bacterial virulence factors and *in vivo* screens for antimicrobials.

Finally, mention will be made of the hijacking of *C. elegans* innate immunity by bacterial virulence factors that are an important aspect of bacterial pathogenicity during infections in mammals.

### ROCKET SCIENCE AT THE TIP OF THE ICEBERG

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Fresh produce, particularly salad leaves that are consumed raw, is becoming an increasingly important source of human infection. Several recent outbreaks of *Salmonella* and *E. coli* O157 infection were traced to consumption of contaminated lettuce and spinach, respectively. Despite the increasing risk to humans little is currently known about the mechanism by which human pathogens bind intact leaf surfaces. The aim of this investigation was to unravel the mechanism underpinning the adhesion of human pathogen to salad leaves.

We developed an intact-leaf attachment model. Wild type and mutant bacterial strains were grown overnight and diluted before leaf inoculation. Inoculated leaves were incubated at room temperature for 1 hour or overnight and washed. A section of the inoculated leaf was fixed for examination by fluorescent and electron microscopes. Another leaf section was used to quantify the level of adherent bacteria.

We found that many human bacterial pathogens bind tightly to salad leaves and remain attached following thorough washes. In particular we found that *E. coli* O157 binds leaves via EspA filaments while enteroaggregative *E. coli* (EAEC) binds the epidermis via AAF pilus and the guard cells of the stomata via flagella. Flagella play a role in leaf attachment of *Salmonella enterica* serovar Senftenberg, but not of *S. Typhimurium*. The mechanisms by which other bacterial pathogens bind leaves remain unknown.

A variety of human pathogens bind avidly to salad leaves, representing a potentially important route of transmission, which should be investigated. Considering that simple

washes do not remove attached bacteria, there is a need to develop new strategies to remove attached bacteria from fresh produce in order to reduce the risk of contamination and pathogen spread.

### INSECT LARVAE AND THEIR USE IN MEASURING FUNGAL PATHOGENESIS

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The insect immune system bears a number of structural and functional similarities to the innate immune response of mammals and as a consequence a strong correlation exists between the response of insects and mammals to microbial pathogens. A wide variety of insects (e.g. *Galleria*, *Drosophila*, *Manduca*) are now used to measure the virulence of fungal pathogens or to assess the potency of novel antimicrobial compounds. The use of insects means that results can be obtained faster and in a more cost effective and ethically acceptable manner than the use of mammals such as mice.

Examination of similarities between neutrophils of mammals and haemocytes of insects demonstrates that both cell types can phagocytose and kill opsonized micro-organisms. The killing mechanism is mediated by the production of superoxide produced by a NADPH oxidase complex that is similar in structure in both cell types. The NADPH oxidase complex in both cell types contains a number of cytosolic proteins (p40<sup>phox</sup>, p47<sup>phox</sup> and p67<sup>phox</sup>) that translocate to the membrane upon stimulation of the cell with PMA or contact with a pathogen. The translocation process can be prevented with the same inhibitors in both cell types. In addition neutrophils and haemocytes undergo degranulation to release proteolytic enzymes into the phagocytic vacuole to kill the pathogen.

### CAENORHABDITIS ELEGANS INTERACTION WITH BACTERIAL TOXINS

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Abstract not received

### SURVIVAL AND REPLICATION OF *LISTERIA MONOCYTOGENES* SCOTT A IN VACUOLES OF *ACANTHAMOEBA CASTELLANII*

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*Listeria monocytogenes* is an intracellular pathogen of man that causes listeriosis, a food borne illness with high case fatality rate particularly in immunosuppressed persons. We have investigated the role of *Acanthamoeba* in the transmission and pathogenesis of *L. monocytogenes* infection using cell culture and electron microscopy. *L. monocytogenes* was co incubated with *Acanthamoeba* at a ratio of 1000:1 and viable bacteria counts were done at time intervals. Results of co culture showed that *L. monocytogenes* persisted and even significantly increased in number in the presence of amoeba and in their by-products particularly, when incubated with agitation ( $P < 0.05$ ), but declined rapidly in cell-free culture. Gentamicin protection assay showed that the number of intracellular bacteria fell sharply by 6 h post infection but then significantly increased after 24 h of infection ( $P < 0.05$ ) and thereafter, the numbers gradually increased. Electron microscopy results confirmed uptake of *L. monocytogenes* which were confined to vacuoles that were mostly large, double membraned and enclosing multiple bacteria at various stages of cell division, consistent with vacuoles formed by autophagy. The findings suggest that *Acanthamoeba* may serve as reservoir for human and animal infection.

### THE *DROSOPHILA MELANOGASTER* MODEL TO STUDY FUNGAL AND BACTERIAL INFECTIONS: NOVEL INSIGHTS INTO INSECT HOST DEFENSE

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*Drosophila* is a model for the study host defense against infections because of its powerful genetics. In a septic injury model, microbes are detected by Pattern Recognition Receptors (PRRs). These PRRs in turn trigger in the fat body NF-kappaB signaling pathways: Toll (Gram-positive bacteria and fungi) and Immune Deficiency (Gram-negative bacteria). Using the entomopathogenic fungus *Beauveria bassiana*, we have discovered that a dual detection system is used to sense fungal infections, namely a PRR-dependent system, and a host protease precursor (Persephone) that becomes activated into a functional protease by virulence factors of fungi. *B. bassiana* appears to have developed a strategy to elude detection by the PRR system, yet still activates the Toll pathway through Persephone.

We have developed an intestinal infection model by feeding flies the potent bacterial pathogen *Serratia marcescens*. To understand the infection from the vantage of the host, we have undertaken a genome-wide screen of the Vienna *Drosophila* RNAi Center collection to identify lines that present an enhanced or decreased survival. While the exact function of many of the genes identified in this screen remains presently unknown, our data indicate that host defense involves many processes that are not limited to classical innate immune response pathways. For instance, we have demonstrated that the JAK/STAT pathway plays an essential role in the regulation of intestinal epithelial homeostasis in response to infection.

#### REAL TIME NON-INVASIVE MONITORING OF BACTERIAL INFECTIONS *IN VIVO*

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*Listeria monocytogenes* is a Gram positive foodborne pathogen with the capacity to invade non-professional phagocytic cells and to grow within the host cell cytoplasm. Bioluminescence imaging approaches allow whole animal imaging in order to determine the localization of the pathogen within the host and for *in vivo* bacterial gene expression profiling. We have developed a suite of vectors that allow either constitutive expression of Lux in *L. monocytogenes* or promoter-dependent Lux expression for monitoring *in vivo* gene expression. We have utilized lux-tagging of *L. monocytogenes* to determine the localization of the pathogen in tumour-bearing mice and have exploited this phenomenon to develop *Listeria* as a means of gene delivery to tumour cells *in vivo*. In addition, creation of virulence gene promoter fusions to lux has allowed us to determine levels of virulence gene expression by *Listeria in situ* in murine and other alternative models of infection. The development of lux vectors and their applications in *L. monocytogenes* will be discussed.

#### MUCOSAL ORGANOTYPIC EPITHELIA AS MODELS OF FUNGAL INFECTIONS

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To reduce reliance upon animal experimentation a number of epithelial organotypic models have been developed to study fungal infections of mucosal surfaces. We have used these organotypic models to study the host-pathogen interactions between oral epithelium and *Candida albicans*. *C. albicans* infection of oral epithelium induced a strong pro-inflammatory cytokine response, which was mediated by the NF-κB and MAPK pathways. Whilst NF-κB activation was linear, MAPK activation was bi-phasic with peaks at 15 min and 2 h, with the second phase constituting MKP-1 phosphorylation (a MAPK phosphatase) and activation of the transcription factor c-Fos. Analysis of *C. albicans* mutants indicated that only hyphal forms activated the second MAPK phase. Cytokine secretion, induced by invading *Candida* hyphae, results in a recruitment of PMNs, which in turn strongly upregulates epithelial TLR4 expression. TLR4 upregulation was independent of PMN-epithelial cell contact and correlated with a protective phenotype against fungal infection. Protection could be reversed by anti-TLR4 neutralizing antibodies and RNAi knockdown of TLR4. The data implicates a pivotal role for 'immunological cross-talk' between *C. albicans*-infected oral epithelium and PMNs, resulting in TLR4-mediated protection against fungal infection. Organotypic epithelial models provide an ideal tool to characterize host/pathogen interactions and to study human infectious diseases.

#### NEW INSIGHTS INTO SUSCEPTIBILITY TO VAGINAL CANDIDIASIS THROUGH A CLINICAL LIVE CHALLENGE MODEL

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Vulvovaginal candidiasis (VVC), caused by the commensal yeast *Candida albicans*, afflicts up to 75% of otherwise healthy women and can be recurrent in 5–8% of women. While *Candida*-specific acquired immunity protects against *Candida* infections at most mucosal sites, in the urogenital tract, traditional cross-sectional clinical studies as well as animal models have shown little to no role for systemic or local adaptive immunity in protection against VVC. Protection instead occurs through innate epithelial cell anti-*Candida* activity. Insofar as these models have been instrumental for studying anti-*Candida* host defenses, they have fallen short for identifying the factor(s) associated with susceptibility to infection. Rather, a provocative yet safe clinical live challenge model, where the natural history of infection can be followed, revealed that symptomatic infections result from a danger response to *C. albicans* by vaginal epithelial cells that promotes an aggressive migration of polymorphonuclear neutrophils (PMNs) into the vaginal cavity. The ensuing inflammatory response causes the symptoms associated with vaginitis without any immune effects on *Candida*. Recent data suggest that calcium-binding proteins (S100A8/A9) are the chemotactic trigger for PMN migration. Our goal is to develop immunotherapeutic strategies to neutralize these calcium-binding proteins that will ultimately reduce the symptoms associated with VVC.

#### DEVELOPMENT OF NOVEL *IN VITRO* MODELS TO STUDY *CAMPYLOBACTER JEJUNI* PATHOGENESIS

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*Campylobacter jejuni* is the leading cause of foodborne diarrhoeal disease, responsible for as many as 500 million cases of bacterial gastroenteritis worldwide each year. Despite the organism's prevalence, understanding of the molecular basis of pathogenesis is still limited, due to three factors:

1. No convenient animal models of infection have yet been established
2. Human volunteer studies are unethical
3. The *in vitro* models described to date have not proved to be totally satisfactory.

To address this, two novel *in vitro* models are being developed. Model one utilizes a Vertical Diffusion Chamber (VDC) to co-culture the microaerophilic *C. jejuni* with human intestinal epithelial cells (IECs) under microaerobic conditions at the apical and aerobic conditions at the basolateral surface. Survival and integrity of IECs cultured under these conditions has been demonstrated by several methods. Furthermore, *C. jejuni* adherence to and invasion of IECs was strongly enhanced in this system. Model two uses *in vitro* cultured human intestinal tissue (biopsies) to study the host response side of the bacterium-host interaction. Production of several pro-inflammatory cytokines by the intestinal tissue was demonstrated in response to challenge with *C. jejuni*. Both models are being used to investigate different aspects of *C. jejuni* pathogenesis.

#### THE CASE FOR MAMMALIAN MODELS OF DISEASE

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The utility and complexity of mammalian models of disease will be discussed in the context of HIV vaccine and microbicide research and development. The case for mammalian models of disease will be addressed through a series of critical questions including: what part does a mammalian model play in the discovery and development of human prophylactic modalities? Is it appropriate to use a model as a gatekeeper to select 'best' candidates? Should a hierarchy of models be used? How can a model be calibrated against humans? Is there an optimal strategy for refining models? How can the 3R's principle be applied? What are the logistic, ethical and environmental aspects to be considered? Can mammalian models be integrated with non-mammalian models to provide holistic solutions? I will propose that the use of mammalian models in the traditional linear product development pathway is a flawed paradigm and that the concept of pre-clinical evaluation should, at least in part, be replaced with a concept of para-clinical evaluation.

# PRODUCTION, FORMULATION AND DELIVERY OF BIOTHERAPEUTICS

## IMPORTANCE OF PROPERLY DESIGNED OPERATION CONDITIONS AND ONLINE MONITORING IN SMALL-SCALE CULTIVATION

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To enhance the speed of process development and the experimental throughput in screening projects it would be desirable to use automated miniaturized parallel micro bioreactors with full measuring and control options. It is obvious that conventional approaches used on the level of jar fermentors cannot directly be transferred to small scale and new concepts have to be developed. Shake flasks and micro titre plates (MTP's) have shown to be a suitable format for small scale fermentations. Setting suitable operational condition is a prerequisite to obtain meaningful results. Techniques will be introduced which allow for on-line measurement of optical density of the culture broth [OD, DOT, pH, NADH and riboflavin fluorescence (Samorski *et al.*, 2005)]. If a fluorescent protein as GFP or its derivatives can be fused to a protein of interest, even the product formation can be monitored on-line. A different technique can be used to assess the oxygen and carbon dioxide transfer rate.

Batch operation mode without pH control is still the general state of the art for cultures performed for screening purposes. This is not sufficient for the initial steps of process development. This consideration has resulted in the development of a new technique, which allows to run the screening in fed-batch operation mode (Jeude *et al.* 2006). The limiting nutrient (e.g. glucose) is encapsulated in a polymer matrix, which slowly releases the nutrient at an adapted rate during the culture. This technique is applicable in shake flasks as well as in MTPs. Dramatic increases in product titre (4- up to 400-fold) were observed compared to conventional batch fermentations.

Frequently mineral media with ammonia as nitrogen source are used in production. As in a screening reactors the pH is not controlled, pH will drop significantly due to the consumption of ammonia by the metabolic activity of the cells. In order to keep the pH in a decent range, polymer based slow release systems have been developed, which contain pH controlling agents like sodium carbonate or urea. Examples of the successful application of this approach (*E. coli* cultures on glycerol and on glucose) are presented.

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## MICROBIOREACTORS FOR EUKARYOTES WITH DISSOLVED OXYGEN CONTROL

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Performance of bioreactors is typically qualified by consideration of transport processes and biochemical conversion [1]. Oxygen transfer to the liquid phase is perhaps the most important transport process, due to both the relatively low solubility of oxygen in water and the high demand from aerobic bioprocesses.

We present a 50 µl volume microfluidic microbioreactor with novel utilization of an oscillating jet, introduced to the bioreactor chamber from an eccentrically placed inlet channel to generate mixing and enhance transfer of oxygen, and examine the feasibility of dissolved oxygen (DO) control during batch fermentations with *Saccharomyces cerevisiae* in YPD media.

The bioreactor was prepared using a multilayer rapid prototyping technique where silicone parts were cast from molds machined with a CNC micromill. Aeration membranes were spin coated on silanized Si wafers, and the four silicone layers were bonded after activation with air plasma. A fluorescent lifetime sensor spot enabled monitoring of DO concentration, which was controlled via a PID scheme that varied the pulse width modulation output of a solenoid valve with nitrogen and air inputs. Cell density correlated linearly with optical density measured vertically through the bioreactor at 600nm wavelength, up to 8cm<sup>-1</sup>.

**Reference** (1) A. Lübbert & S. Bay Jørgensen (2001). Bioreactor performance: a more scientific approach for practice. *J Biotechnol* **85**, 187–212.

## CONTINUOUS CULTURE MICROBIOREACTORS

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In the past years microbioreactor research has come up with many promising solutions to problems existing in fermentation technology: Online optical measurements are available for the most important culture variables such as pH, dissolved oxygen (DO) or optical density (OD) as are techniques to control these variables. Additionally, microbioreactor solutions have been presented which allow for the continuous cultivation of cells including methods for handling liquid flows to and from the reactor:

For the industrial applicability of microbioreactors we believe these techniques have to be taken one step further and integrated into a system where the reactor itself is disposable after one cultivation. Disposability greatly reduces the need for sterilization and cleanup and thus reduces the setup time per experiment; However, it also requires the reactor to be cheap, and thus the reactor has to be easy to fabricate and contain as few parts and sensors as possible. Additionally, it has to deliver reproducible results despite varying handling routines of different users. We have developed a system where one reactor only consists out of one single material and includes a stirrer bar and two sensors. The rest of the setup is located in the surrounding machinery and is reusable.

## DASGIP PARALLEL BIOREACTORS CASE STUDY: HIGH CONTENT SCREENING IN MILLILITRE-SCALE

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The new HTS high content screening system with online measurement, called DASGIP BioLector, and its validation is presented. The technology combines high-throughput screening and high-information content by applying online monitoring of scattered light and fluorescence intensities in continuously shaken microtiter plates (<1 ml). Various examples in calibration of the optical measurements, clone and media screening and promoter characterization are given.

Bacterial and yeast biomass concentrations of up to 50 g/L cell dry weight could be linearly correlated to scattered light intensities. In media screening, the BioLector could clearly demonstrate its potential for detecting different biomass and product yields and deducing specific growth rates for quantitatively evaluating media and nutrients. Growth inhibition due to inappropriate buffer conditions could be detected by reduced growth rates and a temporary increase in NADH fluorescence. A clone screening of 90 different GFP-expressing *Hansenula polymorpha* clones depicted the broad distribution of growth behaviour and an even stronger distribution in GFP expression. The importance of mass transfer conditions could be demonstrated by varying filling volumes of an *E. coli* culture in 96 well MTP.

## AUTOMATED MICROSCALE APPROACHES FOR SCALE-UP PREDICTIONS OF MICROBIAL CELL CULTURES

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Automated microscale processing techniques represent a powerful new approach for high throughput bioprocess design and optimization [1]. Such techniques rely upon parallel microlitre scale experimentation carried out in microtitre plate formats. The generation of quantitative process data at the microlitre scale first requires an understanding of the mixing and mass transfer phenomena within the well. Recently the engineering fundamentals of shaken microwell systems have been the subject of a number of experimental and computational studies. In addition there have been a number of studies on the various bioprocess operations performed in microwell format including fermentation [2] and bioconversion [3]. For microbial processes, advances in automated high throughput screening based on the shaken microwell plate system have provided the solution to significantly increase the number of potential biocatalysts candidates.

This presentation will give an overview of current approaches to the characterization of fundamental phenomena in shaken microwells. The development of a microwell process sequence comprising the fermentation and bioconversion steps will be described using two model systems. The first system addressed is the use of transketolase (TK) catalysed condensation of glycolaldehyde and β-hydroxy-pyruvate to yield L-erythrulose. The second system selected is the Baeyer-Villiger monooxygenase (BVMO) catalysed lactone synthesis. In view of the interest in the development of scaleable bioreactions, for both bioconversions studied it was shown that the microwell results could accurately predict the performance

of the laboratory scale stirred tank bioreactors in which bioprocess development is typically carried out. These results thus confirm the potential for microscale techniques to increase the speed and reduce the costs associated with the process development stage. Finally an efficient approach to bioconversion kinetic model generation will be presented, the method being based on automated microscale experimentation integrated with model driven experimental design [4].

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## CULTIVATION AND BIOTRANSFORMATION IN MICROTITER PLATES

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As a result of the continuous improvement of bioassays and analytical methods, ever smaller microbial culture volumes suffice for microbial screenings, and optimization studies for fermentative and biotransformation processes. The challenge of the last decade has been to miniaturize microbial growth systems, while maintaining the quality of growth and axenicity as traditionally achieved in shake flasks and stirred bioreactors. The use of standard microtiter plates for this purpose is particularly attractive because of the availability of compatible robots, readers, and other equipment.

The presentation will focus on state-of-the-art methods for turning the individual wells from microtiter plates into high-quality 'mini-bioreactors'. Topics include the hydrodynamics, degree of turbulence, and oxygen transfer rates (OTRs) that can be achieved in 24, 48, and 96 well microtiter plates with either square deepwells or round wells, in combination with well-closure systems that are necessary to maintain axenicity, prevent cross-contamination, limit the evaporation rate, and to limit the headspace refreshment rates during vigorous orbital shaking.

The final topic will be biotransformations (mainly hydroxylations) in microtiter plates by (mutant) libraries, with a special focus on reproducibility of the results.

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## DEVELOPING A SCALABLE MODEL OF RECOMBINANT PROTEIN YIELD FROM PICHIA PASTORIS: THE INFLUENCE OF CULTURE CONDITIONS, BIOMASS AND INDUCTION REGIME

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Abstract not received

## PARALLEL MILLILITER-SCALE FERMENTATIONS IN STIRRED-TANK BIOREACTORS

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The stirred-tank reactor is still the most important production reactor in biotechnology. As a consequence many efforts have been published recently to make use of miniaturized stirred-tank bioreactors from microliter- to the milliliter-scale for parallel bioprocess development and optimization. A completely new approach was the development of novel gas-inducing stirrers for stirred-tank bioreactors on a 10 milliliter-scale with high oxygen transfer capabilities of up to  $k_L a = 0.4 \text{ s}^{-1}$ . To ensure an easy parallelization, a magnetic inductive drive was developed which allows for the parallel operation of 48 single-use stirred-tank bioreactors in a bioreaction block. Parallel batch and fed-batch fermentations can be performed with high parallel reproducibility making use of a standard liquid handler for substrate and base additions. The successful scale-down of an industrial production process into the 10 milliliter-scale was demonstrated on the example of the fed-batch process for the microbial production of riboflavin with *Bacillus subtilis*. Recently, reliable 48

times parallel and individual online monitoring and control of stirrer-speed, dissolved oxygen and pH were realized. The automation of 48 single-use stirred-tank bioreactors including DO- and individual pH-control allows now to make use of such a system as a valuable tool in bioprocess development and optimization.

## NEXT GENERATION BIOPROCESSING: SMARTER AND DISPOSABLE

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Early stage bioprocess development is typically carried out in disposable bioreactors with limited or no capability for process monitoring. In contrast, as the process moves to larger scales, well instrumented bioreactors are employed and cell growth conditions are tightly monitored and controlled. This gap clearly needs to be addressed, and we will describe recent advances in low-cost, non-invasive optical sensor technology that make it feasible to instrument bioreactors and other process equipment at any scale throughout the process train. These technologies will go a long way towards enabling Process Analytical Technologies that are being encouraged by regulatory agencies to improve the quality and consistency of biopharmaceutical products.

## ENGINEERING MICRO-ORGANISMS FOR PRODUCTION OF DRUGS AND FUELS

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The richness and versatility of biological systems make them ideally suited to solve some of the world's most significant challenges, such as converting cheap, renewable resources into energy-rich molecules; producing high-quality, inexpensive drugs to fight disease; and remediating polluted sites. Over the years, significant strides have been made in engineering micro-organisms to produce fuels, bulk chemicals, and valuable drugs from inexpensive starting materials; to detect and degrade nerve agents as well as less toxic organic pollutants; and to accumulate metals and reduce radionuclides. The components needed to engineer the chemistry inside a microbial cell are significantly different from those commonly used to overproduce pharmaceutical proteins. Synthetic biology has had and will continue to have a significant impact on the development of these components to engineer cellular metabolism and microbial chassis to host the chemistry. The ready availability of more well-characterized gene expression components and hosts for chemical synthesis, standards for the connection of these components to make larger functioning devices, computer aided design software, and debugging tools for biological designs will decrease both the time and the support needed to construct these designs. Some of the most important tools for engineering bacterial metabolism and their use for production of the anti-malarial drug artemisinin and for production of biofuels are reviewed.

## OPTICAL SENSORS FOR MICRO-SCALE DEVICES

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Optical sensors are established in monitoring bio-processes from medium to large scale. Miniaturized cell and microbial culturing demands significant improvements of the performance of state of the art optical sensors in terms of signal enhancement, response times, opto-electronic read-out systems, fabrication and integration. Optical sensors for dissolved oxygen, pH, carbon dioxide, ammonia and glucose fulfilling these demands are developed in our group. Strategies to improve the signal intensities investigated are the synthesis of new ultra-bright indicators and the use of light harvesting principles. Brightness enhanced sensing films in nanometer thickness and nanosensor particles show higher signals than standard sensors and respond in real-time. Nano sensor particles are attractive for the application in micro-fluidic devices because they can be easily injected to the culture medium and read-out by optical fibres or imaging techniques from outside. A variety of nanosensor particles is presented which form stable suspensions in water and are not uptaken by cells during cultivation. Moreover, magnetic optical sensor particles for in-situ sensor spot formation are presented. These novel nano-sensors can be collected at any desired position in a reaction vessel. In preliminary studies, the new sensors are applied in microfluidic systems and read-out with lifetime imaging or referenced imaging using the colour channels of a CCD camera.



## NANOSCALE CHEMICAL ANALYSIS OF BIOLOGICAL SYSTEMS

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Biological systems can be highly heterogeneous on the nanometer scale. For example, the adhesion of bacteria onto surfaces and the formation of biofilms is governed by nanostructures on the cell surface (e.g. pili) as well as different extracellular polymeric substances (EPS), such as polysaccharides and proteins. Deeper insight into such highly complex systems can for example improve drug design or lead to more efficient biocides against biofilm formation. Therefore, analytical techniques that provide nanoscale imaging and molecular characterization are necessary.

The spatial resolution of spectroscopy and microscopy techniques is often limited to the micrometer or upper nanometer range due to the optical diffraction barrier. On the other hand, highly resolving microscopes, such as the atomic force microscope (AFM), provide only very little chemical information. Our approach to imaging and chemical characterization with high spatial resolution is to combine far-field (confocal microscopy, Raman microscopy) and near-field techniques (AFM, tip-enhanced Raman spectroscopy (TERS)) in such a way that the exact same part of a sample can be analysed by complementary techniques.

The investigation of river-water biofilms by confocal microscopy and AFM has dramatically demonstrated the heterogeneity of the extracellular matrix at the nanometer scale [1]. First model systems for the spectroscopic investigation of biological samples with high spatial resolution were the EPS polysaccharide alginate and the protein cytochrome c. TER spectra of both compounds contain specific marker bands that will be used in further studies for their localization in complex organic matrices [2, 3]. AFM-Raman analysis of a real biofilm was performed in a study on biomineralization. It was found that the bacterium *H. meridiana* can catalyze the formation of dolomite in nature. In order to study this process, *H. meridiana* was grown in laboratory cultures and electron microscopy revealed Ca and Mg-containing mineral nanoglobules located at the cell walls and in the extracellular matrix. Only Raman spectroscopy combined with AFM was able to show that the nanoglobules consist of dolomite due to its spectral signature that is significantly different from other carbonates, such as hydromagnesite or calcite [4]. Our combined AFM-Raman setups are and will be applied to the investigation of biofilm samples, spermatozoa of fishes as well as artificial and real lipid membranes.

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### N-LINKED PROTEIN GLYCOSYLATION: A STARTING POINT FOR GLYCOENGINEERING

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N-linked protein glycosylation is the most frequent post-translation modification in eukaryotic cells. The essential process initiates in the Endoplasmic Reticulum, where an oligosaccharide is assembled on the lipid carrier, dolichylpyrophosphate. In most of the eukaryotes, the oligosaccharide transferred is  $\text{Glc}_3\text{Man}_9\text{GlcNAc}_6$ , however, in protozoa, simpler structures are also found. The covalent linkage of the oligosaccharide to selected asparagine residues of polypeptide chains, characterized by the sequon  $\text{AsN-X-Ser/Thr}$  ( $\text{X}$  = any amino acid residue except proline), is performed by the oligosaccharyltransferase (OST), a complex enzyme that is composed of eight different subunits, all of them membrane embedded proteins.

Recently, it was found that *Campylobacter jejuni*, a Gram-negative bacterium, is able to N-glycosylate proteins as well. A detailed analysis of this prokaryotic post-translational modification became possible due to the functional transfer of the pathway into the model system *Escherichia coli*. This analysis revealed that the bacterial and the eukaryotic process are homologous pathways that are based on the same principles: both pathways utilize isoprenoid lipids as carriers for the assembly of the oligosaccharide in the cytoplasm (requiring a translocation of the oligosaccharide across the membrane) and the  $\text{AsN-X-Ser/Thr}$  is the protein acceptor sequence in both cases. In addition, the same principle of oligosaccharide assembly on the isoprenoid lipid carrier is also found in the pathway of LPS biosynthesis. The experimental system of *E. coli* makes it possible to combine portions of LPS biosynthetic pathways with the process of N-linked protein glycosylation to obtain novel types of N-glycoproteins. In addition, this system can be used to develop genetic tools that are used in for glycoengineering and to produce defined glycoproteins in this bacterial expression system. Recent developments in these areas of research will be discussed.

### N-LINKED PROTEIN GLYCOSYLATION IN *CAMPYLOBACTER* AND *HELICOBACTER* SPECIES

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N-linked glycosylation is a process by which proteins are modified by the attachment of oligosaccharides at defined asparagine residues, within the amino acid sequence  $\text{NXS/T}$ , where  $\text{X}$  is any amino acid except proline. Glycosylation is mediated by an oligosaccharyl transferase enzyme or OSTase. Whilst N-linked glycosylation is an essential and well characterized function in Eukaryotes, only relatively recently have analogous systems been identified in Bacteria, most notably in *Campylobacter jejuni*. In *C. jejuni* a single genetic locus encodes a general glycosylation system, including a gene termed *pglB*, encoding the OSTase. *PglB* enzymes are of particular interest for glycoengineering and access to a broad range of enzymes with different specificities is highly desirable. Genome sequence data reveals *pglB* homologues other *Campylobacter* and *Helicobacter* species. Interestingly, some *Helicobacter* species possess two *pglB* homologues. We have employed an *in vitro* assay for OSTase activity to rapidly characterize the *PglB* mediated glycosylation reaction. Several species of *Campylobacter* and *Helicobacter* were capable of modifying a short peptide containing the  $\text{NXS/T}$  glycosylation sequon. Subsequent mass spectrometry analysis will enable the rapid determination of the corresponding glycan structures, revealing the specificity of different OSTase enzymes.

### CHARACTERIZATION OF BACTERIAL PGLB ENZYMES FOR THE DEVELOPMENT OF GLYCOENGINEERING

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*PglB* is the central enzyme in the N-linked glycosylation pathway of *Campylobacter jejuni* and acts as an oligosaccharyltransferase, transferring the fully synthesized heptasaccharide to acceptor proteins. Previous work found that *CjPglB* can be functionally expressed in *E. coli*, providing an easily tractable system for the generation of recombinant glycoproteins. *CjPglB* is able to transfer a diverse range of sugars, including O-antigen and capsule polysaccharides, which has opened up the possibility for developing novel glycoconjugates of medical and/or commercial interest. This technology is in the preliminary stages of development and one bottleneck encountered is the observed requirement of a 2-acetamido group at the reducing end of the polysaccharide to be transferred. To investigate overcoming this problem, we have carried out extensive searches for alternative *PglB* orthologues, and developed a system for directed evolution of *CjPglB*, with the aim of identifying novel enzymes with alternative sugar specificities. We have identified over 20 *PglBs* from a diverse range of  $\epsilon$ -proteobacteria, including *Campylobacter* spp., *Helicobacter* spp. and bacteria isolated from deep-sea vents. A bank of 11 *PglBs* has been expressed in *E. coli* and do not all function in the same way as *CjPglB*, providing evidence that they may have different sugar specificities that could be utilized in the development of glycoconjugates.

