

## Welcome Letter

**Rhizobacteria** are plant-associated bacteria that are able to colonize and persist on roots. **Plant Growth-Promoting Rhizobacteria (PGPR)** are root associated bacteria that colonize the rhizosphere and improve plant growth when introduced onto seeds, seedpieces, roots, or into soil. PGPR improve plant growth by one or more mechanisms: direct stimulation of plant growth; enhancement of nutrient uptake; suppression of plant pathogens; and/or induction of resistance in plant hosts against pathogens. PGPR are found in a very wide range of genera and some examples include: *Acinetobacter*, *Agrobacterium*, *Arthrobacter*, *Azospirillum*, *Bacillus*, *Bradyrhizobium*, *Burkholderia*, *Cellulomonas*, *Frankia*, *Pantoea*, *Pseudomonas*, *Rhizobium*, *Serratia*, *Streptomyces*, and *Thiobacillus*.

During the last four decades, PGPR research has brought together scientists from multiple disciplines, who have addressed a wide range of topics including: discovery of novel PGPR strains and traits; performance in greenhouse and field trials; production, formulation and delivery of inoculum; registration and commercialization; mechanisms of growth promotion and biocontrol and their molecular and biochemical basis; root colonization and rhizosphere competence traits; role of PGPR in suppressive soils; plant, pathogen and rhizosphere community responses to PGPR; and recombinant PGPR and risk assessment. Now a new chapter in PGPR research has begun with the sequencing of the genomes of several well-studied strains.

The latest developments in PGPR research have been presented at 7 previous PGPR Workshops held around the world: Orillia, Ontario, Canada (1987); Interlaken, Switzerland (1990), Adelaide, Australia (1994); Sapporo, Japan (1997); Cordoba, Argentina (2000); Calicut, India (2003); and Noordwijkerhout, The Netherlands (2006).

The 8<sup>th</sup> International PGPR Workshop is the first time the meeting has been held in the United States. In addition to the topics listed above, this meeting will focus on recent developments in rhizosphere microbial ecology, genomics-enabled biology and functional genomics, and the latest in commercial application of PGPR.

Portland, Oregon was selected as the site for the meeting because it is one of the most eco-friendly cities in America and is a gateway into the spectacular Pacific Northwest, a place of world-class cities and universities, rich diversity of cultures, and some of the friendliest people in America. The meeting organizers would like to acknowledge Dr. Joe Kloepper, who organized the first PGPR Workshop and has been a driving force in their continuing success. We also want to acknowledge Dr. Dieter Haas, whose research contributions have been a major force in shaping contemporary PGPR research.

Welcome to Portland and the **8<sup>th</sup> International Workshop on Plant Growth-Promoting Rhizobacteria!!!**

### Organizing Committee of the 8<sup>th</sup> PGPR Workshop

Dr. David Weller (Chair, USDA, Agricultural Research Service, Washington State Univ., Pullman, WA)

Dr. Linda Thomashow (Co-Chair, Program Committee, USDA, ARS, WSU, Pullman, WA)

Dr. Joyce Loper (Co-Chair, Program Committee, USDA, ARS, Oregon State Univ., Corvallis, OR)

Dr. Timothy Paulitz (Program Committee, USDA, ARS, WSU, Pullman, WA)

Dr. Mark Mazzola (USDA, ARS, WSU, Wenatchee, WA)

Dr. Dmitri Mavrodi (Washington State University, Pullman, WA)

Dr. Blanca B. Landa (Instituto de Agricultura Sostenible, CSIC, Cordoba, Spain)

Ms. Joy Thompson (Events Planner, Washington State University)

The 8<sup>th</sup> International PGPR Workshop is Sponsored by:



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8<sup>th</sup> International  
PGPR Workshop

## Scientific Program May 17-May 22, 2009

### Sunday, May 17

4:00-8:00 pm  
7:00 pm

Registration  
Social: Welcome to the Pacific Northwest

### Monday, May 18

**8:30 am-12:30 pm** *Current Concepts in Rhizosphere Microbial Ecology* (David Weller, chair)

8:30-9:10	<b>A. Hartmann</b> (Germany) Molecular interactions between rhizosphere bacteria and plants mediated by autoinducer signaling compounds
9:10-9:50	<b>Ph. Lemanceau</b> (France) Iron dynamics in the rhizosphere: a case study for understanding interactions among soils, plants and microbes
9:50-10:10	<b>R. Doornbos</b> (The Netherlands) Effects of <i>Arabidopsis thaliana</i> defenses on non-pathogenic rhizosphere bacteria
10:10-10:30	<b>J. Meyer</b> (Switzerland) Impact of genetically modified wheat on the frequency and genetic diversity of root-colonizing pseudomonads associated with soil fertility
10:30-11:00	<b>Break</b>
11:00-11:30	<b>T. Paulitz</b> (USA) Pyrosequencing-A powerful tool for analysis of bacterial communities and the effect of cropping practices
11:30-12:00	<b>B. Glick</b> (Canada) Using proteomic tools to characterize PGPB and rhizosphere
12:00-12:30	<b>L. Newman</b> (USA) Synergistic interactions between poplar and bacteria to improve plant establishment, phytoremediation and sustainable feedstock production on marginal soils
12:30 PM	<b>Lunch provided</b>

<b>2:00 pm -4:40 pm</b>	<b><i>Commercial Application of PGPR Products in North America</i></b> (Joseph Kloeppe, chair)
2:00-2:10	<b>J. Kloeppe</b> (USA) Introduction
2:10-2:40	<b>J. Riggs</b> (USA) Examples of products with PGPR strains registered as biofungicides; Issues related to developing PGPR products in an international chemical company
2:40-3:10	<b>M. Banerjee</b> , L. Yesmin (Canada) Examples of products with S-oxidizing PGPR as crop inoculants alone and with rhizobia; Issues related to developing PGPR products in a seed company
3:10-3:40	<b>P. Cargeeg</b> (Canada) Examples of products with a PGPR strain registered as biofungicide with and without rhizobia; Issues related to PGPR product development in an international rhizobia inoculant company
3:40-4:10	<b>R. Ames</b> (USA) Examples of products containing fermentation extracts of microbes and their metabolites; Issues related to developing a “non-typical” PGPR product
4:10-4:40	Questions from the audience and organizers on product development. Panel discussion with the speakers
4:40-6:30	<b>Refreshments and Poster Session I</b>

**Tuesday, May 19**

- 8:30 am to 12:30 pm** *Functional genomics of PGPR* (Philippe Lemanceau, chair)
- 8:30-9:00 **H. Gross** (Germany)  
Genomic mining for bioactive small molecules in the genomes of *Pseudomonas fluorescens* Pf-5 and *Pseudomonas syringae* pv. *syringae* B728A
- 9:00-9:30 **Y. C. Kim** (Korea)  
Characterization of the *gacA* and *rpoS* proteome of *P. chlororaphis* O6
- 9:30-9:50 **Y. -S. Kwak** (USA)  
A *Saccharomyces cerevisiae* genome-wide mutant screen for sensitivity to 2,4-diacetylphloroglucinol, a biocontrol antibiotic produced by *Pseudomonas fluorescens*
- 9:50-10:10 **A. Sarniguet (France)**  
Hyphosphere and pathorhizosphere effects of the plant pathogenic fungus *Gaeumannomyces graminis* var. *tritici* on growth and gene expression of the biocontrol strain *Pseudomonas fluorescens* PF29ARP
- 10:10-10:30 **G. Lazarovits** (Canada)  
Unraveling the rhizosphere using the *CPN60* genomic marker and a 454 sequencer
- 10:30-11:00 **Break**
- 11:00-11:30 **R. Jackson** (United Kingdom)  
Characterization of a *Pseudomonas fluorescens* SBW25 *fleQ* mutant: influence on motility and gene and phenotype expression
- 11:30-11:50 **T. Kidarsa** (USA)  
Microarray analysis of *Pseudomonas fluorescens* Pf-5 grown on seed surfaces
- 11:50-12:10 **J. van de Mortel** (The Netherlands)  
Unraveling the molecular mechanisms underlying plant growth promotion by *Pseudomonas fluorescens* strain SS101
- 12:10-12:30 **M. Fillion** (Canada)  
Studying PGPR gene expression under soil conditions using TaqMan qRT-PCR assays: comparison of quantification and normalization approaches
- 12:30 pm **Lunch provided**
- 2:00 pm -4:40 pm** *Genomes and Genomics-enabled Biology of PGPR* (Joyce Loper, chair)
- 2:00-2:40 **R. Borriss** (Germany)  
Genomic features of plant-associated *Bacillus amyloliquefaciens* linked with plant growth promotion and biocontrol
- 2:40-3:20 **M. Silby** (USA)  
Genome organization, environmental success, and phylogeny: insights from comparative genomics of *Pseudomonas fluorescens*
- 3:20-3:50 **J. Morrissey** (Ireland)  
Evolution aspects of 2,4 diacetylphloroglucinol synthesis in *Pseudomonas fluorescens*
- 3:50-4:20 **D. Mavrodi** (USA)  
Evolution of the phenazine biosynthesis pathway and diversity of phenazine-producing *Pseudomonas* spp. in dryland wheat-producing areas of Washington State
- 4:20-4:40 **S. Hartney** (USA)  
TonB-dependent receptors of *Pseudomonas fluorescens* Pf-5 and siderophore uptake
- 4:40-6:30 pm **Refreshments and Poster Session II**

**Wednesday, May 20**

**8:30 am-12:00 pm** *PGPR and Rhizosphere Community Responses* (Linda Thomashow, chair)

- 8:30-9:00 **G. Berg** (Austria)  
Antagonist/PGPR interplay with rhizosphere communities
- 9:00-9:30 **B. Landa** (Spain)  
Host plant specificity in 2,4-diacetylphloroglucinol producing *Pseudomonas* spp.: who drives the partner selection?
- 9:30-9:50 **N. Someya** (Japan)  
Effects of the metabolites produced by coexistent microorganisms on antibiotic production by fluorescent pseudomonads
- 9:50-10:10 **N. Bajsa**  
Plant growth-promoting bacteria in soils under crop rotation or continuous cropping
- 10:10-10:30 **J. Kloepper**  
Dynamics of spore germination and colonization of germinating seeds with a *Bacillus pumilus* strain
- 10:30-11:00 **Break**
- 11:00-11:30 **V. Venturi** (Italy)  
Quorum sensing and interkingdom signaling in PGPR *Pseudomonas*
- 11:30-12:00 **L. Pierson** (U.S.A.)  
Quorum sensing and phenazine structure are important for biofilm formation by *Pseudomonas chlororaphis* 30-84
- 12:00 **Lunch provided and Afternoon Excursions**

**Thursday, May 21****8:30 am -12:10 pm**     *Mechanisms of Plant Growth Promotion and Biocontrol* (Mark Mazzola, chair)

- 8:30-9:10            **D. Haas** (Switzerland)  
Krebs cycle intermediates and temperature regulate gac-dependent biocontrol factor expression in *Pseudomonas fluorescens* CHA0
- 9:10-9:40            **J. Raaijmakers** (The Netherlands)  
Global and specific regulation of cyclic lipopeptide biosynthesis in plant growth-promoting *Pseudomonas fluorescens*
- 9:40-10:10          **C. -M. Ryu** (Korea)  
Rhizobacterial volatiles as elicitors of plant defense and growth promotion
- 10:10-10:30        **P. Paré (USA)**  
Rhizobacterial volatiles: an entry into plant growth promotion
- 10:30-11:00        **Break**
- 11:00-11:30        **J. Leveau** (USA)  
Bacterial turnover of auxin: its role in plant growth and health
- 11:30-11:50        **S. Spaepen** (Belgium)  
Auxin signaling in *Azospirillum brasiliense*: a holistic approach
- 11:50-12:10        **L. Rochat** (Switzerland)  
Characterization of indoleacetic acid-responsive mutants of the plant-beneficial bacterium *Pseudomonas fluorescens* CHA0
- 12:10-12:30        **S. Schrey** (Germany)  
Locally and systemically increased resistance against phytopathogens in Norway spruce after root inoculation with a forest streptomycete
- 12:30                **Lunch provided**

**2:00 pm -4:00 pm**     *Plant Responses to PGPR* (Monica Hofte, chair)

- 2:00-2:30            **S. Van der Ent** (The Netherlands)  
Prime time: Beneficial rhizobacteria prime plant defense responses
- 2:30-3:00            **M. Ongena** (Belgium)  
Plant defense reactions stimulated following perception of *Bacillus* lipopeptides
- 3:00-3:20            **D. De Vleeschauwer** (Belgium)  
Bacterial determinants and host immune mechanisms underpinning *Pseudomonas fluorescens*-induced systemic resistance in rice
- 3:20-3:40            **S. -D. Kim** (Korea)  
Drought stress resistance induction and plant growth promotion by the multifunctional PGPR *Bacillus licheniformis* K11 in pepper
- 3:40-4:00            **Break**

**4:00 pm -5:30 pm**     *Progress in the use of PGPR* (Peter Bakker, chair)

- 4:00-4:10            **P. Bakker**  
Introduction
- 4:10-4:30            **L. Ran** (China)  
Biological control of eucalypt bacterial wilt by combination of endophytic and rhizosphere bacteria

- 4:30-4:50 **J. Worapong** (Thailand)  
*Paenibacillus polymyxa* strain TL41, a bacterial endophyte of *Paederia foetida*  
and its potential use for control *Xanthomonas campestris* on lettuce
- 4:50-5:10 **I. Garcia de Salamone** (Argentina)  
Ecophysiology of the response to *Azospirillum* inoculation of cereal crops in  
Argentina
- 5:10-5:30 **C. Ramírez** (Colombia)  
Plant growth promotion by *Bacillus amyloliquefaciens* FZB45 depends on  
inoculum concentration and P-related soil properties
- 7:00 PM **PGPR Banquet**  
Announcement of next meeting venue  
Native American Story Teller: Ed Edmo

**Friday, May 22****8:30 am -12:30 pm***Biotic Interactions in the Rhizosphere* (Blanca Landa, chair)

8:30-9:10

**D. Kobayashi** (USA)*Lysobacter enzymogenes*, a biocontrol agent that establishes pathogenic interactions with a broad range of lower eukaryotes

9:10-9:50

**C. Keel and M. Maurhofer** (Switzerland)

Insecticidal activity in biocontrol pseudomonads

9:50-10:10

**A. Jousset** (Germany)

Predator-prey chemical warfare determines the production of antifungal compounds by a root-associated pseudomonad

10:00-10:30

**P. Frey-Klett** (France)

Mycorrhiza helper bacteria: a particular PGPR group with multiple facets

10:30-11:00

**Break**

11:00-11:20

**P. Okubara** (USA)

PGPR-nematode interactions

11:20-11:50

**D. Weller** (USA)

PGPR: A historical perspective and future prospects

11:50-12:00

**L. Thomashow and D. Weller** (USA)

Concluding Remarks

# **Current Concepts in Rhizosphere Microbial Ecology**

O01

**MOLECULAR INTERACTION BETWEEN RHIZOSPHERE BACTERIA AND PLANTS MEDIATED BY AUTOINDUCER SIGNALING COMPOUNDS****A. Hartmann***Helmholtz Zentrum Muenchen, German Research Center for Environmental Health, Department Microbe-Plant Interactions, D-85764 Neuherberg / Munich, Germany*

N-acylhomoserine lactones (AHLs) of Gram-negative bacteria have been shown to be present in many rhizobacteria and to exert different responses in plants. It has been proposed that bacterial autoinducer signaling compounds are improving the efficiency of cell physiological adaptation. AHLs are the molecular basis for communication within microbial assemblages and even across biological kingdoms to plants. While C6- and C8-acyl homoserine lactones can induce a systemic resistance response in tomato plants, they modulate the phytohormone balance and stimulate root growth in Arabidopsis. Interestingly, it could be shown that C8- and C10-AHLs can be taken up into roots and transported even into the shoots of some plants (Arabidopsis, barley, maize), while they cannot be found in shoots of most legumes because they are effectively hydrolysed by lactonases in the roots. Some plants are known to produce and excrete AHL-mimic substances to interfere with the signals of root colonizing bacteria, since many bacterial pathogens also organize the attack towards plants using these autoinducer or quorum sensing signal compounds.

Recently, modified AHL-signaling substances which have plant derived coumaric acid as acyl side chains were discovered in Gram-negative bacteria. We have found coumaroyl-AHLs in a *Rhizobium radiobacter* strain, tightly associated with the plant growth promoting fungus *Piriformospora indica*. Until now, it is not clear how widespread these coumaroyl-AHLs are in the rhizosphere bacteria and which responses they induce in accompanying rhizosphere microbes and in the plant.

O02

**IRON DYNAMICS IN THE RHIZOSPHERE AS A CASE STUDY FOR ANALYZING INTERACTIONS AMONG SOILS, PLANTS AND MICROBES****Ph. Lemanceau, G. Vansuyt, L. Avoscan, E. Bernaud, T. Corberand, S. Mazurier, C. Mougel***INRA, UMR MSE, Dijon, France*

Iron is an essential element for plants and microbes. However, in most cultivated soils, the concentration of iron available for these living organisms is very low since its solubility is controlled by stable hydroxides, oxyhydroxides and oxides. In the rhizosphere, there is a high demand of iron because of the iron uptake by plants and microorganisms, the density and activity of which are promoted by the release of root exudates. Plants and microbes have evolved active strategies of iron uptake. Iron incorporation by these organisms leads to complex interactions ranging from competition to mutualism. These interactions are under the control of physico-chemical properties of the soils in which they occur, and reciprocally, iron uptake strategies of plants and microbes impact the soil properties. These iron-mediated interactions among soils, plants and microbes impact plant growth and health, and their analysis, together with that of the resulting iron dynamics, is of major agronomic interest. Analysis of the complex interactions among soils, plants and microbes also represents a unique opportunity to advance our knowledge of the rhizosphere ecology. This progress requires merging complementary expertises and study strategies in soil science, plant biology and microbiology. Illustrations of how integration of these approaches allows gaining knowledge in the complex interactions occurring in the rhizosphere will be given.

