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Towards indicators of soil biological quality: use of microbial characteristics

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Preface i
List of participants iii

Contents

Active role of microbial life in soil suppressiveness to *Rhizoctonia solani*.
Yvette Bakker, Francine M.J. van Loon & Johannes H.M. Schneider 1
Biocontrol of *Rhizoctonia solani* on cucumber seedlings by *Trichoderma* spp.
C.M.M. Lucon, C.M. Koike, F.R.A. Patrício & A.S. Santos 7
A potential role for collagen in the attachment of *Pasteuria penetrans* to nematode cuticle
Keith G. Davies & Charles H. Opperman 11
Role of iron-regulated metabolites in *Arabidopsis* root colonization by *Pseudomonas fluorescens* WCS374
M. Djavaheri, J. Mercado-Blanco, L.C. Van Loon & P.A.H.M. Bakker 17
Trichoderma and soil solarization induced microbial changes on plant surfaces
Neta Okon Levy, Y. Elad, J. Katan, S.C. Baker & J.L. Faull 21
Collimonas fungivorans and bacterial mycophagy
Kathrin Fritsche, Johan H.J. Leveau, Saskia Gerards, Sachie Ogawa, Wietse de Boer & Johannes A. van Veen 27
Endophytes as source of efficient biological agents
Rita Grosch, Franziska Faltin, Jana Lottmann, Andreas Kofoet & Gabriele Berg 31
Towards indicators of soil biological quality: use of microbial characteristics
Céline Janvier, François Villeneuve, Véronique Edel-Hermann, Thierry Mateille, Jean Thioulouse, Claude Alabouvette & Christian Steinberg 37
Interactions between arbuscular mycorrhizal fungal hyphae and soil bacteria: Effects of hyphal exudates on bacterial diversity
Jonas F. Johansson, Leslie R. Paul, Björn D. Lindahl, Malin Elfstrand & Roger D. Finlay 41
Physiological and physico-chemical factors modulating ISR elicitor production by *Pseudomonas putida*
Emmanuel Jourdan, Marc Ongena, Akram Adam & Philippe Thonart 45
Multitrophic interactions of *Paecilomyces lilacinus* strain 251 in the rhizosphere of host and non-host plants
Sebastian Kiewnick 53
Commercial research and development of disease-suppressive microorganisms
Matthew S. Krause, Alfons C.R.C. Vanachter & Tom J.J. De Ceuster 63
Enrichment and genotypic diversity of phlD-containing fluorescent *Pseudomonas* spp. associated with crop monoculture
Blanca B. Landa, Olga V. Mavrodi, Kurtis L. Schroeder, Raul Allende-Molar & David M. Weller 67

Combining Brassicaceae green manure with *Trichoderma* seed treatment against damping-off in sugar beet
Stefania Galletti, Pier Luigi Burzi, Eleonora Sala, Simona Marinello & Claudio Cerato 71

Mycotoxin modulation of chitinase gene expression in a plant-beneficial fungus used for biological control
Matthias P. Lutz, Brion Duffy & Geneviève Défago 77

Biological control of soil-borne diseases in flowerbulb cultivation in the Netherlands
M. de Boer, G.J. van Os, V. Bijman & J.M. Raaijmakers 83

Regulation of beneficial traits in antagonistic bacteria
Dmitri Mavrodi, Sharik Khan, Stephen Farrand & Linda Thomashow 89

Antagonistic activity among 2,4-diacetylphloroglucinol (DAPG)-producing fluorescent *Pseudomonas* spp.
Dmitri Mavrodi, Shamil Validov, Olga Mavrodi, Leonardo De La Fuente, David Weller & Linda Thomashow 97

Use of *Serratia plymuthica* to control fungal pathogens in bean and tomato by induced resistance and direct antagonism
Hamid Meziane, Leonid Chernin & Monica Höfte 101

Role of ptsP and orfT genes in root colonization by *Pseudomonas fluorescens* Q8r1-96
Olga Mavrodi, Dmitri Mavrodi, David Weller & Linda Thomashow 107

The potential for *Meloidogyne graminicola* biological control in rice under oxic and anoxic soil environments
Jon Padgham & Richard Sikora 111

Using flow cytometry for in situ monitoring of antimicrobial compound production in the biocontrol bacteria *Pseudomonas fluorescens* CHA0
Patrice de Werra, Aurélie Huser, Eric Baehler, Christoph Keel & Monika Maurhofer . 117

Exploiting the potential of *Pasteuria penetrans* for biological control of root-knot nematodes
Barbara Pembroke & Simon Gowen 123

Decayed *Armillaria mellea* fruiting bodies as source for potential biocontrol agents against root rot disease
Ilaria Pertot, V. Gualandri, F. de Luca, C. Longa & E. Pellegrini 127

Influence on plant growth of *Glomus mosseae* BEG12, *Trichoderma viride* TV1 and *T. harzianum* T39 on grapevine in different environments
Ilaria Pertot, Federica De Luca, Rosaly Zasso & Luca Zulini 131

Molecular investigations of rhizobacteria-induced systemic resistance toward the root-knot nematode Meloidogyne incognita in tomato
K. Schäfer, C. Silva Fabry, R.A. Sikora & R. Hauschild 135

Application of two biocontrol agents to potato roots – a risk assessment approach
Katja Scherwinski, Arite Wolf & Gabriele Berg 141

A chemo attraction in onion & citrus root exudates recognized by *Ditylenchus dipsaci* and *Tylenchulus semipenetrans* in laboratory bioassays
Y. Spiegel, Z. Dagan, M. Bar-Eyal & Y. Kapulnik 147

Heterogeneity of *Fusarium oxysporum* strains isolated as formae specialis radicis-lycopersici
Shamil Z. Validov, Shufan Qi, Tatyana Azarova, Faina Kamilova & Ben J.J. Lugtenberg 151