### LARGE-SCALE SYNTHESIS OF GANGLIOSIDES OLIGOSACCHARIDES BY METABOLICALLY ENGINEERED BACTERIA

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Gangliosides are sialylated glycolipids that are present in all vertebrate cells and that are involved in a number of carbohydrate dependant event of biological recognition and signal transduction. We have previously reported the efficient synthesis of the oligosaccharide portion of GM3, GM2, GM1a and GD2 gangliosides by metabolically engineered *Escherichia coli* strains expressing the appropriate glycosyltransferase genes [1–3]. In these systems the activated sialic acid donor (CMP-Neu5Ac) was generated from exogenous sialic acid, which was transported into the cells by the permease NanT. Since sialic acid is an expensive compound, we have recently developed a more economical process for the large scale synthesis of the GM3 sugar (3'sialyllactose) by using a genetically engineering *E. coli* K12 capable of generating CMP-Neu5Ac using its own internal metabolism [4]. We now report the use of this system for the economical production of GM2 GM1a and GD2 oligosaccharides. In addition we describe different microbial systems for the production of most of gangliosides sugars including GM1b, GD2, GD1a, GD1c and GT1a.

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### DEVELOPMENT OF NOVEL VACCINES AND THERAPEUTICS THROUGH GLYCOENGINEERING IN BACTERIA

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Vaccines based on bacterial polysaccharides have been successfully used for decades. Purified polysaccharides produce a transient immune response. To generate long-term protection, bacterial polysaccharides must be covalently attached to an appropriate protein carrier. Presently, the production of these conjugate vaccines requires intricate synthetic chemistry for obtaining, activating, and attaching the polysaccharides to protein carriers. Production of conjugate vaccines using conventional procedures is complex and the costs are prohibitive for their worldwide utilization. Novel technologies are needed to make possible the globalization of the use of conjugate vaccines for the prevention of bacterial infections. It has recently been established that bacteria are able to glycosylate proteins. Although the attached sugars in naturally occurring glycoproteins are not suitable for vaccination, it has been shown that bacteria can tolerate the manipulation of their glycosylation systems. Therefore, they constitute perfect toolboxes for engineering novel glycan-containing structures that may be used as vaccines. Engineered glycoproteins containing bacterial polysaccharides may constitute a new generation of conjugate vaccines, circumventing most of the disadvantages of the conventional chemical methods, significantly reducing costs, and improving the reproducibility of the conjugates obtained. In this talk, I will present our last advances on the characterization of the bacterial glycosylation systems, including both, N- and O-glycosylations systems. Furthermore, our progress on glycoengineering novel glycoproteins, as well as the initial trials in animal models will be discussed.

## DEVELOPMENT OF NOVEL *FRANCISELLA TULARENSIS* AND *BURKHOLDERIA PSEUDOMALLEI* SUBUNIT VACCINES USING PROTEIN GLYCAN COUPLING TECHNOLOGY

J. CUCCUI<sup>1</sup>, M. KOWARIK<sup>2</sup>, J.L. PRIOR<sup>3</sup>, T. ATKINS<sup>3</sup>, M. WACKER<sup>3</sup> & B.W. WREN<sup>1</sup>

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*Francisella tularensis* and *Burkholderia pseudomallei* are highly pathogenic organisms share the ability to survive intracellularly in host cells and both can be acquired through the aerosol route of infection. Currently, no licensed vaccine exists against either pathogen.

Protein glycan coupling technology (PGCT) uses the capabilities of bacterial oligosaccharyltransferases to attach selected glycan moieties to a given acceptor protein within *E. coli*. Polysaccharide moieties exposed on the cell surface offer a target that the host immune system can recognize. However, purified glycan alone is not sufficient to evoke protection against either organism. By attaching an immunogenic protein carrier it should be able to induce a more favourable T cell dependant response.

We will describe our attempts using PGCT to generate subunit vaccines, demonstrating expression of *F. tularensis* and *B. pseudomallei* O antigen in *E. coli* host cells and our methodology for using this host as the 'work-horse' from which glycoproteins can be purified.

## GLYCOSYLATION OF BACTERIAL S-LAYER PROTEINS: WHERE GLYCOENGINEERING MEETS NANOBIO TECHNOLOGY

CHRISTINA SCHÄFFER, KERSTIN STEINER, ROBIN RISTL, KRISTOF ZARSCHLER, BETTINA JANESCH, SONJA ZAYNI, ANDREA SCHEBERL & PAUL MESSNER

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Self-assembly systems that exhibit nature's manufacturing precision are key elements in nanobiotechnology. Bacterial cell surface (S-) layer proteins, representing the most abundant outermost surface structures of bacteria, are aligned by self-assembly into 2D crystalline arrays with lattice parameters at the nanometer scale. Glycosylation is the major modification of native S-layer proteins. Thus, glycosylated S-layer nanonlattices are unique scaffolds to be tailored by glycosylation engineering for multivalent display of biologically

functional carbohydrate epitopes with ultimate spatial control. In biomimetic approaches, S-layer protein glycosylation converges with other glycosylation pathways. For proof of concept, two types of S-layer neoglycoproteins were produced in *Escherichia coli*. After engineering of one of the natural S-layer protein O-glycosylation sites into a target for N-glycosylation, a heptasaccharide of *Campylobacter jejuni* and the O7 polysaccharide of *E. coli* were co-/ posttranslationally transferred to the S-layer protein by the oligosaccharyltransferase PglB. Electron microscopy revealed that recombinant glycosylation is fully compatible with S-layer protein self-assembly. Strategies for in vivo neoglycoprotein display are under development. Customized nanopatterned, self-assembly neoglycoproteins capable of biostimulation and biotargeting follow the current trend for exploiting means for organizing biological functions at the nanometer level aiming at the development of novel concepts for life and non-life sciences.

## RECOMBINANT BACTERIA EXPRESSING OLIGOSACCHARIDE RECEPTOR MIMICS: DESIGNER PROBIOTICS FOR PREVENTION OF ENTERIC INFECTIONS

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Many microbial pathogens, including those responsible for major enteric infections, exploit oligosaccharides displayed on the surface of host cells as receptors for toxins and adhesins. Thus, blockade of crucial ligand-receptor interactions is a promising therapeutic strategy. One approach is to express molecular mimics of host receptors on the surface of harmless recombinant bacteria capable of surviving in the gut. These designer probiotics bind bacterial toxins in the gut lumen with very high avidity, thereby preventing disease. In the prototypic example, two *Neisseria* galactosyl transferase genes (*lgtC* and *lgtE*) were expressed in an *E. coli* R1 *waaO* mutant, generating a chimeric LPS terminating in Gal $\alpha$ 1-4Gal $\beta$ 1-4Glc-, the receptor for Shiga toxin. The chimeric LPS was incorporated into the *E. coli* outer membrane by the normal assembly route and presented as a closely packed 2-D array of receptors, which bound Stx1 and Stx2 with extraordinary avidity. Administration of the receptor-mimic bacteria to mice infected with highly virulent Shiga toxigenic *E. coli* strains was 100% effective at preventing otherwise fatal disease. Probiotics capable of binding cholera toxin and *E. coli* labile enterotoxin have also been constructed. Protective efficacy in animal models indicates their potential for prevention of travellers' diarrhoea and cholera.

# SIR HOWARD DALTON YOUNG MICROBIOLOGIST OF THE YEAR COMPETITION

## A NOVEL LANTIBIOTIC GENE CLUSTER FROM *MICROBISPOA CORALLINA*

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The increasing occurrence of antibiotic resistance, and frequently multi-drug resistance, in a wide range of bacterial pathogens has led to an urgent need for new antibiotics. Lantibiotics are ribosomally synthesized, post-translationally-modified peptide antibiotics with clinical potential. They are characterized by lanthionine and methyl lanthionine bridges between cysteine and dehydrated serine and threonine residues, respectively, giving lantibiotics their characteristic conformations and stability. Lantibiotics are produced exclusively by Gram-positive bacteria. A newly identified actinomycete species, *Microbispora corallina*, produces a potent lantibiotic, microbisporicin. Microbisporicin shows a high degree of modification including chlorinated tryptophan, dihydroxyproline and aminovinyl-cysteine residues. The first aim of this project was to identify and characterize the biosynthetic gene cluster of microbisporicin and to develop *M. corallina* as a genetic system. This has been followed by deletion analysis and knock-out mutagenesis to determine the minimal gene set for lantibiotic production and the functions of individual genes. Eventually this knowledge may be used to produce derivatives of microbisporicin with improved clinical properties.

## BUNYAMWERA VIRUS UNTRANSLATED REGIONS MEDIATE POLY(A) TAIL-INDEPENDENT TRANSLATION

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The *Bunyaviridae* family of tri-segmented negative strand RNA viruses contains several important human pathogens that cause a wide variety of diseases such as fever, hepatitis, encephalitis, and hemorrhagic fever. Messenger RNAs of bunyaviruses possess a 5' cap structure and contain untranslated regions (UTRs) but lack a 3' poly(A) tail, a common feature of eukaryotic mRNAs. We addressed how efficient translation of viral proteins is achieved by using chimeric viral-like mRNAs consisting of the *Renilla luciferase* gene flanked by the Bunyamwera virus (BUNV) 5' segment UTRs. This approach identified a translation-enhancing element within the 3' UTR that mediated efficient protein synthesis in the absence of a poly(A) tail. The translational activity appeared to be independent of poly(A) binding protein (PABP), indicating that the 3' UTR is not a poly(A) tail surrogate. Furthermore, we found that in BUNV-infected cells the usually cytoplasmically-localized PABP was redistributed to the nucleus. These results suggest that BUNV corrupts PABP function in order to inhibit translation of polyadenylated cellular mRNAs while its own mRNAs are translated in a PABP-independent process.

## FIBRONECTIN-BINDING PROTEIN B VARIATION IN *STAPHYLOCOCCUS AUREUS*

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Fibronectin-binding protein B (FnBPB) of *Staphylococcus aureus* mediates adhesion to fibrinogen, elastin and fibronectin. The gene encoding FnBPB (*fnbB*) occurs in seven out of the nine fully sequenced *S. aureus* genomes. Alignment of FnBPB amino acid sequences indicated substantial variation in the N-terminal fibrinogen and elastin-binding A domain while the C-terminal fibronectin-binding motifs are highly conserved. Four FnBPB variants (isotypes I-IV) were identified based on divergence in the minimal ligand-binding N23 sub-domains, which are 61–81% identical to one another.

Four isotype-specific DNA probes recognizing DNA encoding the highly divergent N23 sub-domains were used to identify *fnbB* variants by DNA hybridization in a *S. aureus* strain collection. Some strains that cluster by phylogenetic analysis contain different *fnbB* variants, whereas other strains that are more divergent contain the same *fnbB* variant. This suggests that *fnbB* genes have been horizontally transferred between strains.

Ligand binding by recombinant FnBPB N23 isotypes I-IV was compared by ELISA-based solid phase assays and by surface plasmon resonance. Each bound to immobilized fibrinogen, elastin and fibronectin dose-dependently and saturably with similar affinities. Binding to fibronectin was surprising because the A domains do not contain any known motifs that mediate binding to fibronectin. Deletion of fourteen C-terminal A domain residues resulted in the loss of fibrinogen-binding function while the ability to bind fibronectin was retained. This suggests that different mechanisms are involved in the binding of the A domain of FnBPB to fibrinogen and fibronectin

Expression of a chimeric protein containing the A domain of FnBPB and the cell wall anchoring domain of ClfA on the *S. aureus* cell surface promoted adhesion to immobilized

fibronectin. This raises the possibility that fibronectin-binding by the A-domain of FnBPB may contribute to *S. aureus* FnBPB-mediated processes *in vivo* such as the internalization into non-phagocytic host cells.

Current experiments aim to localize the fibronectin-binding site in the A domain of FnBPB by studying recombinant truncates and amino acid substitution mutants. Mutants will be constructed based on a comparison with the non-fibronectin-binding A domains of ClfA and FnBPA.

## TOXIN, A PROTEIN-RNA TOXIN-ANTITOXIN AND PHAGE ABORTIVE INFECTION SYSTEM OF *ERWINIA*

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Bacteria are constantly challenged by potentially lethal bacteriophage (phage) infections and have evolved multiple adaptive phage-resistance mechanisms. These include the abortive infection systems, which promote 'altruistic suicide' of an infected cell to protect the clonal population. A cryptic plasmid, pECA1039, of the phytopathogen *Erwinia carotovora* subsp. *atroseptica*, has been shown to encode a two-component phage abortive infection system, ToxIN. This system comprises ToxI RNA and ToxN protein and acts as a toxin-antitoxin system, effective within multiple genera of Gram-negative bacteria and against multiple phage. ToxIN is the defining member of a new class of protein-RNA toxin-antitoxin module and using bioinformatics, we have identified multiple homologues cross-genera. The abortive infection phenotype has been characterized in greater detail and we are undertaking work to couple these data to functional and structural data for the ToxN toxin. This will enable better understanding of the interaction between ToxI and ToxN, the powerful antibacterial activity of ToxN and the nature of the interactions between the three genomes; bacterium, phage and plasmid.

## ADENOVIRUS SEROTYPE 5 HEXON MEDIATES LIVER GENE TRANSFER

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Adenoviruses are used extensively as gene transfer agents, both experimentally and clinically. Upon intravascular delivery however, adenoviruses target liver cells, compromising their potential efficacy. In cell culture adenovirus serotype 5 (Ad5) fibre binds to the coxsackie-adenovirus receptor (CAR). *In-vivo* CAR is not involved in liver transduction, rather an alternate pathway involving virus binding to blood coagulation factors has been demonstrated. We have solved the structure of Adenovirus serotype 5 bound to Factor X (FX), showing that FX binds to the major capsid protein hexon, not the penton or fibre.

## CYPEMYCIN: A NOVEL CLASS OF PEPTIDE ANTIBIOTICS

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Post-translationally modified peptides, such as lantibiotics, are excellent candidates for peptide engineering and rational drug design. The structure and the stability of these molecules is determined by a variety of post-translational modifications. Much interest focuses on unveiling the enzymatic mechanisms that result in the formation of a variety of constituent modified amino acids. Ultimately it should be possible to gather these enzymes into an 'enzyme toolbox' for peptide design and engineering. Thus far, only antibiotics belonging to the lantibiotic family have been considered as potential sources of such enzymes.

Cypemycin is an extensively post-translationally modified peptide antibiotic produced by *Streptomyces* sp. OH-4156. While it lacks lanthionine bridges, and hence is not a lantibiotic, it contains uniquely modified amino acid residues, namely two *allo*-isoleucines and an N-terminal dimethylalanine. In addition, and in common with some lantibiotics, cypemycin possesses four dihydrobutyrine residues and a C-terminal aminovinyl-cysteine moiety. Identification and functional analysis of the cypemycin biosynthetic gene cluster shows that it belongs to a new family of antibiotics. Analysis of cypemycin biosynthesis, using both molecular genetic and biochemical approaches, has revealed novel enzymology that should make a significant contribution to the enzyme toolbox for peptide engineering.

## CONJUGATE VACCINES

### PROSPECTS FOR CONTROL OF PNEUMOCOCCAL DISEASE WITH CONJUGATE VACCINES

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The public health burden of pneumococcal infections is enormous in both developed and resource-poor countries. During the past decade, polyvalent pneumococcal conjugate vaccines have had a large impact on the incidence of pneumococcal disease in places where they have been used. These vaccines have been shown to decrease the incidence of both invasive pneumococcal disease and pneumonia. In the United States, the impact of the heptavalent pneumococcal conjugate vaccine (PCV7) has been observed in both immunized children and, through the phenomenon of herd immunity, unimmunized children and adults. Given the huge burden of pneumococcal infections in resource-poor countries, the public health impact of the widespread use of pneumococcal conjugate vaccines has the potential to be huge. Despite this fact, these vaccines are severely underutilized in the developing world. However, expanded-serotype conjugate vaccines, such as the 13-valent vaccine that will soon be licensed in the United States, will hopefully lead to greater worldwide use of these vaccines. In this presentation, the impact of PCV7 in the United States will be reviewed, as will the results of clinical trials of pneumococcal conjugate vaccines and other data from the developing world.

### A NEW GROUP A MENINGOCOCCAL CONJUGATE VACCINE; PRIMARY PREVENTION TO END MENINGITIS OUTBREAKS IN AFRICA

F. MARC LAFORCE

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Epidemic Group A meningococcal meningitis continues to be a major public health problem in Sub-Saharan Africa. Waves of epidemic Group A meningococcal meningitis occur periodically in Africa and are superimposed on high endemic rates of disease. Reactive vaccination campaigns using meningococcal PS vaccines have not eliminated these epidemics.

The Meningitis Vaccine Project (MVP), a partnership between WHO and PATH, was established in 2001 with Gates Foundation support with the goal of eliminating epidemic meningitis through the development and widespread introduction of an affordable Group A meningococcal (Men A) conjugate vaccine. Using an innovative partnership model (Serum Institute of India; CBER/FDA, Bethesda and SynCoBioPartners, Amsterdam) MVP has developed a Men A conjugate vaccine (*MenAfriVac™*) with a target price of less than \$US 0.50 per dose.

Preclinical development finished in 2004 and the vaccine has been successfully tested in Phase I and Phase II and III clinical trials in India and Africa (1–29 years). The vaccine has been shown to be safe, highly immunogenic and able to prime immunological memory when compared to polysaccharide vaccine. Widespread use of the vaccine is expected to generate broad herd immunity.

MVP has successfully developed and managed a 'push' strategy for the development of an affordable new vaccine product that was of little interest to major vaccine manufacturers. Introduction of the vaccine at public health scale is planned for 2009/2010 and widespread use of the vaccine is expected to eliminate Group A meningococcal meningitis epidemics from Sub Saharan Africa.

### HOW DOES A CONJUGATE VACCINE WORK: THE COMPONENTS OF IMMUNITY TO MenC

ANDREW J. POLLARD

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Meningococcal disease remains one of the leading infectious causes of death in the UK with most cases now caused by serogroup B meningococci but during the 1990s a substantial proportion of cases were caused by group C bacteria. The introduction of the group C meningococcal vaccine (MenC) in 1999 for all children and young people under the age of 19 years led to a dramatic and sustained reduction in disease caused by serogroup C *Neisseria meningitidis* in the UK. Whilst most age groups received a single dose, the infant programme was initially introduced as a 3 dose priming course with no booster. It was found that antibody persisted poorly after infant immunization and, in 2006, the programme was switched to a 2 dose priming course (at 3 and 4 months of age) with a booster at 12 months of age. The success of the programme in producing population protection in the UK

depends on a combination of herd immunity, direct protection through bactericidal antibody and immunological memory. The speed of onset of invasive disease after acquisition of the organism points to the importance of persistent antibody and herd immunity in maintaining protection (rather than memory, which is too slow). Population levels of antibody and herd immunity should wane over time and yet disease rates continue to fall.

### MECHANISMS OF MUCOSAL IMMUNITY IN PROTECTION AGAINST POLYSACCHARIDE ENCAPSULATED BACTERIA

ADAM FINN

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*Abstract not received*

### PERSISTENCE OF PNEUMOCOCCAL PLAIN POLYSACCHARIDE VACCINE-INDUCED MEMORY B CELL HYPORESPONSIVENESS

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**Background** Prior administration of the 23-valent pneumococcal plain polysaccharide (23vP) results in diminished antibody responses to subsequent 7-valent pneumococcal conjugate vaccine (PCV7; Lazarus ISPPD 2008). We have proposed that this phenomenon is mediated by memory B cell depletion. It has been demonstrated that this phenomenon wanes with time elapsed from 23vP administration (Musher; Jackson), with individuals who have been vaccinated with 23vP in the past year showing the lowest IgG responses to PCV.

**Methods** To investigate the influence of 23vP administration on memory B cell responses to PCV7, 98 participants, aged between 50 to 70, were vaccinated with a single dose of PCV7, whilst 50 participants were vaccinated with 23vP, followed by 1 or 2 doses of PCV7. All vaccines were given 6 months apart. Peripheral blood mononuclear cells (PBMCs) were isolated prior to and 28 days after each vaccination. PBMCs cultured for 6 days were used to enumerate antigen specific antibody secreting cells (ASC) to pneumococcal serotypes 4, 6B, 9V, 14, 18C, 19F and 23F in an enzyme linked immunosorbent spot assay (ELISpot).

**Results** The median number of ASCs in participants who received PCV7 six months after 23vP were significantly lower for 5 out of 7 serotypes compared to those who received PCV7 alone. After the second dose of PCV7 the median number of ASCs remained significantly lower for 3 out of 7 serotypes compared to a single dose of PCV7.

**Conclusion** These data provide evidence to support the hypothesis that memory B cells are depleted in the response to a plain polysaccharide vaccine as they are driven to differentiation into plasma cells without replacement of the memory B cell pool. This reduction in the number of serotype specific memory B cells may explain the attenuation of the response to PCV7. Furthermore, the effects of this reduction persist one year after 23vP vaccination although to a lesser extent than at 6 months. This could be due to waning of 23vP induced hyporesponsiveness or recovery of memory B cells by multiple doses of PCV7. These data have implications for potential PCV vaccination strategies in the elderly cohort already vaccinated with 23vP. Optimizing the timing between vaccinations and the number of doses used may help to minimize hyporesponsiveness.

### TOWARDS A LPS-BASED VACCINE TO PROTECT AGAINST MENINGOCOCCAL DISEASE

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Previous studies in our laboratories have identified the potential of LPS-based vaccines to combat meningococcal disease<sup>1</sup>. This was achieved by demonstrating bactericidal activity and the ability to provide passive protection of sera from mice immunized with glycoconjugates prepared by coupling of O-deacylated LPS from a *galE* mutant of *N. meningitidis* strain MCS8 to CRM1197 via the reducing end following dephosphorylation with alkaline phosphatase. Subsequent studies intended to improve and afford a more robust and consistent conjugation strategy failed to produce protective

antibodies. These approaches did however identify the creation of a neo-epitope during the conjugation protocol which dominated the immune response precluding a satisfactory response to the target region. The present study describes a novel conjugation strategy that still targets the terminal glucosamine disaccharide as the point of attachment to the carrier protein. To achieve this goal we have developed the use of amidases produced by the slime mould *Dictyostelium discoideum*. This methodology has facilitated a more robust conjugation strategy which has led to glycoconjugates with much improved carbohydrate loading. These 'high-loading' conjugates were immunized into mice and rabbits and the sera evaluated for cross-reactivity and functional activity.

**Reference** Cox, A.D., Zou W., Gidney, M.A., Lacelle, S., Plested, J.S., Makepeace, K., Wright J.C., Coull, P.A., Moxon, E.R. & Richards, J.C. (2005). Candidacy of LPS-based glycoconjugates to prevent invasive meningococcal disease: developmental chemistry and investigation of immunological responses following immunization of mice and rabbits. *Vaccine*, **43**, 5045–5054.

#### THE CONJUGATION OF *BURKHOLDERIA PSEUDOMALLEI* LIPOPOLYSACCHARIDE TO TETANUS HC FRAGMENT FOR USE AS A VACCINE

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*Burkholderia pseudomallei* is the causative agent of melioidosis, an infectious disease of humans. At present no vaccine is available for human melioidosis. When lipopolysaccharide (LPS) extracted from *B. pseudomallei* K96243 was administered via the intra peritoneal (i.p.) route in a murine model of melioidosis, it was shown to give some protection against an i.p. challenge of 250 mean lethal doses. Consequently, the mean time to death increased from 2.6 days to 17.6 days; with 50% survival 35 days post challenge. Vaccination with LPS alone produces a T cell independent immune response; however it has been reported that conjugation of polysaccharide to protein produces a T cell dependent response. Our aim is to conjugate LPS from *B. pseudomallei* to the tetanus Hc fragment, in order to generate a conjugate vaccine which will stimulate a cell mediated immune response, leading to increased protection against a *B. pseudomallei* challenge. In preliminary experiments to develop our conjugation methodology, whilst conserving valuable LPS, we have conjugated tetanus Hc fragment to the polysaccharide dextran. The evaluated methodology will be extended to LPS, which will be conjugated to tetanus Hc fragment with subsequent trials in the murine model of melioidosis.

#### RECONSIDERATION OF THE PARADIGM FOR PROCESSING AND PRESENTATION OF GLYCOCONJUGATE VACCINES

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The capsular polysaccharides of most pathogenic bacteria are T cell-independent antigens whose conjugation to carrier proteins evokes a carbohydrate-specific response eliciting T-cell help. The generation of glycoconjugate vaccines is one of the great success stories in the biomedical sciences: in most immunized populations, infections with *Haemophilus influenzae* type b, *Streptococcus pneumoniae* (the vaccine types common among children), and *Neisseria meningitidis* (except group B) have nearly been eliminated. However, important problems persist in presently available vaccines, including poor immunogenicity in the elderly. We revisited the insufficiently understood mechanism by which glycoconjugate vaccines activate the adaptive immune system; our aim is to apply this info to the creation of a new generation of improved vaccines. The current mechanistic paradigm is that a B cell specific for production of antibodies to the polysaccharide component takes up the glycoconjugate and presents a peptide from the covalently linked protein to a T cell, which recognizes the peptide in the context of the major histocompatibility class II (MHCII) molecule. Stimulation of the B cell (with consequent production of carbohydrate-specific antibody) and activation of the peptide-recognizing CD4<sup>+</sup> T cell result in T-cell help, which promotes immunoglobulin class switching to IgG and memory responses. Our data show that these complex glycoconjugate molecules are not handled as the traditional paradigm predicts; consequently, the empirical design of current vaccines fails to optimize the potential for immunogenicity.

#### STUDIES ON THE IMMUNOGENICITY OF GLYCOCONJUGATE VACCINES

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Abstract not received

#### GROUP B STREPTOCOCCAL CONJUGATE VACCINES

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*Streptococcus agalactiae* (Group B streptococcus) is an important cause of disease in infants, pregnant women, the elderly and immunosuppressed adults. The disease burden in infants has been well defined in a number of Developed countries and it is clear that active measures are needed to prevent GBS because of its incidence, morbidity and mortality. An effective vaccine is likely to prevent the majority of infant disease (both early and late onset) as well as GBS-related stillbirths and prematurity, to avoid the current real and theoretical limitations of intrapartum antibiotic prophylaxis (IAP) and to be cost-effective. The optimal time to administer such a vaccine would be in the third trimester of pregnancy. The main limitations on the production of a GBS vaccine are not technical or scientific but regulatory and legal. A number of candidates including capsular conjugate vaccines using traditional carrier proteins such as tetanus toxoid and mutant diphtheria toxin CRM197 as well as newly identified GBS-specific proteins such as C5a peptidase; one or more GBS surface proteins; and mucosal vaccines have the potential to be successful vaccines. The capsular conjugate vaccines are the most advanced candidates having already completed phase II human studies including use in the target population of pregnant women. Efficacy trials with GBS vaccines will be large and costly to conduct because disease is relatively infrequent and IAP relatively widespread in many countries. Regulatory approval of GBS capsular conjugate vaccines based on serological correlates of protection may be possible. Changing the regulatory environment to allow the development of a vaccine for use in pregnancy would accelerate the development of this important prevention tool. Alternative strategies such as adolescent vaccination also need further exploration.

#### A *CANDIDA ALBICANS* CONJUGATE VACCINE

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The phosphomannan of the *Candida albicans* cell wall contains a unique  $\beta$ 1,2-mannan antigenic determinant. Investigation into methods to synthesize these  $\beta$ -mannopyranosides provided a series of homo-oligomers subsequently used to characterize monoclonal antibodies that protect mice against challenge with *Candida albicans*. The antibodies were preferentially inhibited by di- and trisaccharide, while larger oligosaccharides were progressively worse inhibitors. Chemical mapping of the binding sites of these antibodies in combination with NMR studies confirm the size and conformation of the epitope and establish the antigen surface contacting the antibody. The data suggested, and subsequent results confirmed, the expectation that a synthetic  $\beta$ 1,2-mannan trisaccharide conjugated to a protein should induce antibodies that recognize the native phosphomannan. A synthetic conjugate vaccine was evaluated in a rabbit model of disseminated candidiasis. Viable *C. albicans* cell counts were significantly reduced in vaccinated animals. A novel approach to synthesis of a chemically defined glycopeptide vaccine has also been developed and shown to provide protection in mice.

#### KINETICS OF ANTIBODY PERSISTENCE OF A COMBINATION MENINGOCOCCAL SEROGROUP C/HAEMOPHILUS INFLUENZAE TYPE B CONJUGATE VACCINE AT 12 TO 14 MONTHS OF AGE IN HEALTHY UK INFANTS PRIMED WITH TWO DOSES OF ONE OF THREE MENINGOCOCCAL GROUP C CONJUGATE VACCINES

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## CONJUGATE VACCINES

(continued)

A booster dose in the second year of life of meningococcal serogroup C (MCC) and *Haemophilus influenzae* type b (Hib) conjugate vaccine was introduced in England and Wales in September 2006 in the form of a combined vaccine, Menitorix. No data were available on whether Menitorix could be given concomitantly with MMR and Prevenar (7-valent pneumococcal conjugate) or on antibody persistence following the booster. In this study children either received Menitorix at 12 to 14 months of age followed by Prevenar and MMR one month later or all three vaccines at the same visit. Antibody persistence to MCC and Hib was followed at 1, 2, 12 and 24 months post-booster. No adverse consequences for either immunogenicity or reactogenicity when Menitorix was administered either separately or together with Prevenar and MMR. For MCC, depending on primary MCC vaccine used 95 to 100% of children were protected 1 month following the Menitorix booster with the greatest magnitude of response seen for those primed with NeisVac-C (MCC-TT). MCC antibody persistence following the booster dose showed the same kinetics as following the primary MCC series. For MCC, 2 years post-booster 27% to 46%, depending on primary MCC, of children remained protected.



# MICROBIAL STRESS AND FOOD PRODUCTION: COPING WITH THE WORK ENVIRONMENT

## POST-GENOMIC ANALYSIS OF *STREPTOCOCCUS THERMOPHILUS* GROWTH IN MILK AND YOGHURT

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*Streptococcus thermophilus* is a thermophilic lactic acid bacterium widely used as starter in the manufacture of dairy products, in particular in yoghurt manufacture in combination with *Lactobacillus delbrueckii* subsp. *bulgaricus*. *S. thermophilus* growth in milk is diauxic. The first exponential phase relies on the utilization of free amino acids and peptides present in milk as well as on formate production by the pyruvate formate lyase that feeds the essential biosynthesis pathway of purines. During the intermediate phase (separating the two exponential phases), the proteinase, responsible for the hydrolysis of caseins into peptides, is induced. The second exponential growth phase of *S. thermophilus* is characterized by a reduced growth rate; some peptides and amino acids transporters and specific amino acid biosynthetic pathways (sulphur and branched-chain amino acids) are up-regulated. This late growth also results in a diversification of carbon sources utilization that likely reflects the harsh conditions encountered by *S. thermophilus*. On the other hand, the cultivation of *S. thermophilus* in milk in the presence of its yoghurt partner, *L. bulgaricus*, results in a growth stimulation of *S. thermophilus* during the 2<sup>nd</sup> phase. The post-genomic study of *S. thermophilus* during this phase evidenced undocumented nutritional exchanges between the 2 yoghurt bacteria (linked to branched-chain amino acids, arginine and purines) and the involvement of iron metabolism probably in response to the deleterious H<sub>2</sub>O<sub>2</sub> production by *L. bulgaricus*. This study revealed a complex and ambiguous relationship between the yogurt bacteria providing evidence that *S. thermophilus* benefits and also protects itself from compounds produced by *L. bulgaricus*.