O03

**EFFECTS OF *Arabidopsis thaliana* DEFENSES ON NON-PATHOGENIC RHIZOSPHERE BACTERIA****R.F. Doornbos, L.C. van Loon, P.A.H.M. Bakker***Plant-Microbe Interactions, Utrecht University, PO BOX 80056, 3508 TB Utrecht, The Netherlands Email: r.f.doornbos@uu.nl*

Pathogen-induced Systemic Acquired Resistance (SAR) and Induced Systemic Resistance (ISR) mediated by non-pathogenic rhizosphere bacteria are phenotypically similar in that upon challenge inoculation, pathogen proliferation and/or activity, as well as disease severity are reduced. To what extent induced plant defenses affect the non-pathogenic microflora in the rhizosphere and phyllosphere is as yet unknown. Using the model plant *Arabidopsis thaliana*, we determined the extent to which bacterial density and diversity are affected on mutants that are deficient in specific resistance signaling pathways. To this end selective plating and denaturing-gradient gel electrophoresis (DGGE) were applied using primers specific for eubacteria and *Pseudomonas* spp.

*Arabidopsis* mutants affected in the salicylic acid (SA) and/or jasmonic acid (JA) signal-transduction pathways developed a rhizosphere microflora that was different in both abundance and diversity as compared to the wild-type control. However, activation of the SA- and JA-defense response by applying the hormones exogenously did not result in such shifts, suggesting no direct effects of induced plant defenses on the indigenous rhizosphere microflora.

In order to study possible effects of selected *Arabidopsis* mutants on specific plant-beneficial *Pseudomonas* spp., rhizosphere colonization by the ISR-inducing *P. fluorescens* WCS417r and non-ISR inducing *P. fluorescens* WCS374r were determined. WCS417r colonization was significantly reduced on the mutant *myb72*, whereas WCS374r had low population densities on both *myb72* and the wild type. MYB72 is a transcription factor expressed in the roots upon colonization by the ISR-inducing pseudomonads and is essential for the expression of ISR. The ecological significance of these results will be discussed.

O04

**IMPACT OF GENETICALLY MODIFIED WHEAT ON THE FREQUENCY AND GENETIC DIVERSITY OF ROOT-COLONIZING PSEUDOMONADS ASSOCIATED WITH SOIL FERTILITY****J. Meyer<sup>1</sup>, C. Keel<sup>2</sup>, M. Maurhofer<sup>1</sup>***<sup>1</sup>Plant Pathology, Institute of Integrative Biology, ETH Zurich, and <sup>2</sup>Department of Fundamental Microbiology, University of Lausanne, Switzerland*

Within the frame of Swiss National Research Programme NRP 59, we study the impact of genetically modified wheat carrying introduced disease resistance genes on soil fertility sustained by plant-beneficial bacteria in greenhouse experiments, open greenhouse experiments and in the field. The major task is to monitor the effect of the transgenic wheat on the frequency and the genetic diversity of two groups of root-colonizing pseudomonads with known plant-beneficial activity: (1) 2,4-diacetylphloroglucinol (DAPG)-producing pseudomonads as an important group of disease-suppressive bacteria and (2) pseudomonads which enhance plant growth by increasing phosphate availability in soil through solubilization of poorly soluble mineral phosphates. For the enumeration of the bacteria, we have chosen the Most Probable Number (MPN) approach and for the diversity study, we developed a Denaturing Gradient Gel Electrophoresis (DGGE) technique specific for the *Pseudomonas pqqC* gene (involved in phosphate solubilization by pseudomonads). First results of the field trials 2008 indicate that there are some differences between transgenic wheat lines and non-transgenic control/sister lines with respect to the frequency and diversity of genotypes of plant-beneficial pseudomonads. Some transgenic lines tend to accumulate less pseudomonads under mildew pressure compared to control lines. However, the differences detected between transgenic and non-transgenic wheat were smaller than differences caused by the age of the plant, or than differences found between commercially available wheat varieties.

O05

**PYROSEQUENCING- A POWERFUL TOOL FOR ANALYSIS OF BACTERIAL COMMUNITIES AND THE EFFECT OF CROPPING PRACTICES****C. Yin<sup>1</sup>, K.L. Jones<sup>2</sup>, D.E. Peterson<sup>3</sup>, K.A. Garrett<sup>4</sup>, S.H. Hulbert<sup>1</sup>, K.L. Schroeder<sup>5</sup>, and T.C. Paulitz<sup>5</sup>**

<sup>1</sup>Department of Plant Pathology, Washington State University, Pullman, WA <sup>2</sup>Savannah River Ecology Lab, Aiken, SC <sup>3</sup>Department of Agronomy, Kansas State University, Manhattan, KS <sup>4</sup>Department of Plant Pathology, Kansas State University, Manhattan, KS <sup>5</sup>USDA ARS and Department of Plant Pathology, Washington State University, Pullman, WA

Pyrosequencing can generate thousands of short sequences from DNA extracted from soil. These sequences can provide taxonomic information about the bacterial communities. We designed primers to the 16s rDNA and amplified DNA from soil samples from a long-term tillage/rotation trial in Kansas for two seasons. This 2 X 2 factorial trial had two rotation treatments (wheat-wheat and wheat-soybean), and two tillage treatments (conventional and no-till), with 4 replicate blocks sampled for each treatment. From the two years of sampling, a total of 20,180 DNA 16S rDNA sequences were generated and a total of 2337 operational taxonomic units (OTUs) were assembled using a 97% similarity cutoff. The most abundant taxonomic group was the phylum Acidobacteria. Other abundant groups were the phylum Gemmatimonadetes, the order Actinomycetales, and the class Alphaproteobacteria. Most of the Acidobacteria were identified to the group level with a high degree of confidence, and different groups showed a preference for different rotation/tillage treatments. For example, Group 1 showed a preference for continuous wheat versus wheat-soybean rotation, but group 4 showed a preference for wheat-soybean rotation. These results were validated with group-specific primers and real-time PCR. This high-throughput sequencing approach can provide taxonomic information about the overall community, and detect community shifts resulting from cropping practices.

O06

**USING PROTEOMIC TOOLS TO CHARACTERIZE PGPB AND RHIZOSPHERE INTERACTIONS****Z. Cheng, B.J. McConkey, B.R. Glick**

Department of Biology, University of Waterloo, Waterloo, Canada

Proteomics is one of the fastest growing areas of biological research. The influence of various environmental stimuli, including plant signals in root exudates, the presence of nickel and salt, on the proteome of the PGPB *P. putida* UW4 and the mutant strain *P. putida* UW4/AcdS<sup>-</sup> lacking a functional ACC deaminase gene were examined using two-dimensional difference in-gel electrophoresis. Furthermore, the combined effects of plant exudates and environmental stresses like nickel and salt were also investigated. Bacterial proteins with significantly altered expression levels in the presence of these stimuli were identified by mass spectrometry. Many of these proteins are involved in nutrient transport and utilization, cell envelope synthesis, stress adaptation, anti-oxidative processes, heavy metal efflux, and transcriptional or translational regulation, and hence may play important roles in environment-bacterial interactions. The genes for some of the *P. putida* UW4 proteins showing the largest changes in expression levels were cloned and then used to create mutants in which the encoded proteins were either over-expressed or not expressed at all. Functional studies of some strains revealed significant changes in plant growth-promoting ability compared to the wild-type *P. putida* UW4, confirming involvement of these proteins in environment-bacterial interactions.

O07

**SYNERGISTIC INTERACTIONS BETWEEN POPLAR AND BACTERIA TO IMPROVE PLANT ESTABLISHMENT, PHYTOREMEDIATION AND SUSTAINABLE FEEDSTOCK PRODUCTION ON MARGINAL SOILS****D. van der Lelie<sup>1</sup>, S.Taghavi<sup>1</sup>, S. Monchy<sup>1</sup>, N. Weyens<sup>2</sup>, L. Odom<sup>3</sup>, A. Hoffman<sup>3</sup>, J. Vangronsveld<sup>2</sup>, and L.A. Newman<sup>1</sup>**<sup>1</sup>Brookhaven National Laboratory, Upton, NY, <sup>2</sup> University of South Carolina, Columbia, SC and <sup>3</sup>Hasselt University, Diepenbeek Belgium

Producing biomass that is tailored toward energy production, but that does not negatively impact food supply is one of the critical socio-economical issues of the proposed US energy program. Poplar is considered as the model tree species for bioenergy feedstock production, phytoremediation and carbon sequestration. As with other plants, poplar lives in close association with microorganisms both in and on its roots. Greenhouse studies have shown that select strains of bacteria have a beneficial effect on the development and growth of poplar in marginal soils.

To better understand the complex interactions between the bacteria and poplar, genomes of four plant growth promoting bacteria, with dualistic rhizospheric and endophytic lifestyles, were sequenced. Genome annotation, analysis of metabolic properties, and comparisons with closely related non-plant associated bacteria resulted in the identification of several unique pathways by which the bacteria can promote plant growth and health. When the microbes are exposed to plant root and leaf extract, we see induction of identified metabolic pathways and production of the plant growth promoting hormones acetoin and butanediol.

In separate studies, we demonstrated in both the lab and the field that inoculation with naturally occurring and engineered bacteria leads to decreased toxicity to toluene, and improved degradation of trichloroethylene. Inoculation with two different bacterial species have resulted in either decreased transpiration or increased metabolism of trichloroethylene within the plant system.

**Commercial  
Application of PGPR  
Products in North  
America**

**O08****SUCCEEDING IN A TRADITIONAL AG-CHEMICAL COMPANY DESPITE THE “SNAKE OIL” / “FOO-FOO DUST” CONCEPTS OF BIOLOGICAL-BASED PRODUCTS****K. Bugg, W. Hairston, J. Riggs***Bayer CropScience, Research Triangle Park, North Carolina, USA*

The number of new chemical pesticide candidates is dwindling due to consolidation of companies and the expense of new chemistry inventions. As fewer “traditional” products are discovered companies are challenging their development personnel to explore new markets or to evaluate “unique” products that once-upon-a-time were not seen as promising prospects. Bayer CropScience has two EPA registered bio-fungicides that are composed of Plant Growth Promoting Rhizobacteria (PGPR). Kodiak<sup>®</sup> Concentrate and Yield Shield<sup>®</sup> Concentrate Biological Fungicides are used as commercial seed treatments. The roads to the market place for Kodiak and Yield Shield have challenged the norm and were not always a straight path, but both products have succeeded in delivering value and efficacy. Bayer CropScience has succeeded at combining “traditional” chemical seed treatments with *Bacillus*-based treatments into a single system that offer extended length of protection and additional modes of actions. Understanding the mechanisms which the individual bacterial strains of Kodiak and Yield Shield use to provide efficacy has been instrumental in the success of each. Future PGPR opportunities are currently being researched that will lead to novel seed treatment products, as well as biological-based products for crop and turf protection markets.

**O09****PROSPECTS OF BACTERIAL GROWTH PROMOTERS IN DIFFERENT CROP PRODUCTION SYSTEMS****M.R. Banerjee and L. Yesmin***Research and Development, BrettYoung Seeds, P.O. Box 99 St. Norbert Postal Station, Winnipeg, Manitoba, R3V 1L5, Canada*

To improve yield potentials, present day crop production is highly dependent on the use of agricultural chemical inputs like fertilizers and pesticides. But there are concerns from the health and environmental perspective regarding their use and application. Because of these concerns, the increased use of microbial inoculants is now viewed as a valuable alternative to chemical treatments and could contribute considerably to attaining the goal of environment friendly sustainable agriculture. Therefore renewed interest in utilizing microbial inoculants has resurfaced. In recent times, PGPR (plant growth promoting rhizobacteria) inoculants are seen as an alternative to chemical treatments in various cropping systems such as field and forage crops as well as fruits and vegetables. We attempted to use naturally occurring sulfur (S)-oxidizing PGPR as crop inoculants in canola (*Brassica napus*), soybean (*Glycine max*), alfalfa (*Medicago sativa*) and corn (*Zea mays*) to enhance the crop performance and production. Numerous field trials showed that this kind of biological treatment with S-oxidizing PGPR could be used as a unique biotechnological tool for better crop production. Our PGPR technology tried to evaluate the feasibility of the potential use of S-oxidizing rhizobacteria as commercial crop inoculants. Hence, the development of commercial growth promoter products like BioBoost for canola (a.i. *Delftia acidovorans*), SoySuperb for soybean (a.i. *Delftia acidovorans* & *Bradyrhizobium japonicum*), AlfaBoost (a.i. *Delftia acidovorans* & *Shinorhizobium meliloti*) and CornBoost for corn (a.i. *Achromobacter piechaudii*) could have been possible.

**O10****USE OF *Bacillus subtilis* STRAIN MBI 600 FOR BIOLOGICAL CONTROL AND ENHANCEMENT OF RHIZOBIAL INOCULANTS****R.D. Piran Cargeeg***Becker Underwood, Saskatoon, Saskatchewan, Canada*

The development of products containing PGPR is dependent upon many parameters. Among the most important factors for consideration are formulation, consistency of end-use performance and registration. Because *Bacillus* species form environmentally resistant endospores and most species are not considered risk agents by federal regulatory agencies in North America and Europe, this is a promising group of PGPR for product development. *Bacillus subtilis* strain MBI 600 is a biological control strain contained in the product Subtilex<sup>®</sup>. The product is an EPA registered biological fungicide labeled for the suppression of disease organisms that invade root systems including *Fusarium*, *Rhizoctonia*, *Alternaria*, and *Aspergillus*. It is labeled for formulating into registered end-use products and for use as a seed treatment (cotton, peanuts, seed and pod vegetables, soybeans, alfalfa, forage and turf grasses, wheat, barley, corn and canola), in-furrow application, or incorporation into peat moss or growing media. MBI 600 is also registered in the product Integral<sup>®</sup>, which is an aqueous preparation. In 2004, Becker Underwood introduced its first product using “BioStacked<sup>®</sup> Technology” which combined the technologies of PGPR and rhizobia. The first BioStacked product, aimed at soybean and peanut, was VAULT<sup>®</sup> for soybean and peanut which contains Integral, advanced strains of rhizobia, a plant activator of nodulation, and an extender for enhancing survival of rhizobia on soybean seed. Examples of benefits with strain MBI 600 will be presented along with a discussion of challenges to producing products containing PGPR.

**O11****COMMERCIAL MICROBIAL COMMUNITY FERMENTATION EXTRACTS IMPROVE FERTILIZER USE EFFICIENCY, SOIL STRUCTURE AND IRRIGATION IN FARMING SYSTEMS****R.N. Ames***Senior Staff Scientist, Advanced Microbial Solutions, Pilot Point, Texas, USA*

Most commercial plant growth promoting rhizobacteria (PGPR) products contain individual bacterial species or assemblages of bacteria which have been produced under aseptic conditions. In contrast, Advanced Microbial Solutions (AMS) manufactures soil amendment products using a fermentation process involving a complex community of microorganisms. The final product contains a subset of the original community as well as microbial by-products extracted from the production system. The biological and biochemical complexity of the product provides the capability to affect both soil and plant growth functions. This approach presents a distinctive performance advantage over previous microbial soil amendment technologies. In today’s economic environment, farmers are faced with concerns for costs of fuels and fertilizers; productivity impacts of reduced soil structure, water availability or low water quality; and regulatory pressures to better manage soil nutrients and reduce environmental impacts. Benefits documented from research with AMS products by independent university, government and private contract research can be grouped into four inter-related functions: reduction in soil salinity, improved soil structure, improved soil moisture and more efficient crop nutrient uptake. Improvements in fertilizer use efficiency and water management are particularly important aspects for agriculture worldwide. Research will be presented showing that AMS products can provide reductions in total salts and improve plant salt tolerance; reduce soil bulk density and compaction; increase water penetration and holding capacity; and provide yield increases with a 10 – 20% reduction in fertilizer application. Thus fermentation extracts from a multi-component microbial community can provide benefits well beyond the scope of typical, more highly targeted PGPR applications.

# **Functional Genomics of PGPR**

O12

**GENOMIC MINING FOR BIOACTIVE SMALL MOLECULES IN THE GENOMES OF *Pseudomonas fluorescens* PF-5 AND *Pseudomonas syringae* PV. *syringae* B728A****H. Gross<sup>1</sup>, N. Smid<sup>1</sup>, M. Kurz<sup>2</sup>, J.E. Loper<sup>3</sup>, M.D. Henkels<sup>3</sup>, B.T. Shaffer<sup>3</sup>, V.O. Stockwell<sup>4</sup>, B. Nowak-Thompson<sup>5</sup>, W. H. Gerwick<sup>6</sup>, A. Burch<sup>7</sup>, S. Lindow<sup>7</sup>**<sup>1</sup>Institute for Pharmaceutical Biology, University of Bonn, Germany, <sup>2</sup>IfMB, University of Bonn, Germany, <sup>3</sup>USDA-ARS, Corvallis, USA, <sup>4</sup>Dept. of Botany and Plant Pathology, OSU, Corvallis, USA, <sup>5</sup>Northland College, Ashland, USA, <sup>6</sup>CMBB/SIO, UCSD, San Diego, USA, <sup>7</sup>Dept. Plant and Microbial Biology, UC Berkeley, USA

Through genome sequence analysis, it had become evident that pseudomonads contain a much higher genetic capacity to produce secondary metabolites than expected, and that many of the products of these genetic pathways remain unknown at the present time. Since numerous microbial secondary metabolites display important biological activities, the exploration and utilization of these compounds is promising and might lead to unique natural products with pharmaceutical or agricultural potential.