Transcription factors in roots and shoots of *Arabidopsis* involved in rhizobacteria-induced systemic resistance
Sjoerd Van der Ent, Maria J. Pozo, Bas W.M. Verhagen, Daniel Bakker, L.C. Van Loon & Corné M.J. Pieterse 157
Concepts of multitrophic interactions
Wim H. van der Putten 163
Microbial diversity in wheat rhizosphere as affected by genetically modified *Pseudomonas putida* WCS358r
Mareike Viebahn, Karel Wernars, Eric Smit, Leendert C. van Loon, Todd Z. DeSantis, Gary L. Andersen & Peter A.H.M. Bakker 167
Disease suppressive soils
David M. Weller 173
Effects of beneficial microorganisms on plants
L.C. van Loon 183
Activity, chemistry and biosynthesis of cyclic lipopeptides produced by *Pseudomonas* species
Maarten J.D. de Kock & Jos M. Raaijmakers 193

Active role of microbial life in soil suppressiveness to *Rhizoctonia solani*.
Yvette Bakker, Francine M.J. van Loon & Johannes H.M. Schneider 1

Abstract: *Rhizoctonia solani* anastomosis group 2-2IIIB causes damping-off, black root rot and crown rot in sugar beet (*Beta vulgaris*). Based on experiences of growers and field experiments, soils can become suppressive to *R. solani*. The fungus may be present in the soil, but the plant does not show symptoms. Understanding the mechanisms causing soil suppressiveness to *R. solani* is essential for the development of environmentally friendly control strategies of rhizoctonia root rot in sugar beet. A bioassay that discriminates soils in their level of disease suppressiveness was developed. Results of bioassays were in accordance with field observations. Irradiation and heating of soil samples eliminated soil suppressiveness, indicating an active role of microbial life in soil suppressiveness to *R. solani*. Furthermore, soil suppressiveness seemed to be built up in presence of *R. solani* that may indicate the involvement of hyperparasites in soil suppressiveness. Preliminary results suggest that for development of soil suppressiveness to *R. solani*, soil microbial life and *R. solani* are important components and to a lesser degree the host. Our research focuses on hyperparasites through bioassays (Cambridge Method), *in vitro* techniques and molecular techniques (PCR-DGGE).

Biocontrol of *Rhizoctonia solani* on cucumber seedlings by *Trichoderma* spp.
C.M.M. Lucon, C.M. Koike, F.R.A. Patrício & A.S. Santos 7

Abstract: Rhizoctonia damping-off in seedlings is one of the most important sanitary problems in a wide range of plant species in Brazil. Species of *Trichoderma* have been shown to be particularly effective to control soil-borne plant pathogens because they can grow very fast in different soils and substrates and use a diverse antagonistic mechanisms to “exclude” the pathogens. In greenhouse assays, 250 *Trichoderma* spp. isolates were evaluated as biological control agents of *Rhizoctonia solani* AG-4 on cucumber seedlings. The results demonstrated that forty seven isolates reduced the cucumber post-damping-off disease from 10 to 100%, and 22 were promising as biological control agents of the pathogen.

A potential role for collagen in the attachment of *Pasteuria penetrans* to nematode cuticle
Keith G. Davies & Charles H. Opperman 11

Abstract: The Gram-positive bacterium *Pasteuria penetrans* is a parasite of root-knot nematodes *Meloidogyne* spp. and has potential as a biological control agent. The bacterium produces endospores that adhere to the cuticle of second-stage juveniles as they migrate through the soil. Immunological heterogeneity of the surface of the endospore is related to host specificity. Recent research has shown the surface of *Bacillus anthracis* contains protein filaments made up of a collagen-like glycoprotein (BclA) the gene of which flanks the rhamnose biosynthesis operon; the length of these filaments in different strains of *B. anthracis* has been shown to be determined by the number of collagen-like (G-x-y) repeats. Similar genes are also present in *B. cereus* and *B. thuringiensis*. A genome sequencing project of *P. penetrans*, has produced 4000 unique sequences and a database of these sequences has been screened for BclA using BLAST. Several collagen-like proteins have been identified in *P. penetrans* that contain G-x-y repeats and these sequences appear to be closely related to those of *Bacillus thuringiensis*. The pre-treatment of endospores with either collagenase or the collagen binding domain of fibronectin inhibited endospore binding to nematode cuticle suggesting that collagen-like proteins are also present on the exosporium surface of *P. Penetrans* and involved in attachment.

Role of iron-regulated metabolites in *Arabidopsis* root colonization by *Pseudomonas fluorescens* WCS374
M. Djavaheri, J. Mercado-Blanco, L.C. Van Loon & P.A.H.M. Bakker 17

Abstract: The plant growth-promoting rhizobacterium *Pseudomonas fluorescens* WCS374 produces several iron-regulated metabolites, including the fluorescent siderophore pseudobactin, salicylic acid (SA), and pseudomonine, a siderophore that contains a SA moiety. To study the functional role of pseudomonine and SA in the colonization of roots of *Arabidopsis thaliana*, genes *pmsA* and *pmsB* of the pseudomonine biosynthesis gene cluster were disrupted using homologous recombination. These mutants, defective in SA and pseudomonine biosynthesis, were further subjected to Tn*5* mutagenesis to generate mutants also deficient in pseudobactin production. The resulting double mutants still appeared to have siderophore activity. The SA/pseudomonine and the double mutants colonized *Arabidopsis* roots to the same extent as the wild type bacterium. Surprisingly, a mutant that produced SA and pseudomonine but lacked pseudobactin colonized the plant roots significantly better. Under the conditions tested production of iron-regulated metabolites does not seem necessary for effective colonization of the *Arabidopsis* rhizosphere by WCS374.