## CRYOTOLERANCE OF LACTIC ACID BACTERIA IS INFLUENCED BY THEIR PASSIVE AND ACTIVE RESPONSES TO COLD STRESS

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Lactic acid bacteria often suffer cold stress, especially when they are frozen for being stored as starters or in culture collections. Depending on the temperature level, cells answer to cold stress by passive and/or active responses, the latter helping them to counterbalance the negative effect of low temperatures.

During freezing, cell deterioration or death occur as a consequence of four kinds of stress that trigger passive responses: Thermal stress induces phase transitions in membrane lipids and modification of membrane fluidity. Due to the formation of ice crystals, osmotic stress leads to a reduction of cellular volume, protein deterioration and changes in intracellular pH and mechanical stress causes destruction of membrane and cellular structures. Solute concentration enhances oxidative reactions of membrane lipids.

When the cold stress is moderate, cells develop some active responses to better resist harsh conditions. By modifying their membrane fatty acid composition, they change their membrane fluidity to adapt to low temperatures. The transient induction of general or stress proteins is reported as a second mechanism. Some of these proteins act as molecular chaperones, which assist the cells for non-covalent folding and unfolding, and prevent proteins from taking conformations that would be inactive. These mechanisms combine to make the cells more cryotolerant.

## REGULATION OF PROTEIN SYNTHESIS IN RESPONSE TO OXIDATIVE STRESS IN THE YEAST *SACCHAROMYCES CEREVISIAE*

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Cells must be able to maintain their intracellular homeostasis in the face of changing conditions. Typically, they respond by invoking complex regulatory mechanisms including global inhibition of translation. This reduction in protein synthesis may prevent continued gene expression during potentially error-prone conditions as well as allow for the turnover of existing mRNAs and proteins whilst gene expression is reprogrammed to deal with the stress. We have analysed the regulation of protein synthesis in response to oxidative stress induced by exposure to H<sub>2</sub>O<sub>2</sub> in the yeast *Saccharomyces cerevisiae*. Our data show that H<sub>2</sub>O<sub>2</sub> causes an inhibition of translation initiation that is dependant on the Gcn2 protein

kinase which phosphorylates the  $\alpha$  subunit of translation initiation factor eIF2. Additionally, our data indicate that translation is regulated in a Gcn2-independent manner; since protein synthesis is still inhibited in response to H<sub>2</sub>O<sub>2</sub> in *gcn2* mutant cells. I will present our data investigating the mechanisms underlying the control of translation in response to oxidative stress conditions.

## COLD RESPONSE IN BAKER'S YEAST

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Recent years have seen a huge growth in the market of industrial yeasts with the need for strains affording better performance or to be used in new applications. Stress tolerance of commercial *Saccharomyces cerevisiae* yeasts is, without doubt, a trait that needs improving. In the bakery sector; the huge growth in frozen dough manufacture, both for bread and pastries, has made it apparent that this organism has problems withstanding temperatures of below 0°C. Such traits are complex, and therefore only in-depth knowledge of their biochemical, physiological and genetic principles can help us to define key factors for strain selection.

Research in this field reported induction of specific cold-stress markers together with the activation of the general stress response, signal transduction pathways and transcription factors that mediate the response to other stressful conditions. This information suggests that some cold-regulated genes are required for resume growth at low temperature, whereas other are involved in protecting cells against additional stressors. Indeed, recent evidences indicate that the main aim of these common stress responses is to protect cells against freeze injury. This view could enable us to identify tools and strategies suitable to improving cold adaptation and freeze tolerance in commercial yeast strains.

## PATHOGENIC *ESCHERICHIA COLI* FACING STRESS: 'I WILL SURVIVE!'

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General stresses occurring during food production, processing and storage have been considered for a long time to prevent colonization by non-desirable micro-organisms. However, contamination by food-borne pathogens such as pathogenic *Escherichia coli* has been shown to occur in a variety of processed food products (fermented sausages, dairy products, and fruit juices) as well as in the well known vectors such as raw and undercooked meat products.

The widely used laboratory strain *E. coli* K-12 is arguably one of the best understood organisms, and is regarded as a model organism in metabolic and genetic studies. However, genome sequencing has revealed that the chromosome of *E. coli* K-12 is as much as one mega base smaller than the chromosomes of pathogenic *E. coli* strains. Much of this 'extra' genomic DNA contains genes of unknown function. Using Phenotype Microarray technology we have observed that pathogenic *E. coli* are more resistant than *E. coli* K-12 to different food processing related stresses, and can utilize different carbon and nitrogen sources. These phenotypic differences can be directly related to genomic and transcriptomic differences between these strains.

## THE EFFECT OF RpoS ON RESISTANCE OF *ESCHERICHIA COLI* TO HIGH HYDROSTATIC PRESSURE

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The general stress response in *Escherichia coli* controlled by the sigma factor  $\sigma^S$  (RpoS) is an adaptive mechanism which results in an increase in resistance to unfavourable conditions. The objective of this study was to investigate the effect of the *rpoS* gene and genes controlled by *rpoS* on pressure resistance. Previously, we showed that the cell membranes of an *rpoS* mutant and its parent strain were damaged as a result of high pressure. However, the parent strain had a more robust cell membrane and could reseal its membrane after decompression whereas the *rpoS* mutant permanently lost its membrane integrity during exposure to high pressure.

# MICROBIAL STRESS AND FOOD PRODUCTION: COPING WITH THE WORK ENVIRONMENT

(continued)

RpoS controls the expression of a large number of genes and it is not known which particular genes are responsible for increased resilience of the cell envelope in stationary phase cells. The effect on pressure resistance of inactivating selected genes controlled by *rpoS* was therefore examined. We found that genes involved in certain cell envelope properties including *cfa* (cyclopropane fatty acyl phospholipid synthase), *nlpl* (new lipoprotein) and *osmB* (osmotically inducible lipoprotein) play a major role in the loss of viability under high pressure. Strains with mutations in these genes showed severe membrane damage after pressurization. Interestingly, it appears that lack of *Nlpl* and *OsmB* prevents cells being able to reseal their membranes after decompression.

We concluded that RpoS-regulated genes bring about physiological changes especially an increase the strength of the cell membrane or envelope, and this then leads to an increase in pressure resistance.

## THE OLIGOPEPTIDE TRANSPORT SYSTEM IS ESSENTIAL FOR THE DEVELOPMENT OF NATURAL COMPETENCE IN *STREPTOCOCCUS THERMOPHILUS* STRAIN LMD-9

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In Gram+ bacteria, oligopeptide transport systems, called Opp or Ami, play a role in nutrition but also in the internalization of signaling peptides involved in quorum sensing (QS) pathways. We aimed at revealing functions that are controlled by QS mechanisms via Ami in *Streptococcus thermophilus*, a bacterium widely used in dairy technology in association with other bacteria. Using a label-free proteomic approach combining 1D electrophoresis with LC-MS/MS analysis, we compared the proteome of the *S. thermophilus* LMD-9 with that of a mutant deleted for the *ami* operon, after growth in a chemically defined medium (CDM) without peptides. We focused on proteins that were no more detected in the *ami* mutant. In addition to the subunits of the Ami transporter, seventeen proteins fulfilled this criterion and, among them, seven were similar to proteins identified as essential for transformation in *S. pneumoniae*. These results led us to find a condition of growth, the early exponential state in CDM that allows natural transformation in *S. thermophilus* LMD-9 to turn on. Furthermore, we demonstrated that the Ami transporter controls the triggering of the competence state through the control of the transcription of *comX*, itself controlling the transcription of late competence genes.

## *OENOCOCCUS OENI* GENOMIC ADAPTATION TO THE WINE ENVIRONMENT

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*Oenococcus oeni* is a minor species among the lactic acid bacteria naturally occurring on grape fruits, but it rapidly becomes predominant in wine and is generally the only detectable species during malolactic fermentation. To identify traits responsible for this so efficient adaptation to the wine environment, we used a panel of genomic approaches to examine *O. oeni* at both species and infra-species levels. Strain typing methods revealed a huge diversity of strains whose genomes are shaped by recombination events and that form at least two well-defined genetic groups. Evidences for genomic plasticity and horizontal gene transfers were confirmed by genome sequence comparisons and by subtractive genomic hybridizations. Differential DNA regions specific of some strains code for enzymes that may help bacteria to cope with the harsh conditions of wine. Taken together our works support the previously suggested idea that *O. oeni* is a fast-evolving species and they provide the first clues about how some strains can better survive in wine. In addition they make it possible for the first time to select the best adapted strains for industrial applications on the basis of genetic traits rather than phenotypic criteria.

## MOLECULAR STUDIES OF GENE EXPRESSION IN THE CHEESE RIPENING YEAST *YARROWIA LIPOLYTICA*

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The adaptation of micro-organisms to their environment depends on both their ability to cope with the physico-chemical characteristics of the cheese and their ability to degrade substrates from their environment, while interacting with other microbial species.

Yeasts are well adapted to cheese, which provides them substrates to grow and develop particular functionalities (eg: flavour production, deacidification) essential for cheese ripening.

Due to its ubiquitous nature, the yeast *Yarrowia lipolytica* has been studied to better understand the adaptive metabolism of this yeast cultivated in pure culture or in association with other micro-organisms also present in cheese.

We first studied key metabolic pathways of cheese ripening, lactate and amino acids catabolisms by pure cultures of *Y. lipolytica*. The combination of molecular, microbial, biochemical and proteomics techniques has helped to show a real preference of this yeast for amino acids consumption.

*Y. lipolytica* was also studied in interaction with two bacteria, *Lactococcus lactis* and *Staphylococcus xylosus*. In the presence of *S. xylosus*, major genes involved in amino acids catabolism are down-regulated in the yeast. Furthermore, the lactate production by both bacteria has an impact on the lactate dehydrogenase gene expression of the yeast, which increases up to 30-fold in the complete association, compared to the *Y. lipolytica* pure culture.

## MITOCHONDRIAL DNA – STRESS INDICATOR OF YEAST DURING BREWERY FERMENTATION

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Brewery fermentations and handling of yeast populations between successive fermentations exposes brewing yeast cells to a number of biological, chemical and physical stresses. It is generally accepted that repitching of yeast in subsequent fermentations leads to an increase in incidence of petite mutations. Eventually this can lead to aberrant fermentation profiles and impaired product quality.

We postulated that brewing yeast mtDNA copy number and / or integrity deteriorated with sequential fermentations. To address this hypothesis we monitored mtDNA copy number using real time PCR and examined sequence integrity by RFLP analysis using restriction enzymes targeted to mtDNA. Cells were also exposed to ethidium bromide to determine the propensity to form petites following mutagen challenge. In addition, the phenotypic profile of the petite mutants was compared to that of the parent strain using phenotypic microarray. Using next generation sequencing approach, we have sequenced the mitochondrial genome of the parent strain and its petite mutant to identify mutational hotspots to be used as indicators of genomic instability using PCR-based techniques.

## STRESS RESPONSE OF WINE YEAST

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Wine yeast suffers adverse conditions throughout its industrial use. Nowadays grape juice is often inoculated with starters in the form of active dry yeast. That biomass production and drying cause an oxidative damage that leads to a decreased fermentative capacity. We have seen that the antioxidant systems, for instance thioredoxin, are necessary for a right performance during yeast production, and a target for genetic improvement. Oxidative damage leads to cellular aging and death. We are currently studying the role of the deacetylases called sirtuins in stress response and longevity in wine yeast during industrial growth.

During grape must fermentation wine yeast deal with many adverse conditions, from hyperosmotic to high ethanol stress. We have developed strategies to overexpress stress response genes under the control of regulatable promoters. Using the stationary phase-induced *SP11* promoter upstream the general stress response transcription factor *MSN2*, we improved some aspects of yeast growth and viability on wine fermentation. We have used a similar approach to increase the expression of stress response genes *HSP26* and *YHR087W*, obtaining a better performance during wine-making. Therefore, manipulation of stress response is a powerful tool to improve industrial fermentation.

## BIOENERGY FUEL SOURCES

### BIOFUELS CHALLENGES AND PROGRESS

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Worldwide demand for mobility is steadily increasing at the same time that concerns are mounting over future climate change. This is stimulating the widespread introduction of biofuels, due to their capacity to ease supply security, their potential to be grown or sourced locally, and their potential to mitigate growth in world CO<sub>2</sub> emissions.

In a number of countries governments are encouraging the production of conventional or 'first generation' biofuels through mandates and incentives. Industries are responding effectively to these obligations, building experience and starting to deliver the anticipated benefits.

However, the prospect of large-scale commercialization of biofuels raises social and environmental issues that will require safeguards. As a leading bio-fuel supplier to global markets, Shell is committed to providing sustainable energy, applying its Sustainability Policies to supplier contracts and championing the development of internationally recognized standards in the supply chain. To scale-up biofuels supply to meet future anticipated demand, alternatives to food-based biomass feedstocks will need to be introduced.

An increasing range of technologies being considered to deliver new lower-carbon pathways from non-food biomass through to advanced biofuel components will be described. This will include, *inter alia*, technologies for commercial scale ligno-cellulosic ethanol, 'biomass-to-liquid (BTL)' components via gasification and Fischer-Tropsch processes and the development of alternative biomass sources such as micro-algae.

### THE ANAEROBIC PRODUCTION OF HYDROGEN AND METHANE: THE NEXT GENERATION BIOFUELS?

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*Abstract not received*

### DERIVING ENERGY FROM WASTE – THE DARK SIDE AND THE LIGHT SIDE OF BIOHYDROGEN PRODUCTION

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We are threatened by an excess of waste and a deficit of fuel. With hydrocarbon resources becoming increasingly limited, major changes in energy systems and fuel usage will be required. Because of its characteristics as a clean energy vector, hydrogen could play a key role in a future carbon-free energy economy. However, hydrogen production is currently dependent upon natural gas, a limited hydrocarbon resource. Therefore, new methods to derive hydrogen fuel from waste are needed.

We focussed on a biological approach because biofuels processes, generally, operate at moderate temperature (30–40°C) and ambient pressure, to produce renewable, carbon-neutral biofuel. Large quantities of organic wastes (e.g. 15MT pa; UK food industry) can provide the feedstock for biofuels production, simultaneously to achieve waste disposal. Therefore, we examined the mechanisms of bio-hydrogen production and identified a promising avenue to produce hydrogen from organic waste. By combining anaerobic fermentation with photofermentation, yields of 8–10 mol H<sub>2</sub>/mol hexose have been demonstrated independently by several authors. Our work addresses the challenge of putting this technology into practice by developing bioreactors for dark fermentation and for photofermentation and by developing integration methods. Lab/pilot-scale experiments are underway to inform future scale-up operations.

### BACTERIAL MEDIATED ELECTRICITY PRODUCTION

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Microbial fuel cells (MFC) are a promising strategy for the conversion of environmental, wastes and renewable biomass into electricity in a carbon neutral fashion. Fundamental to power production in a MFC is the formation of specialized biofilms on an anode surface. For further improvements to occur a further understanding of the power producing biofilms is required. The well studied electricigen, *Geobacter sulfurreducens*, has been a model to

study high current density biofilms due to: a current density equal or greater than all other reported mixed or pure cultures; a genetic system; and being the most commonly recovered microbe from MFC with environmental inoculants. *G. sulfurreducens* current producing biofilms are up to 50 µm thick and spatially heterologous. While all members of the biofilm are metabolically active, an inhibitory proton gradient forms within the biofilm. To further investigate differences within these biofilms a novel strategy was developed to the spatial separation of the biofilm, into an inner and outer layer, to extract RNA. Analysis of the transcriptional differences between the inner and outer layers revealed a large number of genes down regulated in the outer layer with roles in cell metabolism and growth, consistent with the concept that cells at a distance from the anode have lower rates of metabolism. These results are expected to lead to better strategies for optimizing the power output of MFCs and thus broaden their applications.

### NOVEL BIONANOCATALYSTS FOR ELECTRICITY PRODUCTION

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Tomorrow's low carbon hydrogen economy will rely on the use of fuel cells (FC) to convert hydrogen, the energy vector, into electricity. Bacteria can make clean biohydrogen fermentatively via hydrogenase activity. Bacterial hydrogenases can then be used to reduce precious metals like Pd(II) and Pt(IV) to metallic nanoparticles of Pd(0) and Pt(0) which are supported on the bacterial cell surface. Precious metal nanoparticles form the electrodes and catalysts of a fuel cell. Hydrogen is split into protons and electrons at the anode. Electrons go into the circuit and the protons form water by combining with oxygen from the air at the cathode. When the Pd and Pt-coated bacteria are sintered (carbonized) at high temperature they can be used as the electrode materials in a fuel cell, producing as much as, or more electricity than, a commercial FC catalyst. The FC performance of the catalyst relates to the bacterium and hydrogenase which produced the metallic nanoparticle, suggesting some level of biocontrol of nanoparticle fabrication. Precious metals are very expensive. Other studies have shown that bacteria can scavenge precious metals from wastes, hence converting wastes into new catalysts in a single process which utilizes leftover biomass from biohydrogen production processes upstream.

### BACTERIAL HYDROGENASES FOR BIOHYDROGEN TECHNOLOGY

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Hydrogenases are enzymes dedicated to a fascinating chemical reaction: H<sub>2</sub> → 2H<sup>+</sup> + 2e<sup>-</sup>. Hydrogen chemistry holds a central place in microbial metabolism and hydrogenases are utilized by a broad spectrum of micro-organisms ranging from photosynthetic algae through to strictly anaerobic bacteria and extremophilic archaea. Hydrogenases are also diverse in structure and are classified according to their active-site metal content. Thus three main classes, termed [NiFe]-, [FeFe]-, and [Fe]-hydrogenases, have been identified. The ability to understand and control hydrogenases is key to successfully harnessing these enzymes for biohydrogen technology. Production and oxidation of H<sub>2</sub> by hydrogenases is complicated by competition with other gases, in particular O<sub>2</sub>, which is a potent inactivator. Indeed, one major goal of molecular biologists is the identification, or design, of 'oxygen-tolerant' hydrogenases.

*Escherichia coli* produces three [NiFe]-hydrogenases (Hyd-1, -2, and -3). Hyd-1 and -2 are membrane-bound, periplasmically-oriented 'uptake' enzymes dedicated to respiratory hydrogen oxidation. Hyd-3 is a cytoplasmic enzyme that forms part of the formate hydrogenlyase (FHL) complex, which is responsible for hydrogen gas evolution during fermentation. In this work an affinity purification strategy was devised that facilitated the isolation of Hyd-1 and -2. This allowed the electrochemical characterization of these enzymes, which revealed that Hyd-1 was a novel oxygen-tolerant enzyme. In addition, a similar approach enabled the isolation of an intact and active FHL complex for the first time.

### ENABLING BIOENERGY

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Through an unprecedented effort of more than 150 scientists, Novozymes is breaking the barriers for commercialization of lignocellulosic ethanol. Novozymes is developing highly

# BIOENERGY FUEL SOURCES

(continued)

cost effective novel enzyme systems and has launched two state-of-the-art products this year. The development of the enzyme products is done in close collaboration with commercial and academic parties in both the US, Brazil, China and in Europe.

The lignocellulosic material is pre-treated before the enzymatic hydrolysis step in the biomass to ethanol process. The efficacy of this pre-treatment has a large impact on the ease of saccharification by the enzymes but also in many cases on the fermentation of the resulting sugars by the yeast. It is therefore important both from a technical and from an economic point of view to take all three aspects into consideration during the development of improved enzyme cocktails.

## RECOMBINEERING OUR WAY TO SUSTAINABLE FUELS AND CHEMICALS

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A fundamental challenge in strain engineering involves the rewiring of complex phenotypes (i.e. growth rate). Conventional approaches often fall short of expectations -- they rely upon recursive adaptation, which is slow, often results in 'sick' strains, and does not generate the understanding required for further engineering. Quantitative and high-resolution methods capable of exploring the breadth of functions present in a microbial genome are required. We have reported the SCAr Analysis of Library Enrichments (SCALEs) method to quantitatively track 500,000 individual clones throughout laboratory selections and shown that this data can improve understanding of the mechanisms underlying complex phenotypes. We have applied this approach to engineer tolerance to various chemicals, including 3-hydroxypropionic-acid, ethanol, acetate, furfural, naphthol, and various antimicrobials. While this approach has proven useful, it does not detect genes acting in-trans to alter a phenotype or disruptional mutational events. Thus, we initiated the development of a new approach, which we call trackable, recursive, multiplex-recombineering (TRemR), that allows for simultaneous engineering multiple 'up' or 'down' mutations into a single strain as well as the parallel tracking of all of such mutants via molecular barcoding. This talk will describe all of these efforts with a specific focus on biofuels applications.

## ETHANOL FROM LIGNOCELLULOSE

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Pentose sugars, mainly xylose and arabinose, constitute a major fraction of carbohydrates in lignocellulosic agricultural residues such as corn stover, corn hulls, straw and bagasse. Hardwoods cultivated as dedicated energy crops, such as willow and poplar, also contain a significant fraction of pentose sugars. The complete conversion of all carbohydrates is necessary for the economic feasibility of large scale bioethanol processes based on such raw materials. Ethanol fermentation of lignocellulosic feed stocks requires that the raw material is pretreated/fractionated and hydrolyzed. These processes release and generate compounds, weak acids, furans and phenolics, which are inhibitory to the subsequent fermentation process.

Baker's yeast *Saccharomyces cerevisiae* is the preferred fermenting organism in the present fermentation industry. *S. cerevisiae* efficiently ferments hexose sugars, but not pentose sugars. Industrial strains of *S. cerevisiae*, which are able to ferment both xylose and arabinose to ethanol have been developed. Bacterial and fungal pentose pathways have been expressed and evaluated in *S. cerevisiae*. The expression of individual genes has been fine-tuned and genes for targeted metabolic improvements have been identified by genome wide as well as targeted transcription analyses. Individual enzymes have been improved by site-directed and random mutagenesis. Strains have been further improved by adaptation and evolutionary engineering. In parallel, enzymes enabling the detoxification of furan inhibitors have been identified and their genes overexpressed.

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## BEING TOLERANT: CURRENT UNDERSTANDING OF STRESS DURING BIOETHANOL FERMENTATIONS

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Efficient fermentation requires conditions appropriate for ensuring high productivity whilst maintaining yeast viability and vitality. However, optimal conditions for the former can be suboptimal for the latter leading to inconsistent and even 'stuck' fermentations. Whilst optimization and consistency of large scale fermentations from inoculation and dispersal of biomass to product recovery is of critical economic and industrial importance, process innovation requires an effective understanding of the biological constraints and opportunities of the system. During bioethanol fermentations yeast are exposed to fluctuations in oxygen concentration, osmotic potential, pH, ethanol concentration, nutrient availability and temperature. The impact of these stresses on yeast will be discussed with particular reference to osmotic stress, ethanol tolerance, genome stability and fermentation performance.

## BIOFUELS AND SUSTAINABILITY

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The environmental and energy performance of bioenergy production systems varies significantly across forms of bioenergy, feedstocks, conversion processes, and production regions. Life-cycle assessments of bioenergy production systems vary considerably. Most studies indicate that the use of biomass for heat and power generation, preferably combined, tends to be more efficient in terms of fossil energy savings and GHG emission reductions than most forms of first-generation biofuels. Second-generation biofuels offer higher potentials. The implications for the local environment are ambivalent: on the one hand, higher intensification of agricultural production systems can reduce biodiversity and increase soil erosion and water and soil pollution. However the use of innovative bioenergy cropping systems can reduce environmental pressures compared to some food cropping. These reductions in environmental pressure can be made from less nutrient input, enhanced crop diversity and less use of heavy machines. The use of forest residues can support fire prevention measures in Southern Europe. The use of cuttings from grassland can maintain biodiversity-rich grassland and landscape diversity and provide a limited amount of bioenergy. Marine algae are showing potential as a non-land route to biofuel.

## ALGAE AS A SOURCE OF BIOENERGY: PROSPECTS AND CHALLENGES

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Algae are an extremely diverse group of simple aquatic plants, which are responsible for an estimated 50% of global carbon fixation. Until recently, their study at the molecular level has lagged behind that of land plants, but in recent years there has been an enormous increase in interest, not least because they are a potential source of bioenergy. Microalgae offer many advantages as biofuel feedstock, including the fact that their fast rates of growth allow high productivity, and some species can accumulate high levels of potential fuel molecules such as triacylglycerides (TAGs) or hydrocarbons. Moreover, they can be grown on marginal land, in waste-water, or in marine locations, and therefore do not compete with crop plants for agricultural land. There are many challenges that must be overcome to enable commercial algal biofuel production – but we now have the opportunity to overcome these challenges by studying algal biology. Several algal genomes have been sequenced, offering the prospect of data mining to identify key genes in fuel molecule biosynthesis. Tools for transformation and metabolic engineering are well developed for the model green alga *Chlamydomonas reinhardtii*, but these are much more rudimentary, or indeed non-existent, for other species. We need to develop these techniques for biofuel species if we are to take advantage of genomics approaches to the study of microalgae. Another important area that needs to be addressed is to increase our understanding of the metabolism and physiology of microalgal species both in their natural environment, and in dense cultures that will be necessary for large scale biomass production.

## HALOPHYTIC GREEN ALGAE FOR CARBON RECYCLING AND BIOFUEL PRODUCTION

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Alternative, nonfood feedstocks are needed to supply the exponential growth of the US biofuels industry. The current, major limitation of biofuel production is the lack of adequate



feedstocks for biodiesel and ethanol production. Halophytic microalgae provide a potential, non-seasonal, renewable energy alternative to traditional terrestrial crop feedstocks for various reasons. On an annual basis, halophytic microalgae are 10–30 times more productive than terrestrial crop feedstocks, can be grown on marginal lands with brackish or saline water unsuitable for traditional agriculture, can leverage municipal waste water; geothermal and solar resources, and can provide widespread potential for recycling of CO<sub>2</sub> from biomass, coal, and gas-fired power plants. Selecting and analyzing algal feedstocks suitable for biofuels production is a critical need and an important focus for not only basic research, but also for deployment into pilot-scale and demonstration-scale production facilities. Harvesting, extraction and processing technologies are also critical engineering challenges that must be addressed in order to minimize biofuel production costs.

#### INVESTIGATING FACTORS THAT INFLUENCE THE GROWTH OF MICROALGAE AS A SOURCE OF RENEWABLE FUEL

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The aim of the project is to identify and probe parameters that influence growth of high-density microalgal cultures in open-pond and photobioreactor systems, through a combination of biological studies, application of life-cycle analysis and modelling. A key goal is to test the effect on the algal community of growing multiple species together, or through algal-bacterial symbiosis to achieve a robust community. By this we envisage one that attains high productivity with minimal energy inputs and is less prone to contamination by bacteria or competition from other species. Such a community is necessary if industrial scale production of algal biofuels is to become a reality. A bench-top bioreactor has been designed and built in order to conduct initial biological investigations. The bioreactor allows control over essential physical parameters, namely light, temperature and carbon dioxide availability. The growth is semi-continuous. It is an intermediate design between large-scale commercially available photobioreactors that allow continuous culture, and laboratory scale set-ups which are limited to batch cultivation. The reactor allows different treatments to be set up in cultures growing in parallel, so that differences in growth can be compared and accounted for. Initial investigations focus on understanding lipid accumulation in *Chlorella vulgaris* as a result of nitrogen starvation.

#### VITAMIN B12-DEPENDENT GENE EXPRESSION IN THE UNICELLULAR MODEL ALGA CHLAMYDOMONAS REINHARDTII

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Vitamin B12 can affect marine phytoplankton dynamics and thus may be important for oceanic productivity. Approximately half of all microalgal species depend on an external source of vitamin B12, and it has been proposed that many algae can obtain vitamin B12 through a symbiotic relationship with bacteria (Croft *et al.*, Nature 438, 90–93 (2005)). A proteomics approach using two-dimensional difference gel electrophoresis (2D-DIGE) has been used to identify proteins that are differentially regulated when vitamin B12 is present or absent. 11 spots were less abundant when vitamin B12 was present. Four of these spots have been identified, and they correspond to three proteins that are all enzymes involved in the metabolism of one-carbon compounds. One of these is the vitamin B12-independent form of methionine synthase (METE). Together with the vitamin B12-dependent (METH) form, METE is responsible for the adaptation to varying B12 availability in *C. reinhardtii*. Possibly, vitamin B12 dependency in algae arises if only METH is present, but not METE.

#### TRANSCRIPTOMICS OF BOTRYOCOCCUS BRAUNII

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*Botryococcus braunii* is a widespread, colonial microalga (Chlorophyceae). *B. braunii* is unusual in that it synthesizes long-chain, C<sub>25</sub>–C<sub>40</sub>, hydrocarbons with yields of 10–60% of dry biomass. *B. braunii* comprises 3 races, labelled A, B and L, each characterized by the different hydrocarbons produced.

While *B. braunii* races A and B synthesize complex mixtures of various hydrocarbons, race L produces mainly lycopadiene, a C<sub>40</sub>H<sub>78</sub> tetraterpene, and epoxy- and di-epoxy lycopane, the concentrations of which change significantly during growth. We employed a transcriptomic approach to investigate the molecular control of hydrocarbon production in *B. braunii* race L during exponential and stationary growth. In the absence of genomic data, we initially performed cross-species hybridization of *B. braunii* mRNA to the Affymetrix *Arabidopsis thaliana* ATH-1 microarray which contains 22746 oligonucleotide sets. Microarrays were calibrated using *B. braunii* genomic DNA and 7899 sets retained. 230 genes showed differential expression, but the evolutionary divergence between *B. braunii* and *A. thaliana* limited the analysis. To overcome that difficulty, we are currently sequencing and annotating the *B. braunii* transcriptome. cDNAs are annotated by homology using BLAST, assigned gene ontology classifications and EC numbers, scanned for putative domains against the global pfam database and associated with possible metabolic pathways.

This work was supported by the EPSRC and the Nottingham Arabidopsis Stock Centre (NASC).

#### MORE THAN P-VALUES: HOW STATISTICAL MODELLING CAN AID SYSTEMS BIOLOGY

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Systems biology studies usually produce very large amounts of data giving a comprehensive picture of a biological system at a specific organization level (e.g. transcriptomic, proteomic, etc.).

Often, however, this data production is not matched by an adequate data modelling step: results are simply presented as lists of differentially expressed genes, leaving any rationalization to the biological intuition of the researchers. Statistical machine learning techniques allow to integrate the high-throughput data produced with other information about regulatory and pathway structures. This can lead to insightful results and predictions which can be tested by subsequent experiments. In this talk, I will briefly introduce statistical machine learning methodologies, focusing on some recent contributions in the areas of metabolic network modelling and transcriptional regulation.