Both *P. fluorescens* Pf-5 and *P. syringae* pv. *syringae* B728a (*Pss* B728a) were sequenced in 2005 due to their significance in agriculture: Pf-5 is known as a biological control agent because it suppresses crop diseases caused by soilborne plant pathogens whereas *Pss* B728a is known as a plant pathogen that causes brown spot disease on snap bean plants. Bioinformatic analyses of the sequenced genomes revealed a large number of orphan pathways encoding secondary metabolites in addition to known biosynthesis clusters. Using different genomic-guided approaches, we were able to link some of these pathways with natural products, e.g. the new antifungal natural products orfamides A-C and new cytotoxic rhizoxin-derivatives from Pf-5. Furthermore, the genetic locus in *Pss* B728a for the production of the compatible solute N-acetyl-glutaminyl-glutamine-amide could be annotated. For each compound, the biosynthesis, the discovery process as well as the pharmacological and agricultural potential or the physiological role will be discussed.

## O13

CHARACTERIZATION OF THE *gacS* and *rpoS* PROTEOMES OF *Pseudomonas chlororaphis* O6Y.C. Kim<sup>1</sup>, S.H. Han<sup>1</sup>, J.Y. Park<sup>1</sup>, S.A. Oh<sup>1</sup>, A.J. Anderson<sup>2</sup><sup>1</sup>Department of Plant Biotechnology, Chonnam National University, Gwangju, Korea, and <sup>2</sup> Department of Biology, Utah State University, Logan UT84322, UT, USA.

The GacS/GacS two component system in Gram-negative bacteria has been known as a key global regulator involved in many biological processes, and the stationary specific sigma factor, RpoS, is the master regulator of the general stress response. *Pseudomonas chlororaphis* O6 produces secondary metabolites such as phenazines and pyrrolnitrin that are involved in suppression of the growth of plant pathogens, induction of systemic resistance against plant diseases, and resistance to oxidative stress. In order to identify the proteins regulated by the GacS sensor kinase or RpoS, 2-D gel electrophoresis analysis of total proteins from the wild-type and GacS or RpoS mutants was performed, and down-regulated protein spots were characterized by MALDI-TOF and Q-TOF analysis. Genes encoding down-regulated protein spots were cloned by PCR and transcriptional expression of each gene was examined in the wild-type, the GacS mutant, and the complemented GacS mutant by RT-PCR analysis. Real-time RT-PCR analysis indicated that the selected 14 genes were regulated by GacS sensor kinase at the transcriptional level. Compared with the proteome of the *P. chlororaphis* O6 wild-type, 11 protein spots were severely diminished in the *rpoS* mutant cells. RpoS-regulated proteins are related to tryptophan metabolism, such as tryptophan halogenase (PrnA) and tryptophan monooxygenase; oxidative stress, such as peroxidase (AhpC) and glutathione peroxidase (Gpx); secretion, such as the polyamine ABC transporter (PotA), the TonB-dependent outer membrane heme receptor (CirA), and the type I secretion outer membrane protein (TolC); a global regulator, putative serine protein kinase (PrkA); and general metabolism, such as heme oxygenase (HemeO) and S-adenosyl-methyltransferase (MraW). To determine the roles of the GacS-regulated proteins, a *P. chlororaphis* O6 mutant lacking an anthranilate para-aminobenzoate synthesis component I gene (*ascI*), which is involved in the biosynthesis of tryptophan from chorismate, or tryptophan halogenase (*prnA*) involved in biosynthesis of pyrrolnitrin, was constructed by marker exchange mutagenesis. The *AscI* and *PrnA* mutants lost the ability to induce systemic resistance against *Pectobacterium carotovorum* SCCI. Root colonization studies and scanning electron microscopic observation indicated that *P. chlororaphis* O6 did not colonize tobacco roots. We are able to isolate unidentified GacS- or RpoS-regulated proteins and this information will open opportunities to investigate roles and phenotypes of GacS- or RpoS-regulated proteins in biocontrol rhizobacteria.

## O14

***Saccharomyces cerevisiae* GENOME-WIDE MUTANT SCREEN FOR SENSITIVITY TO 2,4-DIACETYLPHLOROGLUCINOL, A BIOCONTROL ANTIBIOTIC PRODUCED BY *Pseudomonas fluorescens***Y.-S. Kwak<sup>1</sup>, S.J. Han<sup>2</sup>, L.S. Thomashow<sup>3</sup>, J. T. Rice<sup>1</sup>, T.C. Paulitz<sup>3</sup>, D. Kim<sup>2</sup> and D.M. Weller<sup>3</sup><sup>1</sup>Dept. of Plant Pathology, Washington State University, Pullman, WA USA; <sup>2</sup>Dept. of Bio and Brain Engineering, KAIST, Daejeon, South Korea; <sup>3</sup>USDA-ARS, Pullman, WA USA

Strains of *Pseudomonas fluorescens* that produce the antibiotic 2,4-diacetylphloroglucinol (DAPG) are biocontrol agents of a variety of soilborne pathogens. DAPG is active against a broad spectrum of organisms ranging from bacteria to higher plants. This suggests that the antibiotic may target basic cellular processes or that there are multiple sites of action. The genetics and regulation of DAPG biosynthesis by *P. fluorescens* have been well studied. However, the effect of DAPG on target pathogens and the host plant has not been well described. We screened a *Saccharomyces cerevisiae* genome-wide deletion mutant pool ( $\approx 5,000$  mutants) as a first step to understanding the mechanism of action of DAPG. The screen identified 231 mutants with increased sensitivity to DAPG, including 22 multi-drug resistance related mutants. These targets included major cellular pathways such as membrane function, reactive oxygen regulation and cell homeostasis. Physiological studies with wild-type yeast validated the results of the mutant screening. Additionally, DAPG showed similar chemical-genetic data fitness with menthol, sodium azide and hydrogen peroxide in a high throughput screening profile. Collectively, these findings suggest that DAPG acts through multiple mechanisms which would make development of resistance in target pathogens unlikely.

## O15

**HYPHOSPHERE AND PATHORHIZOPHERE EFFECTS OF THE PLANT PATHOGENIC FUNGUS *Gaeumannomyces graminis* VAR. *tritici* ON GROWTH AND GENE EXPRESSION OF THE BIOCONTROL STRAIN *Pseudomonas fluorescens* PF29ARP**M. Barret<sup>1</sup>, P. Frey-Klett<sup>2</sup>, A-Y. Guillerme-Erckelboudt<sup>1</sup>, M. Boutin<sup>1</sup> and A. Sarniguet<sup>1</sup><sup>1</sup>INRA, Agrocampus Rennes-Université Rennes 1, UMR1099 BiO3P 'Biologie des Organismes et des Populations appliquée à la Protection des Plantes', 35653 Le Rheu, France<sup>2</sup>INRA, UMR 1136 IAM, 54280 Champenoux, France

Rhizosphere competence is an important prerequisite for biocontrol bacteria. However, whether an effect of the plant pathogenic fungus itself, so-called hyphosphere effect or a combined effect of roots and associated pathogenic fungi, the so-called pathorhizosphere effect, alters bacterial colonization traits remains to be investigated. For this, *in vitro* confrontation bioassays between the take-all fungus *Gaeumannomyces graminis* var. *tritici* (*Ggt*) and the biocontrol bacterial strain *Pseudomonas fluorescens* Pf29Arp were set up to analyse bacterial transcriptional changes induced by the fungal mycelium alone or by the mycelium infecting wheat roots. A Pf29Arp genomic shotgun microarray was constructed and used. During a commensal interaction between the bacteria and the mycelium alone, *Ggt* increased the growth rate of Pf29Arp. Before contact, the mycelium induced bacterial genes involved in the colonization process. At contact, genes encoding proteins of stress response and a patatin-like protein were up-regulated. During interactions on roots, necrosis caused by *Ggt* exerted a stronger effect on gene expression compared to root with ectotrophic *Ggt* and to healthy root. Among these genes some were related to carbon metabolism and oxidative stress, with a highest fold-change on necrosis. Moreover, specific necrosis responsive genes such as a gene involved protein secretion systems were induced.

**O16****UNRAVELLING THE RHIZOSPHERE USING THE *CPN60* GENOMIC MARKER AND A 454 SEQUENCER****G. Lazarovits, R. Ramarathnam, and S.M.Hemmingsen<sup>1</sup>***Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, and National Research Council Plant Biotechnology Institute, Saskatoon, Canada<sup>1</sup>*

The highly complex nature of the microbial communities in the rhizosphere has been a major limitation in trying to differentiate ecosystems where plant growth is enhanced versus one where the microbial community limits plant productivity. Attempts to describe microbial communities by either traditional microbiological or molecular methods have been limited in both scale and precision. The availability of genomics technologies offers an unprecedented opportunity to conduct more comprehensive characterizations of such communities. Hill et al. 2002, (Appl. Envir. Microbiol. 68:3055) described a novel technique for molecular diagnostics of complex microbial ecosystems using a method based on the chaperonin-60 sequence, in combination with high-throughput sequencing. The process involves creating libraries of *cpn60* amplicons generated by PCR of DNA extracts obtained from the rhizosphere. Initially, high throughput sequencing of about 1000-3000 cloned *cpn60* sequences were analyzed. However, by using a 454 sequencer we were able to obtain sequences from 20,000-50,000 PCR amplicons and no cloning was required. The taxonomic origins derived from the sequences ranged from unable to assign an identity, to placement at the genus level, and for some up to 100% DNA sequence identity with a species. However, the reference database at the moment is fairly small at about 7000 bacteria. Results will be presented from tests of this technology using DNA extracts of bulk soil, soil attached to potato roots, and roots washed free of soil. The data indicate that this will become a powerful tool for generating microbial fingerprints for the rhizosphere particularly as genomic identities of organisms increase.

**O17****CHARACTERISATION OF A *Pseudomonas fluorescens* SBW25 *FLEQ* MUTANT: INFLUENCE ON MOTILITY AND GENE AND PHENOTYPE EXPRESSION****R.W. Jackson***School of Biological Sciences, University of Reading, Whiteknights, Reading, UK*

The plant growth promoting rhizobacterium *Pseudomonas fluorescens* survives in soil and on and within plants. The ecological success of these bacteria often has major benefits to plants by suppression of soilborne disease agents and promotion of plant growth. The hypothesis that this occurs via a number of different mechanisms has been directly tested in a number of studies to identify the gene systems important for *P. fluorescens* ecological success in the rhizosphere. With the aid of the newly available genome sequence, almost 150 gene systems are expressed in strain SBW25 in the sugarbeet rhizosphere. Regulatory links controlling expression of some of these genes were also found – the flagellum biosynthesis master regulator, FleQ, was observed to control a wider range of systems than previously seen including flagellum synthesis, swimming and swarming motility, cellulose synthesis and oxidative stress. A combination of suppressor screen, microarray and phenoarray analyses has been used to identify the global influence of FleQ. Our data indicate that FleQ regulates flagellum-dependent swarming motility, but not flagellum-independent sliding motility. Furthermore, a wide range of gene systems, including regulators, enzymes and transporter system genes are influenced by FleQ. Phenoarray analysis indicated the *fleQ* mutant has considerably altered osmotic and pH resistance, dipeptide transport and antibiotic resistance profiles. Taken together, these data indicate that FleQ is a global regulator controlling a wide variety of gene systems and traits that can be analysed to determine their importance in *P. fluorescens* ecological success in the rhizosphere.

**O18****MICROARRAY ANALYSIS OF *Pseudomonas fluorescens* PF-5 GROWN ON SEED SURFACES****T. A. Kidarsa<sup>1</sup>, J. E. Loper<sup>1</sup>**<sup>1</sup>USDA-ARS-Horticultural Crops Research Laboratory, Corvallis, OR, USA

The biological control agent *Pseudomonas fluorescens* Pf-5 suppresses seedling emergence diseases caused by soilborne fungi and Oomycetes. Genes expressed by a biological control agent on seed surfaces determine the outcome of its interaction with target pathogens in the spermosphere, the soil surrounding seed surfaces. Seed exudates provide nutrition for both the biological control agent and the pathogen and can trigger germination and growth of pathogenic propagules. In addition, exudates may influence the spectrum of secondary metabolites produced by the biological control organism, thereby affecting its efficacy. To better understand mechanisms of biological control in the spermosphere, we performed microarray analysis of Pf-5 grown on pea seeds, comparing the transcriptional response of wild-type Pf-5 and derivative strains carrying a mutation in one of the regulatory genes *gacA* or *rpoS*. The *gacS/gacA* two-component regulatory system is required for secondary metabolite production in Pf-5 and *gacS* or *gacA* mutations lead to reduced biological control efficacy in many strains of *Pseudomonas*. In contrast, mutations in the sigma factor *rpoS* can enhance biological control of seedling diseases caused by the Oomycete *Pythium ultimum*. Genes downregulated in the *gacA* mutant include genes for biosynthesis of the secondary metabolites, 2,4-diacetylphloroglucinol, pyrrolnitrin, orfamide A, and rhizoxin analogs and genes encoding a type VI secretion system. Genes involved in iron acquisition were upregulated in the *gacA* mutant. Pyrrolnitrin and rhizoxin biosynthetic genes were also downregulated in the *rpoS* mutant, while pyoluteorin and 2,4-diacetylphloroglucinol genes were upregulated.

**O19****UNRAVELING THE MOLECULAR MECHANISMS UNDERLYING PLANT GROWTH PROMOTION BY *Pseudomonas fluorescens* STRAIN SS101****J.E. van de Mortel, J.M. Raaijmakers**

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The genus *Pseudomonas* not only harbors plant- and human pathogenic species, but also accommodates species that promote plant growth. We investigated the effect of *P. fluorescens* strain SS101 on the growth of *Arabidopsis thaliana*. In soil bioassays, strain SS101 promoted the shoot biomass of *Arabidopsis* by 35%: SS101-treated seedlings showed enhanced shoot and root development, enhanced greening and lateral root formation. To unravel the interaction transcriptome, microarray analyses were performed on *Arabidopsis* colonized by strain SS101. The results showed that over a time course of 18 days, 1179 genes were differentially expressed ( $\geq 2$ -fold, FDR < 0.05) in roots in response to strain SS101, of which 556 genes were up-regulated and 623 genes were down-regulated. In the leaves, 920 genes were differentially expressed with 687 genes up-regulated and 233 genes down-regulated. Subsequent bioinformatic analyses (GeneTrail) showed that many of the genes that were up-regulated in the roots represent genes involved in lateral root formation, unidimensional cell growth, response to auxin and cell wall modification. Genes down-regulated comprised genes involved in iron homeostasis, response to auxin stimulus, cell adhesion and unidimensional cell growth. In leaves, genes that were up-regulated are involved in defense response, cell death, and responses to salicylic acid, jasmonic acid and ethylene. Down-regulated genes were those involved in iron homeostasis and responses to auxin and abscisic acid stimulus. To identify potential mechanisms by which the bacterium promotes plant growth, several biosynthesis and regulatory mutants were tested. So far, however, no specific traits of strain SS101 were identified to play a role in plant growth promotion. For several of the identified plant genes, functional analysis is in progress and the latest results on knock-out and overexpression lines will be presented.

**O20****STUDYING PGPR GENE EXPRESSION UNDER SOIL CONDITIONS USING TAQMAN QRT-PCR ASSAYS: COMPARISON OF QUANTIFICATION AND NORMALIZATION APPROACHES****N. DeCoste and M. Filion***Université de Moncton, Department of biology, Moncton, NB, Canada, E1A 3E9*

PGPR gene expression involved in key functions such as plant growth promoting activities and biological control is not well characterized under complex rhizosphere soil conditions. Recent developments in RNA extraction methodologies and qRT-PCR assays now allow the study of microbial gene expression in soil, but technical difficulties and important considerations still need to be addressed. There is still no clear consensus as to which quantification strategy, absolute or relative, allows the most reliable quantification under soil conditions. Also, little is known about the impact of normalization methods used in these qRT-PCR assays. To address these questions, *Pseudomonas* spp. LBUM300 carrying genes for the production of 2,4-diacetylphloroglucinol (DAPG) and hydrogen cyanide (HCN), was used as a model to study PGPR gene expression under soil conditions. Soil was inoculated with different concentrations of *Pseudomonas* spp. LBUM300, and total soil RNA was extracted after 7 and 14 days for qRT-PCR analyses. Specific real-time PCR primers and TaqMan probes were designed and used in two-step qRT-PCR assays to monitor *phlD* (coding for DAPG) and *hcnC* (coding for HCN) gene expression. Absolute quantification (non-normalized and normalized to total RNA) and relative quantification (using commercially available standards) were compared. Expression of both genes remained detectable for at least 14 days in soil and bacterial dilution had a significant impact ( $P < 0.05$ ) on gene transcripts detected. Both genes demonstrated the same transcriptional trend. Absolute quantification provided reproducible results and a high level of specificity, while the relative approach did not perform well in soil. Normalization to total RNA did not significantly impact quantification. Based on the results obtained, a mainstream methodological approach is proposed for studying PGPR gene expression in soil.