Trichoderma and soil solarization induced microbial changes on plant surfaces
Neta Okon Levy, Y. Elad, J. Katan, S.C. Baker & J.L. Faull 21

Abstract: Both soil solarization and *Trichoderma* reduce disease incidence. In one work we studied the effect of both treatments on rhizosphere populations and in a second work we studied the effect of *Trichoderma* on phyllosphere populations. In both works the effect of the treatments on foliar pathogens was studied. When the roots were in contact with the solarized soil or *Trichoderma* and the foliage was inoculated with pathogens, strawberry, cucumber and common bean grown on treated media showed significant reduction in disease after leaf inoculation with *Botrytis cinerea* or with *Sphaerotheca fuliginea*, hence indicating induced resistance. Attempts were made to find the relationship between indigenous populations of microorganisms in the rhizosphere or in the phyllosphere and disease control. For this purpose we used a molecular approach based on 16S-rDNA and denaturizing gradient gel electrophoresis (DGGE). Bacterial 16S ribosomal DNA (rDNA) extraction from roots and leaves that were subjected to the various treatments was PCR amplified with chosen primers. Amplicons were separated by size and base composition by DGGE in order to fingerprint shifts in the structure of the natural plant-associated microbial communities that may result from the treatments. The soil treatments resulted in changes in the DGGE patterns of rhizosphere and phyllosphere populations. Some bacilli, pseudomonads and actinobacteria were detected and their role in induced resistance is currently tested. *T. harzianum* treatment to leaves or roots resulted in increased variability in the bacterial population inhabiting the leaves. It is suggested that some of the effect exerted by soil solarization and *Trichoderma* are associated with microbial changes.

Collimonas fungivorans and bacterial mycophagy
Kathrin Fritsche, Johan H.J. Leveau, Saskia Gerards, Sachie Ogawa, Wietse de Boer & Johannes A. van Veen 27

No abstract

Endophytes as source of efficient biological agents
Rita Grosch, Franziska Faltin, Jana Lottmann, Andreas Kofoet & Gabriele Berg 31

Abstract: Endophytes are plant-associated bacteria that live inside plants and show neutral or beneficial interaction with the host plants. The biocontrol potential of endophytic bactaria isolated from field-grown potato plants was evaluated in a hierarchical combination of assays using the soil-borne plant pathogen *Rhizoctonia solani* Kühn as target. The pathogen is one of the most important soil-borne pathogens and responsible for yield losses of economically important crops worldwide. Control of *R. solani* is difficult, because of its saprophytic and parasitic properties. Thus, the objective of the study was to select effective biological control agents (BCAs) useful for different crops. The strains were characterized by their antagonistic mechanisms *in vitro* against different anastomosis groups of *R. solani* as well as their effectiveness in disease suppression *in vivo*. In summary, 18 bacteria were selected based on antagonistic activity in dual culture and enzyme activity (chitinase, β-1,3-glucanase, protease) *in vitro*. The disease depression effect of these strains were further tested against *R. solani* on potato, lettuce and sugar beet under controlled cultivation conditions, favourable for the disease development of *R. solani*. Ten BCAs reduced the disease development on potato, seven on lettuce and ten on sugar beet. But only three isolates showed effects against *R. solani* on all three crops. These three bacterial BCAs were tested in field experiments on potato and lettuce. The plant growth of both was significantly diminished by *R. solani* in the field. The application of the bacterial BCAs reduced significantly the disease severity on lettuce and potato. The reduction of disease severity correlated with an increasing yield in the treatments due to the bacterial BCAs on both crops.

Towards indicators of soil biological quality: use of microbial characteristics
Céline Janvier, François Villeneuve, Véronique Edel-Hermann, Thierry Mateille, Jean Thioulouse, Claude Alabouvette & Christian Steinberg 37

Abstract: The aims of this study were to evaluate the impact of 2 cultural practices on diverse soil characteristics, and to establish relationships between these descriptors, in order to identify indicators of the soil biological quality. The experimental plot received either a cattle manure compost amendment, or was treated by biofumigation. Physicochemical and biological data, microbes and nematodes community structure and the soil receptivity to *Rhizoctonia solani* damping-off of carrot were assessed during a whole cultural year. Correspondences were analysed by Principal Component Analysis. Significant changes were observed. Biofumigation induced the strongest effect. The organic amendment resulted in a weaker effect. We are still working on the establishment of relationships for the identification of indicators of the soil biological quality.

Interactions between arbuscular mycorrhizal fungal hyphae and soil bacteria: Effects of hyphal exudates on bacterial diversity
Jonas F. Johansson, Leslie R. Paul, Björn D. Lindahl, Malin Elfstrand & Roger D. Finlay 41

Abstract: In addition to increasing the nutrient absorptive surface area of their host plant root systems, the extraradical hyphae of arbuscular mycorrhizal (AM) fungi also provide a direct pathway for translocation of photosynthetically derived carbon to microenvironments in the soil. This provision of energy-rich compounds, coupled with the relatively large surface area of the hyphae, suggests that mycorrhizal mycelia may be an important niche for bacterial colonisation and growth. What effect mycelial exudates may have on microbial diversity and the activity of the microorganisms is still unknown. Mycelial exudates of *Glomus sp.* MUCL 43205 were collected from split-plate in vitro systems of transformed, mycorrhizal clover roots. A bacterial community extracted from soil was incubated in the presence or absence of exudates to determine their effects on bacterial community structure. Initial and post-exposure bacterial communities were characterised by T-RFLP analysis and 16S DNA sequencing. We also determined bacterial viability using Live/Dead® BacLight™ stain. Both the bacterial diversity and the relative abundance of some individual taxa were higher in the mycorrhizal treatment compared to the non-mycorrhizal treatment. We also found that the proportion of vital bacteria increased in the presence of AM fungal exudates. Initial results suggest that some taxa were exclusively associated with exposure to AM fungal exudates. The results support the idea that AM fungal exudates may influence both soil bacterial diversity and abundance.

Physiological and physico-chemical factors modulating ISR elicitor production by *Pseudomonas putida*
Emmanuel Jourdan, Marc Ongena, Akram Adam & Philippe Thonart 45

Abstract: A better understanding of biotic and environmental factors that regulate the production of active metabolites by beneficial rhizobacteria is crucial for optimising biocontrol under pratical conditions. In this study, we wanted to evaluate the effect of some parameters on the production of the plant defence elicitor synthesized by *Pseudomonas putida* BTP1. This molecule is clearly dependant of the secondary metabolism and chemostat experiments showed that the elicitor is more efficiently produced at a very low cell growth rate. On another hand, the presence of free amino acids in the medium is necessary to obtain an optimal NABD production. A specific positive effect of phenylalanine was evidenced in pulsed continuous cultures suggesting that this residue could play a role as a precursor and/or act as a regulator in the biosynthetic pathway.