#### OUTDOOR CULTURE OF MARINE MICROALGAE IN THE UK: TOWARDS ALGAL PRODUCTION AS AGRICULTURE

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The biological capture of carbon dioxide is an environmentally friendly way to diminish the overall release of CO<sub>2</sub> by man's activities. We are studying the effectiveness of CO<sub>2</sub> capture using marine microalgae growing in outdoor tanks in rooftop cultures which have been unheated through the winter months. Despite this, good algal growth was obtained. We are investigating the use of the resulting biomass as a direct burn fuel, but also as a source of valuable energy rich oils. Technical and energy intensive hurdles such as drying are also being investigated in collaboration with a local power station. We demonstrate that it is not necessarily a requirement to site algal biomass units in tropical countries, and are approaching algal culture as a form of agriculture, which is essential if algae are to be used effectively on a large scale.

#### KEY ISSUES IN DEVELOPING A SUSTAINABLE UK AND EU BIOFUELS POLICY

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Abstract not received

# LIFE IN ANCIENT ICE

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Ice is an excellent matrix for the preservation of microbes and nucleic acids. We have isolated both from glacial ice up to 2 million years old, from the accretion ice (approximately 5,000 years old) above subglacial Lake Vostok, Antarctica, and from more recent Arctic and Antarctic lake ice. Bacteria and fungi were characterized by fluorescence microscopy, culturing, and sequence analyses of ribosomal DNA internal transcribed spacers. More than 1,000 isolates have been cultured, of which approximately 200 have been characterized. While cell concentrations in glacial and accretion ice generally were low, a relatively high proportion of the cells were viable in most ice core sections. None of the tested isolates were psychrophiles, but all were psychrotolerant. All were related to species from cold aquatic or soil environments. The fact that fungi were isolated from subglacial Lake Vostok accretion ice indicates that the lake itself may support heterotrophic microbes, and therefore it may contain a complex and unique ecosystem. Recently, we initiated metagenomics studies of viruses from Arctic and Antarctic lake ice. The viruses have provided clues as to the cellular organisms that inhabit or visit the lakes.

# MICROBIOLOGY OF ANCIENT GREENLAND ICE

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The 3 km deep Greenland ice sheet is the second largest on Earth, covering 1.7 million km<sup>2</sup>. This unique ecosystem has preserved chronologically both microbial life and past climate for the last 110,000 years. Among the six deep ice cores, GISP2 provides the longest high-resolution climate records in the Northern hemisphere. We established a Greenland ice microbial observatory in order to develop a comprehensive picture of the microbial populations in GISP2 samples representing different depths, temperatures, gas and ionic compositions and link microbial diversity and biogeochemistry. The application of microbiological, molecular and geochemical methods with different resolution resulted in major findings: (a) in-depth variations of microbial abundance correlated positively with Ca<sup>2+</sup> concentrations, a proxy of dust with more intensive atmospheric fluxes during colder periods; (b) significant phylogenetic and physiological diversity was found; (c) samples deposited under different climate conditions showed distinct diversity patterns suggesting links to historically prevalent climate characteristics and events and indicating microbial origin; (d) numerous isolates were recovered using improved cultivation strategies and new species were described; (e) trace gases production in ice samples at subfreezing temperatures and evidence for N<sub>2</sub>O and CH<sub>4</sub> producing micro-organisms in ice with trace gas anomalies indicated metabolic activity.

# EXOPOLYMER (EPS) PRODUCTION BY DIATOMS IN SEA ICE

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The brine channels that are a characteristic feature of polar sea ice provide a unique environment for microbial life, with organisms exposed simultaneously to gradients of very low temperatures and high salinity within individual ice flows. Despite these physiological stressful conditions, brine channels support substantial biomass and activity of autotrophic and heterotrophic pro- and eukaryotes. Diatoms are a major component of the microbial community present, and are found associated with substantial quantities of dissolved organic matter, predominantly in the form of extracellular polymeric substances (exopolymers or EPS). Carbohydrate is a major component of the EPS, and there are significant differences in the chemical composition and size structure of EPS within different sea ice sub-habitats. Polar diatom taxa show changes in their production of EPS with changing physiology and conditions, indicating their ability to modify their EPS. EPS can significantly modify the local environment surrounding cells in brine channels and therefore may provide significant survival advantages to microorganisms in sea ice. Whether the patterns in sea ice EPS found *in situ* are a result of altered production pathways by autotrophic micro-organisms, and / or the result of selective degradation or production by heterotrophic organisms is discussed.

# MICROBIAL ACTIVITY AND VIRAL LYSIS IN GLACIAL ECOSYSTEMS

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Up to 10% of the surface area of glaciers may be covered by inorganic and organic debris. Because of their lower albedo compared to the surrounding ice, the debris develops into small water-filled depressions which are known to be colonized by a diverse range of micro-organisms, including viruses, bacteria and algae. Whether microbial communities on the surface of glaciers are actively influencing biogeochemical cycles or are just present in a dormant state has been a matter of debate for long time. We showed that *in situ* photosynthesis and respiration associated with the debris at the surface of glaciers in Svalbard, Greenland and Alps are often comparable to that found in soils in warmer and nutrient richer regions. The net primary production by glacial ecosystems is large enough to contribute to the *in situ* utilization of organic matter and the export of carbon associated with glacial ablation. Viruses most probably play an important role in controlling microbial mortality and hence biogeochemical cycling on glaciers. Such processes are relevant to researchers interested in the possibility of extraterrestrial life and the survival and proliferation of life forms on our early Earth (e.g. during the Snowball Earth).

# THE MCMURDO DRY VALLEY LAKES MICROBIAL OBSERVATORY

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Several lakes have formed in the McMurdo Dry Valleys, Antarctica (77°S) from seasonal glacial runoff and are unique in their constantly cold temperatures, permanent ice covers, absence of wind mixing, and paucity of metazoans. The Dry Valley Lakes Microbial Observatory has focused on Lakes Bonney and Fryxell, using a combination of molecular, conventional and high-throughput culture, and biogeochemical tools to probe prokaryotic diversity and activity. The deep waters of the east and west lobes of Bonney are hypersaline and contain high levels of organic sulfur and nitrous oxide while those of Fryxell are weakly saline but highly reduced. Bacterial productivity is absent in the deep waters of East Bonney, while relatively high rates are present throughout the water columns of West Bonney and Fryxell. SSU rRNA gene libraries showed that Gammaproteobacteria, Firmicutes, and Bacteroidetes predominate in Lake Bonney, while Delta- and Betaproteobacteria, Bacteroidetes, Actinobacteria, and Planctomycetes predominate in Lake Fryxell. Based on *dsrA* and *pufM* analyses, a large diversity of sulfate-reducing bacteria and phototrophic purple bacteria inhabit Lake Fryxell. Cultures of novel purple and sulfate-reducing bacteria showed a cold-active phenotype, and the methane-rich Fryxell sediments contained several archaeal phylotypes. We conclude that there is a strong interaction between the geochemistry of Dry Valley lakes and the prokaryotic diversity they support.

# MICROBIAL BIOGEOCHEMISTRY IN THE ANTARCTIC DRY VALLEYS

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The Antarctic Dry Valleys are regarded as one of the harshest terrestrial habitats on the Earth because of the extremely cold and dry conditions. Despite the extreme environment and scarcity of conspicuous primary producers, the soils contain organic carbon and heterotrophic micro-organisms and invertebrates. The vast majority of the primary producers are microbial and the dry valleys represent one of the few ecosystems that are almost exclusively driven and structured by micro-organisms. Potential sources of organic compounds to sustain soil organisms include *in situ* primary production by algae and cyanobacteria, including lichens, and mosses, plus both spatial and temporal subsidies from lacustrine- and marine-derived detritus. There is, however, little information about the biogeochemical potential of the microbial communities. In this paper we will describe isotopic evidence for the provenance of organic resources in the dry valleys, describe a conceptual model for the distribution and turnover of resources, and examine the biogeochemical repertoire of the microbial communities for the cycling of carbon and nutrients and their responses to change.



## SEX IN A COLD CLIMATE: GENETICS AND VARIATION OF POLAR LICHENS

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Lichens are symbiotic organisms composed of two microbial partners – a fungal 'mycobiont' and a photosynthetic 'photobiont', which may be an alga or a cyanobacterium. Lichens are characterized by their ability to survive and sustain growth in environmentally extreme habitats. In particular they dominate the terrestrial vegetation of polar regions, and are an important component of polar biodiversity. Despite the prevalence, many aspects of lichen physiology and population biology have received relatively little study. This is in part due to the fact that lichenized fungi are perceived as difficult to manipulate under controlled laboratory conditions due to their slow growth rates. However, PCR-based molecular techniques now allow a range of studies to be undertaken which require minimal starting material. This talk will present work focussing on the mycobiont partner; investigating the nature of sexual reproduction and extent of genetic variation in representative lichen-forming fungi from polar environments. In particular, research seeks to determine whether Antarctic lichen-forming fungi have evolved particular characteristic features regarding sexual breeding systems, and whether cryptic speciation and physiological specialization is evident in polar lichenized fungi, which might distinguish them from lichen-forming fungi found in less environmentally extreme regions.

## ARE DARK SEPTATE ROOT ENDOPHYTES BENEFICIAL ASSOCIATES OF PLANTS AT HIGH LATITUDES?

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Fungi forming melanized, septate hyphae in roots are widespread in many ecosystems, but are especially common in cold-stressed habitats, such as those at high latitudes. The functional status of these fungi, commonly known as dark septate endophytes (DSE), has puzzled ecologists for decades. The paucity of mycorrhizal fungi in roots at high latitudes has led some to suggest that DSE might act as surrogate mycorrhizas in cold-stressed ecosystems. Here I present evidence for and against this argument by reviewing and analysing the information available in the literature on the functional status of DSE. Given the available information, it seems unlikely that DSE form mycorrhizal associations *sensu stricto*, since they apparently do not produce plant-fungal interfaces in roots, such as the mantles formed by ectomycorrhizas or the arbuscules formed by arbuscular mycorrhizas. However, it seems plausible that some DSE do act as beneficial associates of plants at high latitudes, possibly when nitrogen is available to roots in organic form. Recent data suggest that DSE may mineralize organic forms of nitrogen in the rhizosphere, making inorganic forms more freely available to roots.

## BACTERIAL DIVERSITY OF VASCULAR PLANTS RHIZOSPHERES FROM MARITIME ANTARCTICA REVEALED BY PYROSEQUENCING

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Antarctica, the Magnoliophyta are represented only by *Deschampsia Antarctica* Desv. and *Colobanthus quitensis* (Kunth) Bartl. (Caryophyllaceae). To our knowledge, this is the first work providing information on bacterial diversity in Antarctic vascular plants rhizosphere and using a pyrosequencing approach to Antarctic ecosystem. We examined bacterial community compositions for five paired samples (samples collected at the same coordinates) of two vascular plant rhizosphere from three different sites at Admiralty bay, King George Island, part of the South Shetlands Archipelago, maritime Antarctica.

The overall phylum, representing 92.23% of the bacterial rhizosphere sequences including the nine phyla that are often encountered in soil (*Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Chloroflexi*, *Verrucomicrobia*, *Bacteroidetes*, *Planctomycetes*, *Gemmatimonadetes*, and *Firmicutes*). However, the phylum distribution presents a peculiar pattern. *Firmicutes* was the most abundant phylum in all analysed samples. Relative abundances of the phyla *Actinobacteria* and *Proteobacteria* had some variation between the samples, and together with *Firmicutes*, were the three most abundant phyla in all analysed samples. *Clostridia* class represented more than 70% of all *Firmicutes* obtained sequences in all analysed samples, which indicate a high anaerobic frequency in these samples, followed by *Bacilli* which represented about 15% of all

*Firmicutes* sequences. The analyses of *Actinobacteria* phyla revealed the high dominance of the order *Bifidobacteriales*. The phylum distribution found in bacterial community associated with Antarctic rhizosphere vascular plants were different from other soils, including Antarctic soils.

## SOIL MICROBIAL COMMUNITY ECOLOGY ALONG THE WESTERN ANTARCTIC PENINSULA

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Soil microbial activities are of central importance to terrestrial ecosystem functioning; however, theoretical relationships between microbial diversity and function remain largely untested. The simplicity (low biomass and diversity) of Antarctic terrestrial microbial communities, relative to those at lower latitudes, provides an opportunity to advance knowledge of these relationships, and the responses of such communities to climate change. The terrestrial ecosystems of the Antarctic Peninsula are among those experiencing the most rapid warming on Earth and are the focus of our study. During the austral summer 2007–08 we collected soils from 70 locations spanning the entire geographical range of the Antarctic Peninsula (53–74°S). A comprehensive set of soil physicochemical (e.g. pH, nutrient status) and biological (DNA, fatty acid, CFE, SIR) analyses have been conducted and functional rates such as decomposition and nitrification have been measured. Constrained/unconstrained multivariate ordinations and a range of other ecological statistical techniques are being employed to interpret the data. We will present the outcome of these analyses in relation to the following hypotheses: 1) microbial diversity declines with increasing latitude; 2) microbial diversity is linked to the distribution of resources; and 3) microbial diversity is linked to rates of ecosystem processes such as carbon mineralization and nitrification.

## METAGENOMICS OF ANTARCTIC MARINE ECOSYSTEMS

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Spatial patchiness in marine surface bacterioplankton populations was investigated in the Southern Ocean, where the Antarctic Circumpolar Current meets the islands of the Scotia Arc and is subjected to differences in terrestrial input, upwelling of nutrients and seasonal phytoplankton blooms. Results from an analysis of 16S rRNA gene sequences from seven independent clone libraries across a latitudinal gradient showed a high degree of consistency between communities, but also highlighted some potential differences. As distinct microbial communities in Antarctic marine ecosystems might constitute an important gene pool, which plays a unique and diverse role in global biogeochemical cycling and production, a metagenomic library was constructed and sequenced. Here we present the results of a biodiversity assessment using the original clone library approach and comparing it to 65,000 16S rRNA fragments from 1.4 million GS-FLX titanium sequencing reads from the Antarctic marine metagenomic library.

## LITTLE LIFE ON POLAR EARTH: MICROBIAL DIVERSITY AND ACTIVITY IN CRYOCONITE HOLES

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The cryosphere presents some of the most challenging conditions for life on earth. Nevertheless, biota can survive in glacial systems in niches such as cryoconite holes. Cryoconite holes are water-filled depressions on glacial surfaces containing a mat of dark granular material covering their base, providing a niche for multi-trophic biotic communities to survive and representing a hotspot for primary production and biogeochemical cycling. This research has combined molecular investigations of microbial diversity together with radioisotope-labelling-based determination of photosynthesis/respiration to investigate the structure, diversity and biological activities of the microbial/faunal communities present within cryoconite holes. T-RFLP analysis of rRNA genes revealed global variation in the structure of bacterial and eukaryotic communities. Subsequent sequence analysis revealed considerable diversity amongst bacteria (e.g. *Cyanobacteria*, *Actinobacteria*, and *Proteobacteria*) and

eukaryotes (eg. Cercozoa, Ciliophora and Tardigrada). Photosynthetic activity measurements suggested net autotrophy under both high- and low-light intensities, whilst respiration rates remained constant irrespective of light intensity. This study demonstrates that cryoconite holes offer a niche for a diverse and active microbial community, within an otherwise ruthless environment.

### THE BIODIVERSITY OF CYANOBACTERIA IN ANTARCTIC MICROBIAL MATS

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The biodiversity and distribution of cyanobacteria from microbial mat samples of Antarctic lakes and meltwaters was studied in 4 different regions: Eastern Antarctica, McMurdo Dry Valleys, Antarctic Peninsula and Transantarctic Mountains. The survey was carried out with clone libraries and Denaturing Gradient Gel Electrophoresis based on rRNA sequences (16S rRNA and the Internal Transcribed Spacer). Moreover, 60 strains were isolated and genotypically characterized. For the analysis, OTUs (Operational Taxonomic Units) were defined as groups of 16S rRNA sequences sharing more than 97.5% similarity.

A total of 65 OTUs were identified among our sequences, of which a majority has not been recorded for non-Antarctic sites (absent from databases). This suggests a high degree of endemism.

The four samples from the most hostile biotopes (Transantarctic Mountains) had a particularly poor diversity (from 2 to 5 OTUs). This is in contrast with the higher diversity found in the other regions, varying from 4 to 12 OTUs. Multivariate analyses have shown that salinity and depth (correlated to light) were important factors to explain the distribution of cyanobacterial OTUs. In almost each sample studied, new OTUs were found. Thus, the bulk of the cyanobacterial genomic diversity in Antarctic biotopes still remains to be discovered.

## BACTERIAL CELL WALLS

### CELLULAR ARCHITECTURE AND DYNAMICS IN GRAM-POSITIVE BACTERIA

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The bacterial cell wall is essential for the maintenance of viability and shape determination. The major cell wall structural constituent is peptidoglycan, which forms a single macromolecule around the cell. This molecule is dynamic being synthesized, modified and hydrolysed to allow for cell growth and division. The use of atomic force microscopy has recently revealed novel peptidoglycan architecture in *Bacillus subtilis* involving apparent cables of material running across the cell cylinder. Division septa also show an elegant and complex architecture of cables. In contrast, *Staphylococcus aureus* has very different peptidoglycan architecture as a result of its alternative mode of growth and division. *S. aureus* divides in 3 sequential planes, thus requiring information to be carried across the generations to allow this to occur with fidelity. Our AFM analysis has revealed novel peptidoglycan features with the capability of conferring this intergenerational signal for division site localization. Our approach is being extended to other organisms to identify if common peptidoglycan architectural solutions are employed to answer the engineering problems encountered during bacterial growth and division.

### ROLE OF PENICILLIN-BINDING PROTEINS IN CELL ELONGATION AND DIVISION IN *ESCHERICHIA COLI*

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The peptidoglycan sacculus is the shape-maintaining and stress-bearing structure located in the cell envelope of most bacteria. It consists of glycan strands that are cross-linked by short peptides. *Escherichia coli* has a thin, mainly single-layered sacculus. Growth and division of the bacterial cell calls for a well-regulated enlargement of the sacculus by incorporation of the precursor lipid II by membrane-bound peptidoglycan synthases, the Penicillin-binding proteins (PBPs). We have studied the activities of peptidoglycan synthases from *E. coli* and their interactions with peptidoglycan hydrolases, cell division proteins and cell elongation proteins. Our studies support a model according to which the sacculus is enlarged by multi-enzyme complexes composed of peptidoglycan synthases and hydrolases which are controlled (or positioned) by proteins associated with the bacterial cytoskeleton. In rod-shaped bacteria like *E. coli*, there are different peptidoglycan synthesis complexes active in cell elongation and cell division. Newborn cells elongate by insertion of new material into the lateral wall in an MreB- and PBP2-dependent way. Shortly before cell division, FtsZ and other early cell division proteins appear to locate PBP2-containing peptidoglycan synthesis complexes to mid-cell resulting in a brief period of cell elongation from mid-cell position. Cell division occurs when the complete divisomal ring including the late cell division proteins has assembled and requires the activities of PBP3-containing peptidoglycan synthesis complexes.

### CRYSTAL STRUCTURE OF MEMBRANE PROTEIN INVOLVED IN BACTERIAL CELL WALL SYNTHESIS

ANDREW LOVERING

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Bacteria rely upon their cell walls for shape, support, protection and numerous other essential roles e.g. involvement in cell division. These functions also represent an excellent target for the development of antimicrobials, as evidenced by the wealth of clinically-used compounds that inhibit peptidoglycan synthesis. Understanding cell wall synthesis is therefore critical for the development of new drugs to use against resistant 'superbugs', and also paramount in terms of unraveling the details of bacterial physiology. We are interested in the biosynthetic steps of cell wall formation, with particular focus on those occurring at the membrane interface. Following our recent studies detailing the mode of action of peptidoglycan synthases (Lovering et al. – 2007, *Science* **315**; 2008, *J Mol Biol* **383**; 2008, *Curr Opin Struct Biol* **18**), we report here the solubilization, purification and structure of another member of the cell wall biosynthetic apparatus, and describe its mode of interaction with the lipid bilayer and phospholipid substrates.

### THE ROLE OF PEPTIDOGLYCAN IN HOST-MICROBE INTERACTIONS

IVO BONECA

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The peptidoglycan (PGN) is an essential, unique and major component of the bacterial cell wall. The essential nature of PGN has made it the target of several classes of successful antibiotics such as  $\beta$ -lactams and glycopeptides since assembly of this macromolecule is coordinated with essential processes such as cell growth, shape and division. PGN synthesis is accomplished presumably by protein complexes involving PGN synthetases (or penicillin-binding proteins) and PGN hydrolases. Hence, bacteria constantly remodel and shed their PGN layer into their environment. Released PGN fragments are an excellent trademark of actively growing bacteria and are exploited by higher eukaryotes to sense the presence of bacteria. Mammals sense released PGN fragments by the intracellular innate immune receptors Nod1 and Nod2 to mount an adequate response to bacteria, particularly on mucosal surfaces. In fact, Nod2 loss-of-function polymorphisms are a risk factor for developing chronic inflammatory bowel diseases such as Crohn's disease. This presentation will address the role of peptidoglycan metabolism both in the context of host/pathogens and host/commensals interactions.

### STREPTOMYCES COELICOLOR AS A MODEL SYSTEM FOR CHARACTERIZING RESISTANCE TO ANTIBIOTICS THAT TARGET THE BACTERIAL CELL WALL

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The development of resistance to existing antibiotics, coupled with a sustained decline in the success rate of the discovery of new ones, is leading to a point in the future where many infections will essentially be untreatable by the compounds that are available. A fundamental understanding of bacterial antibiotic tolerance and resistance mechanisms will be an important part of any future strategy aimed at solving the growing problems with treating microbial infections. I am using *Streptomyces coelicolor* as a model system to investigate the mechanisms involved in sensing, responding and adapting to the presence of one important class of antibiotic compounds, those that target cell wall biosynthesis. The bacterial cell wall is crucial for normal cell growth, and many antibiotics that target the cell wall find use in the treatment of infectious diseases. *Streptomyces* spp. produce 70% of known antibiotics and are the ultimate source of most antibiotic resistance genes, making them a particularly attractive model for this work.

I will present results from an extensive transcriptome profiling study in which DNA microarrays were used to characterize the response at the transcriptional level when growing cultures of *Streptomyces coelicolor* are challenged with three antibiotics (vancomycin, bacitracin and moenomycin A) that target distinctly different stages of cell wall biosynthesis. Analysis of the data has identified sets of genes that are induced only in response to each individual antibiotic, or in response to all the antibiotics tested. These genes are likely to be involved in adaptation/resistance to the drugs, the latter representing a generalized response to cell wall damage and the former the antibiotic-specific responses. I am currently working on the further characterization of a selected number of these genes aimed at defining and understanding their role in the adaptive response to the antibiotics, and in cell envelope homeostasis, and I will present some preliminary data from these studies.

### STRUCTURE AND TENTATIVE FUNCTION OF A HETEROTRIMER OF PROTEINS CENTRAL TO THE DIVISION PROCESS OF *STREPTOCOCCUS PNEUMONIAE*

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*Streptococcus pneumoniae* (the pneumococcus) divides with parallel planes, a process that involves about 10 conserved proteins. This set of proteins includes DivB (FftQ in Gram negatives), FtsL and DivC (FtsB) that are central to the process of bacterial division but whose function is not known. The *divB* gene is not essential for pneumococcus growth in rich medium, but the  $\Delta divB$  mutant forms chains and a small fraction of enlarged cells with defective septa. The deletion mutant does not grow in a chemically defined medium. In the absence of DivB and when protein synthesis is blocked by erythromycin, the partner FtsL is rapidly degraded, pointing to a role of DivB in stabilizing FtsL. Deletion of *divB* increases the susceptibility to  $\beta$ -lactams, a phenotype which is enhanced in a resistant strain. This latter observation supports a role for DivB in cell wall synthesis. The three proteins form a complex at the division site. We have analysed the structure of the DivB-FtsL-DivC complex by NMR, small-angle neutron and X-ray scattering, and interaction studies by surface plasmon resonance. This work relied on soluble truncated recombinant version of

## BACTERIAL CELL WALLS

(continued)

the proteins. We found that the beta-sheet side of the 'bean'-shaped central beta-domain of DivIB interacts with the C-terminal regions of the FtsL-DivIC dimer. We propose that the interaction with DivIB leaves the FtsL-DivIC coiled-coil region, either alone or together with the transmembrane regions, exposed to interact with other proteins.

### MYCOBACTERIAL CELL WALLS

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In spite of effective antibiotics to treat tuberculosis in the late 1950s and early 1960s we begin the new millennium with tuberculosis (TB) currently the leading cause of death from a single infectious agent, killing more than three million people worldwide each year. Thus, an understanding of drug-resistance mechanisms, the immunobiology of cell wall components to elucidate host-pathogen interactions and the discovery of new drug targets are now required for the treatment of TB. Above the plasma membrane is a classical chemotype IV peptidoglycan to which is attached the macromolecular structure, mycolyl-arabinogalactan via a unique diglycosylphosphoryl bridge (often referred to as the mAGP complex). Clearly, cell wall assembly is a profitable choice for the design of novel anti-TB agents. In addition, new targets within the complex may be identified as well as establishing the mode of action of existing agents, mechanisms of drug resistance and the immunomodulatory role of cell wall constituents. In summary, the presentation will discuss the assembly of the mAGP, its associated lipids and the site of action of several major anti-TB drugs.

### HOW TO LIVE WITHOUT A WALL: L-FORMS OF *BACILLUS SUBTILIS*

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The ability of many conventional walled bacteria to metamorphose into wall-deficient variants called L-forms, sometimes reversibly, has been investigated on and off for over 70 years. Most early reports of L-forms were in clinical situations, typically involving isolates from patients on  $\beta$ -lactam therapy. The difficulty of propagating L-forms and the lack of molecular techniques with which to characterize and classify L-forms led to considerable confusion in the literature and failure of the field to achieve mainstream status. We have revisited the L-form problem using the well characterized and highly tractable model bacterium, *Bacillus subtilis* (Leaver et al., 2009, *Nature*). We developed a method for rapidly and reproducibly generating L-forms from genetically tagged strains of *B. subtilis*. We showed that a single point mutation in the *ispA* gene is sufficient to predispose cells to the L-form transition and confirmed that the cell lines generated had the various properties expected of classical L-form cells. Remarkably, we found that L-forms no longer require the normally essential FtsZ-dependent cell division apparatus. Instead they proliferate by various events involving extrusion of membrane-bound tubes or vesicles, followed by resolution into multiple progeny.

# CONTRIBUTION OF THE GLOBAL N CYCLE TO GLOBAL PROCESSES

## RHIZOBIUM–LEGUME SIGNALLING EVENTS LEADING TO THE FORMATION OF SYMBIOTIC NITROGEN-FIXING NODULES

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The induction by rhizobia of nitrogen-fixing nodules on legumes involves rhizobial attachment and signal exchange between the bacteria and the plant. Attachment mediated via a *Rhizobium leguminosarum* polysaccharide and a legume lectin expressed on root hairs is very important for legume infection. Also required for attachment are other surface polysaccharides, some of which are affected by bacterial quorum-sensing regulation. There is also attachment activated by a plant component, which appears to be a secreted arabinogalactan protein; this appears to be relatively non-specific. Rhizobia activate gene expression in legumes by producing 'Nod-factor' signals that are chitin oligomers with several decorations that are important for host specific nodulation. Using bacterial and plant mutants, we have identified three separate signalling outputs that occur after recognition of the Nod-factors by plant receptor-kinases. One induces root hair deformation; this entraps the bacteria leading to initiation of infection. Two other outputs result in changes in intracellular calcium in root hairs; one of these induces perinuclear calcium oscillations whereas the other induces an influx of calcium at the root-hair tip. The former is coupled to the induction of legume genes required for nodulation, while the other seems to be primarily involved in the initiation of the infection events.

## THE CONSEQUENCES OF AMMONIA OXIDIZER DIVERSITY FOR SOIL ECOSYSTEM FUNCTION

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Ammonia oxidizers convert ammonia to nitrite, the first step in the nitrification process. This process controls the rate of nitrification in most environments and it is therefore a key control point in the global nitrogen cycle. In the soil, ammonia is derived from decomposition of organic matter; urea- or ammonia-based fertilizers and animal waste. It therefore controls rates of fertilizer loss, nitrate pollution, nitrous oxide production and the form of inorganic nitrogen available to plants. The major recent advances in our understanding of ammonia oxidation have arisen through greater knowledge of ammonia oxidizer diversity and community structure. Laboratory studies on a limited number of strains have also demonstrated metabolic diversity in ammonia oxidizing bacteria and their potential for contributing to ecosystem processes under different environmental conditions. A major challenge is mapping this physiological diversity onto the high levels of phylogenetic diversity indicated by molecular studies of ammonia oxidizer diversity. Molecular techniques now enable *in situ* assessment of physiological activity and of the conditions that favour particular phylogenetic groups. The requirement and consequences of ammonia oxidizer diversity for soil ecosystem function will be considered using information on diversity from laboratory, field and microcosm studies.

## THE ECOPHYSIOLOGY OF ARCHAEAL AMMONIA OXIDIZERS

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The isolation of the first ammonia-oxidizing archaeon *Nitrosopumilus maritimus* provided the organismic link between an abundant phylogenetic group, the marine group I.1a crenarchaeotes, and the nitrogen cycle. Subsequent molecular surveys using archaeal ammonia-monooxygenase subunit A genes demonstrated that ammonia-oxidizing archaea (AOA) form a quite diverse micro-organism group and may even represent the predominant ammonia oxidizers in certain marine and terrestrial habitats. Both, archaeal and bacterial ammonia oxidizers share the same chemolithoautotrophic physiology by gaining energy via aerobic ammonia oxidation to nitrite and fixing inorganic carbon as sole carbon source. However, recent studies on *N. maritimus* revealed differences between biochemical pathways operating in AOA and in AOB. For instance, all known AOB use the Calvin-cycle for carbon assimilation while a modified 3-hydroxypropionate-pathway was found to operate in AOA. As AOB oxidizes ammonia via the intermediate hydroxylamine, homologue genes to the bacterial hydroxylamine oxidoreductase could not be identified in the genome of *N. maritimus* pointing to a different mechanism for ammonia oxidation.

The cultivation of novel AOA, like a thermophilic enrichment from Yellowstone hot spring and a marine strain from the German Wadden, provide direct evidence for archaeal nitrification in natural habitats. By combining genetic, biochemical and microbiological studies we aim to define physiological properties that enable AOA to successfully compete with their bacterial counterparts in nature.

## ECOLOGY AND EVOLUTION OF NITRITE OXIDIZERS: INSIGHTS FROM IN SITU TOOLS AND ENVIRONMENTAL GENOMICS

HOLGER DAIMS

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The chemolithotrophic oxidation of nitrite to nitrate is catalyzed by the, phylogenetically heterogeneous, functional group of nitrite-oxidizing bacteria (NOB). Among these organisms, the genus *Nitrospira* is most diverse and abundant in many kinds of natural and engineered ecosystems. Their environmental distribution indicates that major *Nitrospira* lineages are adapted to different ecological niches. As the majority of *Nitrospira* is uncultured, much less has been known about these organisms than about cultured NOB such as *Nitrobacter*.