**Genomes and  
Genomics – enabled  
Biology of PGPR**

## O21

**GENOMIC FEATURES OF PLANT-ASSOCIATED *Bacillus amyloliquefaciens* LINKED WITH PLANT GROWTH PROMOTION AND BIOCONTROL****X.H. Chen<sup>1</sup>, C. Rückert<sup>2</sup>, B. Baumgarth<sup>2</sup>, and R. Borriss<sup>1</sup>***1) Bacterial Genetics, Institute of Biology, Humboldt University Berlin, Chausseestr. 117, 10115 Berlin, Germany.**2) Center for Biotechnology (CeBiTec), Bielefeld University, Universitätsstr. 27, 33615 Bielefeld, Germany*

Plant-associated *B. amyloliquefaciens* strains are distinguished from other representatives of *B. amyloliquefaciens* by their ability to actively colonize the plant rhizosphere, to stimulate plant growth, and to suppress competing phytopathogenic bacteria and fungi. Due to their biofertilizer and biocontrol properties they are becoming increasingly important as a natural alternative to chemical pesticides and other agrochemicals. In order to reveal the specific genomic features linked with the properties beneficial for plant growth we have already sequenced the whole genome of FZB42, one of the representatives of this group, which is economically exploited as a natural biofertilizer and genetically amenable (1). Direct comparison of the genomes of FZB42 and the other *Bacillus* strains, including *B. subtilis* 168, revealed 310 genes as being unique for FZB42 and possibly involved in plant-bacterium interactions. In order to reduce further the number of those candidate genes we have determined now the whole genome sequence of the type strain *B. amyloliquefaciens* F (DSM7) as the closest relative of FZB42 without plant colonizing ability. Within the DSM7 genome the number of giant gene clusters for nonribosomal synthesis of secondary metabolites (lipopeptides and polyketides) was found to be dramatically reduced. In contrast to FZB42, no gene clusters involved in synthesis of fengycin, macrolactin, difficidin and the nrs antibiotics exist, underlining the importance of those secondary metabolites for the plant-associated life-style of FZB42. In addition, we have included further representatives of that interesting taxonomic group in our analysis by using microarray-based comparative genomic hybridisation (M-CGH). Our preliminary results revealed that significant differences in the genomes of plant-associated and non-associated *B. amyloliquefaciens* strains do exist: whilst representatives of the plant-associated ecotype are distinguished by only 100 genes, around 300 genes are different between the representatives of both groups.

(1) Chen et al. *Al.* 2007. *Nature Biotechnol.* 25:1007-14

O22

**GENOME ORGANIZATION, ENVIRONMENTAL SUCCESS, AND PHYLOGENY: INSIGHTS FROM COMPARATIVE GENOMICS OF *Pseudomonas fluorescens*****M.W. Silby<sup>1</sup>, A.M. Cerdeño-Tárraga<sup>2</sup>, S.R. Giddens<sup>3</sup>, R.W. Jackson<sup>4</sup>, S.B. Levy<sup>1</sup>, P.B. Rainey<sup>5</sup>, N.R. Thomson<sup>2</sup>**

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The genome sequences from *P. fluorescens* strains Pf0-1, SBW25, and Pf-5 have been compared. At a genome-wide scale, comparison reveals compartmentalized genomes where the majority of conserved coding sequences are clustered around the origin of replication, and extensive reciprocal recombination around the replication terminus is apparent. Only 61% of genes are conserved; this diversity suggests the *P. fluorescens* pan genome is very large. Genes expressed in natural environments are likely to be important for success in those environments, and that success is critical for applications such as biocontrol. Comparative genomics using data from a comprehensive screen for environmentally-induced genes in SBW25 shows that in *P. fluorescens*, ecological success depends on both conserved and specialized functions. Further analysis of additional genes that promote environmental fitness supports the concept that in many cases these strains have found different solutions to similar problems. Phylogenetic analysis of *P. fluorescens* and other *Pseudomonas spp.* using a whole genome approach resulted in relatively low support for classification of *P. fluorescens* into a single species. Closer examination of orthologous genes lends credence to the idea that Pf0-1, SBW25, and Pf-5 shouldn't be grouped into one species. Ironically, the comparison of multiple genomes from the same species, enabling unprecedented insight into critical aspects of the biology of that species, is also leading to the conclusion that in some cases we may not be comparing the same species at all.

**O23****EVOLUTION ASPECTS OF 2,4 DIACETYLPHLOROGLUCINOL SYNTHESIS IN *PSEUDOMONAS FLUORESCENS*****J. Morrissey, J. Moynihan, E. Boyd, and F. O’Gara***Microbiology Department and BIOMERIT Research Centre, University College Cork, Ireland*

Pseudomonads produce many different secondary metabolites and while some are known to improve ecological fitness, and others are important for host colonisation or virulence, the function and evolutionary origins of many remain undetermined. We are interested in the polyketide 2,4, diacetylphloroglucinol (DAPG) that is produced by some isolates of *P. fluorescens*. This metabolite is of particular interest because of its antifungal properties and strains that produce DAPG are associated with natural disease-suppressive soils and with biological control applications. Surveys indicate that approximately 1% of strains of *P. fluorescens* produce this metabolite, with some suggestions that frequencies may be higher in certain niches or in association with some plant genotypes. For these reasons, we decided to explore the evolutionary history of the DAPG biosynthetic cluster, initially taking advantage of genomic sequence information from two producing strains F113 and Pf5, and later expanding our study to a geographically diverse collection of 36 strains of *P. fluorescens*. This analysis covered both sequence polymorphisms within the key *phlD* gene, and genomic organisation of the biosynthetic cluster. In parallel, we analysed housekeeping genes to reconstruct species phylogeny. We found that the DAPG biosynthetic cluster is ancestral in *P. fluorescens* and is not a recent horizontal acquisition. It appears to have been lost once in the lineage that has given rise to the majority of (DAPG-negative) strains of *P. fluorescens* and, within DAPG-positive strains, intragenomic rearrangements have resulted in the DAPG cluster being present in at least three distinct genomic loci. These correspond to different phylogenetic clades indicating that these rearrangements are also ancient. Expanding the analysis to include additional data on *phlD* in the literature suggests that additional rearrangements may have occurred. The genomic analysis also identified an additional gene in the cluster, though the function of this gene remains unknown. Although the sequences of genes within the *phl* gene cluster are highly conserved, some differences in putative regulatory regions can be discerned. We are interested in knowing how the gene cluster is regulated in different phylogenetic lineages and our recent work addresses this question.

O24

## EVOLUTION OF THE PHENAZINE BIOSYNTHESIS PATHWAY AND DIVERSITY OF PHENAZINE-PRODUCING *Pseudomonas* SPP. IN DRYLAND WHEAT-PRODUCING AREAS OF WASHINGTON STATE

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Phenazines are versatile secondary metabolites of bacterial origin that function as signaling compounds and contribute to the ecological fitness and pathogenicity of the producing strains. A 2007-2008 survey of commercial dryland fields in central Washington State (annual precipitation <15 in) revealed unexpectedly high populations of phenazine-producing *Pseudomonas* strains on non-irrigated cereals grown within an area of about three million acres that is roughly bounded by 46.8° and 47.9° N and 117.5° to 119° W. The presence of phenazine-producing *Pseudomonas* spp. correlated with high levels of the antibiotic phenazine-1-carboxylic acid that were detected on roots of field-grown wheat and barley. Genotyping using the key phenazine biosynthesis gene *phzF* as a molecular marker revealed that phenazine-producing strains from Washington State soils may form two new species that belong to the *P. fluorescens* species complex. The investigation of distribution, diversity and genomic context of phenazine genes was further extended using a collection of 82 strains of *Pseudomonas*, *Pectobacterium*, *Burkholderia*, *Brevibacterium*, and *Streptomyces* of diverse geographic, environmental and clinical origins. Contrasting phylogenies inferred from sequences of *phzF* and housekeeping genes (16S, *recA*, *rpoB*, *atpD*, and *gyrB*) suggested the involvement of horizontal gene transfer in the evolution of the *phz* biosynthetic pathway in some lineages of bacteria, but at the same time revealed a high degree of conservation of the core phenazine genes in *Pseudomonas* spp.

O25

## TONB DEPENDENT RECEPTORS OF *Pseudomonas fluorescens* PF-5 AND SIDEROPHORE UPTAKE

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TonB-dependent receptors (TBDRs) are outer membrane proteins with essential roles in iron uptake by Gram-negative bacteria. The biological control strain *Pseudomonas fluorescens* Pf-5 has 45 predicted TBDRs in its genome, which far exceeds the number of TBDRs in most published bacterial proteomes. Bioinformatic analysis confirmed all 45 TBDRs have conserved domains with shared functionality, such as the plug and receptor domains. 18 TBDRs were found to have the N-terminal extension domain characteristic of transducers, a subclass of TBDRs that typically initiate a signaling pathway involving anti-sigma factors and extracytoplasmic function (ECF) sigma factors. The remaining 27 TBDRs lack the N-terminal extension domain and linked sigma factors, but may function as receptors for a variety of substrates. Phylogenetic analysis of the transducers and receptors aid in assigning putative functions and implicate horizontal gene transfer as a mechanism for acquisition by Pf-5. Five of the 18 putative transducers are related to TBDRs that function in the uptake of ferric-pyoverdines; the fluorescent siderophores produced by *Pseudomonas* spp.; these are being tested as ferric-pyoverdine receptors in Pf-5. Cross-feeding assays between different strains of *Pseudomonas* and Pf-5 receptor mutants in a pyoverdine-pyochelin deficient background indicate specificity of receptors for different pyoverdine structures. Additionally, Pf-5 is able to utilize other siderophores and iron containing compounds not made by *Pseudomonas*. TonB dependent receptors may have important roles influencing the environmental fitness of *P. fluorescens* Pf-5.

**PGPR and  
Rhizosphere  
Community  
Responses**

O26

**ANTAGONIST/PGPR INTERPLAY WITH RHIZOSPHERE COMMUNITIES****G. Berg<sup>1</sup>, C. Zachow<sup>1</sup>, K. Scherwinski<sup>1</sup>, M. Cardinale<sup>1</sup>, R. Grosch<sup>2</sup>, and R. Tilcher<sup>3</sup>**

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To use micro-organisms to control plant pathogens, to enhance plant growth and protect them against abiotic stress are environmentally friendly alternatives in agriculture. Although originating from plant-associated microenvironments themselves, beneficial bacteria, if applied to plants in adequate numbers, may perturb indigenous microbial populations and the important ecological functions associated therewith. Therefore, possible non-target effects of the applied antagonists on ecologically important soil-microbes need to be considered. Methods as well as examples from literature and our own research are summarized. Regarding the latter, a study which analysed the effect of biological control agents (BCAs) on non-target microbes in the field will be presented. Whereas the bacterial BCAs *Serratia plymuthica* HRO-C48 (RhizoStar<sup>®</sup>) and *Streptomyces* sp. HRO-71 (RhizoVit<sup>®</sup>) were applied to control the pathogen *Verticillium dahliae* on strawberry and potato, the bacterial strains *Pseudomonas trivialis* 3Re2-7 (Salavida<sup>®</sup>), *P. fluorescens* L13-6-12, *S. plymuthica* 3Re4-18 and the fungal antagonists *Trichoderma reesei* G1/8 and *T. viride* G3/2 were introduced to control *Rhizoctonia solani* on lettuce and potato. As the analysed BCAs belong to different microbial groups like gram positive (HRO-71) and gram negative (HRO-C48, L13-6-12, 3Re2-7, 3Re4-18) bacteria or the ascomycota (G1/8, G3/2) and originated from different micro-habitats like the rhizosphere or the endorhiza, general conclusions could be drawn from our results. After BCA treatment we did not observe any long-term effect on the plant-associated microbes in any tested pathosystem. This was confirmed by results from other studies. Therefore, no sustainable risks could be seen for the indigenous micro-organisms. In addition, it is necessary to assess the potential in human pathogenicity. For this procedure, an assay with the nematode *Caenorhabditis elegans* was developed. Our new findings may help to improve the development as well as the registration procedures of future microbial inoculants.

O27

**HOST PLANT SPECIFICITY IN 2,4-DIACETYLPHLOROGLUCINOL PRODUCING *Pseudomonas* SPP.: WHO DRIVES THE PARTNER SELECTION?****B. B. Landa***Institute of Sustainable Agriculture, CSIC, P.O. Box 4084, 14080 Córdoba, Spain.*

Plants have evolved strategies of stimulating and supporting specific groups of antagonistic microorganisms in the rhizosphere as a defense against diseases caused by soilborne plant pathogens. Strains of *Pseudomonas* spp. producing the polyketide antibiotic 2,4-diacetylphloroglucinol (*phlD*<sup>+</sup>) are some of the most effective plant growth-promoting rhizobacteria (PGPR) that have been implicated worldwide in natural soil suppressiveness and in controlling several root and seedling diseases. Many studies have documented the effect of plant species on microbial communities, especially on specific groups of bacteria, and specialized relationships seem to exist between certain non-symbiotic biocontrol PGPR and the host plant. In fact, some studies have shown that plants may initiate and maintain sophisticated mutualistic relationships with *phlD*<sup>+</sup> *Pseudomonas* spp. Thus, some *phlD*<sup>+</sup> genotypes show affinity or preference for the roots of certain crops at the species and cultivar level. Although over 30 different *phlD*<sup>+</sup> genotypes have been identified worldwide so far using diverse molecular techniques, little is still known concerning the abiotic and biotic factors that govern the presence and abundance of specific *phlD*<sup>+</sup> genotypes in a certain soils or environments, as well as their superior colonization of certain host plants. This talk will focus on the biogeography and likely host plant specificity of *phlD*<sup>+</sup> *Pseudomonas* spp. While it is important to know which *phlD*<sup>+</sup> genotypes are where, the more interesting question is why they are found there and not somewhere else. In other words, what determines the match between a *phlD*<sup>+</sup> genotype and a host plant in a specific environment?

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O28

**EFFECTS OF THE METABOLITES PRODUCED BY COEXISTENT MICROORGANISMS ON THE ANTIBIOTIC PRODUCTION BY FLUORESCENT PSEUDOMONADS****N. Someya<sup>1</sup>, T. Yoshida<sup>2</sup>, M.T. Noguchi<sup>2</sup>, H. Sawada<sup>3</sup>, K. Tsuchiya<sup>4</sup>**

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Certain rhizospheric fluorescent pseudomonads produce antibiotics such as 2,4-diacetylphloroglucinol (DAPG). These antibiotic-producing pseudomonads are utilized as effective biocontrol agents against phytopathogens. However, in an agricultural environment the production of antibiotics by these microorganisms is frequently influenced by various biotic and abiotic factors. It has been reported that certain metabolites, termed inhibitors, such as fusaric acid or phenylacetic acid, suppress antibiotic biosynthesis in pseudomonads and they are produced by coexistent microorganisms including phytopathogens. To investigate the biocontrol activity of pseudomonads under agricultural conditions, we collected antibiotic-producing fluorescent pseudomonads from various plant rhizospheres and assessed their DAPG production under the influence of inhibitors and related compounds. The inhibitory effect of the above metabolites on DAPG productivity was variable depending upon the isolates examined. Phylogenetic analysis based on the 16S rDNA gene revealed that inhibitor-sensitive and inhibitor-insensitive isolates belonged to different clusters. DAPG biosynthesis in inhibitor-sensitive isolates was partially restored by addition of iron. Antibiotic-producing pseudomonads play critical roles in disease suppression when they are applied as biocontrol agents. It is important to select the optimum inhibitor-insensitive isolates for successful biocontrol of phytopathogens.

O29

**PLANT GROWTH-PROMOTING BACTERIA IN SOILS UNDER CROP ROTATION OR CONTINUOUS CROPPING****N. Bajsa<sup>1,2\*</sup>, G. Azziz<sup>1</sup>, H.C. Coutinho<sup>3</sup>, A.S. Rosado<sup>4</sup>, A. Arias<sup>1</sup>**<sup>1</sup>Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay. <sup>2</sup>Facultad de Ciencias, UdelaR, Montevideo, Uruguay. <sup>3</sup>Embrapa Solos, Rio do Janeiro, Brasil. <sup>4</sup>Universidade Federal do Rio de Janeiro, Brasil. \*nbajsa@iibce.edu.uy

Crop rotation has been incorporated by Uruguayan farmers as a sustainable agricultural practice, based on its effects on crop productivity, soil physicochemical parameters and pathogen populations. However, less is known about the impact of this management strategy on plant-growth promoting bacteria (PGPB). The objective of this work was to assess the impact of continuous cropping of grain crops or its rotation with pastures on the structure of PGPB communities. Soil samples were taken in autumn and spring during 2 years, from long-term field assays. Cultivable bacterial populations were determined by plate counting. A higher number of heterotrophs, actinobacteria and fluorescent *Pseudomonas* was detected in systems with higher proportion of pastures than in continuous cropping systems. *Bacillus* spp. populations did not show a consistent response related to management. Bacterial diversity was studied by DGGE analysis of PCR-amplified 16S rDNA gene from total soil DNA, including nonculturable organisms. Analysis of the *Pseudomonas* community was more sensitive to detect changes in species composition than the analysis of the *Bacteria* domain, although those changes were not related to rotation intensity. The results of this work will contribute to the knowledge of edaphic bacteria, and to the designing of management strategies that would prevent soil degradation processes towards the conservation of agroecosystems and its sustainable functioning.