Multitrophic interactions of *Paecilomyces lilacinus* strain 251 in the rhizosphere of host and non-host plants
Sebastian Kiewnick 53

Abstract: The facultative egg pathogenic fungus *Paecilomyces lilacinus* is one of the most widely tested biocontrol agents for control of plant parasitic nematodes. The commercial strain 251 (PL251) is undergoing registration procedures in the EU and US and is commercially available as BIOACT® WG in several countries. To better understand the multitrophic interactions of PL251 in the rhizosphere, dose-response experiments were conducted to evaluate the relationship between the antagonist dose and biocontrol efficacy and fungal persistence. The importance of host- or non-host plants, nematodes, mutualistic fungal endophytes, and mycorhiza for biological efficacy and unwanted side effects caused by the application of the biocontrol fungus was also investigated. It could be demonstrated that persistence and consequently the biocontrol efficacy of PL251 is not, unlike other nematophagous fungi, linked to the presence of the target nematode nor the host plant. Furthermore, some nematode host plants seem to provide unsuitable conditions in their rhizosphere resulting in rapid decline of fungal density and in some cases reduced efficacy of the antagonist. In contrast to other nematophagous fungi, rhizosphere competence is not a key factor for the biocontrol efficacy of PL251. Multiple applications did increase the persistence of the fungus in soil which was correlated with excellent control of root-knot nematodes under field conditions.

Commercial research and development of disease-suppressive microorganisms
Matthew S. Krause, Alfons C.R.C. Vanachter & Tom J.J. De Ceuster 63

No abstract.

Enrichment and genotypic diversity of phlD-containing fluorescent *Pseudomonas* spp. associated with crop monoculture
Blanca B. Landa, Olga V. Mavrodi, Kurtis L. Schroeder, Raul Allende-Molar & David M. Weller 67

Abstract: Fluorescent *Pseudomonas* spp. producing the antibiotic 2,4-diacetylphloroglucinol (2,4-DAPG) play a key role in take-all decline, which develops when a field is continuously cropped to wheat or barley after a take-all outbreak, and also contribute to the disease suppressiveness of other soils. In this study, we showed that over many years of monoculture, the crop species grown in a field enriches for genotypes of 2,4-DAPG producers from the reservoir of genotypes naturally present in the soil that are especially adapted to colonize the rhizosphere of the crop grown.

Combining Brassicaceae green manure with Trichoderma seed treatment against damping-off in sugar beet.
Stefania Galletti, Pier Luigi Burzi, Eleonora Sala, Simona Marinello & Claudio Cerato 71

Abstract: In recent years different biological approaches of varying degrees of efficacy have been developed with the aim of controlling plant diseases. One of these is represented by the green manure of *Brassicaceae* plants, which provides a natural fumigation based on the defensive myrosinase-glucosinolate system. After tissue damage, glucosinolate enzyme-derived compounds are released with a toxic effect on phytopathogenic fungi. Another approach is represented by the utilisation of biological control agents, like the soil saprophytic fungi *Trichoderma* spp. which was shown to be effective against several soil-borne diseases through different mechanisms. The aim of this work was to evaluate the combined effects of biofumigation and *Trichoderma* treatment on the control of damping-off in sugar beet.

A commercial mixture of *Brassica napus* varieties selected against different soil pathogenic fungi was sown at the dose of 10 kg ha^{-1}on a soil highly infested by *Pythium* and *Rhizoctonia* in central Italy. Sixty days after growing, an estimated biomass of 67 t ha^{-1} of green material was incorporated into the soil by a rotating harrow at 20 cm depth. After one month samples of amended and unamended soil were collected and utilised for an experiment in greenhouse to evaluate the protective effect against sugar beet damping-off, in a randomised block design with six replicates (pots), twenty seeds per pot. The effect of two different *Trichoderma* strains applied to sugar beet seeds was evaluated in comparison to untreated seeds both on amended and unamended soil. Such strains were previously selected for *in vitro* activity against *Pythium* spp. and *Rhizoctonia* spp.

Factorial ANOVA analysis highlighted a statistically significant effect of the green manuring on the number of healthy plants as well as of *Trichoderma* seed treatment, which showed an adjunctive protective effect after *Brassica napus* green manuring.

Mycotoxin modulation of chitinase gene expression in a plant-beneficial fungus used for biological control
Matthias P. Lutz, Brion Duffy & Geneviève Défago 77

Abstract: Biocontrol agents such as *Trichoderma* were applied in many different environments. These habitats were potentially also occupied by mycotoxin producing fungi. This indicates that *Trichoderma* will likely encounter an array of mycotoxins. But little is known how these fungal toxins affect biocontrol efficacies. Any competitive advantage conferred by mycotoxins would complicate efforts to control mycotoxigenic fungi. We examined the influence of important mycotoxins on expression of *Trichoderma* chitinase genes using *ech42-goxA* or *nag1-goxA* reporter gene fusions in *T. atroviride* P1. Production of chitinases, such as the ECH 42 endochitinase and the N-acetyl-β-glucosaminidase NAG1, is a primary mechanism of action for *T. atroviride* P1. Recently, the molecular interaction between the *Fusarium* mycotoxin deoxynivalenol (DON) was evaluated in detail. It was found that DON down-regulates *nag1* gene expression in the fungal antagonist *Trichoderma atroviride* P1. Using a broader approach, additional mycotoxins that are prevalent in various crops and environments were evaluated *in vitro* for their ability to interact with the antagonistic fungus *Trichoderma atroviride* strain P1. Three different patterns were identified with certain mycotoxins having no impact on expression of either chitinase gene (patulin, fumonisin B1, and beauvericin); others reduced significantly *nag1*, but not *ech42* expression (DON, ochratoxin A), and a third group induced *ech42*, but had no influence on *nag1* expression in *T. atroviride* P1 (aflatoxin and α-zearalenol). As sole mycotoxin beauvericin reduced significantly *Trichoderma* growth. Implications for the understanding of ecological functions of mycotoxin production and for deployment of biocontrol strategies are discussed.