By applying molecular *in situ* methods and metagenomics, an unexpected diversity of related *Nitrospira* was detected in nitrifying activated sludge, where several *Nitrospira* populations co-exist on small spatial scales. At least some of these populations differ in ecophysiological traits, and evidence for genetic recombination among closely related *Nitrospira* strains has been recorded.

Recently, environmental genomics revealed that *Nitrospira* differ from other NOB (e.g. *Nitrobacter*) in key metabolic pathways including the basic features of autotrophic NOB, which are nitrite oxidation, inorganic carbon fixation, and the electron transport chain for aerobic respiration. Together with phylogenetic analyses, these data complement and extend current hypotheses on the evolution of nitrite-oxidizing bacteria.

## SOIL MICROBIAL SOURCES OF NITROUS OXIDE: CURRENT STATE OF KNOWLEDGE AND FUTURE CHALLENGES UNDER A CHANGING CLIMATE

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Soils are a major source of the greenhouse gas nitrous oxide ( $N_2O$ ), which can be produced during several microbial processes: denitrification, ammonia oxidation, nitrifier denitrification and nitrate ammonification. These processes may occur simultaneously in different microsites of the same soil but there are uncertainties surrounding the regulation of  $N_2O$  production and reduction and uncertainty as to which process is predominantly contributing to measured emissions. It is essential to resolve this for the development of targeted mitigation strategies for  $N_2O$ . Recent technical advances now facilitate source partitioning between all known microbial sources, and here selected examples will be presented demonstrating how this has advanced our knowledge on the significance of each process in different management systems under a changing climate, enabled us to more closely link measured processes to the underpinning microbiology, and elucidated flexibilities in microbial function. This offers us exciting opportunities for manipulating the rhizosphere microbial population to lower net  $N_2O$  emission, and the challenges we face before this can be fully realized are discussed.

## MOLECULAR ECOLOGY OF DENITRIFIERS

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Denitrifying micro-organisms are capable to reduce soluble oxidized nitrogen compounds into gaseous  $N_2O$  or  $N_2$  for energy conservation. The denitrification process has received a lot of attention because it accounts for significant losses of nitrogen fertilizer from agricultural soils. Denitrification is also responsible for the emission of  $N_2O$ , an important greenhouse gas with a global warming potential 250 times higher than that of carbon dioxide. Understanding the relationships between denitrifier community ecology and N-fluxes by denitrification is therefore important for a better management of N-input in agriculture and mitigation strategies. Indeed, the last decade several molecular methods have been developed and extensively applied to study the diversity, the abundance and the distribution

# CONTRIBUTION OF THE GLOBAL N CYCLE TO GLOBAL PROCESSES

(continued)

of the denitrifying community in the environment. This talk will summarize our current knowledge of this functional community and of its environmental controls and present some preliminary steps toward bridging denitrifier community ecology and denitrification process.

## NOVEL PERSPECTIVES ON NITROGEN CYCLING IN OCEANIC OXYGEN MINIMUM ZONES

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About 20–40% of oceanic nitrogen-loss occurs in the so-called oxygen minimum zones (OMZs). The Peruvian and Arabian Sea OMZs, are two out of three such major OMZs. Increasing evidence indicates that anammox, instead of heterotrophic denitrification, is the predominant N-loss pathway in these OMZs; yet the sources of nitrite and ammonium are not entirely clear. At the same time, the exact N-loss pathway in the Arabian Sea has not been clearly identified. We used a combination of  $^{15}\text{N}$ -incubation experiments, qualitative and quantitative functional gene expression analyses, to differentiate between N-loss due to anammox and denitrification, and to identify and quantify active nitrogen transformations co-occurring with these N-loss processes, and the key microbial players involved. We found that in both OMZs, anammox was the predominant N-loss pathway. The majority of nitrite for anammox came from nitrate reduction, whereas up to one-third came from aerobic ammonia oxidation. Anammox bacteria acquired ammonium from the degradation of organic matter associated with nitrate reduction, but also from dissimilatory nitrate reduction to ammonium. Intriguingly, there was no evidence of N-loss in the central-northern Arabian Sea, previously considered the main region of N-loss. Instead, substantial N-loss due to anammox was detected in the Omani upwelling area.

## DENITRIFYING METHANOTROPHY: METABOLISM, BIOCHEMISTRY AND ULTRASTRUCTURE OF THE RESPONSIBLE BACTERIA

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Recently, a microbial consortium was found to couple anaerobic methane oxidation to denitrification [Raghoebarasing et al. 2006]. The consortium consisted of a bacterium representing a phylum without cultivated representatives and an archaeon distantly related to known methanogenic/methanotrophic archaea. Later, it appeared that the bacterium also oxidizes methane in the absence of the archaeon.

Because this bacterium divides only once per month, it has not yet been isolated in pure culture. However, with metagenomics, transcriptomics, proteomics, biochemical analysis,  $^{15}\text{N}$  isotope labeling and electron microscopy of the enrichment culture, it was still possible to characterize the physiology of the novel denitrifying methanotroph.

Surprisingly, we found a combination of anaerobic and aerobic pathways, which were both found to be expressed and functional.

**Reference** Raghoebarasing, A.A., Pol, A., van de Pas-Schoonen, K.T., Smolders, A.J., Ettwig, K.F., Rijpstra, W.J., Schouten, S., Sinninghe-Damste, J.S., Op den Camp, H.J., Jetten, M.S. & Strous, M. (2006). A microbial consortium couples anaerobic methane oxidation to denitrification. *Nature* **440**, 918–921.



## CLINICAL MICROBIOLOGY WORKSHOP MENINGITIS

### MECHANISMS OF *NEISSERIA MENINGITIDIS* RESISTANCE AGAINST COMPLEMENT-MEDIATED KILLING

CHRISTOPH M. TANG  
Imperial College London

*Abstract not received*

### DEXAMETHASONE IN TREATMENT OF ACUTE BACTERIAL MENINGITIS: PROS AND CONS

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Neuronal injury following bacterial meningitis is primarily due to the host inflammatory response, attenuation of which should lead to improved outcome. To this end, steroid adjuvant therapy has been investigated widely for over 40 years but results from trials provide conflicting evidence and the debate continues.

In animal models, steroids reduce the intensity and duration of the inflammatory response but no mortality data are available. In humans, the utility of steroids may depend on several factors including age, infecting organism, timing of steroid therapy, severity of illness and locale of presentation. Guidelines from several wealthy countries broadly recommend steroid adjuvant therapy but there is considerable variation in clinical practice. It is likely that guidelines will require continuing revision in light of emerging evidence and the changing epidemiology of bacterial meningitis.

Although there is no trial evidence of an excess of adverse events in patients receiving steroid adjuvant therapy, there is concern over the effect of steroids on CSF penetration of certain antibiotics and over the potential for harm when the diagnosis of bacterial meningitis has not been microbiologically confirmed.

Although steroids may have a role, early diagnosis and appropriate antibiotic therapy remain the cornerstones of successful management.

### BACTERIAL NITRIC OXIDE DETOXIFICATION PREVENTS HOST CELL S-NITROSTHIOL FORMATION: A NOVEL MECHANISM OF BACTERIAL PATHOGENESIS

JAY R. LAVER, TÂNIA M. STEVANIN, MARGARET E. LEE, AMY DEHN LUNN, SARAH L. MESSENGER, JAMES W.B. MOIR, ROBERT K. POOLE & ROBERT C. READ  
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S-nitrosylation is an important mediator of multiple nitric oxide (NO)-dependent biological processes, including events such as apoptosis and proinflammatory signaling. Dysregulation of S-nitrosylation has been implicated in a number of chronic conditions. Many pathogenic bacteria possess NO detoxification mechanisms, such as the nitric oxide reductase (NorB) of *Neisseria meningitidis* and the flavohemoglobins (Hmp) of *Salmonella enterica* and *Escherichia coli*, which protect the micro-organism from nitrosative stress. Expression of meningococcal NorB influences host cell physiology; modulating cellular cytokine output and influencing entry into apoptosis. We tested the hypothesis that bacterial NO detoxification mechanisms are capable of influencing the concentration of host cell S-nitrosothiol (SNO).

We developed a SNO-rich infection model using the macrophage cell line, J774.2; which produces a prolonged enrichment of SNO upon exposure to cellular agonists. We demonstrate that infection with NorB-expressing *Neisseria meningitidis* or Hmp-expressing *Salmonella* or *E. coli* results in a reduced abundance of host-cell SNO comparative to uninfected cells. We postulate that bacterial mechanisms of NO detoxification act to prevent formation of 'unstable' SNO, by the efficient removal of NO. This data suggests a role for host-cell SNO dysregulation in acute conditions such as microbial infection or septicemia, the pathophysiological consequences of which may be profound.

### HOST PATHOGEN INTERACTIONS IN DEVICE-RELATED *STAPHYLOCOCCUS EPIDERMIDIS* MENINGITIS

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Toll-like receptors (TLRs) are key components of the innate immune system that are activated in response to microbial and host-derived factors. They have potentially important

roles in the host response to infection associated with insertion of a medical device. *Staphylococcus epidermidis* is a frequent cause of neurosurgical device-related meningitis. Expression of the *icaADBC* operon product, polysaccharide intracellular adhesion (PIA), and the ability to produce biofilm are important virulence factors of *S. epidermidis*. Recent studies have linked PIA to activation of human TLR2 in primary and transformed human astrocytes with PIA time- and dose-dependently inducing expression of the proinflammatory cytokines IL-8, IL-6 and MCP-1. Interestingly levels of these cytokines are also elevated in human cerebrospinal fluid (CSF) associated with *S. epidermidis* infection compared to normal CSF. Taken together these data implicate PIA as an important immunogenic component of the *S. epidermidis* biofilm that can regulate proinflammatory cytokine production from human astrocytes, in part, via TLR2. This has implications for the optimal management of staphylococcal neurosurgical infection.

### MICROBIAL AND HOST FACTORS INVOLVED IN SEVERITY OF MENINGOCOCCAL DISEASE

ROBERT C. READ  
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*Abstract not received*

### SURROGATE MARKERS FOR VACCINE EFFICACY AGAINST MENINGITIS-CAUSING ORGANISMS

RAY BORROW  
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In order to allow licensure of vaccines without the need for conducting expensive, laborious efficacy trials, it is important to have laboratory markers of immunity that can reliably predict clinical protection in the field. Such markers, termed surrogates of protection, are derived from evidence that the presence of the immune marker consistently predicts clinical protection in the individual and that the specific antibody that is being measured is actually mediating the protection observed. The term correlate of protection is used to denote a laboratory measure that is correlated with protection, and therefore with the surrogate, but may not be directly measuring the antibody that is mediating protection. For *Haemophilus influenzae* type b, from the Finnish vaccine trials, a level of 0.15 µg/mL for short term protection and 1.00 µg/mL for long term protection have been internationally accepted. The classic studies by Goldschneider *et al.* in 1969 clearly established a serum bactericidal antibody (SBA) titre of ≥4 utilizing human complement as a correlate of protection against meningococcal serogroup C infection. This, together with the subsequent experience with capsular polysaccharide and OMV vaccines, lead to SBA being considered as a generic surrogate of protection against meningococcal disease irrespective of serogroup. For licensure of pneumococcal conjugate vaccines, it was demonstrated that in infants receiving a 7-valent pneumococcal conjugate vaccine at 2, 4 and 6 months of age, more than 97% achieved antibody levels of 0.20 µg/mL which correlated with the observed protective efficacy of 97.3%. This antibody threshold was then raised to 0.35 µg/mL when data became available from controlled trials in other countries. For conjugate vaccines, immune memory can no longer be considered a correlate of long term protection and antibody persistence is a more appropriate correlate.

### TYPE IV PILUS BIOLOGY: TOWARDS A COMPLETE PICTURE IN *NEISSERIA MENINGITIDIS*

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The widespread role of pili as colonization factors has long been recognized in Gram-negative and more recently in Gram-positive bacteria, making the study of these hair-like filaments a perennial hot topic for research. No other pili are found in as many or as diverse bacteria as type IV pili (Tfp) probably because of their unique ability to promote multiple and strikingly different phenotypes such as attachment to surfaces, aggregation, motility etc. However, after two decades of investigations we know relatively little about the molecular mechanisms of Tfp biogenesis and/or the functions that they mediate. One of the underlying reasons is paradoxically the fact that the various aspects of Tfp biology have been studied in several model species, generating sometimes conflicting, but always incomplete

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results. We have therefore started a systematic functional analysis of Tfp biology in a single and well-defined genetic background, the human pathogen *N. meningitidis*, in order to get a complete picture. Recent discoveries, which will be discussed here, bring us a few steps closer to understanding how these filaments are produced and how they mediate some of the functions that made them one of the most widespread virulence factors in the bacterial world.

#### HAEMOPHILUS INFLUENZAE TYPE B AND NEISSERIA MENINGITIS TYPE C VACCINATION – RATIONAL AND OUTCOMES

MARY E. RAMSAY

Health Protection Agency, London

*Abstract not received*

## FRED GRIFFITH REVIEW LECTURE

### FROM SPORES TO ANTIBIOTICS VIA THE CELL CYCLE

JEFF ERRINGTON

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Spore formation in *Bacillus subtilis* is a superb experimental system with which to study fundamental problems of cellular development and differentiation. Work begun in the 80's and ongoing today has led to an impressive understanding of the temporal and spatial regulation of sporulation, and the functions of many of the several hundred genes involved. Early in sporulation the cells divide in an unusual asymmetric manner; to produce a small prespore cell and a much larger mother cell. Aside from developmental biology, this modified division has turned out to be a powerful system for investigation of cell cycle mechanisms, including the components of the division machine, the mechanism whereby it is correctly positioned in the cell, and how division is coordinated with replication and segregation of the chromosome. Insights into these fundamental mechanisms have provided opportunities for discovery and development of novel antibiotics.

## MICROBIOLOGY OUTREACH PRIZE LECTURE

### SCHOOLS, SPIDERMAN AND SUPERBUGS; COMMUNICATING MICROBIOLOGY AT PRIMARY LEVEL

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Schoolchildren have all heard of bacteria and can quote TV adverts for major probiotics and disinfectants, but how much do they actually know? Through funding from Aimhigher and the Wellcome Trust, over 800 year five and six pupils have been engaged in the Superhero Superbug project, learning about how superbugs evolve, why they are so super and how we can tackle them. This presentation demonstrates how some of these complex issues have been communicated and presents some of the children's perceptions of the next emerging superbug.

### HW01/01 THE EVOLUTIONARY DIVERSITY OF MICROBIAL LIFE IN UK FOREST SOILS

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Advances in molecular techniques have allowed for better investigation into the microbial diversity of many natural environments revealing many novel branches on the tree of life. These approaches have in turn given us a better understanding of how microbial communities have evolved to occupy very different niches. We investigated a range of forest soil types to assess diversity of both prokaryotes and microbial-eukaryotes. We looked at both the total community DNA and RNA (as cDNA) portion of the community using denaturing gradient gel electrophoresis, clone library analysis and 454 sequencing of DNA and RNA using primer sets targeting 16S SSU and 18S SSU sequences. The results describe a complex microbial community dominated by specific prokaryote, fungal and protozoan lineages. Our analysis also enabled us to identify a large diversity of previously undescribed microbial life occupying numerous novel branches on the tree of life. Much of this diversity was present in low amplicon number, previously termed 'the rare biosphere' within marine environments. In conclusion, we report a new diagnostic method for exploring whole microbial communities from soil environments.

### HW01/02 INFERRING THE PHYLOGENY OF THE KINETOPLASTIDS: A COMPARATIVE GENOMICS STUDY USING MAXIMAL GENOME CONTENT WHEN FEW TAXA ARE AVAILABLE

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Attempts to resolve the phylogeny of the human parasitic kinetoplastids has produced conflicting data. Here we describe a novel method of reconstructing species phylogeny using whole genomes and few taxa. We calculated a phylogeny for every gene family present in *T. brucei* to recover reliable gene markers for large scale analysis, identifying 75 gene families. All 75 datasets were then concatenated resulting in an alignment of 36,278 characters and subjected to six different phylogenetic methodologies and alternative topology tests. We then constructed a reduced dataset with additional species in order to break the long-branch outgroup. Meanwhile eight trees showed evidence of reciprocal kinetoplastid paralogue rooting, negating the need for an outgroup and were analysed using the same methodologies. We then subjected all phylogenetic datasets to analyses with serial stripping of fast evolving sites. Throughout our results we recovered near total support for the monophyly of *T. brucei* and *T. cruzi* pinpointing the major branching order of the kinetoplastids. We then used these results to map gene loss events across the kinetoplastid phylogeny demonstrating, in contradiction to most models of parasite evolution, large scale gene loss coupled to the transition from an intracellular to an extracellular parasite.

### HW01/03 EVOLUTION OF DNA REPLICATION IN EUKARYOTES

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All cellular organisms have double-stranded DNA genomes. Cells must ensure their genomes are replicated with extreme precision and only once during a single cell cycle. DNA replication is thus a complex and strictly regulated process. Current research on eukaryotic DNA replication is derived principally from studies of yeasts and animals, which cover a small fraction of the whole eukaryotic diversity, and few studies have been

carried out for other eukaryotes. The recent availability of abundant genome sequences for eukaryotes and modern bioinformatic methods have provided the opportunities to study the evolution of eukaryotic DNA replication. We have applied comparative genomics and phylogenetic analysis to map ancient patterns of evolution by pinpointing gains, losses and duplication events within over 70 DNA replication gene/protein components across 35 eukaryotes, covering five of six eukaryotic supergroups. Our studies have constructed a taxonomic distribution of the core DNA replication proteins across eukaryotes and identified ancient conserved protein components. We have revealed a potential model of the DNA replication machine in the last common ancestor of eukaryotes. This knowledge will enhance our understanding of the evolution of eukaryotic DNA replication and the tree of life.

### HW01/04

Withdrawn

### HW01/05 WHAT ARE THE FUNCTIONS OF PROTEINS RESEMBLING GLYCOLYTIC ENZYMES IN AFRICAN TRYPANOSOMES?

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*Trypanosoma brucei* has evolved to survive in the mammalian bloodstream and tsetse fly digestive tract, and has subsequently streamlined its metabolism to suit these niche environments. The pathway of glycolysis is well-characterized in *T. brucei*, and several of the glycolytic enzymes are uniquely compartmentalized in peroxisomes, but in a recent genomic analysis, we have identified several proteins which resemble glycolytic enzymes. These proteins contain either putative degenerate active sites or unexpected targeting determinants. We have identified an enolase-like protein, four phosphoglycerate mutase-related proteins, as well as GAPDH- and phosphoglycerate kinase-related proteins, which when tagged localize to the trypanosome flagellum. Here, we will report the results from gene-specific RNAi experiments and speculate on likely functions for this family of trypanosome proteins, which are clearly homologous to glycolytic enzymes. Finally, we complemented this experimental work with *in silico* investigations into the distribution of proteins resembling glycolytic enzymes in other unicellular eukaryotes. Our findings parallel those for some glycolytic enzymes in other eukaryotic lineages which have acquired additional moonlighting functions in unusual cellular compartments.

## POSTERS

## PUTTING MICROBES TO WORK

HW02/01

Withdrawn

detection only takes 3 hours. The biosensor ADPI\_recA\_lux performed extraordinary stability and robustness: it can, without any pretreatment, maintain a rapid and sensitive response to the toxic compounds after storage at 4°C for 15 days. The biosensor *Acinetobacter baylyi* ADPI\_recA\_lux could be valuable in providing a simple, rapid, stable, quantitative, robust and costly-efficient approach for the detection of toxicity in environmental samples.

**Keywords** luxCDABE, biosensor, *Acinetobacter baylyi* ADPI, recA, mitomycin C (MMC), chemotoxicity, genotoxicity,

#### HW02/04 PUTTING MICROBES TO WORK: BIOREMEDIATION MICROCYSTIN REMOVAL STRATEGIES FOR WATER PURIFICATION

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Cyanobacteria are a common and naturally occurring component of most aquatic ecosystems. Under favourable environmental conditions (nutrients, light, and temperature) they develop mass growths known as blooms, producing inherent toxins causing toxicity to Animal and humans. Among them microcystins, globally occurring cyclic heptapeptide hepatotoxins are more concerned because of its diversity of more than 80 variants. Unfortunately, these toxins are not removed by conventional water treatments. One of the most exciting areas that hold promise for a successful and cost effective solution is bioremediation of cyanotoxins. Recent work carried out, resulted in a successful isolation and characterization of novel bacteria capable of degrading microcystin-LR. In our current research a wide range of microcystins (MC-LR, LF, LW, LY, RR and Nodularin) was tested on new bacterial isolates to determine the degradation of each toxin at a concentration of 10 µg/ml. Results show the degradation capability of novel isolated bacteria against chemically diverse microcystins including their potential for use in a cost effective approach to remove microcystins from natural waters. Future work will seek to study the degradation mechanisms, by-products and their fate in environment.

HW02/05

Withdrawn

HW02/06

Withdrawn

#### HW02/02 CHARACTERIZATION OF A NOVEL ELECTROGENIC BACTERIUM FROM AN ACETATE-FED MICROBIAL FUEL CELL

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Microbial fuel cells (MFCs) represent a novel way of generating electricity. MFCs can utilize a wide variety of substrates including the waste products of industrial processes and so have potential for the treatment of wastewater. MFCs harness the ability of electrogenic bacteria to transfer electrons to insoluble electron acceptors. Electrogens have been identified amongst the  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -Proteobacteria as well as amongst the Acidobacteria and the Firmicutes. We have isolated two species of  $\epsilon$ -Proteobacteria from an acetate-fed MFC and have characterized these by 16S rRNA analysis and biochemical tests. These two species formed a major component of the microbiological consortium in the MFC.

One isolate, from the anode surface, was shown to be highly electrogenic when grown in pure culture with acetate as its sole carbon source, and it rapidly generated a strong electronegative electrode potential. The substrate metabolism of the two bacteria was studied by experimental analysis of substrate utilization and metabolic analysis of the pathways for intermediary metabolism from genomic data. The data were consistent with utilization of a reductive TCA cycle. The utilization of this knowledge to improve MFC efficiency with different carbon substrates and the potential for modelling carbon utilization in the MFC-containing consortia of bacteria able to degrade particular xenobiotic compounds will be discussed.

#### HW02/03 CHROMOSOMALLY-BASED BIOSENSOR FOR CHEMOTOXICITY AND GENOTOXICITY DETECTION

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Toxicity detection of environmental contaminants requires a simple, sensitive, rapid and reliable method to assess health risk of environmental samples. Chemical analysis is not ideal for assessing toxicity of environmental samples containing complex chemical mixtures and it is difficult to assess bioavailability of those contaminants. We developed a chromosomally based biosensor ADPI\_recA\_lux by fusing a luxCDABE with recA gene of *Acinetobacter baylyi* ADPI. The biosensor enables us to detect toxicity of a variety of chemicals and environmental samples. The detection limit of mitomycin C and benzo[a]pyrene were 60 and 400 pM which is even lower than common GC/MS and HPLC detection limit. Since the biosensor expresses a constant baseline, it can detect both chemotoxicity and genotoxicity which are caused by enzyme inhibition and DNA damaging. The biosensor

## POSTERS

### MICROBIAL POLYSACCHARIDES

HW03/01

*Withdrawn*



## ALTERNATIVE MODELS TO STUDY MAMMALIAN PATHOGENS

**HW07/01 ACANTHAMOEBA CASTELLANII AS A MODEL FOR BACTERIAL INFECTION AND AS AN AID TO BACTERIAL SURVIVAL WITHIN THE ENVIRONMENT**

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The *Acanthamoeba* genus is one of the most common protozoa found in soil and is also frequently found in fresh water. With this widespread dispersal there are plenty of opportunities for these protozoa to encounter and predate bacterial species. Since the early 1980's the interaction between amoebae and *Legionella pneumophila* has been studied and has demonstrated the ability of *Legionella* to survive and even replicate within this host. Similar mechanisms for invasion and resistance to phagocytosis were used for both invasion of the amoeba and mammalian host cells, suggesting the potential for use as a model of infection. However, it has also been shown that co-incubation with amoebae can actually increase bacterial survival within the environment.

In this study we exploit this model organism to examine bacterial species such as *Listeria monocytogenes*, *Campylobacter jejuni* and *Yersinia pseudotuberculosis*. We compare the survival of wild type strains and defined mutants to identify genes specific for survival within mammalian hosts as well as those required for survival within the environment.

**HW07/02 TRANSCRIPTIONAL ANALYSIS OF SALMONELLA ENTERICA IN AVIAN CELLS**

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*Salmonella enterica* is an intra-cellular pathogen which is capable of inducing disease in a wide range of host species. Contaminated poultry meat and eggs are common sources *Salmonella* inflammatory enteritis in humans. There is a paucity of information on the immune responses of chickens to different serotypes of *Salmonella* at the cellular or molecular level. The invasion and immune responses of chicken kidney cells (CKCs) by six different strains of *Salmonella* were assessed using gentamicin assay and quantitative RT-PCR. Avian typhoid salmonellae were less invasive in comparison with the non-typhoidal serovars. Nitric oxide was detected 6 hours post infection with *S. Typhimurium*, *S. Enteritidis*, *S. Hadar* and *S. Infantis*. Inflammatory cytokines were not induced by *S. Pullorum* or *S. Gallinarum* infection. *S. Typhimurium*- and *S. Enteritidis*-infected CKCs expressed IL-6 and IL-8 more than *S. Hadar* and *S. Infantis*. Further studies will be carried out to investigate the gene expression profile for the other regulatory cytokines.

**HW07/03***Withdrawn*

#### HWII/01 DEVELOPMENT AND EVALUATION OF A CAPSULAR POLYSACCHARIDE-TETANUS TOXOID CONJUGATE VACCINE TO PROTECT AGAINST *BURKHOLDERIA PSEUDOMALLEI* INFECTION

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*Burkholderia pseudomallei* is the causative agent of melioidosis, which causes high mortality in endemic areas. *B. pseudomallei* is known to express three surface polysaccharides, a capsule (CPS), lipopolysaccharide (LPS) and an exopolysaccharide (EPS). The CPS is well characterized and a known virulence factor; having shown immunogenicity and protection in murine models. Generally, polysaccharides do not confer T cell-dependent immunity and therefore immunity is short-lived. By covalently linking polysaccharide to protein, the polysaccharide moiety of the conjugate becomes T cell-dependent and immune memory is induced. The aim of this project is to develop and evaluate capsular polysaccharide-tetanus toxoid conjugates as vaccine candidates to protect against *B. pseudomallei* infection. Attempts to produce a source of recombinant *B. pseudomallei* capsule have included screening genomic libraries for clones containing the CPS gene cluster. A clone was identified and subsequent efforts have focused on optimizing expression of CPS in this clone. Another approach taken was to chemically synthesize the capsular polysaccharide to produce both a hexamer and an octamer. Future work will focus on conjugation of chemically synthesized or wild type *B. pseudomallei* CPS to tetanus toxoid. Any resultant conjugates will be evaluated using a murine model.

## POSTERS

## MICROBIAL STRESS AND FOOD PRODUCTION: COPING WITH THE WORK ENVIRONMENT

### HW12/01 A HIGH-THROUGHPUT METHOD FOR MEASURING MICROBIAL GROWTH AT ELEVATED HYDROSTATIC PRESSURE

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Hydrostatic pressure is an interesting and important source of microbial stress, of relevance both to deep sea microorganisms in the natural environment and to food sterilization. One major challenge in the study of the effects of hydrostatic pressure is the limited number of samples which can be placed into a typical pressure vessel. Pressure vessels are restricted in size due to both safety and cost issues. To overcome some of these issues, we have developed a high-throughput method using standard 96-well microtiter plates and a plate reader, which allows the simultaneous analysis of a large number of microbial cultures using a single pressure vessel. This method allows us to measure optical density as well as fluorescence and luminescence, for up to 960 samples (ten 96-well plates) at pressures ranging from 0.1 MPa to 60 MPa.

We present high resolution data on the growth of three wild-type strains of the deep sea bacterium *Photobacterium profundum* over a range of hydrostatic pressures and NaCl concentrations. Each strain shows a different optimum hydrostatic pressure with *P. profundum* SS9 having dual NaCl optima of 250 mM and 500 mM NaCl at 45 MPa but only a single optima of 350 mM at 10 MPa.

The method has also been used for screening mutant libraries: we present preliminary work on the screening of an *E. coli* single gene deletion library for growth at elevated hydrostatic pressure. This data shows that genes responsible for DNA replication and repair as well as cell replication and stress factors are essential for *E. coli* to replicate at 31 MPa. Genes identified include as essential include *dnaK*, *dnaT*, *rimM* and *holC*.

### HW12/02 EFFECTS OF A RECOMBINANT $\beta$ -D-GLUCAN PRODUCING PROBIOTIC ON THE QUALITY OF STIRRED-TYPE YOGHURT

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The effects of *in situ* produced  $\beta$ -D-glucan by probiotic *Lactobacillus paracasei* NFBC 338 on the physical and rheological properties of low-fat yoghurt fermented in combination with a commercial non-exopolysaccharide producing yoghurt culture (CH-1) was investigated, and data compared with control yoghurts made with non- $\beta$ -D-glucan producing probiotic

*Lactobacillus paracasei* NFBC 338 and starter culture as above. Cultures were inoculated into 1.4% (w/v) reconstituted skim milk supplemented with 5% (w/v) sucrose in a Labfors 3 fermenter and pH declined from 6.4 to 4.6 over 6 h. A temperature of 37°C was found to be optimal for the desired level of ropiness, which was reduced during fermentation at higher temperatures.  $\beta$ -D-Glucan production had no effect on culture growth and viability during fermentation and viable counts exceeded the standard required for probiotic yoghurts (FAO/WHO, 2003). Rheological measurements showed that the final value of the storage modulus ( $G'$ ) for  $\beta$ -D-glucan enriched yoghurt was consistently higher than control probiotic yoghurt after 8 h fermentation (37°C, 0.5% strain), and this along with the comparison of frequency sweeps (0.1–100 rad/s) indicated the formation of a stronger gel in the former compared with the latter product. Probiotic viability and quality characteristics of the yoghurts during shelf-life storage for 28 days are currently under investigation

### HW12/03 DEVELOPMENT OF A POLYCLONAL ANTIBODY AGAINST OPUCA IN CHICKENS FOR MEASURING THE ACTIVITY OF $\sigma^B$ IN *LISTERIA MONOCYTOGENES*

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Effective multiplication in many different environments and resistance at the early stages of infection within the host are crucial to the transmission and invasion of the foodborne pathogen *Listeria monocytogenes*. The alternative sigma factor ( $\sigma^B$ ) plays a significant role in listerial stress tolerance and many components of  $\sigma^B$  regulon have been identified. Using Western blotting, we observed that individual mutations of four genes under  $\sigma^B$  control with unknown function (*lmo0796*, *lmo0913*, *lmo2391* and *lmo2748*) do not have any influence on the levels of  $\sigma^B$  and also on the levels of two proteins predicted to participate in  $\sigma^B$  regulation – RsbW and RsbV. However, the proposed  $\sigma^B$  regulation system suggests that the activity of  $\sigma^B$  could still be affected. Growth experiments with a range of concentrations of NaCl confirmed the presence of an alternative defence against osmotic stress controlled by  $\sigma^B$  as growth of the  $\Delta$ sigB strain is impaired while the  $\Delta$ opuCA strain is not affected by salt concentrations up to 1.8 M. OpuCA which is responsible for the uptake of compatible solute carnitine and whose expression is controlled by  $\sigma^B$ , may be a good candidate protein to act as reporter of  $\sigma^B$  activity. Two chickens were immunized with the purified OpuCA protein to produce polyclonal antibodies. Chicken sera were tested for specificity by Western blotting and used for investigating the activity of  $\sigma^B$  during osmotic stress. Our data suggest that this antibody will be the useful tool in determining the activity of  $\sigma^B$  in the stress response of *L. monocytogenes*.