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O30

**DYNAMICS OF SPORE GERMINATION AND COLONIZATION OF GERMINATING SEEDS WITH A *Bacillus pumilus* STRAIN****J.W. Kloepper<sup>1</sup>, L. De La Fuente<sup>1</sup>, C. Ramírez<sup>1</sup>, M. Burkett-Cadena<sup>1</sup>, J.A. McInroy<sup>1</sup>, I.B. Sorokulova<sup>2</sup>, O. Pustovyy<sup>2</sup>, and V. Vodyanoy<sup>2</sup>**<sup>1</sup>Dept. of Entomology & Plant Pathology; <sup>2</sup>College of Veterinary Medicine, Auburn Univ., Auburn, Alabama, USA.

Endospore formation by bacilli PGPR allows the production of highly stable formulations for use as seed treatments. However, in contrast to pseudomonad PGPR, with bacilli, little is known about the dynamics of spermosphere and root colonization. This study addresses the key question: What are the dynamics of endospore germination on planted seeds? Spores of PGPR strain *B. pumilus* INR-7 were evaluated *in planta* and with a light microscope using annular dark field illumination to enhance resolving power. With microscopy, the early spore germination steps were evaluated by selecting 33 spores/sample time and using Image-Pro Plus software to calculate the percentage distribution of length and volume. Under ideal conditions in tryptic soy broth, the distribution of cell length increased from 70% < 1.2  $\mu\text{m}$  at time 0 to 90% > 1.2  $\mu\text{m}$  after 50 min. Spores in seed exudates of lentil or soybean showed delayed responses, with increases in length and volume being evident after 6 hr. Colonization dynamics on soybean seed were evaluated in a bioassay in which INR-7 routinely elicits growth promotion. Spores of the wild-type strain and 2 rif-resistant mutants, all of which showed a similar colonization pattern, were inoculated onto seeds at  $10^6$  or  $10^3$  spores/seed. Populations of spores and vegetative cells were quantified, by plating with and without pasteurization, at 0, 6, 12, 24, 36, 48, 54, and 72 hr. Radicals emerged starting at 48 hr, and root systems were present by 72 hr. From 6 – 12 hr after planting, spores applied at  $10^6$ /seed decreased to  $10^1$ /seed, while vegetative cells increased from 0 to  $10^6$ /seed, indicating a high percentage of initial spore germination. Vegetative cells then apparently sporulated, as spore counts increased to  $10^7$ /seed, while vegetative decreased to  $10^1$ /seed. More work is underway to clarify the role of vegetative cells and spores in colonization.

**O31****QUORUM SENSING AND INTERKINGDOM SIGNALING IN PGPR *Pseudomonas*****V. Venturi, I. Bertani, L. Steindler, and S. Subramoni***International Centre for Genetic Engineering & Biotechnology (I.C.G.E.B.) Padriciano 99, 34012 Trieste Italy*

Bacteria in the wild mainly live in close association with many different bacteria and eukaryotic hosts, meaning that they constantly need to be aware of their neighbors. It is now recognized that bacteria communicate and behave/synchronize as a community employing a level of gene regulation involving intercellular communication (quorum sensing, QS) organized by the production and detection of small signal molecules. QS has been studied in many bacterial species and shown to provide a significant advantage to a community of bacteria by adapting to environmental conditions and often enhancing its defense capabilities against other microorganisms or eukaryotic resistance mechanisms. In Gram-negative bacteria, *N*-acyl homoserine lactones (AHLs) are to date the most commonly used signal molecules being produced by a synthase enzyme belonging to the LuxI protein family. A transcriptional regulator belonging to the LuxR family then forms a complex with the cognate AHL at threshold levels, altering the transcriptional activity of target genes. A major question our research is addressing is if and how PGPR *Pseudomonas* employ AHL QS to establish a beneficial association with plants. In addition, is AHL QS involved in inter-species and inter-kingdom communication and is there any interplay between beneficial and pathogenic bacteria? We are studying these aspects of bacteria-bacteria and bacteria-plant communication using the rice plant, rhizosphere pseudomonads and rice-pathogenic bacteria as working models. Our results thus far have demonstrated that most rhizosphere *Pseudomonas* do not communicate via AHL QS, whereas rice pathogenic bacteria do and regulate virulence associated factors. Finally some pseudomonads are able to respond to exogenous signals produced by other bacteria and/or to communicate with the plant by responding to a plant compound via a protein which is highly related to the quorum sensing LuxR family.

**O32****QUORUM SENSING AND PHENAZINE STRUCTURE ARE IMPORTANT FOR BIOFILM FORMATION BY *Pseudomonas chlororaphis* 30-84****L.S. Pierson III, E.A. Pierson, VSR K. Maddula, M. Vyas***Dept. of Plant Sciences, Division of Plant Pathology & Microbiology, The University of Arizona, Tucson, AZ.*

*Pseudomonas chlororaphis* strain 30-84 produces two major phenazine (PZ) compounds, phenazine-1-carboxylic acid (PCA) and 2-hydroxy-phenazine-1-carboxylic acid (2OHPCA). The production of these PZs is responsible for two behaviors critical for biological control, inhibition of the fungal take-all pathogen *Gaeumannomyces graminis* var. *tritici* (*Ggt*) and persistence of strain 30-84 in the rhizosphere in the presence of the indigenous microbial community. The role of PZs in these two behaviors suggested they serve a competitive role. However, PZ production is regulated at multiple levels, including quorum sensing (QS) by the PhzR/PhzI system, inconsistent with a strictly competitive role. Using single-pass flow cells, we demonstrated that QS and PZ production are essential for biofilm formation. In a PZ biosynthesis structural mutant, increased copies of the PhzR/PhzI QS system failed to restore biofilm formation, while constitutive expression of the PZ biosynthesis genes resulted in earlier biofilm formation. To probe the roles of different PZ structures, we constructed derivatives of *P. chlororaphis* that produced only PCA or more efficiently converted PCA into 2OHPCA while maintaining normal QS regulation. These derivatives produced altered ratios of the PZs although the total amount of PZ produced was similar. The PCA-only derivative produced thicker, denser biofilms than the wild type. In comparison, the 2OHPCA enhanced strain demonstrated earlier cell adhesion as compared to the wild type or the PCA-only derivative. The 2OHPCA enhanced strain also produced a thicker biofilm than the wild type, but contained fewer cells as compared to the PCA-only derivative. The PZ-altered derivatives also differed in their ability to inhibit *Ggt in vitro*. Preliminary microarray data suggested that PZs themselves serve as signaling mechanisms. Our findings demonstrate that PZs play multiple roles in the lifestyle of *P. chlororaphis* strain 30-84.

# **Mechanisms of Plant Growth Promotion and Biocontrol**

O33

**KREBS CYCLE INTERMEDIATES AND TEMPERATURE REGULATE GAC-DEPENDENT BIOCONTROL FACTOR EXPRESSION IN *Pseudomonas fluorescens* CHA0****K. Takeuchi, B. Humair, C. Reimmann, C. Keel, C. Dubuis, J. Rolli, D. Haas***Department of Fundamental Microbiology, University of Lausanne, CH-1015 Lausanne, Switzerland*

In the plant-beneficial biocontrol strain *Pseudomonas fluorescens* CHA0, the Gac/Rsm signal transduction pathway positively controls the production of antifungal biocontrol factors (secondary metabolites and exoenzymes) via the up-regulation of the small RNAs RsmX, RsmY and RsmZ. These small RNAs sequester the RNA-binding proteins RsmA and RsmE, which are translational repressors of genes involved in biocontrol. In batch culture, this process typically occurs after exponential growth (also termed the trophophase) when cells reach high population densities and grow slowly (i.e., during the idiophase). Both extracellular and intracellular signal molecules favor the onset of the idiophase. Mutational analysis in strain CHA0 has shown that an imbalance in the tricarboxylic acid cycle can strongly affect the expression of RsmXYZ and hence that of biocontrol factors. In particular, RsmXYZ expression appears to be stimulated by high fumarate levels and hindered by high isocitrate levels, indicating links between primary and secondary metabolism. Moreover, high incubation temperatures (> 30°C) inhibit the function of the GacS/GacA two-component system, by strengthening the action of the RetS sensor, an antagonist of the GacS sensor. These findings open up new perspectives in the understanding of biocontrol mechanisms in *P. fluorescens*.

O34

**GLOBAL AND SPECIFIC REGULATION OF CYCLIC LIPOPEPTIDE BIOSYNTHESIS IN PLANT GROWTH-PROMOTING *Pseudomonas fluorescens*****J. M. Raaijmakers and I. de Bruijn***Laboratory of Phytopathology, Wageningen University, 6709 PD Wageningen, The Netherlands*

Cyclic lipopeptide biosurfactants (CLPs) play important roles in motility, colonization, biofilm formation and antimicrobial activity of a variety of microorganisms. CLPs are synthesised by large nonribosomal peptide synthetases. In PGPR-strain *Pseudomonas fluorescens* SS101, biosynthesis of the CLP massetolide is governed by three NRPS genes designated *massA*, *massB* and *massC*. In contrast to most other CLP gene clusters, *massA* and *massBC* are not organized in a single operon but are physically and transcriptionally disconnected. To identify the regulatory network of CLP biosynthesis in *P. fluorescens*, a variety of approaches was taken including random and site-directed mutagenesis followed by cloning, sequencing and functional analysis. Both global and pathway-specific regulatory genes were identified, including the GacA/GacS two-component system and LuxR-type transcriptional regulators. Overexpression of the identified *luxR* gene resulted in up-regulation of *mass* gene expression and increased massetolide production. In contrast to several other CLP-producing *Pseudomonas* strains, no evidence was found for *N*-AHL-mediated quorum sensing in *P. fluorescens* SS101. In addition to the global and pathway-specific regulators, several novel regulatory genes were identified, including the ClpP protease. The role of this serine protease in CLP-biosynthesis was confirmed by site-directed mutagenesis, genetic complementation and expression analyses. An overview will be given of the versatile functions of CLPs and the complexity of the genetic network underlying CLP-biosynthesis in beneficial *Pseudomonas fluorescens*.

**O35****RHIZOBACTERIAL VOLATILES AS ELICITORS OF PLANT DEFENSE AND GROWTH PROMOTION****C.-M. Ryu<sup>1</sup>, H. Yi<sup>1</sup>, M.A. Farag<sup>2</sup>, P.W. Paré<sup>2</sup>, and J.W. Kloepper<sup>3</sup>**<sup>1</sup>Bio-Industry and Bioenergy Research Center, KRIBB, Daejeon, S. Korea; <sup>2</sup>Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, TX USA; <sup>3</sup>Department of Entomology and Plant Pathology, Auburn University, Auburn, AL USAEmail: [cmryu@kribb.re.kr](mailto:cmryu@kribb.re.kr)

Some plant growth-promoting rhizobacteria (PGPR), in the absence of physical contact with plants, stimulate plant growth and elicit induced systemic resistance (ISR) via volatile organic compound (VOC) emissions. Gas chromatographic analysis of VOCs collected from the PGPR strains *Bacillus subtilis* strain GB03 and *B. amyloliquefaciens* strain IN937a reveals consistent patterns in VOC emissions. The two most abundant compounds, 2,3-butanediol and 3-hydroxy-2-butanone, are consistently emitted from GB03 and IN937a while these metabolites are not released from DH5 $\alpha$ . Of several Arabidopsis mutant lines tested for regulatory control of ISR against *Erwinia carotovora* subsp. *carotovora*, only the ethylene-insensitive line (*ein2*) did not exhibit an amelioration of disease symptoms when Arabidopsis plants were pre-treated with GB03 volatiles. Cytokinin was revealed to have a critical role in bacterial VOC-elicited plant growth promotion. To assess potential utilization of PGPR VOCs for crop plants, volatile blends from GB03 and IN937a were applied to pepper and tobacco roots. The survival capacity of 2,3-butanediol null bacterial mutants was significantly reduced in proximity with plant roots. These reduced bacterial survival rates suggest that in addition to bacterial VOCs' triggering plant growth and ISR in plants, such chemicals provide protection for PGPR via chemical signaling within the host plant. Our results suggest that 2,3-butanediol produced by *B. subtilis* may serve in dual functions to elicit indirectly ISR on the foliar parts and directly the production of plant antimicrobial compounds on the root system and to act as a protecting agent for bacterial cells against the compounds. (The project was supported by BioGreen21, RDA, S. Korea)

**O36****RHIZOBACTERIAL VOLATILES: AN ENTRY INTO PLANT GROWTH PROMOTION****H. Zhang, X. Xie, M. Kim, M. Aziz and P.W. Paré**

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Plant growth-promoting rhizobacteria are naturally occurring soil microorganisms that colonize roots and stimulate plant growth. Although such beneficial bacteria have been applied to a wide range of agricultural crops for the purpose of growth enhancement, the biochemical complexity of such plant-microbe interactions have limited our mechanistic understanding of bacterial-induced plant growth promotion. Using a simplified experimental design in which the bacterial stimulus is limited to volatile components, we have begun to characterize initial chemical signals from the commercial strain *Bacillus subtilis* GB03 as well as down-stream molecular and physiological plant responses involved in inducible growth promotion in Arabidopsis. Here we will provide biochemical evidence that GB03 regulates photosynthesis through modulation of endogenous sugar/ABA-signaling to increase photosynthetic efficiency as well as chlorophyll content thereby establishing a regulatory role for soil symbionts in plant acquisition of energy.

O37

**BACTERIAL TURNOVER OF AUXIN: ITS ROLE IN PLANT GROWTH AND HEALTH****J. Leveau***Department of Plant Pathology, University of California, Davis CA 95616, USA*

Culture-dependent and -independent approaches were used to determine the abundance and diversity associated with bacterial recycling of the plant hormone indole 3-acetic acid (IAA). Bulk and rhizosphere soils as well as other plant material collected from different sources contained significant numbers of bacteria that were able to use IAA as a sole source of carbon and energy, showing that IAA recycling is not an uncommon property among soil and plant-associated bacteria. For many of these it was confirmed by PCR that they harbor DNA sequences that resemble *iac* genes for IAA turnover in model strain *Pseudomonas putida* 1290. Similar *iac* carriers were also identified among those bacterial species for which a genome sequence is now available. Several were tested and confirmed to be able to grow on IAA, showing that IAA degraders have representatives in the  $\beta$ - and  $\gamma$ -Proteobacteria as well as the high G+C Gram-positive bacteria. Interestingly, not all of these originated from soil or plant environments. Based on complementation of *Pseudomonas* strains that lacked one or more of the *iac* genes, a cultivation-independent method was developed for the isolation of DNA fragments that encode IAA turnover. Several such fragments were sequenced to reveal the genetic diversity of genes underlying bacterial IAA catabolism. This data set provides the basis for a genetic and molecular approach to the impact of bacterial IAA turnover on plant functioning and growth promotion and its role in plant-microbe interactions.

O38

**AUXIN SIGNALING IN *Azospirillum brasilense*: A HOLISTIC APPROACH****S. Spaepen<sup>1</sup>, J. Vanderleyden<sup>1</sup>***<sup>1</sup>Centre of Microbial and Plant Genetics, K.U.Leuven, Heverlee, Belgium*

The term phytostimulation has been used to indicate the direct plant growth promoting effect upon *Azospirillum* inoculation. Since then, many reports have described the bacterial production of the phytohormone indole-3-acetic acid (IAA) in different species as a possible mechanism to explain their plant growth promotion.

The microbial biosynthesis of IAA and its regulation has intensively been studied in *Azospirillum* and other plant-associated bacteria and revealed that synthesis occurs via different pathways, with the occurrence of more than one pathway in the same bacterial strain. The biosynthesis can be subject to complex regulation, including positive feedback regulation by IAA itself. Recently, it has been shown that IAA is used as a signaling molecule in bacteria and other micro-organisms.

In view of these results, we postulate that IAA synthesis by PGPB such as *Azospirillum* serves multiple functions. We use *Azospirillum brasilense* Sp245 as a model system to apply various approaches, including focused genetic and biochemical techniques. Recently, we have implemented high throughput technologies such as transcriptome analysis of free-living *A. brasilense* and of *Azospirillum*-colonized *Arabidopsis* roots, and proteome analysis of free-living *Azospirillum* cells, aiming to gain more insights in the role of IAA and other mechanisms of direct plant growth promotion. We will report on the proteome analysis of *A. brasilense* Sp245 with focus on IAA-induced proteins.