Biological control of soil-borne diseases in flowerbulb cultivation in the Netherlands
M. de Boer, G.J. van Os, V. Bijman & J.M. Raaijmakers 83

Abstract: Flowerbulb production in the Netherlands is threatened by several soil borne diseases. Root rot caused by *Pythium* spp., Augusta disease caused by Tobacco necrotic virus (TNV), and bare patch disease caused by *Rhizoctonia solani* are major problems. Biological control methods are being investigated at Applied Plant Research Flowerbulbs.
The biosurfactant producing *Pseudomonas fluorescens* strain SS101, was shown to cause the lysis of zoospores of oomycetes. In bioassays, SS101 suppressed Pythium root rot in hyacinthus, iris and crocus under controlled and under field conditions. In several expanded field trials with hyacinths, the efficacy of biocontrol varied by field and by year. Augusta disease in tulips is transmitted via zoospores of the fungal vector *Olpidium brassicae*. Adding strain SS101 to the standard fungicide solution, which is used for bulb drench prior to planting, resulted in a 46% reduction of the disease incidence compared to a bulb drench without the antagonist. The application of SS101 is currently repeated and further optimized in new experiments.
Verticillium biguttatum is an obligate mycoparasite of *R. solani*. The antagonist infects the sclerotia and the hyphae of its host. *R. solani* anastomosis group (AG) 2-2IIIB causes stem and bulb infection in lily, resulting in reduction of bulb yield and quality. In lab experiments, the antagonist completely suppressed the hyphal growth of *R. solani* AG2-2IIIB through soil at 24°C. In field trials, a spore suspension of *V. biguttatum* was applied as a bulb drench and a soil treatment prior to planting, resulting in significant control of *Rhizoctonia* with artificial inoculum as well as with natural infestation.

Regulation of beneficial traits in antagonistic bacteria
Dmitri Mavrodi, Sharik Khan, Stephen Farrand & Linda Thomashow 89

Abstract: The *phz* operon of *Pseudomonas fluorescens* 2-79, which produces the antibiotic phenazine-1-carboxylic acid (PCA), is preceded by *phzR* and *phzI*, a pair of quorum-sensing members of the *luxR-luxI* gene family. Quantitative analyses showed that strain 2-79 produces six acyl-homoserine lactone (HSL) signaling components, of which *N*-(3-hydroxyhexanoyl)-L-HSL is the most abundant. Deletion of *phzR* and *phzI* led to the loss of production of PCA as well as all acyl-HSLs. Expression of *phzI* in *Escherichia coli* or the PCA nonproducer *P. fluorescens* 1855 enabled the synthesis of all six acyl-HSLs normally produced by strain 2-79. A reporter strain of 2-79 bearing *phzA* and *phzR* fused respectively to *lacZ* and *uidA* required PhzR and the addition of acyl-HSLs for maximum expression of both gene fusions. Analyses with synthetic acyl-HSLs revealed that the *phzA*::*lacZ* fusion responded with highest sensitivity and greatest magnitude to *N*-(3-hydroxy hexanoyl)-L-HSL. When exposed to extracts of culture supernatants from strain 2-79 containing normal ratios of all six acyl-HSLs, the reporter responded to N-(3-hydroxy-hexanoyl)-L-HSL but not to the other five acyl-HSLs. Mapping of the transcriptional start sites for the divergently-oriented *phzA* and *phzR* genes showed that the putative -35 element of the *phzA* promoter is situated downstream from and adjacent to an 18-bp almost-perfect inverted repeat, the *phz*-box, which resembles the binding sites of other members of the LuxR family. Disrupting the *phz*-box abolished PhzR-dependent activation of *phzA* and *phzR*. We conclude that PhzI of strain 2-79 synthesizes 3-OH acyl-HSLs and, unlike the *N*-hexanoyl-L-HSL-based *phz* systems of *P. aureofaciens* 30-84 and *P. chlororaphis* PCL1391, strain 2-79 recognizes *N*-(3-hydroxy-hexanoyl)-L-HSL as its quorum-sensing signal. We conclude further that PhzR, together with its quormone, activates expression of *phzA* and *phzR* and that this activation requires the *phz*-box present in the divergent promoter region. Considering the differences in spacing of the *phz*-box relative to the -35 elements of the *phzA* and *phzR* promoters, we also suggest that PhzR activates transcription of these genes by different mechanisms.

Antagonistic activity among 2,4-diacetylphloroglucinol (DAPG)-producing fluorescent *Pseudomonas* spp.
Dmitri Mavrodi, Shamil Validov, Olga Mavrodi, Leonardo De La Fuente, David Weller & Linda Thomashow 97
Abstract: Certain strains of *Pseudomonas fluorescens* that produce the antibiotic 2,4-diacetylphloroglucinol (2,4-DAPG) can colonize roots and suppress soilborne pathogens more effectively than other strains that otherwise are almost identical. We recovered DNA fragments from the superior root colonizer *P. fluorescens* Q8r1-96 by suppression subtractive hybridization with the moderate colonizer strain Q2-87 in order to identify genetic differences that might account for superior colonization activity. One clone from Q8r1-96 exhibited similarity to a pore-forming bacteriocin and resides in a 23-kb pyocin-like gene cluster that includes a functional two-gene lysis module and a bacteriophage tail assembly. Treatment of strain Q8r1-96 with mitomycin C caused production of phage tail-like particles. Subsequent screening in vitro revealed that bacteriocin activity was common among 2,4-DAPG-producing isolates representing 17 distinct genotypes. Such activity may contribute to strain competitiveness and persistence in vitro and in the rhizosphere.

Use of *Serratia plymuthica* to control fungal pathogens in bean and tomato by induced resistance and direct antagonism
Hamid Meziane, Leonid Chernin & Monica Höfte 101
Abstract: We investigated the potential of *S. plymuthica* strain IC1270 to control the fungal leaf pathogens *Colletotrichum lindemuthianum* and *Botrytis cinerea* by induced resistance and direct antagonism. Bean and tomato plants grown in soil treated with IC1270 cells showed an enhanced resistance to the above pathogens. Mutant IC1270-P1 deficient in Prn production was as effective as the parental strain, indicating that there is no evidence for a role of Prn in induced resistance to *B. cinerea* in bean. However, IC1270-C7 mutant deficient in chitinase production was significantly less effective than the parental strain, suggesting the possible involvement of chitinolytic activity in induced resistance in bean plants. Leaf application with strain IC1270 decreased the number of *B. cinerea* spreading lesions from 92% in the control to 64%, and from 78% to 48% in bean and tomato, respectively, indicating that IC1270 is also a direct antagonist of this fungus. In addition, studies on *Serratia* metabolites that are involved in direct antagonism showed that pyrrolnitrin and chitinases are required.