### HW13/01 BIOMINERALIZED PALLADIUM IS AN EFFECTIVE HYDROGENATION CATALYST

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Efficient catalysts remain a goal in the field of reaction engineering. This study aimed to develop a new heterogeneous Pd catalyst based on biologically mineralized palladium (Bio-Pd). *Desulfovibrio desulfuricans* was used to reduce Pd(II) to nanocrystalline Pd(0) held on the bacterial surface, with the biomass providing support and preventing coalescence of the palladium nanoparticles. Palladized biomass had catalytic activity in a range of applications including reductions, oxidations and hydrogenations. Processing of Bio-Pd under various conditions developed a supported palladium catalyst with a variety of Pd crystal morphologies (examined using environmental scanning electron microscopy) and thus potentially different catalytic properties. Various biomass-derived Pd preparations with different Pd loadings were evaluated in the hydrogenation of 2-pentyne and in the Heck reaction with iodobenzene and ethyl acrylate as substrates. The best preparations of Bio-Pd gave an enhanced rate and selectivity in 2-pentyne hydrogenation as compared to commercial catalysts. The catalytic performance of the native and processed bionanomaterials was different in the Heck reaction. Differences in the morphology of the metal particles related to the processing, and also changes in the metal-support interactions, will be discussed.

### HW13/02 BIOFILM ADHESION AND NON LINE-OF-SIGHT HYDROXYAPATITE BIOCOATING USING A *SERRATIA* SP.

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Biofilms are important in fouling and industry. Extracellular polymeric substance (EPS) hydrogel adheres biofilm to solid substrata. This study focused on adhesion of a *Serratia* sp. biofilm to various supports (polyurethane foam, porous glass, polypropylene and titanium alloy). A phosphatase enzyme 'tethered' within the EPS mediates hydroxyapatite (HA) biomineral growth via enzymatic liberation of inorganic phosphate into HA crystals when supplied with Ca<sup>2+</sup>. The crystallite size of bio-HA (by X-ray diffraction analysis) was substantially smaller (<50 nm) than chemically-made HA. Non-invasive magnetic resonance imaging visualized the biofilm-HA coating of surfaces within 3-D matrices. A novel micromanipulation technique was used to estimate the adhesive strength (related to the roughness of the surfaces) for native and dried biofilms, naked and mineralized with HA. Dried biofilm had adhesive strength ~20 times higher than fully hydrated biofilm and the adhesive strength of HA-biofilm was much higher than that of biofilm alone. Nanoscale bio-HA was visualized under an Environmental Scanning Electron Microscope as a > 80 µm layer of HA-biofilm coating on a titanium-disc. The biofilm system provides a non line-of-sight coating method and also a method for manufacturing nanoscale HA, which has better mechanical properties as a potential bone replacement material, than commercial HA.

### HW13/03 BIORECOVERY OF PRECIOUS METALS FROM WASTES AND CONVERSION INTO FUEL CELL CATALYST FOR ELECTRICITY PRODUCTION

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Bacteria, e.g. *Desulfovibrio desulfuricans* and *Escherichia coli*, can harness hydrogenase activity to the recovery of precious metals from wastes, reducing (e.g.) Pd(II) to Pd(0) (Bio-Pd(0)). Precious metal waste leachates are highly aggressive and are not physiologically-compatible. First, nanoscale Bio-Pd(0) was made on bacterial cells enzymatically under permissive conditions and then these nanoparticle 'seeds' were used as a chemical catalyst for reduction of Pt(IV) and Au(III) to Pt(0) and Au(0) respectively. Metals biorecovered into nanoparticles were catalytically active. A proton exchange membrane fuel cell was constructed using commercial anode catalysts in parallel with metallized bacteria using precious metals recovered from pure metal solutions and from wastes. The power outputs using pure 'bioelectrodes' and those fashioned from pure metals and from wastes were compared. 'Biorefining' of precious metals into energy materials can remove the need for expensive

precious metal refining. We show a simple two step method for converting wastes into fuel cell materials.

### HW13/04 BIORECOVERY OF URANIUM FROM MINEWATERS INTO PURE MINERAL PRODUCT AT THE EXPENSE OF PLANT WASTES

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Perceived problems of nuclear fuel fabrication, use and treatment limit the acceptability of nuclear power as an alternative to fossil fuels. Contamination occurs in nuclear fuel processing and reprocessing but it also occurs at source via run-offs from current and historic mining activities. The price of uranium (U<sub>3</sub>O<sub>8</sub>) in the 1990s was US\$10/lb but is currently US\$58/lb, peaking in 2007 at US\$135/lb. With the potential global expansion of nuclear power as an alternative to fossil fuels the market and strategic values of U are set to rise. In the 1990s a new biotechnology was demonstrated for recovery of U from minewaters as pure hydrogen uranyl phosphate (HUP). An economic assessment (assuming negligible value of U) showed that the single limiting factor as a waste treatment process was the cost of the phosphate feed supplement using an organophosphate feedstock (glycerol 2-phosphate) which contains 1 mol/mol phosphate. We describe the use of phytic acid (inositol phosphate; 6 mol phosphate/mol), a ubiquitous plant waste, to support the removal of uranium as HUP by an immobilized cell reactor and shift the focus away from just bioremediation to encompass also value product manufacturing from wastes, and hence resource efficiency.

### HW13/05 HYDROGEN PRODUCTION BY MUTANT STRAINS OF *ESCHERICHIA COLI*

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Hydrogen technology offers potentially a clean energy supply. Fermentative processes offer attractive options for bio-hydrogen production from biomass. Biomass, an abundant renewable resource, could support sustainability of the future hydrogen economy. However, fermentation of cellulosic feedstocks represents a bottleneck since enzymatic breakdown is slow. Cellulose depolymerization to simple sugars using thermochemical pre-treatment is shown as a potential method to convert cellulose to a fermentable sugar feedstock that can be fermented into hydrogen. *E. coli* is a well characterized facultative anaerobe that is potentially attractive for such biotechnological application. Anaerobically, *E. coli* converts sugars to H<sub>2</sub> and other soluble products via the mixed acid fermentation. The metabolic pathway and hydrogenase activity of *E. coli* were genetically manipulated. Several mutants were evaluated to determine the best strain of *E. coli* in terms of its ability to produce hydrogen and to make organic acids as the feed for a downstream photo-fermentation process that converts organic acids into additional H<sub>2</sub>.

### HW13/06 MICROARRAY AND PROTEOMIC ANALYSES OF TRUE FUEL CELL SYSTEMS OF *GEOBACTER SULFURREDUCTENS*

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Elucidating the mechanisms by which *Geobacter sulfurreducens* transfers electrons to the anodes of microbial fuel cells is expected to aid in the optimization of these systems. Whole-genome microarray and proteomic analysis of biofilms growing on anodes in microbial fuel cells with small anodes that prevent several electrochemical limitations revealed higher expression of the outer surface c-type cytochromes OmcS and OmcT, versus cells grown on the anode surface with fumarate as the electron acceptor. Down-regulated genes included those indicative of stress and regulatory function. Microarray analysis of the effect of low (10Ω) versus high resistance (10000Ω) indicated when resistance was low, lipoprotein and hypothetical genes were up-regulated. When resistance was high, expression of the genes for the periplasmic cytochromes, PpcB and PpcC as well as transport and biosynthesis proteins were up-regulated. Surprisingly there was no apparent increase in the expression of genes for pili or the outer-membrane c-type cytochrome OmcZ, which are highly expressed in during growth at high current densities on anodes held at an artificially high potential with a potentiostat. These results demonstrate that the physiological state of cells in anode biofilms can differ dramatically depending on the conditions for power production.

### HW13/07 COMMERCIALIZING CELLULOSIC ETHANOL

CLAIRE MERCIER

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Globally, momentum is building to deliver commercially viable advanced biofuels and in particular cellulosic ethanol. There are a number of technically feasible routes but the challenge remains the delivery of a commercially viable solution to market. For microbially generated cellulosic ethanol, three major hurdles are the high capital cost, the high cost of enzymes used to deliver a fermentable sugar stream and the ability of ethanologens to efficiently convert these mixed sugars into ethanol.

TMO has developed a thermophilic ethanologen (TM242) which effectively reduces the barriers to commercialization. This presentation will illustrate TM242's innate ability to utilize multiple oligomeric sugars in addition to monomeric hexose and pentose sugars. The impact of this is our ability to reduce enzyme loadings partnered with a relatively mild pretreatment that significantly reduce capital and operating costs. TMO have built the UK's first operational cellulosic ethanol facility and we will update on progress.

*The fuel of TMOrow, here, now.*

### HW13/08 GLYFINERY – A BIOREFINERY FOR THE SUSTAINABLE AND INTEGRATED PRODUCTION OF LIQUID BIOFUELS, GREEN CHEMICALS AND BIOENERGY FROM GLYCEROL

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Biofuels are of increasing importance as we aim to reduce our dependence on fossil fuels and find alternative, sustainable energy sources. The most common biofuel alternative to fossil fuels in Europe is biodiesel where production has accelerated from close to 500,000 tons in 1998, to 6 million tons in 2006, and is expected to further increase to 10 million tons in 2010. The biodiesel produced is a renewable, biodegradable biofuel, produced from plant oils and animal fats.

One concern with biodiesel processes is that they generate a large surplus of the by-product glycerol. With the increasing focus on sustainable alternatives to fossil fuels, a global glut of glycerol is occurring. This surplus represents a considerable waste of potential energy and resources with much of the surplus being disposed of by incineration. A potential new, alternative technology for glycerol processing can be biological conversion of glycerol into liquid biofuels, green chemicals and bioenergy in fermentation processes. The GLYFINERY project provides an integrated solution for sustainable management of glycerol, a solution being not only environmentally friendly but also economically feasible. The concept incorporates three product streams to alcohols, green-chemicals and biogas directly from the crude glycerol waste.

#### HWI4/01 WHAT DRIVES MICRO-ECOSYSTEM STRUCTURE AND FUNCTION IN ANTARCTIC SOILS: A TOP-DOWN BOTTOM-UP MODELLING APPROACH

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The Antarctic Peninsula is one of the fastest warming regions of the planet where daily soil temperature fluctuations about 0°C are common. With annually increasing temperatures, profound changes in future microbial community composition, nutrient cycling and energy flow are likely to occur. We investigate the potential impacts of climatic and environmental factors on soil microbial communities using samples collected from sites along a latitudinal gradient (53–74°S). Soils were chemically analysed and biologically profiled using ELFA analysis. We created a conceptual model of how environment and resource drivers impact on soil microbial community structure and test it using Structural Equation Modelling with the ELFA data and environmental characteristics of sites.

#### HWI4/02 STUDY OF *FLAVOBACTERIUM* STRAINS ISOLATED FROM ANTARCTIC AQUATIC AND TERRESTRIAL SAMPLES

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The microbial diversity on Antarctica is largely under-explored. As part of the AMBIO-project that aims to explore bacterial distribution patterns in Antarctica, nine samples, both terrestrial and aquatic, from different regions were investigated. Isolations were made using several conditions and strains were then subjected to rep-PCR fingerprinting as a fast screening to eliminate duplicate isolates. Cluster analysis of fingerprint patterns using BioNumerics software revealed a number of clusters (cut-off level 80%) of similar strains and a number of separate isolates. Representatives were used in partial 16S rDNA sequencing to obtain a first approximate identification. The results show a large diversity, distributed over the major phylogenetic groups and only little overlap between the samples. We focussed on the genus *Flavobacterium* because several of the isolated clusters and strains that were found in this genus show low similarity values with neighbouring sequences in the EMBL-database and thus may represent new species. The 16S rDNA sequence was completed and fatty acid analysis as well as some phenotypic tests were performed. A housekeeping gene, *gyrB*, is being sequenced and, if required DNA-DNA hybridizations will be carried out, to establish whether these isolates can be described as new species.



## POSTERS

## CONTRIBUTION OF THE GLOBAL N CYCLE TO GLOBAL PROCESSES

**HW17/01 MOLECULAR ANALYSIS OF CRENARCHAEAL AUTOTROPHY IN SOIL AND SEDIMENTS**

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Carbon isotopic measurements of crenarchaeal lipids extracted from sediments and ocean waters suggest that up to 83% of the production of these crenarchaeal communities result from autotrophic CO<sub>2</sub> fixation. Recent cultivation of an ammonia-oxidizing, carbon-fixing crenarchaeon, *Nitrosopumilus maritimus*, confirmed that autotrophy exists in mesophilic crenarchaea, and analysis of its genome suggests that CO<sub>2</sub> fixation is achieved through the 3-hydroxypropionate/4-hydroxybutyrate assimilation pathway. The aim of this study was to develop a PCR-based approach to characterize crenarchaeal CO<sub>2</sub> fixation potential in the environment. PCR primers targeting specifically the crenarchaeal genes of the 4-hydroxybutyryl-CoA dehydratase, a key enzyme of *N. maritimus* CO<sub>2</sub> fixation pathway, were designed, based on sequences obtained from crenarchaeal genomes and metagenomic fragments. PCR amplifications performed on DNA extracts from a grassland soil and estuarine sediments yielded specific products of 430 bp that were subsequently cloned and sequenced. Phylogenetic analysis of the sequences confirmed that they were derived from organisms placed within crenarchaeal lineages. The gene fragment targeted by our primers was also shown to be suitable for denaturing gradient gel electrophoresis (DGGE) fingerprinting. In conclusion, the newly designed primers enable a PCR-based analysis of a crenarchaeal carbon fixation genes which, combined with stable isotope probing, provides a useful tool to investigate crenarchaeal autotrophy.

**HW17/02 IS SOIL pH THE MAJOR FACTOR DETERMINING THE PHYLOGENETIC DISTRIBUTION OF TERRESTRIAL AMMONIA-OXIDIZING ARCHAEA AND BACTERIA?**

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Nitrification is a central component of the global nitrogen cycle. Ammonia oxidation, the first step of nitrification, is performed both by ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA) in terrestrial ecosystems. Published studies based at

several locations have indicated that soil pH may be a critical factor in controlling the size and abundance of AOA and AOB populations. To determine whether soil pH or other environmental factors are major drivers at a global scale, ammonia monooxygenase sub-unit A (*amoA*) and 16S rRNA gene sequences were retrieved from public databases together with the corresponding published environmental data (including soil management, vegetation and soil pH). Phylogenetic analyses were performed and results indicate that the geographical origin (at a global scale) does not determine the presence of specific populations. Furthermore, this meta-analysis supported observations made at small spatial scales and site-specific analyses, specifically the importance of pH in determining the structure of ammonia-oxidizing communities.

**HW17/03 CRENARCHAEAL NITRIFICATION IN AN ACIDIC FOREST PEAT SOIL**NEJC STOPNISEK<sup>1</sup>, CÉCILE RANGIN<sup>2</sup>, SPELA HOFFERLE<sup>1</sup>, GRAEME NICOL<sup>2</sup>, INES MANDIC-MULEC<sup>1</sup> & JIM I. PROSSER<sup>2</sup><sup>1</sup>Dept of Food Science & Technology, Chair of Microbiology, Biotechnical Faculty, University of Ljubljana, Vecna pot 111, 1000 Ljubljana, Slovenia; <sup>2</sup>Institute of Biological & Environmental Sciences, University of Aberdeen, Cruickshank Building, St Machar Drive, Aberdeen AB24 3UU

*Crenarchaeota* are found in marine and terrestrial environments where they frequently outnumber bacterial ammonia oxidizers, in terms of abundances of *amoA* genes, a key functional gene that encodes ammonia monooxygenase subunit A. This study aimed to determine the relative importance of crenarchaeal and bacterial ammonia oxidizers for nitrification in an acidic forest peat soil from Ljubljana marsh, Slovenia. 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 in soil microcosms during incubation for 20 days after addition of different concentrations of ammonia. Phylogenetic analysis, based on archaeal 16S rRNA genes, showed high diversity among crenarchaea, with two dominant clades belonging to *Crenarchaeota* groups 1.1c and 1.3. Denaturing gradient gel electrophoresis profiles of *amoA* gene transcripts demonstrated changes in the community structure of ammonia oxidizing crenarchaea, but not bacteria, during incubation, providing evidence of nitrification activity of particular phylotypes. Furthermore, qPCR quantification of crenarchaeal *amoA* demonstrated crenarchaeal, but not bacterial ammonia oxidizer growth. Thus, our findings suggest that crenarchaea dominate ammonia oxidation in this acidic soil.

**FM/01 REGULATION OF THE NITRITE REDUCTASE ANIA BY FNR IN NEISSERIA MENINGITIDIS**JAMES EDWARDS<sup>1</sup> & JAMES MOIR

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*Neisseria meningitidis* encodes a truncated denitrification pathway, allowing for nitrite to be used as an alternative electron acceptor when environmental oxygen concentrations are low. Regulation of the expression of the nitrite reductase AniA is controlled by several transcriptional regulators, one of which is the oxygen sensor FNR.

Working in an anaerobic environment we have shown that purified FNR (NMB0380) can bind the promoter sequence of *aniA* and that upon exposure to oxygen the 4Fe4S cofactor of FNR rapidly undergoes a switch to a 2Fe2S form that is unable to bind DNA. These findings are consistent with those seen with the *E. coli* FNR. However, we find that the rate at which oxygen treated FNR dissociates from DNA *in vitro* is slower than that of wild-type *E. coli* FNR but similar to an *E. coli* FNR\* (aerobically active) mutant. This probably reflects the physiology of *N. meningitidis* which is unable to grow anaerobically, and therefore has evolved a higher tolerance to oxygen. We are currently interested in the role of the transcriptional repressor NsrR (NMB0437) that binds to a site overlapping that of FNR and the role this protein takes in displacing DNA bound FNR.

**FM/02 THE CRYSTAL STRUCTURE OF A FAMILY GH-25 LYSOZYME FROM BACILLUS ANTHRACIS IMPLIES A NEIGHBOURING-GROUP CATALYTIC MECHANISM WITH RETENTION OF ANOMERIC CONFIGURATION**

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Lysozyme is a generic term given to describe hydrolytic enzymes which cleave the  $\beta$ -1,4-glycosidic bond between *N*-acetylmuramic acid (NAM) and *N*-acetylglucosamine (NAG) in the carbohydrate backbone portion of bacterial peptidoglycan. Lysozymes are found in many of the sequence-based families of glycoside hydrolases ([www.cazy.org](http://www.cazy.org)) where they show considerable structural and mechanistic diversity. Lysozymes from glycoside hydrolase family GH25 adopt a  $(\alpha/\beta)_5(\beta)_3$ -barrel like fold with a proposal in the literature that these enzymes act with inversion of anomeric configuration; the lack of a suitable substrate, however, means that no group has successfully demonstrated the configuration of the product. The 3-D structure of the GH25 enzyme from *Bacillus anthracis* at 1.4 Å resolution shows that the active-centre is extremely similar to those from GH18, 20, 56, 84 and 85 implying that, in the absence of evidence to the contrary, GH25 enzymes act with net retention of anomeric configuration using a neighboring-group catalytic mechanism common to this 'super-family' of enzymes.

**FM/03 STOMP: STOCHASTIC MODELLING FOR PROKARYOTES**ROSALIND J. ALLEN<sup>1</sup>, DOV J. STEKEL<sup>2</sup> & A. JAMIE WOOD<sup>3</sup><sup>1</sup>University of Edinburgh, EH9 3JZ; <sup>2</sup>University of Nottingham, B15 2TT; <sup>3</sup>University of York, YO10 5YW

StoMP is a 3-year research network funded by BBSRC under the 'Mathematical Tools for Systems Biology' initiative. Its objectives are to bring together UK microbiologists and modellers, nucleate productive collaborations and foster the development of a vibrant community at the modelling-microbiology interface. StoMP organized a well-received training workshop in Birmingham in 2009 and a research conference in Edinburgh in 2009. A number of more focused 'satellite' meetings are planned for 2009–10. New members are welcome to join at any time ([www.stompnet.org](http://www.stompnet.org)). This poster will present a review of StoMP's recent activities and plans for the future.

**FM/04 DISSECTING THE FUNCTION OF THE OUTER MEMBRANE PROTEINS OF THE GASTRIC PATHOGEN HELICOBACTER PYLORI**MATTHEW MCCLUSKEY<sup>1</sup>, JENI LUCKETT<sup>1</sup>, BARRIE KELLAM<sup>2</sup> & KIM R. HARDIE<sup>1</sup><sup>1</sup>School of Molecular Medical Sciences, <sup>2</sup>School of Pharmacy, University of Nottingham, Centre for Biomolecular Sciences, University Park, Nottingham NG7 2RD

*Helicobacter pylori* infects half of the world's population and causes a range of gastric infections including gastroenteritis, peptic ulcers and gastric cancer. The ability for this organism to adhere to the gastric epithelium is an important first step in its colonization and subsequent infection. Adhesion is accomplished by utilizing a range of binding adhesins, which

are currently poorly understood. The Blood group Antigen Binding adhesin, BabA, binds to the ABO blood group antigen Lewis-B (Le<sup>b</sup>), and is one of the best studied outer membrane proteins of *H. pylori*. BabA belongs to the *H. pylori* Outer membrane Protein (Hop) family, which are characterized by an N-terminal signal peptide and C-terminal membrane anchor that surround a central functional domain likely to be exposed on the cell surface. This architecture is reminiscent of the autotransporter proteins. Here we describe the construction of molecular tools incorporating Hop functional domains and autotransporter secretion signals that will enable a systematic analysis of Hop function.

**FM/05 THE METABOLIC IMPACT OF SILVER(I) ON SHEWANELLA ONEIDENSIS**HUI WANG<sup>1</sup>, NICHOLAS LAW<sup>1</sup>, GERALDINE PEARSON<sup>1</sup>, BART E. VAN DONGEN<sup>1</sup>, ROY GOODACRE<sup>2</sup> & JONATHAN R. LLOYD<sup>1</sup><sup>1</sup>School of Earth, Atmospheric & Environmental Sciences & Williamson Research Centre of Molecular Environmental Science, University of Manchester, M13 9PL; <sup>2</sup>School of Chemistry, University of Manchester, Manchester Interdisciplinary Biocentre, 131 Princess Street, Manchester M1 7DN (Corresponding author: Jonathan R. Lloyd; Email [jon.lloyd@manchester.ac.uk](mailto:jon.lloyd@manchester.ac.uk); Tel. 0161 275 7155; Fax 0161 306 9361)

Anaerobic culture *Shewanella oneidensis* MR-1, reduced toxic Ag(I) forming nanoparticles of elemental Ag(0), as confirmed by X-ray diffraction analyses. The addition of 1–50  $\mu$ M Ag(I) had limited impact on growth, but 100  $\mu$ M Ag(I) reduced both the doubling time and cell yields. At this higher concentration of added silver, transmission electron microscopy showed the accumulation of elemental silver particles within the cell, while at lower concentrations the metal was exclusively reduced and precipitated outside the cell. Whole organism metabolite fingerprinting, using the method of Fourier transform infrared spectroscopy analysis of cells grown at a range of silver concentrations, confirmed that there were significant physiological changes at 100  $\mu$ M silver, with principal component-discriminant function analysis (PC-DFA) loading plots highlighting changes in the following functional groups: notably lipids, Amide I, II and nucleic acids. Molecular analyses confirmed a dramatic drop in cellular yields of both the phospholipid fatty acids and their precursor molecules at high concentrations of silver, suggesting that structural integrity of the cellular membrane was compromised at high silver concentrations, resulting in intracellular accumulation of the toxic metal.

**Keywords** *Shewanella oneidensis* MR-1, silver, biocide, metal toxicity, Fourier transform infrared spectroscopy, metabolomics, phospholipids, fatty acids

**FM/06 THE THREE-DIMENSIONAL STRUCTURE OF THE TYPE IV PILUS BIOGENESIS MEMBRANE PROTEIN PilC FROM THERMUS THERMOPHILUS**

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Type IV pili are retractable fibres found on the surface of many Gram-negative bacteria. These pili mediate diverse cellular functions, including host-cell adhesion, surface motility, biofilm formation, cell signalling and DNA uptake. PilC is an integral inner membrane protein and a central component of the type IV pilus biogenesis system. The aim of this project is to determine the structure and characterize the function of this membrane protein. PilC from *Thermus thermophilus* was expressed in *E. coli*, extracted from the membrane by detergent extraction and purified by metal ion affinity chromatography. It was shown to be tetrameric by native polyacrylamide gel electrophoresis and size exclusion chromatography. A three-dimensional (3-D) electron microscopy reconstruction of the PilC multimer was determined using single particle analysis and cryo-electron microscopy. It revealed an elongated structure with C2 or C4 symmetry. A soluble N-terminal domain from PilC was also expressed and purified: the crystal structure showed two helical N-domains, forming an asymmetric dimer. We propose that PilC plays a major role in type IV pilus formation by interacting with other cytoplasmic or periplasmic components of the biogenesis system.

**FM/07 INVESTIGATION OF A POTENTIAL PROTEIN GLYCOSYLATION SYSTEM IN HELICOBACTER PULLORUM**LAURA E. YATES<sup>1</sup>, REBECCA H. LANGDON<sup>1</sup>, DENNIS LINTON<sup>2</sup> & BRENDAN W. WREN<sup>1</sup><sup>1</sup>London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E 7HT; <sup>2</sup>University of Manchester, Faculty of Life Sciences, Michael Smith Building, Oxford Road, Manchester M13 9PT

In 2002, the first bacterial N-linked protein glycosylation system was identified in *Campylobacter jejuni*. Further *pglB*s were identified by degenerate PCR in two species of *Helicobacter*, *H. pullorum* and *H. canadensis*. The genomes of these two organisms were subsequently sequenced, and found to contain orthologues for all the genes within the *Campylobacter* system, although not in a single locus. Of particular interest was the discovery that the genomes contain two genes which appear similar to the oligosaccharyltransferase, *pglB*. To determine whether this *Helicobacter* system is in fact involved in protein glycosylation, we plan to carry out complementation studies in *Escherichia coli*. (The *Campylobacter jejuni* glycosylation system has previously been shown to be functional when expressed in *E. coli* together with an appropriate glycoprotein acceptor). We will attempt to restore glycosylation in *E. coli* constructs lacking functional *C. jejuni pglC* or *pglE* genes by complementation with the respective *Helicobacter* gene. We then aim to investigate the significance of the two *pglB* genes, and determine whether both are required for glycosylation or whether one is sufficient.

#### FM/08 ELUCIDATING THE FUNCTION OF THE *mce* GENE CLUSTER IN *STREPTOMYCES COELICOLOR*

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*Streptomyces coelicolor* is a non-pathogenic Gram positive soil-dwelling bacterium. It is a member of the actinomycetes, an order which includes human pathogens *Mycobacterium tuberculosis*, *M. leprae* and *Nocardia farcinica*. The *mce* (mammalian cell entry) operon is a conserved cluster of nine core genes found in varying copy number throughout the actinobacteria. *S. coelicolor* contains a single copy of the operon. Studies in *M. tuberculosis* indicate that the *mce* region is involved in invasion of and survival within macrophages during lung infections, while bioinformatic analysis suggests that the operon encodes an ABC importer-like system.

The *S. coelicolor mce* operon consists of a putative ATPase, two transmembrane domains and six *mce* genes (A-F) which have predicted substrate-binding functions. Disruption mutants in the ATPase, both transmembrane domains, and *mceB*, *D* and *E* have been created along with a complete cluster knockout. Mutants were screened for phenotypes using several different approaches including interaction with amoebae. RT-PCR and GFP localization studies have been undertaken to establish expression profiles. The results suggest that the *S. coelicolor mce* operon may play an important role in survival of these organisms in the soil environment. Further work is being undertaken to elucidate the biochemical mechanism of the *mce* cluster.

#### FM/09 UNRAVELLING CYANOBACTERIAL SIGNALLING COMPOUNDS

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Cyanobacterial blooms are a great concern, because of the toxins they produce. These toxins pose a threat to human health and safety. Of particular interest are microcystins. In previous studies it has been found that the presence of a non-toxic competitor (*Planktothrix agardhii*) increases the toxin production in the toxic cyanobacterium *Microcystis aeruginosa*. It is believed that a signalling compound released by *P. agardhii* is responsible for this. Applying a novel stable isotope (<sup>15</sup>N) labelling protocol allows the determination of de novo toxin.

This was successfully used to determine de novo synthesis in *M. aeruginosa* in the presence of extracts from *P. agardhii*. The results suggest that there is a cyanobacteria allelochemical that is capable of strongly affecting the toxin production in another cyanobacterium. It has the capability of increasing toxin production. Most surprisingly, however, it was found that there were strong inhibiting effects inherent in the signalling compound-free spent medium.

#### FM/10 INVESTIGATING THE ROLE OF LIPOPOLYSACCHARIDE ALTERATIONS IN THE *SINORHIZOBIUM MELILOTI*-ALFALFA SYMBIOSIS

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*Sinorhizobium meliloti* forms a beneficial interaction with the legume alfalfa and the *BacA* protein has been shown to be essential for *S. meliloti* persistence within the plant cell. An *S. meliloti bacA* null mutant has a 50% reduction in its lipopolysaccharide (LPS) very-long-chain fatty acid (VLCFA) content. This led to the hypothesis that the altered LPS could account for the symbiotic defect of this mutant. To investigate this further, we constructed and characterized *S. meliloti* mutants lacking either *AcpXL* (VLCFA acyl-carrier protein) or *LpxXL* (VLCFA acyl-transferase). Despite lacking the LPS VLCFA in their free-living state, these mutants could form a successful symbiosis but produced aberrantly shaped bacteroids that appeared to be prematurely senescing. This shows that the LPS-VLCFA modification plays an important role in bacteroid development. Since the *acpXL* and *lpxXL* genes form part of a cluster of genes predicted to be involved in LPS VLCFA biosynthesis, we also constructed and are characterizing mutants in these genes and have shown that they are also involved in the biosynthesis of the LPS-VLCFA modifications and show similar phenotypes to the *acpXL* and *lpxXL* mutants. This research will help to understand the role of VLCFA-modified LPS in establishing and maintaining the *Sinorhizobium*-legume symbiosis.