O39

**CHARACTERIZATION OF INDOLEACETIC ACID-RESPONSIVE MUTANTS OF THE PLANT-BENEFICIAL BACTERIUM *Pseudomonas fluorescens* CHA0****L. Rochat<sup>1</sup>, M. Péchy-Tarr<sup>1</sup>, P. de Werra<sup>2</sup>, M. Maurhofer<sup>2</sup>, C. Keel<sup>1</sup>**<sup>1</sup>*Department of Fundamental Microbiology, University of Lausanne and* <sup>2</sup>*Plant Pathology, Institute of Integrative Biology, ETH Zurich, Switzerland*

Plants communicate with many different organisms in the rhizosphere. There is evidence that this cross-talk takes place via signals present in root exudates released by the plant. Among the root-associated microorganisms that may be influenced by these plant signals are the plant growth-promoting rhizobacteria that have the ability to protect their plant host against soil-borne pathogens. In the present study, the behaviour of one such biocontrol bacterium, *Pseudomonas fluorescens* CHA0, was investigated in regard of effects of plant-produced phenolics on the expression of biosynthetic genes for antifungal compounds contributing to the strain's biocontrol activity. Among the investigated plant phenolics, the phytohormone indole-acetic acid (IAA) was of particular interest as it stimulated the expression of biosynthetic genes for 2,4-diacetylphloroglucinol (DAPG) and other antifungal compounds. To get better insight into the molecular basis of the IAA-mediated effect on antifungal gene expression, a library of transposon insertion mutants of *P. fluorescens* CHA0 was screened for altered responsiveness to IAA with respect to DAPG biosynthetic gene expression. An IAA-hypersensitive mutant, in which DAPG biosynthetic gene expression was hyperstimulated, was selected for an in-depth study. The molecular characterisation of this mutant pointed out a potential involvement of a putative efflux pump and a linked MarR-type transcriptional regulator in the phenolic-mediated effects on antifungal gene expression. In particular, the transcriptional regulator was identified as a novel potent control element of antifungal activity in *P. fluorescens* CHA0.

O40

**LOCALLY AND SYSTEMICALLY INCREASED RESISTANCE AGAINST PHYTOPATHOGENS IN NORWAY SPRUCE AFTER ROOT INOCULATION WITH A FOREST STREPTOMYCETE****S.D. Schrey<sup>1</sup>, N.-A. Lehr<sup>1,3</sup>, R. Hampp<sup>1</sup>, M.T. Tarkka<sup>2</sup>**<sup>1</sup>*University of Tübingen, Institute of Microbiology, Physiological Ecology of Plants, Tübingen, Germany;* <sup>2</sup>*UFZ-Helmholtz-Centre for Environmental Research, Department of Soil Ecology, Halle, Germany;* <sup>3</sup>*University of Helsinki, Department of Forest Ecology, Helsinki, Finland*

Filamentous soil bacteria belonging to the actinomycetes are commonly described for their antagonistic potential against plant pathogens and other microbial species. Interactions involving increased defense responses in host plants leading to suppression of plant disease development have thus rarely been detailed.

We will present a study on the mechanisms of disease suppression by *Streptomyces* sp. GB 4-2 against *Heterobasidion* root and butt rot in Norway spruce seedlings. GB 4-2 promoted mycelial growth of the phytopathogenic fungus, germination rate of fungal spores, extension of germ tubes and early colonization of outer cortical layers of the plant root. Reduced colonization of the inner cortical cell layers was accompanied by the induction of cell wall appositions, and increased xylem formation in the vascular cylinder emerged after bacterium-fungus co-inoculation. Bacterial treatment led to decreased water content in roots and needles and increased photosynthetic yield (Fv/Fm) and peroxidase activities in needles. The infection of needles by *Botrytis cinerea* was reduced by bacterial pre-treatment.

Complex interactions of GB 4-2 with Norway spruce and *Heterobasidion* were discovered. The bacterium promoted the growth of the phytopathogenic fungus but induced plant defense responses. Host responses indicate that GB 4-2 induces both local and systemic defense response in Norway spruce.

# **Plant Responses to PGPR**

## O41

**PRIME TIME: BENEFICIAL RHIZOBACTERIA PRIME PLANT DEFENSE RESPONSES****Sj. Van der Ent<sup>1,2</sup>, M.J. Pozo<sup>1,3</sup>, J. Ton<sup>1,4</sup>, and C.M.J. Pieterse<sup>1,2</sup>**

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*Arabidopsis* develops broad-spectrum disease resistance in response to root-colonization by nonpathogenic *Pseudomonas fluorescens* WCS417r bacteria. This induced systemic resistance (ISR) requires expression of the transcription factor (TF) gene *MYB72* in the roots and an intact response to the plant hormones jasmonic acid (JA) and ethylene. WCS417r does not trigger direct changes in defense-related gene-expression in above-ground plant parts, but rather primes the innate immune system, which results in an augmented response to subsequent pathogen attack. To study a putative regulating role of transcription factors in WCS417r-mediated priming, we performed a qPCR-based genome-wide screen for WCS417r-responsive TF genes. This screen demonstrated that WCS417r induced TF genes that have been related to the regulation of jasmonate (JA)-dependent defenses, such as *ERF1* and *MYC2*. Interestingly, promoter analysis of WCS417r-primed genes also pointed to a regulating role of *MYC2*. The relevant promoter regions were enriched for the *cis*-element CACATG, which serves as a docking site for *MYC2*. Moreover, ISR was abolished in mutants that were disrupted in *MYC2*, demonstrating a necessity of *MYC2* in ISR. These data suggest that JA-related TFs, amongst which *MYC2*, accumulate upon WCS417r-treatment and amplify pathogen-triggered defense-gene expression. Remarkably, WCS417r also renders *Arabidopsis* more resistant to the biotrophic oomycete *Hyaloperonospora arabidopsidis*, though JA-regulated defenses are not effective against this pathogen. Microscopical analysis indicated that WCS417r-treatment leads to a higher incidence of impenetrable callose-rich papillae at *H. arabidopsidis* infection sites. Collectively these data demonstrate that WCS417r primes distinct defense mechanisms of *Arabidopsis*, hereby enabling the plant to respond adequately to subsequent pathogen encounter.

O42

**PLANT DEFENSE REACTIONS STIMULATED FOLLOWING PERCEPTION OF *BACILLUS* LIPOPEPTIDES****M. Ongena, G. Henry, A. Adam, E. Jourdan, P. Thonart***Walloon Centre for Industrial Biology, Gembloux Univ. of Agricultural Sciences/University of Liege, Belgium*

The beneficial rhizobacterium *Bacillus subtilis* produces antimicrobial cyclic lipopeptides (cLPs) from the surfactin, fengycin and iturin families. We have recently shown that some of these cLPs can stimulate ISR in plants and have further investigated the defense mechanisms that could be stimulated in plant cells following their perception. In tobacco suspension cells, surfactin induced defense-related early events such as extracellular medium alkalinization coupled with ion fluxes and reactive oxygen species production but addition of the lipopeptide in the micromolar range is not associated with any marked phytotoxicity. The occurrence of these events is closely related to Ca<sup>2+</sup> influx, phospholipase activity and dynamic changes in protein phosphorylation. Surfactin also stimulated some typical defense enzymes and modified the pattern of phenolics produced by the elicited cells. In whole plants, lipopeptide-overproducing *Bacillus* isolates also induces some defense reactions, notably the accumulation of antifungal compounds and the activation of the so-called oxylipin pathway leading to the synthesis of a wide array of biologically active secondary metabolites. Key enzymes of this pathway, i.e. lipoxygenase and lipid hydroperoxidase, are stimulated concomitantly with the reduction of disease symptoms at the macroscopic level suggesting that they actively contribute to the global defensive response of the host plant lighted following *Bacillus* cLP perception. The test of various cLP homologues also revealed that their perception by plant cells is dictated by structural clues both in the acyl moiety and in the cyclic peptide part. Although the presence of specific receptors cannot be excluded, our results suggest that these amphiphilic molecules may interact with plant cell membranes in a way sufficient to induce some transient disturbances in the plasma membrane which could in turn lead to elevated defensive responses.

O43

**BACTERIAL DETERMINANTS AND HOST IMMUNE MECHANISMS UNDERPINNING *Pseudomonas fluorescens*-INDUCED SYSTEMIC RESISTANCE IN RICE****D. De Vleeschauwer<sup>1</sup>, P.A.H.M. Bakker<sup>2</sup>, M. Höfte<sup>1</sup>**<sup>1</sup>Laboratory of Phytopathology, Ghent University, Belgium and <sup>2</sup>Plant-Microbe Interactions, Utrecht University, The Netherlands

Compared to the relative wealth of molecular information on PGPR-inducible pathogen resistance (ISR) in dicots, little is known about the bacterial traits and host defense responses underpinning ISR in cereals. Here, we show that inoculation of rice, a monocot model plant, with the PGPR strain *Pseudomonas fluorescens* WCS374r protects naïve leaves from subsequent infection by the rice blast pathogen *Magnaporthe oryzae*. Using various mutant and transgenic rice lines, evidence is brought forward demonstrating that this WCS374r-ISR operates through a jasmonate/ethylene-regulated signaling mechanism, whilst acting independently of salicylic acid (SA). Additionally, bacterial mutant analysis pinpointed a pseudobactin-type siderophore as a crucial determinant for ISR elicitation. Feeding rice roots with purified WCS374r pseudobactin primed distal leaves for potentiated expression of an infection-inducible multifaceted cellular defense response, comprising among others the rapid recruitment of phenolics at sites of attempted pathogen entry, concerted expression of an array of structural defenses, and a timely hyperinduction of H<sub>2</sub>O<sub>2</sub>. Exogenously administered SA, however, alleviated this pseudobactin-induced priming for enhanced defense, while pseudobactin treatment antagonized SA-responsive gene transcription, the latter suggesting negative crosstalk among the SA- and pseudobactin-modulated resistance routes. Interestingly and consistent with analysis of pseudobactin-negative mutant derivatives, application of pseudobactin siderophores from other resistance-inducing PGPR, such as *P. aeruginosa* 7NSK2 or *P. putida* WCS358, failed to cause any substantial disease reduction. The potential involvement of the methionine pathway in pseudobactin-induced ISR and the possible role of siderophore-inflicted iron depletion in this process will be discussed.

**O44****PLANT GROWTH PROMOTION, BIOLOGICAL CONTROL AND DROUGHT STRESS RESISTANCE INDUCTION BY INDIGENOUS MULTI-FUNCTIONAL PGPR****J.-H. Lim and S.-D. Kim\****Department of Applied Microbiology, College of Natural Resources, Yeungnam University, Gyeongsan, 712-749, Korea*

Plant tissues produce ethylene under the environmental stress, including stress caused by drought and high salt. An increased concentration of ethylene in plants can cause inhibition of plant growth. The ACC (1-aminocyclopropane-1-carboxylic acid) deaminase produced by PGPR can reduce the plant's ethylene concentration by cleaving the ethylene precursor ACC, causing stimulated plant growth by reducing environmental salt and drought stress. The multi-functional PGPR *B. licheniformis* K11 has ACC deaminase. *B. licheniformis* K11 can also produce auxin, antifungal  $\beta$ -glucanase, and siderophore, and the strain has a mechanism for solubilization of insoluble phosphate. Non-treated pepper died on the fifteenth-day under drought conditions, while pepper plants treated with *B. licheniformis* K11 tolerated the drought stress. Even under the high salt stress of 50mM NaCl, pepper plants can subsist when treated with *B. licheniformis* K11. Protein and RNA expression patterns on pepper roots were investigated using two Dimensional (2-D) gel electrophoresis and DD-PCR (Differential Display PCR) methods under the drought conditions for 15 days. As the result of 2-D gel electrophoresis, 6 kinds and 8 proteins were identified; pathogenesis-related protein 10; adenosine kinase isoform 1T; vacuolar H<sup>+</sup>-ATPase A1; dehydrin-like protein; early nodulin ENOD18; S-adenosylmethionine synthetase. Four genes of ACCO gene, sHSP gene, vacuolar H<sup>+</sup>-ATPase A1 gene, and dhn gene showed higher levels of expression transcripts in non-treated pepper compared to water-treated pepper and *B. licheniformis* K11-treated pepper. The changes in protein and RNA patterns are attributed to *B. licheniformis* K11, which caused drought stress resistance in pepper plants. The auxin and ACC deaminase producing PGPR *B. licheniformis* K11 might reduce the drought and high salt stress in saline region or caused and induced by use of agrochemicals.

# **Progress in the use of PGPR**

## O45

**BIOLOGICAL CONTROL OF EUCALYPT BACTERIAL WILT BY A COMBINATION OF ENDOPHYTIC AND RHIZOSPHERE BACTERIA****L.X. Ran<sup>1</sup>, H. E<sup>1</sup>, Z.N. Li<sup>1</sup>, H.P. Li<sup>1</sup>, P.A.H.M. Bakker<sup>2</sup>, L.S. Thomashow<sup>3</sup>**<sup>1</sup>Forestry College, Agricultural University of Hebei, China; <sup>2</sup>Plant-Microbe Interactions, Utrecht University, Utrecht, The Netherlands; and <sup>3</sup>USDA-ARS, Root Disease and Biological Control Research Unit, Pullman, Washington, USA

Eucalypt bacterial wilt, caused by *Ralstonia solanacearum*, has led to great yield losses in South China. Breeding of resistant species or clones was the main strategy for disease suppression. However, it has been reported that resistance decreased after successive planting or subculturing for 3 or 4 years. Therefore, endophytic bacterial strains CN015r and CN017r, rhizosphere *Pseudomonas* strain WCS417r and its genetically modified derivatives, strain L3d6 and L2c9 constitutively producing 2,4-diacetylphloroglucinol, with stronger growth inhibition and less antagonism to *R. solanacearum*, respectively, were tested alone or together for disease suppression. When applied alone, all strains significantly reduced the percentage of wilted seedlings both in hydroponic system and potting soil system except for L2c9. Suppression effect in a potting soil bioassay by combinations of CN015r+WCS417r and CN015r+L3d6 was comparable to the effects of single strains, and in a hydroponic system it was observed that these two combinations showed less effectiveness than individual strains. In another set of potting soil bioassays, CN017r +WCS417r and CN017r+L3d6 significantly reduced the percentage of wilted seedlings as compared to the control, but not with comparison to the effect of individual strains. However, when performed in a hydroponic system, the combination of CN017r+WCS417r resulted in an enhanced suppression effect as compared to single strains whereas increased disease suppression by the combination of CN017r+L3d6 was not observed. This result indicated that suppression effects not only depended on the specific combinations of endophytic and rhizosphere bacteria, but also relied on the types of bioassays.

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## O46

***Paenibacillus polymyxa* STRAIN TL41, A BACTERIAL ENDOPHYTE OF *Paederia foetida* AND ITS POTENTIAL USE FOR CONTROL OF *Xanthomonas campestris* ON LETTUCE****J. Worapong<sup>1</sup>, M. Srisaisup<sup>1</sup>, and P.A. Okubara<sup>2</sup>**<sup>1</sup>Department of Biotechnology, Faculty of Science, Mahidol University, Rama 6 Rd., Payathai, Bangkok, Thailand 10400; <sup>2</sup>USDA-ARS, Root Disease and Biological Control Research Unit, Pullman, Washington, USA

The present study addresses a potential use of the endophytic bacterium *Paenibacillus polymyxa* strain TL41, isolated from the aggressive climbing vine *Paederia foetida*, as a biocontrol agent of *X. campestris* on lettuce both *in vitro* and under greenhouse conditions. An eight-hour-old potato dextrose broth culture of *P. polymyxa* TL41 strongly inhibited the growth of *X. campestris* on Mueller Hinton agar. Thoroughly mixing of 25 mL containing  $8.3 \times 10^7$  CFU of *P. polymyxa* TL41 with 500 g soil infested with  $4.3 \times 10^7$  CFU *X. campestris* in 25 mL restored seed germination, and growth of 8-week-old lettuce plants equaled the noninfested control. In these greenhouse experiments, infected *X. campestris* lettuce populations were stunted with dark brown spots on leaves. Besides its *in vitro* antibacterial activity, strain TL41 was able to inhibit a number of fungal pathogens, including *Alternaria alternata*, *Colletotrichum gloeosporioides*, *C. capsici*, *Fusarium* sp., *F. equiseti*, *Phytophthora palmivora* and *Pythium ultimum* on potato dextrose agar. Our results provide promising information on this endophytic bacterium in the control of a broad spectrum of plant pathogens.