Role of ptsP and orfT genes in root colonization by *Pseudomonas fluorescens* Q8r1-96
Olga Mavrodi, Dmitri Mavrodi, David Weller & Linda Thomashow 107
Abstract: *Pseudomonas fluorescens* Q8r1-96 produces 2,4-diacetylphloroglucinol (2,4-DAPG) and suppresses take-all disease of wheat caused by *Gaeumannomyces graminis var. tritici.* Strain Q8r1-96 is representative of the D-genotype of DAPG producers, which aggressively colonize and maintain large population sizes on the roots of wheat, pea, and sugarbeet. In order to study the role of *ptsP* and *orfT* in colonization of the wheat rhizosphere, clones containing these genes were identified in a Q8r1-96 genomic library, sequenced, and used to construct gene replacement mutants of Q8r1-96. PtsP plays a role in organic nitrogen utilization and the role of the *orfT* gene is unknown. Mutants in these genes were characterized phenotypically for 2,4-DAPG production, motility, fluorescence, colony morphology, exoprotease and hydrogen cyanide production, carbon and nitrogen utilization, and the ability to colonize the rhizosphere of wheat grown in a natural soil. Colonies formed by the mutant *orfT* did not differ from those of Q8r1-96, whereas those of the *ptsP* mutant exhibited altered colony morphology, increased fluorescence, and decreased motility. In addition, the *ptsP* mutant produced decreased amounts of exoprotease and phloroglucinol compounds as compared to the wild type. The *ptsP* mutant was impaired in wheat root colonization, whereas the *orfT* mutant was not impaired. However, both mutants were less competitive than *P. fluorescens Q8r1-96* in the wheat rhizosphere when introduced into the soil in mixed inoculation with the parental strain.

The potential for *Meloidogyne graminicola* biological control in rice under oxic and anoxic soil environments
Jon Padgham & Richard Sikora 111

Abstract: The rice root-knot nematode (*Meloidogyne graminicola)* is a serious pest in rice producing areas of South and Southeast Asia. A biological control system for *M. graminicola* is being developed at the University of Bonn using bacteria recovered from rice roots grown in upland and lowland rice soils of Bangladesh, Myanmar, and Taiwan. One of these bacteria, *Bacillus megaterium*, was found to have high activity against *M. graminicola* in the rice root resulting in a greater than 40% reduction in nematode galling severity and J2 penetration compared with noninoculated controls. *B. megaterium* also reduced attraction of the nematode to rice. This paper will discuss modes of action through which *B. megaterium* impacts *M. graminicola* and strategies for how biocontrol can be combined with floodwater management practices in rice to control nematode damage.

Using flow cytometry for in situ monitoring of antimicrobial compound production in the biocontrol bacteria *Pseudomonas fluorescens* CHA0
Patrice de Werra, Aurélie Huser, Eric Baehler, Christoph Keel & Monika Maurhofer 117

Abstract: *Pseudomonas fluorescens* strain CHA0 is able to protect plants against a variety of pathogens, notably by producing the two antimicrobial compounds 2,4-diacetylphloroglucinol (DAPG) and pyoluteorin (PLT). The regulation of the expression of these compounds is affected by many biotic factors, such as fungal pathogens, rhizosphere bacteria as well as plant species. Therefore, the influence of some plant phenolic compounds on the expression of DAPG and PLT biosynthetic genes has been tested using GFP-based reporter, monitored by standard fluometry and flow cytometry. *In situ* experiments were also performed with cucumber plants. We found that several plant metabolites such as IAA and umbelliferone are able to modify significantly the expression of DAPG and PLT. The use of flow cytometry with autofluorescents proteins seems to be a promising method to study rhizobacteria-plant interactions.

Exploiting the potential of *Pasteuria penetrans* for biological control of root-knot nematodes
Barbara Pembroke & Simon Gowen 123

Abstract: Commercial horticulture needs to develop pest management practices that are safe to the producer, consumer and environment. Root-knot nematodes are one of the production constraints for which a biological management strategy is urgently needed as a replacement for the relatively toxic nematicides. *Pasteuria penetrans* is an obligate bacterial parasite of these nematodes that could be deployed in the field by commercial growers and smallholders. Refinements in the system of mass-production of *P. penetrans* are dependent on a thorough understanding of the biology of the parasite and its host. This paper highlights technical issues concerning the production of *P. penetrans* spores that need to be addressed if this bacterium is to become an established biological control agent of root-knot nematodes.

Decayed *Armillaria mellea* fruiting bodies as source for potential biocontrol agents against root rot disease
Ilaria Pertot, Valeria Gualandri, Federica De Luca, Claudia Longa & Elisabetta Pellegrini 127

Abstract: *Armillaria mellea* is the causal agent of root rots of several perennial woody plants. On grapevine *A. mellea* infections cause reduction of plant vigor, poor grape quality and, in the last stage, the death of the plant. Chemical control is not effective and the available control measures are base on long rotations with non-susceptible crops. The use of biocontrol agents could be an additional tool to prevent infections in the new plantations. Several microorganisms were shown to be antagonistic to *A. mellea*, in controlled conditions both *in vitro* and *in planta*, but in the field their effect is usually weak and short lasting. To identify highly aggressive hyperparasites, 14 partially degraded *A. mellea* fruiting bodies were collected in infected vineyards in northern Italy (Trentino) and 109 microorganisms were isolated and identified. Their potential biocontrol activity against *A. mellea* was preliminary screened in vitro in dual culture on Petri dishes. Then the most effective microorganisms were tested on wood portions inoculated with the pathogen and then tested on *A. mellea* artificially infected strawberry plants. Filamentous fungi and bacteria were more frequent than yeasts on *A. mellea* fruiting bodies. *Aspergillus*, *Penicillium* and *Alternaria* species, even if effective in controlling the disease, were not selected because unsuitable as biocontrol agents. Few microorganisms (*Gliocladium* sp., *Trichothecium roseum*, *Cladosporium cladosporioides*, an unidentified bacterium, an unidentified *Actinomyces* and a sterile mycelium) showed a promising antagonistic activity against the pathogen.