#### FM/11 NOVEL *nodE*-MEDIATED SIGNALLING SPECIFICITY DURING NODULATION OF PEAS BY *RHIZOBIUM LEGUMINOSARUM*

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Legume nodulation requires *Rhizobium*-made nodulation (Nod) factors, which are chitin oligomers carrying an N-linked acyl group and other substituents (such as acetate or sulphate) that determine which legumes (pea, alfalfa) that can be nodulated. In *Rhizobium leguminosarum* biovar *viciae* (which nodulates peas), *nodE* determines a C18:4 N-linked acyl group on the Nod-factor; *nodE* mutants carry a C18:1 acyl group instead. Some strains of *R. leguminosarum* also carry *nodX*, which determines an acetyl group on the Nod factor. The *nodX* gene enables nodulation of pea varieties (such as cultivar Afghanistan) that are resistant to nodulation by most *R. leguminosarum* strains; this resistance to nodulation in peas segregates as a single locus (*SYM2*). Surprisingly a *nodX*-containing strain of *R. leguminosarum* carrying a mutation in *nodE* was unable to nodulate cv. Afghanistan peas. Using an introgression line of pea, the resistance to nodulation (by the *nodE* mutant) was shown to map near *SYM2*. Other lines of pea are nodulated poorly by the *nodE* mutant; we are using genetic mapping to determine if this resistance also maps to *SYM2*. This should determine if resistance to nodulation of *nodE* and *nodX* mutants of *R. leguminosarum* is determined by one or two loci in pea.

#### FM/12 A NOVEL MECHANISM OF COUPLING QUORUM SENSING TO GENE REGULATION IN *RHIZOBIUM LEGUMINOSARUM*

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Rhizobia are important for their ability to form nitrogen-fixing nodules on legume plants. In several rhizobia, this symbiosis has been shown to be influenced by quorum-sensing gene regulation, which allows them to regulate their gene expression in a population dependent way. Typically, the quorum-sensing systems consist of two genes. One encodes an enzyme that synthesizes signalling molecules (acyl homoserine lactones or AHLs), and the other encodes a transcriptional regulator that can regulate specific genes in response to the AHLs made.

In *Rhizobium leguminosarum* bv. *viciae*, which nodulates peas, the *cin* quorum sensing system is involved in regulating exopolysaccharide synthesis and processing and also acts as a master regulator for the control of the other quorum sensing systems. The *cin* system is unusual because it contains a third gene, encoding a small protein without homology to proteins outside of rhizobia. This protein, CinS, acts independently of AHLs and we provide evidence that it functions via a novel mechanism by directly interacting with a transcriptional regulator.

## POSTERS

### FUNDAMENTAL MICROBIOLOGY

(continued)

#### FM/13 ISOLATION OF A NITRILASE GENE FROM NOVEL BACTERIAL STRAINS, SCREENING ENVIRONMENTAL SOILS FOR THE GENE BY REAL-TIME PCR AND EXPRESSION OF THE NITRILASE FROM *RHODOCOCCUS ERYTHROPOLIS* AJ270 IN *ESCHERICHIA COLI*

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Nitrile metabolism has been extensively studied in numerous bacterial species, due to the potential application of the associated enzymes for biotransformation and bioremediation. Four isolates, *Microbacterium* sp. AJ115, *Rhodococcus erythropolis* AJ270, AJ300 and ITCBP, are known nitrile-metabolizers, and harbour both a two-step nitrile hydratase/amidase enzyme system and a one-step nitrilase system. The isolates are from two different geographic locations but contain identical nitrilase (*nit*) genes, suggesting the possibility of this gene being widespread in the environment due to horizontal gene transfer. Real-time PCR analysis has also indicated that the *nit* gene in these strains is carried on an unstable, variable copy number mobile genetic element. Environmental soil samples have been screened for *nit* using real-time PCR, resulting in the isolation of several novel *Burkholderia* sp. that harbour the *nit* gene. Several *Burkholderia* sp. that harbour a very similar nitrilase gene have also been isolated. Cloning and expression of the nitrilase gene from strain AJ270 in *E. coli* has allowed the determination of nitrilase activity. The enzyme appears to be very substrate specific with phenylacetone nitrile the only substrate identified to date.

#### FM/14 *BURKHOLDERIA MULTIVORANS* GENES INDUCED IN SOIL ENVIRONMENT

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In order to understand behavioral and adaptive strategies of *B. multivorans* ATCC 17616 in soil environment, we conducted IVET (*in vivo* expression technology) screening to identify that are not expressed under laboratory conditions but specifically induced in the soil environment. The screening of a large-scale library consisting of about 50,000 clones and subsequent identification of the loci, we obtained a list of 350 mls (*B. multivorans* ATCC 17616 loci induced in soil). These 350 loci were classified into functional categories and found to be biased to four categories, transport and metabolism (50%), transcriptional regulator (10%), stress response (10%), and cell wall modification (10%). These mls were scored for i) number of times found from the library, ii) expression in liquid media supplemented with soil extracts. For some of the mls we constructed mutants and tested for their ability to compete with the wild-type cells *in vivo*. We found that although anthranilate dioxygenase gene (*andAc*) is not induced by the soil extracts *andAc* is essential to survive in the soil environment. These results suggest that the versatile catabolic ability of ATCC 17616 plays important role in living and surviving in the soil environment.

**ID/01 VACCINATION AGAINST *DERMANYSSUS GALLINAE* IN POULTRY WITH BM86 AND SUBOLESIN RECOMBINANT PROTEINS**DAVID HARRINGTON<sup>1</sup>, MARIO CANALES<sup>2</sup>, JOSÉ DE LA FUENTE<sup>2,3</sup>, CARLOS DE LUNA<sup>1</sup>, KAREN ROBINSON<sup>4</sup>, JONATHAN GUY<sup>1</sup> & OLIVIER SPARAGANO<sup>1</sup><sup>1</sup>School of Agriculture, Food & Rural Development, Newcastle University, Newcastle upon Tyne NE1 7RU; <sup>2</sup>Instituto de Investigación en Recursos Cinegéticos IREC (CSIC-UCLM-JCCM), Ronda de Toledo s/n, 13005 Ciudad Real, Spain; <sup>3</sup>Dept of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK 74078, USA; <sup>4</sup>Institute of Infection, Immunity & Inflammation, Centre for Biomolecular Sciences, University of Nottingham, University Park, Nottingham NG7 2RD

*Dermanyssus gallinae* has a worldwide distribution and is considered to be the most serious and economically significant ectoparasite affecting egg-laying poultry in Europe. Recombinant Bm86 and subolesin proteins derived from *Boophilus microplus* ticks and *Aedes albopictus* mosquitoes were used to immunize poultry in an attempt to control *D. gallinae* *in vitro*. Immunization with subolesin and Bm86 stimulated different profiles of IgY response, whilst Bm86 but not subolesin were recognized by IgY on western blots. Orthologues for Bm86 were not found in *D. gallinae* by PCR, but a 150 bp fragment aligned with mammalian akirin 1 and a 300 bp fragment aligned with *Amblyomma hebraeum* were amplified by subolesin PCR. *D. gallinae* mortality after feeding was 35.1% higher ( $P = 0.009$ ) in the Subolesin group and 23% higher (not significant) in the Bm86 compared to the Control group. Thus it can be concluded that immunization with recombinant subolesin can stimulate a protective response in laying hens against *D. gallinae*.

**ID/02 INVESTIGATION OF THE ACTIVITIES OF FILAMENTOUS FUNGI IN THE POST-HARVEST SPOILAGE OF SWEET POTATO (*IPOMEA BATATAS*)**

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Sweet potato samples were stored at three different temperatures, 13°C, 21°C and 29°C for a period of four weeks. Samples were collected over the storage period and analysed on potato dextrose agar (PDA) for the presence of filamentous fungi. Sub-culturing was carried out until axenic cultures were obtained. Biomolecular methods which include fungal genomic DNA extraction, the polymerase chain amplification of ribosomal DNA, as well as sequence determination were employed in the characterization and identification of the fungal isolates. Results revealed six (6) different fungi, namely; *Penicillium chrysogenum*, *Mucor circinelloides*, *Penicillium expansum*, *Cladosporium cladosporioides*, *Penicillium brevicompactum* and *Penicillium crustosum*. To confirm the abilities of these organisms to cause tissue deterioration in sweet potato, they were re-inoculated on to fresh sweet potato samples. All the isolates produced evidence of tissue deterioration in both whole tubers and slices of sweet potato.

**ID/03 CHARACTERIZATION OF *ENTEROCOCCUS FAECALIS* OGIRF EXCRETED PROTEINS**

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*E. faecalis* is an opportunistic pathogen capable of causing wound infections, bacteraemia, endocarditis and urinary tract infections. *E. faecalis* recently gained notoriety due to transfer of vancomycin resistance to *S. aureus*. Virulence mechanisms are poorly understood in this bacterium and understanding its physiology is pivotal in identifying novel drug targets.

Gelatinase (GelE) is a protease that regulates the excreted protein repertoire of *E. faecalis* including SalB. SalB was previously shown to be up-regulated under stress conditions, particularly bile salt stress, and mediates binding to ECM molecules.

The excreted protein profile of *E. faecalis* OGIRF was altered in a *salB* deletion mutant and stress related proteins were shown to be up-regulated relative to the wild type using 2D DIGE and MALDI-TOF analysis. Fluorescence microscopy of growing cells showed cell separation anomalies that were exacerbated in a *gelE/salB* mutant. Histidine-tagged SalB was over-expressed in *E. coli* and purified and exhibited peptidoglycan (PG) hydrolytic activity as evidenced by renaturing gels and RP-HPLC/Mass spectroscopy.

Taken together, these data suggests that SalB is post-translationally regulated by GelE and is an endopeptidase active on the PG cross-linking and plays an important role in cell wall dynamics.

**ID/04 MULTIDRUG-RESISTANT NON-TYPHOIDAL SALMONELLAE IN BACTERAEMIC PATIENT FROM DEMOCRATIC REPUBLIC OF CONGO AND UGANDA**MANAR NAJIM AL-MASHHADANI<sup>1</sup>, JOHN CHEESBROUGH<sup>2</sup>, MARTIN O'KONG<sup>2</sup>, NEIL FRENCH<sup>2</sup> & CHRISTOPHER M. PARRY<sup>1</sup><sup>1</sup>Dept of Medical Microbiology & Genitourinary Medicine, University of Liverpool, L69 3GA; <sup>2</sup>London School of Hygiene & Tropical Medicine, WC1E 7HT

Background Non-Typhoidal Salmonellae (NTS) are a frequent cause of community-acquired bacteraemia in children under 5 years and adults with HIV infection in sub-Saharan Africa. Resistance to antimicrobial agents in NTS contributes to morbidity and mortality.

**Methods** Disc susceptibility and minimum inhibitory concentration testing were performed, using CLSI guidelines, on blood isolates of NTS, collected from children in Kimpese, Democratic Republic of Congo (DRC) (1998–1992; N=252) and adults in Entebbe and Kampala, Uganda (1996–1998; 2002–2003; N=105).

**Results** The majority of isolates at both locations were serovar Typhimurium or Enteritidis and resistance to the commonly used antibiotics was common. In the DRC resistance in Typhimurium to amoxycillin was 80%, co-trimoxazole 81% and gentamicin 75% with the resistance pattern ASSuTmGK occurring in 55%. In Enteritidis isolates resistance to amoxycillin were 80%, chloramphenicol 81% and tetracycline 84% with the pattern ACTSSuTm occurring in 48%. In Uganda, resistance in Typhimurium increased between 1996–8 and 2002–3 for amoxycillin (53–85%), chloramphenicol (3–54% and co-trimoxazole (53–83%). In 2002–3 54% were ACSSuTm resistant. For Enteritidis resistance increased for amoxycillin (53–92%) and co-trimoxazole (30–93%) but declined for chloramphenicol from 47 to 25%. In 2002–3 85% of isolates were resistant to ATSSuTm. All isolates were susceptible to extended spectrum cephalosporins but 5% of 2002–3 Uganda isolates had reduced susceptibility to ciprofloxacin.

**Conclusion** Resistance to commonly used antimicrobials is common in invasive NTS but varies according to location, time and serovar. Low level resistance to ciprofloxacin is emerging in Uganda.

**ID/05 GENETIC BASES OF FLUOROQUINOLONE RESISTANCE IN NON-TYPHOIDAL SALMONELLA ISOLATES FROM LIVERPOOL, UK BETWEEN 2003 AND 2007**

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**Introduction** Ciprofloxacin is commonly used for treating invasive non-typhoidal salmonellosis (NTS). Low-level (MIC 0.1–1.0 µg/ml; CipL) and high-level resistance (>1.0 µg/ml; CipR) is emerging due to point mutations in the genes encoding the target enzymes DNA gyrase and topoisomerase IV and also the acquisition of the plasmid mediated *qnr* genes.

**Methods** Disc diffusion tests and MICs using the CLSI methods were performed on a selection of NTS isolates from faeces or blood from patients attending the Royal Liverpool University Hospital (2003–2007). Point mutations in *gyrA* and *parC* and the *qnr* genes were detected by PCR and sequencing. Strains were typed by serotyping, phage typing and PFGE (using XbaI & SpeI).

**Results** 311 NTS isolates were studied. Serovar Enteritidis the most common (65%). CipL was detected in 72/311 (23%) and CipR in one isolate. 70/73 (96%) of these isolates were also nalidixic acid resistant. Resistance to other antibiotics was 13% or less. All serovar Enteritidis PT1 and PT21 (29), Virchow (6) and 7/11 Newport isolates had CipL resistance. In 22/29 of the Enteritidis isolates a *gyrA* point mutation (GAC to TAC) causing Asp87Tyr was detected. These isolates shared an identical PFGE pattern. A *qnr* gene was detected in three isolates (*qnrS* gene in Enteritidis (1), Corvallis (1); *qnrB* in Typhimurium (1)).

**Conclusion** CipL was the most common resistance phenotype in NTS isolates in Liverpool and this has implications for the treatment of invasive disease. CipL was associated with particular serovars, phage types and point mutations in *gyrA*.

**ID/06 IDENTIFICATION OF GLYCOPROTEINS IN THE BIOTREAT AGENT *BURKHOLDERIA PSEUDOMALLEI***A.E. SCOTT<sup>1</sup>, T.P. ATKINS<sup>1</sup>, S.M. TWINE<sup>2</sup> & J.L. PRIOR<sup>1</sup><sup>1</sup>Biomedical Sciences Dept, Dstl Porton Down, Salisbury SP4 0JQ; <sup>2</sup>National Research Council Institute for Biological Sciences, Ottawa, ON, Canada



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*Burkholderia pseudomallei* is the causative agent of melioidosis, a debilitating disease with mortality rates often exceeding 40% despite appropriate antibiotic treatment. This, combined with a relatively low infectious dose through the aerosol route and its presence on the CDC list of bio-threat agents has led *B. pseudomallei* to be considered a potential biological warfare agent. A range of proteins from *B. pseudomallei* have been tested as vaccine candidates. However, despite homologues of these proteins being efficacious as vaccines for other bacteria, these proteins do not elicit protection against *B. pseudomallei*. Glycoproteins are molecules which contain both protein and sugar. In many cases the glycan moiety significantly alters the immunogenicity of the protein such that an immune response mounted against the naked protein would not recognize the native glycoprotein. Thus, identification of glycoproteins in *B. pseudomallei* will generate a list of putative vaccine candidates for further study which may elicit a more effective immune response and therefore a more efficacious vaccine. In this poster we detail the isolation and identification of glycoproteins of *B. pseudomallei* and subsequent downstream characterization of these glycoproteins.

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#### ID/07 CAPSULE SACCHARIDE BIOSYNTHESIS IN *BURKHOLDERIA PSEUDOMALLEI*

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The bacterium *Burkholderia pseudomallei* causes melioidosis, a common cause of community acquired infection in Thailand and North-East Australia. This bacterium has been identified as a potential bioterror agent, as it requires complicated treatment and shows high mortality in patients presenting with severe infections. Consequently, there is a strong desire to prepare a vaccine for melioidosis. Current research suggests that the strongest vaccine candidates are conjugates of major polysaccharides with key protein virulence factors.

This study examines the proteins involved in the biosynthesis of the capsule polysaccharide of *B. pseudomallei*, one of the two major polysaccharides of this organism. The polysaccharide is a homopolymer of 2-O-acetyl-6-deoxy- $\beta$ -D-manno-heptopyranose. This saccharide is synthesized from sedoheptulose-7-phosphate, an intermediate in the ubiquitous pentose phosphate pathway.

**Key point:** bioinformatic analysis of the genes in the capsule biosynthetic cluster suggests a pathway for the biosynthesis of the precursor GDP-2-O-acetyl-6-deoxy  $\beta$ -D-manno-heptopyranose. The relevant proteins have been recombinantly expressed and purified. We show that these proteins can perform their proposed functions, and so provide evidence for the route of capsule biosynthesis in *B. pseudomallei*.

#### ID/08 THE ROLE OF *CAMPYLOBACTER JEJUNI* GLYCOPROTEINS DURING BACTERIAL INTERACTIONS WITH HUMAN INTESTINAL EPITHELIAL CELLS

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The *Campylobacter jejuni* N-linked protein glycosylation (pgl) system, the first identified example of this modification in a bacterial pathogen, has been shown to be an important factor in pathogenicity. Over 30 functionally diverse *C. jejuni* proteins have been shown to be glycosylated by the pgl system, however their role in pathogenesis remains poorly understood. Two of these glycoproteins are Cj0420 (putative periplasmic protein) and Cj0843c (putative secreted lytic transglycosylase). Initial studies have demonstrated that 11168H Cj0420 and Cj0843c mutants are hyper-motile and have reduced ability to colonize chicks. An investigation into the ability to adhere to and invade Caco-2 intestinal epithelial cells showed that both mutants have a hyper-adherent and hyper-invasive phenotype in comparison to the wild-type strain. Studies to investigate the host response to both mutants are in progress. Recently a separate study has shown that Cj0843c is up-regulated in the presence of physiologically relevant concentrations of bile salts and that such conditions appear to induce virulence gene expression. Further studies are in progress to study the effect of bile salts on gene expression in both mutants, with the aim of further understanding the hyper-invasive phenotype and to elucidate the role of both glycoproteins in pathogenesis.

#### ID/09 AN INVESTIGATION INTO THE EFFECTS OF MANUKA HONEY ON PROTEIN EXPRESSION IN METICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* USING 2D ELECTROPHORESIS

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**Background** Manuka honey inhibits MRSA, but the mechanism of action is unknown. Using 2-D gel electrophoresis, we investigated whether protein expression was affected by manuka honey.

**Methods** EMRSA-15 (NCTC 13142) was cultivated at 37°C for 4 hours in TSB with and without 10% (w/v) manuka honey, and in TSB with 10% (w/v) artificial honey (to determine if results seen were due to sugars). Cells were harvested, treated in a bead beater and cell free extracts containing 150  $\mu$ g of protein were separated in the first and second dimensions on pH 3–10 IPG strips. After staining with Simply blue safe stain, images were recorded using UVP AutoChemi gel doc system and analysed using PDQuest Basic. Triplicate samples were analysed.

**Results** Spot patterns from honey treated cells were distinct from those of untreated cells and cells treated with artificial honey. Compared to untreated samples, 82% and 70% of proteins matched for artificial honey and manuka honey, respectively. One protein missing after honey treatment compared to untreated and artificial treatments was provisionally identified as FabI, an enoyl-acyl carrier protein.

**Conclusion** Changes in protein expression in manuka honey treated MRSA were not caused by sugars alone. Implications of down regulation of FabI must be further investigated.

#### ID/10 CHARACTERIZATION OF THE ROLE OF NITRATE REDUCTASE SYSTEMS DURING *SALMONELLA TYPHIMURIUM* INFECTION

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Following infection via the natural oral route in mammals, *Salmonella* is exposed to a range of reactive nitrogen species, including nitrate, during the enteric phase of infection. *S. Typhimurium* possesses three distinct nitrate reductase systems, NarGHJ<sub>L</sub> (NR-A), NapA (Nap) and NarZYWV (NR-Z).

Consistent with the anaerobic nature of the gastrointestinal tract, the ability of nitrate to serve as a terminal electron acceptor during anaerobic bacterial growth, and the relatively high levels of nitrate in the mammalian gut, we hypothesize that bacterial infection, occupation of the mammalian gastrointestinal tract and nitrate dissimilation by NR-A, Nap and NR-Z are linked.

Using continuous culture and microarray technology, we have determined the global gene expression profile of an array of *Salmonella* nitrate reductase mutants, in both nitrate rich and limiting conditions. This transcriptomic data, together with a dissection of the properties of these nitrate reductase systems made possible through both *in vivo* and alternative *in vitro* methods, is giving further insight into such a connection.

Assessment of the relationship between nitrate reductase and gut colonization is providing an integrated view of the importance of nitrate reduction during gut colonization by enteric pathogens, and could identify potential targets for novel therapeutics or vaccines.

#### ID/11 INVESTIGATION OF A miniTn10 MUTAGENESIS SYSTEM IN *CLOSTRIDIUM DIFFICILE*

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*Clostridium difficile* is the leading cause of nosocomial antibiotic-associated diarrhea causing a spectrum of diseases from mild-self limiting diarrhoea to life threatening pseudomembranous colitis. The ability of this Gram-positive organism to produce potent cytotoxins and form spores is known to play a significant role in pathogenesis. A lack of efficient tools to manipulate DNA and inadequate *in vivo* model systems has limited investigation of other potential virulence factors and our understanding of regulation of expression of virulence factors. The conjugative transposon Tn916 has been used to generate random insertions

in *C. difficile* however this system has several potential limitations highlighting the need for alternate random mutagenesis systems. A *Tn10* transposon derivative known as mini*Tn10* has been successfully used to generate insertion mutant libraries in *B. subtilis*. Here we investigate the use of an unstable plasmid to deliver the mini*Tn10* transposon through conjugation with *E. coli*.

ID/12

Withdrawn

ID/13

Not presented

#### ID/14 COMPARATIVE PROTEOMIC ANALYSIS OF THE OUTER MEMBRANE OF MANNHEIMIA HAEMOLYTICA ISOLATES FROM CATTLE AND SHEEP

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The Gram-negative bacterium *Mannheimia haemolytica* is the causative agent of bovine and ovine pneumonic pasteurellosis. The species consists of genetically distinct subpopulations that are differentially adapted to either cattle or sheep. In this study, the outer membrane (OM) proteomes of seven *M. haemolytica* isolates and one *M. glucosida* isolate from diseased cattle and sheep were investigated to identify outer membrane proteins (OMPs) likely to be involved in virulence and host-adaption. A comprehensive analysis of a serotype A1 *M.*

*haemolytica* proteome was first carried out using bioinformatic approaches to predict OMPs in the proteome. Comparative proteomic approaches were then used to examine OMP expression in the eight isolates. OMPs were extracted by Sarkosyl solubilization of the inner membrane and separated using 1-D SDS-PAGE. Proteins were identified using MALDI-TOF-TOF MS/MS and compared with those identified by the prediction methods in order to confirm their location in the OM. Forty three proteins were identified, of which 31 were confidently predicted to be OMPs. This study is the first comparative analysis of the OM proteome of *M. haemolytica* isolates from different host species and will lead to an improved understanding of the involvement of OMPs in host-adaption and virulence.

#### ID/15 COMPARATIVE OUTER MEMBRANE PROTEOMIC ANALYSES OF PASTEURRELLA MULTOCIDA ISOLATES FROM DIFFERENT HOST SPECIES

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The Gram-negative bacterial pathogen *Pasteurella multocida* causes serious economically important infections of domesticated animals. Very little is known about the role of *P. multocida* outer membrane proteins (OMPs) in host-specificity and virulence. This study aimed to compare the OM proteome of eight representative *P. multocida* isolates associated with diseased cattle, sheep, pigs and poultry. The approach was to first predict putative OMPs using bioinformatic techniques and then identify proteins recovered from the OM by proteomic methods. Approximately eighty proteins were confidently predicted as OMPs by text mining and filtering of the predicted data. The OMPs of *P. multocida* isolates grown in complex growth medium were obtained by Sarkosyl extraction. The OM peptides were prepared by in-gel and in-solution tryptic digestion methods and identified by MALDI-TOF-TOF MS and LC-ESI-Q/TOF MS. Approximately 60% of the confidently predicted OMPs were identified by these techniques. Certain proteins were identified in isolates from all host species whereas other proteins were unique to one or a few isolates. This study represents the first comparative OM proteomic analysis of *P. multocida* isolates from different host species and will shed light on how the OM proteome of this pathogen is adapted to these different species.

ID/16

Not presented

#### ID/17 PHASE VARIATION OF LPS-MODIFICATION GENES IN SALMONELLA TYPHIMURIUM

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*Salmonella* serovars are a major cause of gastroenteritis, affecting both humans and livestock. Since the 1940s we have known that some *Salmonella* serovars undergo phase variation

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(previously referred to as form variation) of their O-antigens. It is now known that the genes responsible for O-antigen modification are the *gtrABC* glycosyltransferases, however until now the mechanism of phase variation was unknown. One of the *S.Typhimurium gtr* promoters contains two putative OxyR binding sites overlapping four GATC sequences – the target sequence for Dam methylase. This research shows that phase variation of these genes is dependant on both Dam methylase and the global regulator OxyR. OxyR is thought to act as both an activator and a repressor of transcription, depending on which site in the promoter it binds to, whilst DNA methylation ensures the expression state remains heritable.

ID/18

Not presented

were identified and differentiated by biochemical tests. Antibigrams for all isolated species were determined using Muller Hinton agar. All results obtained from this survey were analysed using SPSS software.

**Results** Of the 1509 samples, 986 (65.3%) were positive for pathogenic bacteria, 170 (17.2%) of which were from men and 816 (82.8%) from women. *E. coli* was the most prevalent with 591 cases (58.7%), followed by *Enterobacter* 115 (11.4%) and *Klebsiella* 88 (8.8%). Data analysis revealed that the correlations between the WBC, RBC, nitrite, crystal, and protein were significantly higher in culture-positive samples. Of the antibiotics tested, isolated species were most sensitive to amikacin and most resistant to ampicillin.

**Conclusions** The present study revealed a correlation between pyuria and bacteruria; however, it should be noted that the clinical signs and the presence of WBC in urine could not be used to confirm the UTI. In addition, since different bacterial species are able to cause UTI, in order to administer proper treatment while controlling improper use of antibiotics, thorough testing, including UA and UC together with antibiogram, is strongly recommended.

**Keywords** Urine analysis, UTI, urine culture

ID/20

Not presented

#### ID/19 CORRELATION BETWEEN URINE ANALYSIS AND URINE CULTURE IN THE DIAGNOSIS OF URINARY TRACT INFECTION IN YAZD CENTRAL LABORATORY

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**Background** The misdiagnosis of urinary tract infection (UTI) may lead to kidney deficiency and even pyelonephritis. Since different species may cause this disease, urine culture (UC) and antibiogram of the isolated species should be performed and results compared to urine analysis (UA) parameters to obtain the best diagnosis.

**Methods** The urine specimens from 1509 patients (1195 women and 314 men) were processed for UA, UC and antibiogram. First of all, the sterile urine samples were cultured using differential media, including EMB and blood agar. After 24 hr incubation, the colonies

**MDE/01 ULTRASONIC DNA TRANSFER TO GRAM-NEGATIVE AND GRAM-POSITIVE BACTERIA**

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In environmental microbiology, the most commonly used methods of bacterial DNA transfer are conjugation and electroporation. However, conjugation requires physical contact and cell-pilus-cell interactions; electroporation requires low-ionic strength medium and high voltage. These limitations have hampered bacterial DNA transfer in industry practice. We have employed a standard low frequency 40 kHz ultrasound bath to successfully transfer plasmids (broad host plasmid pBBR1MCS2) into *Pseudomonas putida* UWC1, *Escherichia coli* DH5 $\alpha$  and *Pseudomonas fluorescens* SBW25, and pDA71 into *Rhodococcus* sp. RC92, RC291 and RC89 separately. We have also successfully transferred a large plasmid 83 kb pDTG1 into UWC1. Under optimal conditions: ultrasound exposure time of 10 s, 50 mM CaCl<sub>2</sub>, temperature of 22°C, plasmid concentration of 0.8 ng/ $\mu$ l, cell population of  $2.5 \times 10^9$  CFU/ml and reaction volume of 500  $\mu$ l, the efficiency of ultrasonic DNA transfer can reach  $10^{-5}$  transformants per cell. Low frequency ultrasound DNA delivery (UDD) can be readily scaled up, allowing for a broad application not only in laboratory conditions but also in industrial scale.

**MDE/02 THE ROLES OF UNCULTURABLE BACTERIA IN HORIZONTAL GENE TRANSFER AND BIODEGRADATION A TOLUENE CONTAMINATED GROUNDWATER**

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Horizontal gene transfer (HGT) plays important role in the evolution of bacteria and bacteria in nature can acquire new traits by HGT to rapidly adapt changing environment. Unculturable prokaryotes as an unseen majority may play key roles in both horizontal gene transfer and biodegradation. Groundwater from five boreholes representing background, light, medium and heavy toluene contamination was sampled from an aquifer in south England. We isolated five toluene degraders by directly inoculating groundwater onto selective plates which toluene was a sole carbon source. However, the comparison of cell DAPI-staining counting and agar plate counting indicates that the vast majority of bacteria (> 99%) are yet to be cultured. Hence, we employed a culture-independent approach: FISH-Raman-SIP (fluorescent in-situ hybridization – Raman microspectroscopy – stable isotope probing) to investigate unculturable toluene degraders and their functional genes. Using this approach, we identified three groups of unculturable bacteria (*Pseudomonas* sp., *Variovorax* sp., and *Agrobacterium* sp.) involved in mediating of toluene biodegradation and linked them with gene functionality without the bias. This study demonstrates the unculturable bacteria play key roles not only in HGT but also biodegradation *in-situ*. It helps a revelation of the secret lives of unculturable microorganisms in natural environment.

**MDE/03 A NOVEL, BIOCIDES-FREE APPROACH TO THE PREVENTION OF CORROSION AND MARINE BIOFOULING**J. GITTENS<sup>1,2</sup>, R. AKID<sup>1</sup>, T.J. SMITH<sup>2</sup>, H. WANG<sup>1</sup> & J.C. EARTHMAN<sup>3</sup>

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Existing anti-fouling paints that release biocides such as copper are used worldwide and cause extensive damage to aquatic life. Biofouling can lead to microbially-induced corrosion (MIC) of metals by promoting the formation of destructive biofilms containing corrosion-causing bacteria such as sulphate-reducing bacteria, which form a corrosive metabolic by-product, H<sub>2</sub>S. Corrosion-causing biofilms are often resistant to destruction by biocides since the biofilm bacteria are protected by a matrix of exopolymeric substances (EPS). Paradoxically, other biofilms that contain protective bacteria such as *Pseudomonas fragi* and *Paenibacillus polymyxa*, can actually inhibit corrosion. Sol gel technology and immobilized micro-organisms have been combined in a unique coating that inhibits corrosion on an aluminium alloy 2024-T3 and is a low-cost, effective and environmentally friendly. The results of a viable colony count have shown that they are not compromised by solvent concentration of up to 50% v/v and a low pH of < 3. Experiments in the laboratory, as well as field trials in a variety of marine/estuarine environments have shown that the coatings loaded with appropriate bacterial cells or spores show resistance to biofilm formation and corrosion inhibition relative to the control.