O47

## ECOPHYSIOLOGY OF THE RESPONSE TO *Azospirillum* INOCULATION OF CEREAL CROPS IN ARGENTINA

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*Azospirillum* is one of the most studied PGPR and one that is able to promote the growth of many crops around the world. *Azospirillum* can produce plant growth regulators, increase the development of roots, and fix atmospheric nitrogen (N<sub>2</sub>), among other mechanisms. *Azospirillum* associates with crop plants and produces direct beneficial effects on growth and plant nutrition, and is an ecological and economical alternative which is gaining acceptance in Argentina and internationally. Because of its agricultural impact, ecophysiology studies conducted under field conditions for over two decades are reviewed here. The experiments were carried out to assess the behavior of *Azospirillum* strains on biological and grain yield, nitrogen (N) nutrition and associated microbial communities of wheat, maize and rice plants. Wheat-*Azospirillum* association was studied in two periods: 1986-1990 and 2004-2006. The analysis of data from trials of wheat, showed a significant strain-genotype-environment interaction. Depending on the bacteria-plant combinations, yield increases due to inoculation ranged from 5 to 34%. Inoculation with *Azospirillum* on average for all experiments significantly increased the production of wheat biomass by 21% while N fertilization increased by only 5%. Similar results were observed in a series of experiments (1987-1995 and 2002) carried out to study the yield, ecophysiology, and biochemistry of N assimilation of maize crops. Certain *Azospirillum* strains previously selected for their ability to fix N *in vitro* were used for these experiments. In all cases we observed a significant interaction corn genotype-strain-fertilization. The responses obtained for yield, grain N, absorbed N and nitrate reductase activity were categorized on the basis of the ratio: [Value inoculated/Value not inoculated\*100]. The genotypes used were categorized into three types of inoculation response: no response, negative response and positive response. Yield increases due to *Azospirillum* inoculation ranged from 2 to 94%, and demonstrated that the potential response of maize genotypes was highly significant. The *Azospirillum* strains showed a great capacity to associate with maize plants but the level of colonization varied among different plant genotypes. Estimates of the percentage of N derived from biological nitrogen fixation (BNF) in this association, using the technique of isotope dilution <sup>15</sup>N varied significantly among maize genotypes and the type of inoculum. It was observed that certain strain-genotype combinations yielded 1-1.5 g per plant derived from BNF. The rice-*Azospirillum* association was studied in the period 2006-2008. *Azospirillum* inoculation with experimental inoculants increased aerial biomass by 35% and 50% at tillering and grain-filling stages, respectively. Plant N-content was increased on average 35% and the estimated BNF was on average 6 kg ha<sup>-1</sup>. Microbial physiological activity obtained by principal component analysis of the community-level physiological profiles showed different activity of the rhizosphere communities among inoculation treatments. From our data it can be concluded that *Azospirillum* inoculation increased the quantity and density of roots in the inoculated plants of wheat, corn and rice. The variables studied and the results obtained showed the ability of certain strains of *Azospirillum* to alter the ecophysiology of these important crops under field conditions. It was observed that the strain-plant-environment interaction was relevant to the inoculation response obtained. The variability in the capabilities of both *Azospirillum* strains and associated plants needs to be adjusted and enhanced, including alternative mechanisms in order to improve the levels of inoculation response at field conditions.

O48

**PLANT GROWTH PROMOTION BY *Bacillus amyloliquefaciens* FZB45 DEPENDS ON INOCULUM CONCENTRATION AND P-RELATED SOIL PROPERTIES****C.A. Ramírez<sup>1,2</sup> and J.W. Kloepper<sup>1</sup>**<sup>1</sup>Department of Entomology and Plant Pathology, Auburn University, Auburn, Alabama, USA and <sup>2</sup>Instituto de Biología, Universidad de Antioquia, Medellín, Colombia.

Increasing the consistency of growth promotion by PGPR requires knowledge of specific mechanisms and how these are influenced by environmental and agronomic factors. However with the bacilli PGPR, little is known about the mechanisms actually involved in beneficial plant-bacterial interactions and how they are affected by soil conditions. Previous reports showed that phytase production contributes to plant growth promotion by *Bacillus amyloliquefaciens* FZB45 in a soilless, sterile, phosphorus-limited system; however, in soil, phosphorous availability is affected by many factors such as fixation, precipitation, and immobilization. Therefore, the objectives of this study were to 1) determine the roles of phytase production by FZB45 on growth promotion and P-uptake in a soil system and 2) evaluate the influence of inoculum concentration. The model plant system was Chinese cabbage (*Brassica rapa*, Kaboko Hybrid), a mycorrhizal independent plant. A soil was selected with known P-related properties (P-fixation capacity, P content with three extractants, and low organic matter content). Two inoculum concentrations ( $10^6$  and  $10^8$  spores/seedling) were evaluated under four P regimes: no P addition, inorganic P ( $P_i$ , 15 mg/kg soil), and two levels of phytate (equivalent to 15 and 90 mg  $P_i$ /kg soil). Although there was a significant interaction between inoculum concentration and P regime, bacterial inoculation only increased fresh shoot weight and plant  $P_i$  content at the high rate of phytate and the low rate of FZB45. The lack of growth promotion with the high rate of FZB45 suggests that a factor other than phytase is also involved. In growth pouch tests FZB45 caused a hormone-like promotion of root growth at the low but not high rates, and production of IAA was confirmed *in vitro*. More work is required to understand the interaction of IAA and phytase on plant growth promotion.

# **Biotic Interactions in the Rhizosphere**

O49

***Lysobacter enzymogenes*, A BIOCONTROL AGENT THAT ESTABLISHES PATHOGENIC INTERACTIONS WITH A BROAD RANGE OF LOWER EUKARYOTES****D.Y. Kobayashi***Department of Plant Biology & Pathology, Rutgers, The State University of New Jersey, New Brunswick New Jersey USA*

*Lysobacter enzymogenes* is a  $\gamma$ -proteobacterium belonging to the family Xanthomonadaceae. The species is known as a prolific producer of lytic enzymes and secondary metabolites and a potent antagonist of other microorganisms. Recently, strains of *L. enzymogenes* have been reported to control a number of plant diseases. Efforts to characterize biocontrol mechanisms have indicated that *L. enzymogenes* establishes pathogenic interactions with a number of lower eukaryotic hosts; the bacterium can colonize and replicate internally within host cells before initiating lysis and release of its population into the surrounding environment. The *L. enzymogenes* genome sequence has revealed the presence of several genes associated with bacterial pathogenesis, including those encoding multiple hydrolytic enzymes and biosynthetic pathways associated with secondary metabolite production, as well as genes encoding type III, type IV and type VI secretion systems. The presence of these effector translocation systems, which have functional importance in bacterial pathogenesis of higher plants and animals, suggest *L. enzymogenes* utilizes complex, sophisticated virulence mechanisms to infect its hosts. Infection assays developed for the fungus *Magnaporthe oryzae*, the nematode *Caenorhabditis elegans*, the moss *Physcomitrella patens*, and the unicellular alga *Chlamydomonas reinhardtii* have provided model host systems to conduct genome scale investigations involving interactions with *L. enzymogenes*. These studies are expected to provide new insights into microbial antagonism and identify novel host molecular targets for disease control.

O50

**INSECTICIDAL ACTIVITY IN BIOCONTROL PSEUDOMONADS****C. Keel<sup>1</sup>, M. Maurhofer<sup>2</sup>***<sup>1</sup>Department of Fundamental Microbiology, University of Lausanne and <sup>2</sup>Plant Pathology, Institute of Integrative Biology, ETH Zurich, Switzerland*

Root diseases and pests are a serious problem in agricultural crops, causing each year important yield losses. Biological disease and pest control with microbial agents applied to soil or plant material has evolved as a promising alternative strategy. Plant-beneficial fluorescent pseudomonads that operate in the rhizosphere are well-characterized for their antifungal activity that helps them protect plants against root diseases caused by pathogenic fungi. Recently, we have made the exciting discovery that some disease-suppressive *Pseudomonas fluorescens* strains exhibit also potent insecticidal activity. Anti-insect action is linked to a genomic locus encoding a novel large protein toxin that we have termed Fit for *P. fluorescens* insecticidal toxin. The Fit toxin is related to potent insect toxins of *Photorhabdus luminescens*, a mutualistic bacterium of insect-invading nematodes. A first survey of the occurrence and molecular diversity of the insect toxin locus in root-associated pseudomonads indicates that the *fit* genes may be present in specific subgroups of these bacteria that include the well-characterized biocontrol agents CHA0 and Pf-5. In our current work, we investigate the molecular characteristics of the Fit toxin locus, focusing on regulatory elements and signals that control toxin production and transport. To get insight into the (agro)ecological function of the *Pseudomonas* insect toxin, we monitor the biological activity of Fit in bioassays involving insects and rhizosphere microanimals and try to identify accessory *Pseudomonas* products that sustain anti-insect action of the toxin. The occurrence of antifungal and anti-insect activities in root-colonizing pseudomonads highlights not only the impressive arsenal of features that these bacteria possess to manipulate their rhizosphere habitat, but points also to new possibilities to protect the health of agricultural crops.

## O51

**PREDATOR-PREY CHEMICAL WARFARE DETERMINES THE PRODUCTION OF ANTIFUNGAL COMPOUNDS BY A ROOT-ASSOCIATED PSEUDOMONAD****A. Jousset<sup>1</sup>, L. Rochat<sup>2</sup>, C. Keel<sup>2</sup>, S. Scheu<sup>1</sup>, M. Bonkowski<sup>3</sup>**<sup>1</sup>Technische Universität Darmstadt, Institute for Zoology, Schnittspahnstr. 3, 64287 Darmstadt, Germany;<sup>2</sup>University of Lausanne, Department of Fundamental Microbiology, Biophore Building, 1015 Lausanne,Switzerland;<sup>3</sup> University of Cologne, Abt. Terrestrische Ökologie, Weyertal 119, 50931 Köln, Germany.

The production of antifungal exoproducts by root-associated pseudomonads is a core component of their biocontrol ability in soil. Interestingly, the same toxins play a central role for the fitness of the bacteria, especially by protecting them against microfaunal predators. Predation is a major selective pressure on soil bacterial communities and promotes toxic phenotypes with biocontrol potential. We tested the effect of predation by the amoeba *Acanthamoeba castellanii* on the production of extracellular toxins by the biocontrol agent *Pseudomonas fluorescens* CHA0. We used GFP-based reporter fusions to track the expression of the biosynthetic genes for toxic exoproducts 2,4-diacetylphloroglucinol (DAPG), pyoluteorin, pyrrolnitrin (PRN) and hydrogen cyanide (HCN) that contribute to the capacity of this bacterium to suppress fungal root diseases. We tested the effect of chemical cues of the predator on the expression of the bacterial biocontrol genes in a batch assay. In addition, we measured the effect of the predator itself on pseudomonad gene expression *in vitro* and on the roots of barley. Bacteria reacted rapidly to predator cues by increasing the expression of the DAPG, PRN, and HCN biosynthetic operons. Predators could however partly reverse this reaction as they reduced the expression of the DAPG biosynthetic genes on the roots and in the batch experiment. The results suggest that rhizosphere bacteria adapt the production of toxic metabolites in response to biotic stresses, and that predators can disarm their prey by interfering with their toxicity. Therefore, the interaction between soil bacteria and their predators determines not only the fitness of biocontrol strains, but also the production of antifungal compounds in the rhizosphere.

## O52

**MYCORRHIZA HELPER BACTERIA: A PARTICULAR PGPR GROUP WITH MULTIPLE FACETS****P. Frey-Klett<sup>1</sup>, A. Deveau<sup>1,2</sup>, A. Diedhiou<sup>1</sup>, A. Cusano<sup>1</sup>, M. Barret<sup>3</sup>, S. Uroz<sup>1</sup>, J. Garbaye<sup>1</sup>, A. Sarniguet<sup>3</sup>**<sup>1</sup>INRA, UMR1136 INRA-Nancy Université "Interactions Arbres/Micro-organismes", Centre de Nancy, 54280 Champenoux, FRANCE, <sup>2</sup> Department of Microbiology and Immunology, Dartmouth Medical School, Hanover, NH 03755, USA and <sup>3</sup>INRA, UMR1099 "Biologie des Organismes et des Populations appliquée à la Protection des Plantes", 35 653 Le Rheu Cedex, FRANCE

In natural environments, mycorrhizal fungi are surrounded by complex bacterial communities which modulate the mycorrhizal symbiosis. Among them, the so-called "mycorrhiza helper bacteria" (MHB) refer to the bacteria which either assist mycorrhiza formation or interact positively with the functioning of the symbiosis. These bacteria directly or indirectly promote plant nutrition, health and growth. Evidences for MHB exist in diverse mycorrhizal systems, such as arbuscular and ectomycorrhizal symbioses. The development of nucleic-acid-based methods and especially genomics has yielded new insights into the taxonomy of MHBs, into the detection of endofungal bacteria which may form a tri-partner symbiosis within the mycorrhizal association, as well as into the mechanisms that control the ecology of these bacteria and their helper effect. The presentation will draw an overview of the present knowledge about this particular group of PGPR with multiple facets. It will discuss future research priorities regarding the fruitful concept of MHB and the developing scientific field of fungal-bacterial interactions.

O53

**PGPR-NEMATODE INTERACTIONS****P.A. Okubara<sup>1</sup>, H. Aly<sup>2</sup>, O. Mavrodi<sup>3</sup>, N. Walter<sup>1</sup>, E. Riga<sup>3</sup>, R. Bonsall<sup>3</sup> and C. Taylor<sup>2</sup>**<sup>1</sup>USDA-ARS, Root Disease & Biological Control Research Unit, Pullman, WA; <sup>2</sup>Donald Danforth Plant Science Center, St. Louis, MO; <sup>3</sup>Department of Plant Pathology, Washington State University, Pullman, WA

We seek to explore inhibitory mechanisms used by *Pseudomonas* spp. for the suppression of nematodes. As a first step, we isolated *Pseudomonas* spp. from novel sources, including water from the Missouri and Mississippi Rivers, herbarium specimens, and soils from agricultural production sites. In an in vitro *Caenorhabditis elegans*-based screen of 500 candidate bacteria, 63 inhibitory strains were of the genus *Pseudomonas* as determined from Blastn analyses of 16S DNA. These strains were tested for motility and for production of HCN, exoproteases, extracellular polysaccharides, siderophores, surfactants and the antifungal metabolites DAPG and PCA. Most strains were motile and produced extracellular polysaccharides, suggesting that they have the potential to migrate to and associate with nematodes or plant roots. Nineteen of the 63 strains were lethal to the root-knot nematode (RKN) *Meloidogyne incognita*. Three of these 19 were inhibitory to *M. chitwoodi* and one to *M. hapla* in vitro, and 12 reduced in planta populations of the soybean cyst nematode *Heterodera glycines*. No single strain was effective against all types of nematodes or against all *Meloidogyne* species. Although nematode-lethal *Pseudomonas* share common biochemical characteristics, no single characteristic accounted for the nematicidal activities of all strains. Population densities of most of the RKN-lethal strains did not exceed  $\log 4.5$  CFU  $\text{g}^{-1}$  soil on soybean roots after four planting cycles, indicating that persistence of these strains in the soybean rhizosphere was relatively weak. Our findings suggest that *Pseudomonas* spp. harbor multiple nematicidal mechanisms, and that effective biological control of nematodes will encompass both species-targeted activities and rhizosphere performance.

# Poster Presentations

Poster abstracts are ordered very approximately by subject area. Posters numbered 1-34 are presented in Poster Session I; those numbered 35-72 are in Poster Session II.

**P01****ASSESSMENT OF PGPR-BASED INOCULANTS FOR IMPROVING PLANT USE EFFICIENCY OF FERTILIZERS****A.O. Adesemoye<sup>1</sup>, H.A. Torbert<sup>2</sup>, and J.W. Kloepper<sup>1</sup>**<sup>1</sup>Department of Entomology & Plant Pathology, Auburn University and <sup>2</sup>USDA ARS National Soil Dynamics Lab., Auburn, Alabama, USA.

Environmental problems occurring with nutrient run-off from agriculture are prompting research in integrated nutrient management using multiple inputs, including microbial agents. Field and greenhouse tests were designed to test the overall hypothesis that PGPR or their combinations with arbuscular mycorrhiza fungi (AMF) would lead to increased nutrient uptake and allow reduced rates of fertilization. Microbial inoculants used included a mixture of PGPR strains (*Bacillus amyloliquefaciens* IN937a and *Bacillus pumilus* T4), a commercial PGPR formulation, and AMF, *Glomus intraradices*. Field evaluations of inoculants were done in a split-split RCB design across two tillage systems (no-till/conventional till) and two fertilization regimes (poultry litter/chemical fertilizers). In the greenhouse, factorial experiments were conducted with chemical fertilizer and Hoagland solution. Also, depleted ammonium sulphate ( $^{15}\text{NH}_4)_2\text{SO}_4$  was used in  $^{15}\text{N}$  tracer experiments. Results supported the overall hypothesis and indicated that inoculants promoted plant growth, enhanced yield, and led to removal of significantly higher amounts of N, P, and K from field plots with corn and greenhouse trials with tomato. For example, inoculants with 75% of recommended rate of fertilizer had growth parameters that were statistically equivalent to full fertilizer rate without inoculants. In another greenhouse result, the difference in atom %  $^{15}\text{N}$  per gram of plant tissues was significantly higher with PGPR inoculation than without PGPR. In the field, grain yields ( $\text{kg ha}^{-1}$ ) for inoculants were 7,717 for AMF, 7,260 for PGPR+AMF, 7,313 for PGPR, and 5,725 for the control. The results suggest that PGPR can be used as inputs in integrated nutrient management strategies.

**P02****PROSPECTS OF BACTERIAL GROWTH PROMOTERS IN DIFFERENT CROP PRODUCTION SYSTEMS****M.R. Banerjee and L. Yesmin**

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See oral presentation abstract 09.