Influence on plant growth of *Glomus mosseae* BEG12, *Trichoderma viride* TV1 and *T. harzianum* T39 on grapevine in different environments
Ilaria Pertot, Federica De Luca, Rosaly Zasso & Luca Zulini 131

Abstract: Root rot caused by *Armillaria mellea s. s.* and poor plant growth due to immediate replant is serious and increasing problem in some grapevine growing areas in Trentino, Italy. Since arbuscular mycorrhizal fungi (AMF) are known to increase plant growth and resistance to certain wilt and root pathogens and some strains of *Trichoderma viride* and *T. harzianum* can act as biocontrol agents and have some effect on plant growth, their effectiveness on grapevine replant disease was evaluated both in field and greenhouse controlled conditions. The degree of plant growth enhancement caused by *Glomus mosseae* BEG12 (AMF), *T. viride* TV1 and *T. harzianum* T39 is strongly related to the different environments. Reduced effect of all the tested organisms was present in optimal growing conditions in greenhouse trials. The best approach to be used to study the environmental factors that influence the plant growth enhancement of the three organisms tested was discussed.

Molecular investigations of rhizobacteria-induced systemic resistance toward the root-knot nematode Meloidogyne incognita in tomato
K. Schäfer, C. Silva Fabry, R.A. Sikora & R. Hauschild 135

Abstract: A number of rhizobacteria have been shown to posses the ability to induce systemic resistance against the root-knot nematode *M. incognita* on tomato. Systemic resistance in these cases is characterised by a reduction in the numbers of galls and eggs produced per root-system in comparison to untreated root-systems. Differences between the bacteria strains with regards to their spectrum of action could be detected. However, the changes in plant metabolism that are linked to increased resistance are still unknown. In the present study, mRNA was extracted from the roots of induced and non-induced plants and the fragments of differently expressed genes enriched by subtractive suppressive hybridization. Results on the identity of these genes will be presented and possible benefits for agricultural practice will be discussed.

Application of two biocontrol agents to potato roots – a risk assessment approach
Katja Scherwinski, Arite Wolf & Gabriele Berg 141
Abstract: The impact of the two *Verticillium* antagonists *Serratia plymuthica* HRO-C48 and *Streptomyces* sp. HRO-71 on the rhizosphere communities of potato was assessed in two consecutive field trials in Germany. The abundances of rhizobacteria as well as their proportion of *Verticillium* antagonists were determined. Additionally the culture-independent method of PCR-single strand conformation polymorphism analysis was applied to investigate culturable and non-culturable bacterial communities, respectively. No statistically significant differences between treatments and control plants were observed for the abundances of culturable rhizobacteria as well as for the proportion of in vitro *Verticillium* antagonists. The species composition of the non-target antagonistic bacteria did not differ between treated and untreated plants. By using the culture-independent method, only transient differences between the bacterial communities were observed with no correlation to the treatment. Computer-assisted comparison of total bacterial community fingerprints revealed only seasonal changes in the microflora. Nevertheless, the impact of treatment on the non-target microflora was lower than those of the growth stage of the plant or climatical factors.

A chemo attraction in onion & citrus root exudates recognized by *Ditylenchus dipsaci* and *Tylenchulus semipenetrans* in laboratory bioassays
Y. Spiegel, Z. Dagan, M. Bar-Eyal & Y. Kapulnik 147
No abstract.

Heterogeneity of *Fusarium oxysporum* strains isolated as formae specialis radicis-lycopersici
Shamil Z. Validov, Shufan Qi, Tatyana Azarova, Faina Kamilova & Ben J.J. Lugtenberg 151
Abstract: *Forl (Fusarium oxysporum f. sp. radicis-lycopersici*) strains causing TFRR (tomato foot and root rot) isolated from greenhouses in The Netherlands were analyzed on the basis of the sequence of the IGS (intergenic spacer region) and of their virulence. Comparison of IGS revealed four clades of *Forl* strains. Strains shown to be most aggressive towards tomato were classified in four clades indicating that virulence is not a property of a certain group. Comparison of IGS sequences of *Forl* with those published in GenBank of *Fol* (*Fusarium oxysporum f. sp. lycopersici*) and of other eight formae specialis clustered *Fol* in groups with *Forl* strains. Strain PD87/245 is closely related to *F.o. f. sp. cyclaminis* on the basis of its IGS sequence. There is an indication that *Forl* strains differ in genetic origin.

Transcription factors in roots and shoots of *Arabidopsis* involved in rhizobacteria-induced systemic resistance
Sjoerd Van der Ent, Maria J. Pozo, Bas W.M. Verhagen, Daniel Bakker, L.C. Van Loon & Corné M.J. Pieterse 157

Abstract: Plants possess inducible resistance mechanisms through which they can regulate their defense response to pathogen attack. Colonization of *Arabidopsis thaliana* roots by non-pathogenic *Pseudomonas fluorescens* WCS417r bacteria triggers a jasmonate- and ethylene-dependent induced systemic resistance (ISR) that is effective against a broad range of foliar pathogens. In the roots, the transcriptional activity of a large number of genes is altered upon colonization by WCS417r. To investigate the role of WCS417r-responsive, root specific genes in ISR signaling, we screened T-DNA insertion lines of a subset of these genes. Bioassays revealed that *AtMYB72*, a transcription factor gene specifically induced in the roots upon colonization by WCS417r, is essential for activation of ISR. The *myb72* knockout mutant was incapable of mounting WCS417r-mediated ISR against the challenging pathogens *Pseudomonas syringae* pv. *tomato* DC3000 and *Hyaloperonospora parasitica*. Analysis of *AtMYB72* gene expression revealed that ethylene is an important regulator of *AtMYB72*. This was supported by the finding that AtMYB72 was found to physically interact with the ethylene-regulatory protein EIL3 in a yeast two-hybrid assay. Transcript profiling revealed that ISR-expressing leaves are primed for augmented expression of predominantly jasmonate- and ethylene-responsive genes. Promoter analysis of these primed genes showed overrepresentation of an *AtMYC2* binding motif, suggesting a regulatory role for this transcription factor in ISR. Further evidence for the involvement of *AtMYC2* in ISR arose from bioassays showing that *AtMYC2* knockout mutants were not able to show ISR after root colonization by WCS417r, while their level of basal resistance was comparable to that of wild-type Col-0.