**MDE/04 LIPOPOLYSACCHARIDE DIVERSITY IN HAEMOPHILUS PARAINFLUENZAE**

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*Haemophilus parainfluenzae* (Hp) and *H. influenzae* (Hi) are closely-related members of the Pasteurellaceae family and are common commensal bacteria of the human nasopharynx. Whilst Hi is frequently implicated in meningitis, otitis media and respiratory tract infections, reports of pathogenic behaviour by Hp are very rare. Lipopolysaccharide (LPS) is a key component of the gram-negative cell wall, and its structure influences the ability of *Haemophilus* to proliferate and evade immune clearance in the host. The LPS characteristics of 21 Hp carriage isolates were surveyed and compared to known Hi LPS structures. Homologues of the Hi LPS inner core synthesis genes, but not the phase-variable outer core genes, were detected in Hp by PCR and Southern analyses. The Hi LPS outer core includes phosphocholine, which mimics host cell molecules; immunoblotting indicated that phosphocholine is absent in Hp.

Tricine SDS-PAGE demonstrated the presence of O-antigen sugar chains in a minority of the Hp strains, an unusual feature for *Haemophilus*, and analysis of isogenic mutant strains suggests that the O-antigen may influence bacterial-host interactions. Hp is a successful commensal but appears to lack the flexibility of adapting its LPS by phase variation of gene expression, potentially limiting its range of host niches.

**MDE/05 USING MICROBIAL MICROCOSMS TO INVESTIGATE PROKARYOTIC BIOGEOGRAPHY OF FRESHWATER ENVIRONMENTS IN SCOTLAND**EULYN PAGALING<sup>1</sup>, FIONA STRATHDEE<sup>1</sup>, ROSALIND ALLEN<sup>2</sup> & ANDREW FREE<sup>1</sup>

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Biogeography aims to explain the spatial patterns of diversity. In this study, we investigated whether microbial populations sampled from different environments produced similar communities when subjected to the same environmental growth conditions. We sampled from 11 freshwater lochs and one pond in Scotland. The lochs had different chemical compositions, pH, and altitudes and were at various geographic distances. Sediment and water were sampled and used to inoculate sterilized microcosms. These microcosms were based on the Winogradsky column, which consisted of pond sediment and water from Blackford pond in Edinburgh. They were 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 microbial communities that developed in the columns were dependent on the source material. These results suggest 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.

**MDE/06 THE IMPACT OF AMMONIA DEPOSITION ON THE ACTIVITY AND COMPOSITION OF AMMONIA- AND METHANE-OXIDIZING COMMUNITIES IN FOREST SOILS**EMMA THORPE<sup>1</sup>, DAVE NEDWELL<sup>1</sup>, ELENA VANGUELOVA<sup>2</sup> & CORINNE WHITBY<sup>1</sup>

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Nitrification is important in the global nitrogen cycle and involves the oxidation of ammonia to nitrite and nitrate. Ammonia oxidation, mediated by ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) is catalysed by the enzyme ammonia monooxygenase (AMO), encoded by the *amoA* gene. AMO of AOB is evolutionarily related to pMMO encoded by the *pmoA* gene in methane-oxidizing bacteria (MOB). This research aims to analyse the effects of increased ammonium deposition on AOB, AOA and MOB community structure and activity. Hockham, Heath, at Thetford Forest consists of both Corsican pine (*Pinus nigra*) and Beech (*Fagus sylvatica*) species and has a gradient of nitrate and ammonia across a 300 m transect. Nitrification potentials from both Pine and Beech mineral soils were measured and for Beech were significantly higher at 20 m (22.72 nmol NO<sub>3</sub><sup>-</sup> produced gdw<sup>-1</sup> h<sup>-1</sup>) compared to at 300 m (0.69 nmol NO<sub>3</sub><sup>-</sup> produced gdw<sup>-1</sup> h<sup>-1</sup>). Lower nitrification potentials were measured at Pine 20m and 300 m compared to Beech.

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Significant methane oxidation potentials (MOP) were also found for Pine and Beech mineral soils and Pine litter soils at 300 m. PCR amplification of the 16S rRNA and *amoA* genes for AOB and *amoA* genes for AOA was performed and AOB *amoA* genes were detected in most soils. Based on 16S rRNA genes, changes in AOB community diversity were also observed along the transect. These results show that ammonia deposition may increase nitrification but inhibit methane-oxidation and has implications for forest management in mitigating the impacts of organic pollution in the future.

#### MDE/07 ON THE RELATIVE CONTRIBUTION OF ARCHAEA AND BACTERIA TO AMMONIA OXIDATION IN AGRICULTURAL SOIL

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Recent findings have indicated that Archaea may play a major role in the oxidation of ammonia in the environment. Based on the presence of the prokaryotic functional marker gene *amoA*, putative ammonia-oxidizing archaea (AOA) generally appear to be more abundant in soil than ammonia-oxidizing bacteria (AOB). However, the relative contribution of AOA to nitrification activity in soil remains to be elucidated. In addition, it is not clear whether there is a distinct separation of these two groups with respect to ecological niche. We have addressed the hypothesis that AOA prefer lower ammonia concentrations than AOB, i.e. different fertilizer regimens with contrasting rates of ammonia supply will result in distinct nitrifier communities. In order to investigate the contribution of both AOA and AOB to nitrification in agricultural soils, plots of a grassland site (Palace Leas, Northumberland, UK) amended with organic (manure) and chemical (ammonium sulphate) fertilizer for over 100 years, were sampled. Quantitative PCR and DGGE analysis of gene and transcript abundance (16S rRNA and *amoA*) revealed clear differences indicating that long-term fertilizer regimens did select for distinct community structures. However, experiments using soil microcosms did not provide any evidence that there was a differential short-term response of AOA and AOB populations to different nitrogen amendments. In microcosms amended with organic and/or chemical fertilizer, profiles of community structures and transcriptional activity revealed no evidence to indicate that AOA or AOB were more active in one particular set of conditions i.e. when exposed to a slow release of mineralized nitrogen or a high concentration of ammonium-based fertilizer.

#### MDE/08 SOIL PH REGULATES THE ABUNDANCE AND DIVERSITY OF GROUP 1.1c CRENARCHAEOTA

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Archaeal communities in many forest soil systems are dominated by a distinct crenarchaeal lineage Group 1.1c. These Crenarchaeota have also been found consistently in other acidic soils including grassland, moorland and alpine soils. To determine the effect of soil pH on Group 1.1c crenarchaeal community size and composition, soil samples were collected across a pH gradient from 4.5 to 7.5. The abundances of Group 1.1c Crenarchaeota, total Crenarchaeota and total bacteria were assessed by quantitative PCR (qPCR) targeting 16S rRNA genes and the diversity of Group 1.1c crenarchaeal community was investigated by denaturing gradient gel electrophoresis (DGGE) and phylogenetic analysis of 16S rRNA gene sequences. The abundance of Group 1.1c Crenarchaeota declined as the pH increased, whereas total Crenarchaeota and Bacteria showed no clear trend. Community diversity of Group 1.1c Crenarchaeota was influenced by pH with different DGGE bands dominating at different pH. The abundance of Group 1.1c Crenarchaeota was also quantified in 13 other soils representing various habitats, soil types and pH. These results exhibited the same trend i.e. Group 1.1c Crenarchaeota were more abundant and represented a greater proportion of total Crenarchaeota in the most acidic soils. The results indicate strong pH selection for Group 1.1c soil Crenarchaeota.

#### MDE/09

Withdrawn

#### MDE/10 EVOLUTION OF THE VIRULENCE PLASMID IN *SHIGELLA SONNEI*

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*Shigella sonnei* has emerged as the major cause of bacillary dysentery in developed and transitional countries. To understand the pathogenic evolution, we have applied Solexa sequencing and comparative genomics on the virulence plasmid (VP) of a global collection. We identified ten (VP1-10) regions of the VP with potential informative SNPs in comparison with the reference VP from complete genome Ss046, and tested these regions in 119 isolates. While VP1-10 were amplified by PCR from 24 isolates, one or more regions could not be amplified from the remaining isolates, suggesting large scale rearrangements has occurred in the VP. The nucleotide sequences of VP3, 5 and 7 have been uniform and only one SNP was observed for one strain in VP2 region. We also observed a deletion of 360 bp fragment in four isolates in VP6. The remaining regions appear to be primarily evolving by clonal diversification because they are only separated by one or two SNPs. However, imports have also been observed in few isolates for these regions from closely related *Escherichia coli* and in one case from *Salmonella enterica*. This is the first study demonstrating the dynamic nature of the VP by genetic rearrangements and recombination in *S. sonnei*.

#### MDE/11 EFFECT OF PHENANTHRENE CONCENTRATION ON MICROBIAL COMMUNITY DYNAMICS IN A POLLUTED SOIL

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Polycyclic aromatic hydrocarbons are a group of organic compounds of great interest from an environmental perspective. Due to their abundance and persistence in natural environments, as well as their deleterious effects on living organisms they have been rated priority pollutants by environmental protection agencies worldwide. Natural degradation of PAHs occurs within the soil, with rates of disappearance depending upon contaminant concentration and previous soil exposure.

The aim of this study was to examine the effect of phenanthrene (a 3 ring PAH) on soil microbial community structure over time. Two concentrations of phenanthrene (200 mg kg<sup>-1</sup> and 2000 mg kg<sup>-1</sup>) were used to amend a soil with a history of PAH contamination and microbial community dynamics were compared with an unamended soil. The rate of disappearance of phenanthrene was assessed by means of GC-FID. Microbial activity and counts were monitored using dehydrogenase activity and most probable number, respectively. The influence of different concentrations of phenanthrene on microbial community structure was assessed by a culture independent technique, T-RFLP. The effect of the PAH on the abundance of a key functional gene in the phenanthrene metabolic



pathway was also assessed using real-time PCR. Both concentrations of phenanthrene were readily removed from the pre-exposed soil. Microbial community profiles differed in amended and non-amended soil and clear differences could also be observed in bacterial community structure at different concentrations of phenanthrene. The abundance of a key functional gene varied over time, with highest levels detected during the period associated with maximum degradation.

#### MDE/12 DEHALOGENATION OF CHLORINATED ORGANIC POLLUTANTS BY BACTERIAL GLUTATHIONE S-TRANSFERASES

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Glutathione S-transferases are prokaryotic and eukaryotic proteins involved in the cellular detoxification and excretion of a large variety of compounds. In our laboratory, a specific bacterial GST, BphK<sup>LB400</sup> isolated from *Burkholderia xenovorans* LB400, has been studied and was shown to be capable of dehalogenating chlorinated organic substrates, rendering them less toxic. BphK<sup>LB400</sup> was over-expressed in *E. coli* and the dehalogenating activity of BphK<sup>LB400</sup> towards a number of synthetic chlorinated organic compounds associated with varying levels of ecotoxicity, including chlorobenzoates (PCB metabolites) and commonly used pesticides, was measured biochemically.

Site-directed mutagenesis at a number of highly conserved amino acid positions in BphK<sup>LB400</sup> was carried out to study the effect of mutation on catalytic activity, and mutants with an increase in dehalogenating activity towards a number of the substrates tested were identified. Laboratory scale phytoremediation studies suggest that BphK<sup>LB400</sup> (wildtype and mutant) is capable of dehalogenating toxic synthetic chlorinated organic compounds in soil, protecting the plants from the effects of these chemicals.

Molecular techniques are currently being used in the laboratory to identify bacterial GST sequences in genomic DNA isolated from environmental soil samples contaminated with chlorinated organic compounds.

BphK<sup>LB400</sup>, and other bacterial GSTs, could have potential in bioremediation of contaminated soil.

#### MDE/13 IDENTITY AND ABUNDANCE OF AMMONIA-OXIDIZING CRENARCHAEOTES IN THE GERMAN WADDEN SEA

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The cultivation of the mesophilic crenarchaeote *Nitrosopumilus maritimus* and subsequent molecular studies have demonstrated the important role of ammonia-oxidizing archaea (AOA) in the global nitrogen cycle. The isolation of a novel AOA from a tidal channel indicated archaeal nitrification in the German Wadden Sea (site Janssand). Strain Jan I affiliates with the genus *Nitrosopumilus* and represents the first mesophilic AOA isolated from a natural environment. Here, we studied the distribution of archaeal and bacterial nitrifiers at site Janssand by combining MPN cultures with molecular techniques. While antibiotic-free cultures revealed the complete oxidation of ammonia to nitrate by bacterial nitrifiers, accumulation of nitrite and presence of AOA were found in tubes amended with streptomycin. 16S rRNA gene analysis revealed the predominance of a single archaeal phylotype that affiliated with the genus *Nitrosopumilus* and showed a close relatedness to strain Jan I. MPN cultures further indicated an up to 100-fold higher abundance of AOB compared to AOA in the surface sediment. Archaeal clone libraries from the surface sediment confirmed a low abundance of AOA, suggesting that AOA do not significantly contribute to nitrification at site Janssand. Nevertheless, selective cultivation resulted in proof of the presence and finally in the identification of AOA.

#### MDE/14 DEGRADATION OF CYLINDROSPERMOPSIN BY TITANIUM DIOXIDE PHOTOCATALYSIS

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Cyanobacteria are a group of oxygenic phototrophic bacteria that exhibit an enormous diversity and occupy widespread habitats including brackish, marine and fresh water. A major concern among these organisms is their toxin production; under suitable circumstances

they can reach high biomass level and for in toxic algal blooms. The toxins produced are secondary metabolites and are referred to as cyanotoxins. Cyanotoxins affect not only the aquatic ecosystem but also impact the health of human population who utilize the water resource. The cyanotoxin cylindrospermopsin, produced by *Cylindrospermopsis raciborskii* is hepatotoxic and a strong protein inhibitor. The increasing occurrence of cylindrospermopsin is major concern particularly within drinking water supplies. The investigation of more effective technologies of water treatment is therefore of great importance. This study details the potential degradation of purified cylindrospermopsin using titanium dioxide photocatalysis treatment method. TiO<sub>2</sub> photocatalysis has been known to degrade a wide range of organic pollutants, but very little information is available regarding the removal of toxicity of cylindrospermopsin. Current applications of TiO<sub>2</sub> photocatalysis have used fine powder of TiO<sub>2</sub> which is difficult to remove from the treated water. In this investigation novel granular TiO<sub>2</sub> is evaluated to assess its application in the removal of cylindrospermopsin from the drinking water.

#### MDE/15 PHOTOAUTOTROPHIC COMMUNITIES ASSOCIATED WITH GEOTHERMAL SPRINGS IN TAI-AN, TAIWAN

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Carbon cycle in the Earth including carbon dioxide fixation and organic matter decomposition plays an important role in the global geochemistry. Some studies reported that heterotrophic micro-organisms were often found in bicarbonate hot springs. What kind of organic carbon sources providing these micro-organisms is an issue worthy of further research. The assumption of this study is that some of the autotrophic microbial communities using inorganic carbons – carbon dioxide, bicarbonate and carbonate might be organic carbons providers to drive the carbon cycle. The main purpose of this study was to find out members utilizing inorganic carbon source in hot springs and to analyse the genetic, phylogenetic and physical characters of the members in order to understand the basis of the carbon cycle. Four strains TA01, TA02, TA03 and TA04 were isolated from the bicarbonate hot springs in Tai An. The pH value and the temperature of the springs were 8.48 and 50°C. Based on 16S rRNA gene sequence comparison, both strains TA02 and TA03 were clustered to *Methanothermobacter* (98%). Strain TA04 and strain TA01 were closely to *Owenweeksia* spp. in the order of Flavobacteriales (84%) and cyanobacteria *Thermosynechococcus elongatus* BP-1 (99%) respectively. Additionally, strain TA01 has similarity of 82.4% with *T. elongatus* BP-1 in *cpcBA*-IGS (phycocyanin intergenic spacer sequences). Strain TA01 could grow at the temperature of 30–60°C with the optimal temperature of 50°C and the pH range of 8.5 to 10. It was implicated that strain TA01 might be the provider of organic carbon owing to photosynthesis in the carbon cycle at Tai-An hot springs.

**Keywords** cyanobacteria, geomicrobiology, hot springs, thermophilic bacteria, *Thermosynechococcus elongatus*

#### MDE/16 ISOLATION AND CHARACTERIZATION OF THRAUSTOCHYTRID-LIKE MICRO-ORGANISMS PRODUCING FATTY ACIDS FROM THE MARINE SEDIMENTS IN NORTHWESTERN COAST, TAIWAN

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Thraustochytrids, a group of unicellular heterotrophic fungoid-like protists, are abundant in a variety of marine environment. A recent industrial interest in thraustochytrids was associated with the extraction of polyunsaturated fatty acids (PUFAs), carotenoids and Co-enzyme Q10 from the cells. Thraustochytrid-like strains producing PUFAs will be purified and identified and serve as model organisms to understand the PUFAs-biosynthesis mechanism in this study. In the preliminary experiments, samples were collected from northwestern coast in Taiwan, and several marine microorganisms include *Thraustochytrium* spp., *Schizochytrium* spp. and *Ulva* spp. are identified by 18S-ribosomal DNA gene sequence analysis. Grown on glucose/seawater at 22 °C, *Thraustochytrium* spp. strain FJ-01 was found with 10.4 g/L of biomass and 19.2% of lipid content containing 16.2% DHA. The lipid vacuole in the cell of *Thraustochytrium* spp. strain FJ-01 was analysed with 64.48% by confocal microscope. *Thraustochytrium* spp. FJ-01 seems to be an ideal candidate for studies on biosynthetic molecules of polyunsaturated fatty acids and commercialization.



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#### MDE/17 CHARACTERIZATION OF CO<sub>2</sub>-UTILIZING METHANOGENESIS BY A METHANOGENIC CONSORTIUM FROM MARINE SEDIMENTS IN KINGMEN

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The methanogenesis catalyzed by microbial processes is generally considered to be one of the major driving forces for carbon cycling in marine sediments. The catabolic methanogenesis can be contributed by 3 pathways: CO<sub>2</sub>-reducing, methylotrophic, and acetoclastic pathways. Many published research reports reveal that CO<sub>2</sub>-reducing pathway is a commonly observed methanogenesis in the marine facies sediment environments. But very few information about the potential capacity of CO<sub>2</sub>-reducing methanogenesis by the methanogenic consortia has been obtained. In this study, we analysed the effect of temperature and pH factors on CO<sub>2</sub>-reducing methanogenesis of a methanogenic consortium TP-01 from the marine sediments in Kingmen in an attempt to understand the potential capacity of methanogenesis. The preliminary results showed that the optimal temperature and pH for methane generation capacity of the methanogenic consortium TP-01 are 40°C and pH 8 with the evolution of 22.18 μmole-CH<sub>4</sub>/ml and 18.65 μmole-CH<sub>4</sub>/ml, respectively. The methanogenic consortium TP-01 was also characterized with archaeal methanogens and bacteria by the analysis of 16S rRNA and *mcrA* genes using PCR techniques. From the study, these results implicate that the methanogenic communities in the marine facies sediment environments bear the great potentials of CO<sub>2</sub>-reducing methanogenesis.

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**Keywords** carbon dioxide, geomicrobiology, marine sediment, methanogenesis

#### MDE/18 ABUNDANCE AND COMMUNITY STRUCTURE OF SPHINGOMONADS IN PLANT RESIDUES AND SOIL

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Sphingomonads are a group of bacteria widely distributed in the environment. In spite of increasing interest in the diversity and distribution of sphingomonads in the field, detailed information on them has not yet been accumulated. We examined the abundance and the diversity of sphingomonads in soil and the decaying leaf residues of plants, by real-time PCR and denaturing gradient gel electrophoresis (DGGE) analyses. It was shown that sphingomonads generally accumulate in plant residues in high proportions, comprising approximately 15% of the total bacteria, and form a community structure characteristic to residual plant species. At least within the time period studied, the abundance of sphingomonads in plant residues changed, but the community structure was basically maintained. The proportion of sphingomonads to total bacteria in the soil was about 1.7% on average. The community structure of sphingomonads in soil differed to some extent between sampling points, and was generally different between samples collected at different times. The sequences of 55 DGGE bands including 28 different motility types with duplication, originating from the residues of eight plant species, illustrated the diversity of sphingomonads in the residue samples. This study provides valuable information on the behaviour of sphingomonads in soil systems.

**TM/01 MUTAGENESIS OF SOLUBLE METHANE MONOOXYGENASE TO INVESTIGATE SUBSTRATE SPECIFICITY AND ENTRY TO THE ACTIVE SITE**TIM NICHOL<sup>1</sup>, MALCOLM LOCK<sup>1</sup>, THOMAS J. SMITH<sup>1</sup> & J. COLIN MURRELL<sup>2</sup><sup>1</sup>Biomedical Research Centre, Sheffield Hallam University, Sheffield S1 1WB; <sup>2</sup>Dept of Biological Sciences, University of Warwick, Coventry CV4 7AL

The ability of soluble methane monooxygenase (sMMO) to oxidize methane to methanol at ambient temperature using dioxygen as the oxidant, as well as to cooxidize a wide range of adventitious substrates, has made it an attractive possibility for biocatalytic and bioremediation applications. Nonetheless, the catalytic versatility of sMMO has significant limitations, in particular the inability to oxidize large polyaromatic hydrocarbons such as triaromatic pollutants phenanthrene or anthracene. Previous site-directed mutagenesis highlighted a critical residue Leu110 in determining regioselectivity with mono- and di-aromatic substrates. However, neither wild-type nor the Leu110 mutants could oxidize the triaromatic pollutants phenanthrene and anthracene, probably because steric factors prevent access to the active site. Using an adaptation of the homologous expression system developed previously, we have created a system accommodating random mutagenesis of the active site of sMMO using various error prone PCR strategies. Mutant libraries have been screened for activity against triaromatic compounds using a high-throughput colorimetric assay. This system may allow for directed evolution of sMMO towards the oxidation of triaromatic compounds and identification of residues which restrict substrate access to the active site. Additionally molecular modelling has been used to design site-directed mutants to investigate possible routes that substrates may take into the enzyme.

**TM/02 MODELLING METABOLIC SWITCHING IN DIFFERENTIATING BACTERIUM *STREPTOMYCES COELICOLOR***

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*Streptomyces* are common soil bacteria that are of major biotechnological importance due to their ability to produce secondary metabolites, some with antibiotic activities. The linear chromosome of the model organism *S. coelicolor* A3(2) has been fully sequenced. When growing in liquid cultures, the individual hyphae of the filamentous *S. coelicolor* tangle together forming hyphal aggregates and eventually dense pellets. Growing in a pellet form creates challenges to industrial fermentations. The penetration of nutrients and oxygen to the core of the pellet becomes limited as the pellet size increases. To understand the filamentous growth and to offer solutions for bioprocesses, we have developed a continuum model of pellet formation relating growth to oxygen and glucose profiles and antibiotic production. The model predicts high biomass concentration at the edge of the pellet where oxygen and nutrients are freely available. The antibiotics are produced at a zone just below the surface of the pellet. For bioprocesses, this means directing valuable nutrients to a large amount of cells that do not produce secondary metabolites. We are currently updating the continuum model and developing a discrete model. Together they will provide a tool for deeper understanding of morphological and metabolic heterogeneity of the pellet.

**TM/03 DEVELOPMENT OF A POTENTIALLY NOVEL VACCINE AGAINST *STREPTOCOCCUS SUIS* SEROTYPE 2**CHEN TAN<sup>1,2</sup>, JON CUCCU<sup>1</sup>, HUANCHUN CHEN<sup>2</sup> & BRENDAN W. WREN<sup>1</sup><sup>1</sup>London School of Hygiene & Tropical Medicine, London WC1E 7HT; <sup>2</sup>State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, PR China

*Streptococcus suis* is an important swine pathogen, and 35 *S. suis* serotypes have been identified based on differences in their capsular antigens. *S. suis* serotype 2 (SS2) is the most frequently isolated serotype worldwide and can cause meningitis, septicemia, arthritis, endocarditis, pneumonia, and even acute death not just in swine but also humans.

Many types of vaccines have been developed against SS2, but are invariably unsatisfactory due to low immunogenicity. In this project, we will transfer the SS2 capsular polysaccharide to an immunogenic cell surface protein by Protein Glycan Coupling Technology (PGCT) that exploits the ability of recently identified bacterial oligosaccharyltransferases to link glycans to proteins in laboratory strains of *E. coli*.

We have isolated SS2 from diseased pigs and identified the capsular polysaccharide biosynthesis coding region. In this presentation, we will describe our attempts to obtain

this region and achieve its expression in *E. coli*. We will discuss our methods and how we propose to generate a glycoprotein in this host organism and test its effectiveness as a novel conjugate vaccine against *S. suis*.

**TM/04 MONITORING ENZYME-CATALYSED POLYSACCHARIDE SYNTHESIS ON A SURFACE**CARLA CLÉ<sup>1</sup>, A. PATRICK GUNNING<sup>2</sup>, KARL SYSON<sup>1</sup>, LAURA BOWATER<sup>1</sup>, ROBERT A. FIELD<sup>1</sup> & STEPHEN BORNEMANN<sup>1</sup><sup>1</sup>Dept of Biological Chemistry, John Innes Centre, Norwich Research Park, Norwich NR4 7UH;<sup>2</sup>Institute of Food Research, Norwich Research Park, Norwich NR4 7UA

Cell walls and starch granules are strategically important biological materials not least because of their role in biofuel production. These complex biological systems involve insoluble substrates and products so classical solution state enzymology has limited utility in understanding their biosynthesis and degradation. New methods are required in order to quantitatively measure enzyme action at surfaces. Using a model system, we have demonstrated the real-time measurement of transglucosidase activity on a surface using surface plasmon resonance (SPR) spectroscopy. We initially obtained evidence that the glucanucrase alternansucrase was capable of extending dextran in solution. We then monitored alternan synthesis on a dextran surface with this enzyme. Conditions were used where surface polymer synthesis rates were a function of enzyme concentration and proportional to the extent of enzyme binding to the surface. The  $K_m$  for sucrose on the surface was indistinguishable from that in solution. A method to determine the turnover number of the enzyme on the surface was also developed. The presence of new amorphous polysaccharide was observed optically, detected by lectin binding and imaged by atomic force microscopy. This new surface method will have utility in a wide range of surface carbohydrate enzyme systems that mimic the biological system of choice.

**TM/05 SYNTHESIS OF PREBIOTIC GALACTO-OLIGOSACCHARIDES FROM LACTOSE USING WHOLE *BIFIDOBACTERIUM* CELLS AS THE BIOCATALYST**

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The aim of this work was to investigate the effects of temperature and lactose concentration on galactooligosaccharide (GOS) synthesis, using *Bifidobacterium bifidum* NCIMB 41171 as the biocatalyst. The synthesis reactions were carried out in 100 ml bottles using different temperatures (from 40° to 60°C) and lactose concentrations (from 35% to 51% w/w). Samples were taken every 4 h for up to 24 h and analysed for their carbohydrate content by High Performance Liquid Chromatography (HPLC) and High Performance Anion Exchange Chromatography coupled with Pulsed Amperometric Detector (HPAEC-PAD). The results showed that the purity yield (defined as the percentage of GOS based on total carbohydrates) increased as the temperature increased from 40°C to 60°C. This could be explained by the fact that one of the galactosidases, namely BbgIV, which from previous research was shown to have a key role in GOS synthesis, has an optimum temperature of 50°C. The yield also increased as lactose increased from 35% to 43% w/w when the temperature was set between 40°C and 50°C. This could be due to the fact that the higher the lactose concentration the higher the probability of it acting as a receptor for the galactosyl moieties. However, lactose concentrations higher than 43% w/w resulted in decreased yields. This was possibly because the numbers of intact cells in these cases, as measured microscopically using the Live/Dead assay, were reduced, as a result of the higher osmotic pressure.

**TM/06 BACTERIAL BIOTRANSFORMATION OF MYRCENE FROM HOPS**

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Myrcene (7-methyl-3-methylene-1,6-octadiene) is an acyclic monoterpene accounting for a relatively large fraction of monoterpenes extracted from the essential oils of hop plants (*Humulus lupulus*), and it is therefore a relatively inexpensive starting material for biotransformations targeting more commercially attractive derivatives. A wide range of microbial systems have been studied towards the biotransformation of terpenes for uses in flavour and fragrance, as well as pharmacological precursors, however limited interest has been shown in bacterial biotransformations involving myrcene. *Rhodococcus erythropolis* MLT1 isolated from a selective enrichment using material from *Humulus lupulus* and surrounding soil is able to utilize myrcene as a sole carbon source for growth

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(continued)

when provided in the vapour phase. Activity for the biotransformation of myrcene to the fragrant monoterpene geraniol is observed, and is inducible when the organism is grown on myrcene, representing a potentially desirable industrial biotransformation.

TM/07

Not presented

### TM/08 HETEROLOGOUS EXPRESSION OF GLYCOSYLTRANSFERASE FROM *PEDIOCOCCUS PARVULUS* 2.6 AND IMPROVED STRESS TOLERANCE OF PROBIOTIC *LACTOBACILLUS PARACASEI* NFBC 338

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The glycosyltransferase enzyme, responsible for microbial beta glucan production catalyses the conversion of sugar nucleotides to beta glucan. In this study, the *gtf* gene encoding glycosyltransferase from *Pediococcus parvulus* 2.6 has been heterologously expressed in *Lactobacillus paracasei* NFBC 338, and when grown in the presence of glucose (7%), the recombinant strain displayed a 'ropy' phenotype. Scanning electron microscopy (SEM) revealed strands of polysaccharide linking neighbouring cells and when grown in MRS (7% glucose), there was a two-fold increase in viscosity of the beta glucan producing *Lactobacillus paracasei* NFBC 338 in comparison to non-beta glucan producing control strain. We analysed the ability of the beta glucan producing *Lactobacillus paracasei* NFBC 338 to survive both technological and gastrointestinal stresses, compared to the control strain. Interestingly, stress tolerance assays revealed that production of exopolysaccharide significantly protects the probiotic strain during heat, acid, simulated gastric juice and bile stress conditions. This study demonstrates that the ability of food cultures to produce beta glucan confers a technological advantage in terms of survival during food processing and gastric transit.

### TM/09 RATIONAL OPTIMIZATION OF *STREPTOMYCES LIVIDANS* TOWARDS AN IMPROVED HOST CELL FOR HETEROLOGOUS PROTEIN PRODUCTION

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Among the bacterial systems applied as host cells for the production of industrially relevant proteins, the Gram-positive bacterium *Streptomyces lividans* is considered an interesting candidate thanks to its innate high secretion capacity, its low endogenous protease activity and the extensive knowledge on its fermentation processes. It has been shown already that several proteins are secreted in acceptable levels by this host. On the other hand, many heterologous proteins, mainly of eukaryotic origin, are so far only poorly secreted. Our goal is to optimize this organism as a host cell for the production of heterologous proteins.

Transcriptome and proteome analysis of strains of *S. lividans*, producing different selected proteins in varying quantities, is carried out to identify genes/proteins that are up- or down regulated under secretion stress. Relevant genes are selected for overexpression and/or deletion in order to relief secretion stress aiming to improve protein production.

A first gene selected was a sigma factor. Its overexpression in and deletion from the different production strains is performed and the impact on secretion yield is analysed.

It will be shown that this approach can be useful in the framework of strain improvement.

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