Concepts of multitrophic interactions
Wim H. van der Putten 163

Abstract: The development of the concept of multitrophic interactions has been strongly driven by aboveground biocontrol research. Biocontrol studies below ground have contributed far less to ecological concept development. Here, I discuss some of the principles of the multitrophic interactions concept. Then, I argue that above and belowground biocontrol studies should become more integrated, as plants are exposed to both above and belowground enemies at the same time. Control of one natural enemy, either above or below ground, may influence plant exposure to other enemies in the same or in the opposite subsystem.

Microbial diversity in wheat rhizosphere as affected by genetically modified *Pseudomonas putida* WCS358r
Mareike Viebahn, Karel Wernars, Eric Smit, Leendert C. van Loon, Todd Z. DeSantis, Gary L. Andersen & Peter A.H.M. Bakker 167

Abstract: Introduction of genetically modified micro-organisms (GMOs) in the environment can lead to perturbations of soil ecosystems. To monitor possible disturbances by GMOs, the impact of *Pseudomonas putida* WCS358r and two genetically modified derivatives, which constitutively produce phenazine-1-carboxylic acid (PCA) or 2,4-diacetylphloroglucinol (DAPG), on the rhizosphere microflora of wheat was studied. The GMOs were introduced into soil as a wheat seed coating in 1999 till 2002. Every year the same treatment was applied in the same field plots. To compare a possible impact of the GMOs to that of common agricultural practice, a crop rotation of wheat and potato was incorporated in the field experiment and evaluated against continuous wheat cropping. The microflora was studied by extracting DNA directly from rhizosphere soil and subsequently amplifying rDNA using bacteria-specific primers. The resulting amplicons were separated using denaturing gradient gel electrophoresis (DGGE). In the first year all bacterial treatment had a transient impact on the indigenous bacterial microflora. After repeated introduction the DAPG-producing GMO had a significant effect on the bacterial microflora as from the second year, and the PCA-producing GMO after the fourth introduction. Cropping potato had a clear long-term impact that continued to exist in a subsequent wheat crop. The impact of the GMOs was never larger than that of changing crop from wheat to potato.
Rhizosphere samples of control wheat plants and of plants treated with wild-type bacteria or the GMOs were also analyzed using an Affymetrix GeneChip® containing 16S rDNA sequences of approximately 9000 bacterial operational taxonomic units. Depending on the year and the treatment differences were observed in occurrence of specific bacterial groups.

Disease suppressive soils
David M. Weller 173

Abstract: Suppressive soils have been described worldwide for a broad spectrum of plant pathogenic fungi, bacteria and nematodes. Classical approaches employed to identify the microorganisms and mechanisms involved in suppression have changed little during the last four decades. However, when classical approaches are used in combination with sophisticated techniques of molecular ecology, it is possible to more rapidly define and characterize the agents and mechanisms responsible for specific suppression. Mechanisms of suppressiveness operating in several suppressive soils are discussed below.

Effects of beneficial microorganisms on plants
L.C. van Loon 183

Abstract: Non-pathogenic soilborne microorganisms can promote plant growth, as well as suppress diseases. Plant growth promotion is taken to result from improved nutrient acquisition or hormonal stimulation. Disease suppression can occur through microbial antagonism or induction of resistance in the plant. Several rhizobacterial strains have been shown to act as plant growth-promoting bacteria through both stimulation of growth and induction of systemic resistance (ISR), but it is not clear in how far both mechanisms are connected. ISR results from the specific recognition of (a) bacterial determinant(s) by (a) plant receptor(s) on the roots and is manifested as a reduction of the number of diseased plants or in disease severity upon subsequent infection by a pathogen. ISR is effective against both soilborne and foliar pathogens and its demonstration requires spatial separation of the inducing bacterium and the challenging pathogen. The spectrum of diseases to which ISR confers enhanced resistance overlaps partly with that of systemic acquired resistance (SAR), which is induced as a result of limited infection by pathogenic microorganisms. Both ISR and SAR represent a state of enhanced basal resistance of the plant that depends on the signaling compounds jasmonic acid and salicylic acid, respectively, and for which sensitivity to ethylene is required. Pathogens are differentially resisted by these resistance signaling pathways, which can explain the differential effectiveness of ISR and SAR against biotrophic and necrotrophic pathogens, as well as their additive effects against pathogens that activate both resistance signaling pathways.

Activity, chemistry and biosynthesis of cyclic lipopeptides produced by *Pseudomonas* species
Maarten J.D. de Kock & Jos M. Raaijmakers 193

Abstract: Soil-borne diseases caused by oomycete and fungal pathogens are major yield-limiting factors in the production of food, fibers and ornamental crops. Biological control of plant diseases with antagonistic microorganisms offers an attractive alternative or supplement to currently used control measures. Studies in different laboratories have led to the identification of antagonistic strains of *Pseudomonas* species with biocontrol activity. The biocontrol activity of several of these *Pseudomonas* strains is determined, in part, by the production and secretion of metabolites with antibiotic activities. Among the antibiotic metabolites, cyclic lipopeptides (CLPs) have received considerable interest. CLPs are diverse molecules that contain a peptide moiety linked to a fatty acid tail. Specific biocontrol activity of CLPs against both oomycete and fungal pathogens has been reported and specificity is presumably related to the amino acid sequence and/or the length of the fatty acid tail. CLPs are produced by multi-enzyme complexes composed of several nonribosomal peptide synthetases with distinct modules. The current knowledge of the diversity and biosynthesis of multiple CLPs produced by antagonistic and plant pathogenic *Pseudomonas* species will be discussed.