**P03****PLANT GROWTH PROMOTING RHIZOBACTERIA IN DISEASE MANAGEMENT AND GROWTH OF BAST FIBRE CROPS – THE CURRENT CONCEPTS****A. Bandopadhyay<sup>1</sup>, A. K. Bandopadhyay<sup>2</sup>, S. K. Bhattacharya<sup>2</sup> and M.S. Reddy<sup>3</sup>**<sup>1</sup>Molecular and Applied Plant Pathology Laboratory, Botany Department, Calcutta University<sup>2</sup>Biocontrol laboratory, Central Research Institute for Jute and Allied Fibres, Barrackpore, India<sup>3</sup>Plant Pathology and Entomology Department, Auburn University, USA

Challenge inoculations of *Pseudomonas fluorescens* strain Psf11, *P. glume* PsglM85, *P. striata* Pst1 and *Azotobacter chroococcum* Azbc2 showed potential in disease control, biomass and fibre production in jute and sunnhemp crops. A consortium of Azbc2 and Pst1 reduced jute root rot caused by *Macrophomina phaseolina* by 23-40%, enhanced plant biomass by 11-42% and fibre yield by 18-40%. *Rhizobium* Rjc2 and Pst1 combined reduced wilt caused by *Fusarium udum* by 40%, enhanced nodulation by 45% and yield by 26% in sunnhemp. A residual effect achieved 13.4q ha<sup>-1</sup> yield in the succeeding mustard crop. Talc- and kaolin-based seed dressing with 2% CMC and soil and flyash-based soil inoculants with 5% molasses formulations of *P. fluorescens*, *P. striata* and *Azotobacter* (1:1:1) were evaluated. 10g kg<sup>-1</sup> seed dressing and 1kg soil inoculants mixed with 5kg soil-organic matter in a soil drench on 0.05-hectares 7-15 DAS suppressed jute root rot and sunnhemp wilts in farmers' fields. Biocontrol mechanism implied chitinase, pectinase and protease enzymes produced by PGPRs. Volatiles of Psf11 and PsglM85 inhibited *Macrophomina sclerotia* 58-62%. The principal component was HCN. The nonvolatile antibiotic phenazine from Psf11 inhibited pathogens up to 73.3%. Siderophore production was maximum at 38.2µM ml<sup>-1</sup> in Psf11, increased in near neutral to higher pH and was thermostable at 125°C. Azbc2, Psf11 and Pst1 produced IAA 38.5-158.4µg ml<sup>-1</sup>. Phosphorus solubilization by Pst1 was 0.445 mg P ml<sup>-1</sup>. Studies indicated use of near isogenic PGPR strains consortium with biofertilizer and growth regulators for multiple benefits of pathogen suppression, plant nutrient supply and growth promotion. Molecular characterization of functional genes and biopesticide from genetically improved PGPR may improve sustainable farming.

**P04****REDUCED CARBON UTILIZATION, COMPETITIVE COLONIZATION OF THE SPERMOSPHERE, AND DISEASE SUPPRESSION BY *Enterobacter cloacae*****D.P. Roberts, L.F. McKenna, and J.S. Buyer**

USDA-ARS, Sustainable Agricultural Systems Laboratory, Beltsville, MD 20705

De Wit Replacement series and disease suppression experiments with a collection of nutritional mutants of *E. cloacae* 501R3 are being used to determine the role of reduced carbon compounds found in seed exudate during beneficial activities by this bacterium. Mutants A-11, M2, and M43 contain single mini-Tn5 Km insertions in *pfkA*, *sdhA*, and *aceF*, respectively. A-11 was severely impaired in growth on almost all carbohydrates, M2 was severely impaired in growth on almost all amino acids and organic acids, and M43 was severely impaired in growth on almost all carbohydrates, amino acids, and organic acids detected in cucumber seed exudate. Replacement series experiments with 501R3 and A-11 where initial inoculum levels were above carrying capacity, essentially at carrying capacity, and below carrying capacity, indicated that the importance of carbohydrate in cucumber seed exudate for competitive seed colonization decreases with increased initial population size. Preliminary experiments with 501R3 and M2 provided similar results. Initial disease suppression assays comparing A-11 and 501R3 demonstrated that A-11 provided levels of control of *Pythium ultimum* damping-off of cucumber that were similar to those of the parental strain, 501R3. These experiments suggest a large degree of nutritional flexibility for *E. cloacae* during beneficial activities in the spermosphere. Future experiments with M2, M43, and additional partially characterized mutants of 501R3 will be performed to further examine the role of nutritional flexibility in competitive seed colonization and in disease suppression under various conditions in the spermosphere.

P05

### EFFECTS OF THE COMMERCIAL INOCULANT SOILBUILDER WITH ORGANIC FERTILIZERS AND THE CONTRIBUTION OF MICROBIAL METABOLITES TO GROWTH PROMOTION

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SoilBuilder (SB) is a soil amendment product consisting of a mixed microbial community together with microbial metabolites produced during proprietary fermentation process. SB differs from other PGPR-based products by nature of the diversity of microbes present and the presence of biologically active metabolites. The objectives of this study were 1) determine if plant growth promotion by SB was compatible with organic fertilizers and 2) assess the capacity of the microbial and metabolite fractions of the product to elicit growth promotion in defined bioassays. For objective 1, multiple experiments were conducted with 4 ornamental plants: holly, juniper, yew, and miniature roses, testing organic fertilizers with and without SB. Additional tests were conducted with various rates of SB and organic fertilizers on transplanted tomato and pepper. The overall results showed that significant plant growth promotion previously observed with SB using inorganic fertilizers (higher in N content than organic fertilizers) consistently occurred with organic fertilizers. Root growth promotion was particularly evident in these studies. For objective 2, bioassays, each with a different host plant, were used: drenching of trays seeded with Chinese cabbage or radish in a peat-based commercial planting mix and drenching of 4-wk-old pepper seedlings during transplanting into field soil. Treatments included SoilBuilder at the label rate, a filter sterilized metabolite fraction, a microbial fraction collected on a 0.2 µ filter, and a control. Data were collected 3 wks after treatment on shoot and root weights as well as root architecture. Overall, the results indicated that growth promotion was elicited by the complete product and that both the microbial and metabolite fractions contributed; however, the relative contribution of the metabolite fraction was greater than that of the microbes.

P06

### DIVERSITY AND BIOLOGICAL CONTROL OF *Sclerotium rolfsii*, CAUSAL AGENT OF STEM AND POD ROT OF GROUNDNUT

**L.N. Cuong<sup>1,2</sup>, M. Kruijt<sup>1</sup>, and J.M. Raaijmakers<sup>1</sup>**

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Groundnut (*Arachis hypogaea* L.) is an important crop in Vietnam but its production is hampered by several diseases. Among the fungal diseases, stem and pod rot caused by *Sclerotium rolfsii* is one of the most devastating with yield losses up to 25%. To date, however, little is known about the diversity of *S. rolfsii* populations associated with groundnut in Vietnam. Knowledge of the genetic and phenotypic diversity within pathogen populations is essential for the development of effective and sustainable control strategies. In Vietnam, plant disease management strategies are mostly based on personal experience of the local farmers and systematic approaches to develop efficient control methods is lacking. Current methods to control *S. rolfsii* on groundnut include rotation with non-host crops and the frequent use of fungicides. However, these cultural and chemical control measures are not very effective and most fungicides are too expensive for local farmers. Given that *S. rolfsii* has a wide host range and survives as persistent sclerotia in soil or in plant residue, the strategies to control this pathogen should be based on a combination of different strategies, including biological control. To this end, we have isolated beneficial bacteria from the stem base and roots of groundnut plants in Vietnam and tested their activity against *S. rolfsii* *in vitro* and in greenhouse bioassays. Results will be presented on the diversity and identity of groundnut-associated bacteria with activity against *S. rolfsii*.

P07

**DEVELOPMENT OF A GREENHOUSE SHEATH BLIGHT DISEASE ASSAY IN RICE FOR EVALUATION OF SELECTIVE PGPR STRAINS AS BIOCONTROL AGENTS****K. Vijay Krishna Kumar<sup>1</sup>, M.S. Reddy<sup>1</sup>, K.K. Lawrence<sup>1</sup>, D.E. Groth<sup>2</sup>, M. Miller<sup>3</sup>, and J.W. Kloepper<sup>1</sup>**<sup>1</sup>Department of Entomology & Plant Pathology, Auburn University, AL 36849, <sup>2</sup>LSU Ag Center Rice Research Station, Rayne, LA 70578, and <sup>3</sup>Department of Biological Sciences, Auburn University, AL 36849.

Rice sheath blight caused by *Rhizoctonia solani* is an economically significant disease in all rice-growing areas of the world. The objectives of this study were to 1) develop a reproducible whole-plant disease assay, 2) develop a detached leaf assay, and 3) begin screening PGPR for potential biocontrol activity. For objective 1, small *R. solani* sclerotia (<0.5 mm diam.) were inoculated on sheaths of 30-day-old plants 2 cm above the water line in the greenhouse. Immature, mature, and aged sclerotia were used, and the disease was assessed after 6 days. Water-soaked lesions appeared as soon as 48 h with immature sclerotia. On leaf sheaths, a single and uniform lesion was produced by immature sclerotia, whereas mature and aged sclerotia produced 1-2 lesions. The mean lesion size with immature sclerotia was 1.1 cm<sup>2</sup> and it ranged from 0.7 - 0.8 cm<sup>2</sup> with mature and aged sclerotia. Disease was quantified by calculating the highest relative lesion height (HRLH), where HRLH = (total height of all lesion/total plant or leaf height) X 100. With immature and mature sclerotia, the HRLH ranged from 28.7 - 30.6% with immature and mature sclerotia. For objective 2, leaves were placed in trays, inoculated with sclerotia, and incubated in moist chambers. Lesion size was assessed after 7 days. Immature sclerotia produced significantly larger lesions (57.5 mm) than mature and aged sclerotia (48-49 mm). In objective 3, strains of *Bacillus* and *Paenibacillus* spp. were evaluated in the detached leaf assay. Ten strains resulted in significantly reduced lesion sizes compared to the control. The HRLH for effective PGPR ranged from 3 to 75%, compared to 100% for the controls. *B. subtilis* (AP301) is the superior strain with a HRLH of 2.9%. Effective PGPR strains are now being evaluated in the whole plant disease assay.

P08

**THE EFFECT OF *Pseudomonas fluorescens* STRAIN Q2-87 IN PATHOGEN INHIBITION AND GROWTH PROMOTION OF SLASH PINE SEEDLINGS****J.Y. Chen<sup>1</sup>, S.S. Cai<sup>1</sup>, J.M. Hu<sup>1</sup>, L.S. Thomashow<sup>2</sup>, D. M. Weller<sup>2</sup>**<sup>1</sup>Hubei Forestry Academy, Wuhan, China and <sup>2</sup>USDA-ARS, Root Disease and Biocontrol Research Unit, Pullman, Washington, USA

*Pseudomonas fluorescens* strain Q2-87 showed significant antagonistic activity against the damping-off pathogens of slash pine (*Pinus elliottii*), including *Rhizoctonia solani*, *Alternaria alternata* and *Fusarium oxysporum*. *In vitro* assays showed that strain Q2-87, which has an inhibition index higher than that of other biocontrol strains (*P. fluorescens* strain HT5-1, FFL1R8, 2-79, Pf-5, 1M1-96, Q37-87, MVP1-4), has the strongest antagonistic activity against the three pathogens. Rhizosphere colonization experiments in the greenhouse demonstrated that strain Q2-87 colonized the roots of slash pine efficiently, and during 10-75 days the population density of Q2-87 in the rhizosphere increased from  $4.36 \times 10^6$  CFU/g to  $1 \times 10^9$  CFU/g (the summit value), and then decreased. In a transplantation experiment, immersing the seedling roots in a bacterial suspension of strain Q2-87 inhibited the pathogens, reduced the incidence of damping-off, and increased the survival rates of the seedlings. On day 184 after the seedlings were transplanted, the survival rate of the seedlings immersed in the suspension of Q2-87 (84%) was significantly higher than that of the control immersed in water (72.4%). In sum, *P. fluorescens* strain Q2-87 not only has strong antagonistic ability against the damping-off pathogens of slash pine, but also raises the seedling survival rates to attain the effect of preventing disease and promoting growth.

P09

### SELECTION OF *Pseudomonas* STRAINS THAT ARE ANTAGONISTIC TO *Lecanicillium fungicola*, THE CAUSAL AGENT OF DRY BUBBLE DISEASE IN *Agaricus bisporus*

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Pathogenic fungi cause major losses in the cultivation of *Agaricus bisporus* (the white button mushroom). The most important pathogen of *A. bisporus* is *Lecanicillium fungicola*. During the last decade *L. fungicola* has become less sensitive to the only fungicide that is still approved in mushroom production. Therefore alternative control strategies need to be developed. We aim for applying antagonistic pseudomonads, a dominant bacterial group in casing soil. To develop effective control, knowledge on the ecology of the pathogen is indispensable. Disease symptoms depend on the timing of infection during development of the fruiting body and vary from small necrotic lesions to a totally deformed fruiting body. The interaction between the pathogen and its host occurs in the casing soil that is applied in a layer on top of the compost. *L. fungicola* spore germination was studied on differentially treated casing soil. The general microbial activity in casing inhibits germination, autoclaving the casing significantly increased spore germination. It was observed that at high *L. fungicola* spore densities, germination is significantly lower compared to that at low spore densities, suggesting the presence of a self-inhibitor. The implications of these findings for both the critical interaction between *Lecanicillium* and *Agaricus* and the possibilities to interfere in this interaction using antagonistic pseudomonads will be discussed.

P10

### SYNERGY SOLUTION: ENHANCING GROWTH OF LEGUMES WITH BACTERIAL CONSORTIA

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In many parts of North America use of rhizobium inoculants for growing leguminous crops (leguminosae family) is quite prevalent, especially with crops like soybean (*Glycine max* L.), alfalfa (*Medicago sativa* L.) and peas (*Pisum sativum*). Frequent use of inoculant can easily establish a background level of rhizobium population and, for example, can be seen in many soybean fields of mid-western parts of USA where *Bradyrhizobium japonicum* establishment is quite common. The same scenario is also seen in the alfalfa fields in Canadian prairies. For this reason many marketplace rhizobium inoculant products do not enhance the crop productivity at the desired level. The present investigation reports a unique strain of sulfur (S)-oxidizing plant growth promoting rhizobacteria (PGPR), *Delftia acidovorans* RAY209, that was found to be compatible with different nitrogen (N)-fixing rhizobia and shown to enhance plant performance via increased root nodulation, shoot and root biomass and ultimately increase in yield. Performance of this PGPR in association with rhizobium was tested in the laboratory and under field conditions with *Bradyrhizobium japonicum* (for soybean), *Sinorhizobium meliloti* (for alfalfa) and *Rhizobium leguminosarum* (for peas). Several years of trials showed positive results on soybean and alfalfa production under field conditions and promising results on pea yields under growth room conditions. The results suggest that if these legumes are inoculated with the respective bacterial consortia, a potential yield increase can be achieved. Therefore, this study accomplishes a new approach of boosting legume production via the synergistic association of rhizobia and S-oxidizing PGPR *Delftia acidovorans* RAY209 that leads the development of commercial legume growth promoters.

**P11*****Achromobacter xylosoxidans* WSM3457 ENHANCES NODULATION OF *Medicago truncatula* WHEN SYMBIOTIC EFFECTIVENESS IS CHALLENGED BY SUB-OPTIMAL RHIZOBIAL INOCULUM****S. Fox, G. O'Hara, W. Reeve, and L. Bräu***Centre for Rhizobium Studies, School of Biological Sciences and Biotechnology, Murdoch University, Murdoch, Western Australia, 6150, Australia*

The use of legumes in Australian cropping and pasture systems is often constrained by environmental conditions detrimental to the survival of inoculant rhizobia. While there are published reports of PGPR enhancing legume nodulation, there has been no work that specifically investigated if PGPR enhance nodulation in situations where low-density rhizobial cell numbers challenge the establishment of an effective symbiosis. We had previously determined that an indole-acetic-acid (IAA) producing soil isolate *Achromobacter xylosoxidans* WSM3457, increased nodule scores, nodule weights and root mass on the model legume *Medicago truncatula*, when co-inoculated with low cell numbers ( $10^4$  cfu/ml) of *Sinorhizobium medicae* WSM419. Increased nodulation in the crown region of the *M. truncatula* root system suggested that there might be enhanced early nodulation with co-inoculation. Given that WSM3457 produces IAA we hypothesised that production of this plant growth regulator in the rhizosphere may promote early root development of the medic and thereby enhance early colonisation and nodule initiation by *S. medicae* WSM419. Two experiments were conducted to investigate this theory. Early root development of *M. truncatula* and the rate of nodule initiation were followed for 21 days following inoculation. The data revealed that while the rate of nodule initiation was enhanced by co-inoculation this was not due to increased *M. truncatula* root development or root hair density. These results suggest that the rhizobacteria WSM3457 may be enhancing very early molecular interactions between the rhizobia and legume. We have also recently initiated further studies to determine if this enhanced early nodule initiation imparts an advantage to rhizobia when competing with other, ineffective rhizobia.

**P12****PROMOTION OF ALFALFA GROWTH BY *Pseudomonas fluorescens* STRAINS APPLIED AS SEED INOCULANT****M. L. Yanes<sup>1</sup>, P. Vaz<sup>1</sup>, L. Quagliotto<sup>1</sup>, N. Bajsa<sup>1,2</sup>, E. Dibar<sup>3</sup>, H. Varela<sup>3</sup>, N. Altier<sup>4</sup>, A. Arias<sup>1</sup>***<sup>1</sup>Instituto de Investigaciones Biológicas Clemente Estable. Montevideo, Uruguay; <sup>2</sup>Facultad de Ciencias, Universidad de la República. Montevideo, Uruguay; <sup>3</sup>Facultad de Ingeniería, Universidad de la República. Montevideo, Uruguay; <sup>4</sup>Instituto Nacional de Investigación Agropecuaria. Canelones, Uruguay.*

Alfalfa establishment is the most critical step to achieve long stand persistence and high quality forage. Seedling diseases considerably affect forage yield. Diseases can be reduced through the application of antagonistic microorganisms. Five fluorescent *Pseudomonas* strains were selected from a collection of 701 isolates taken from the rhizosphere of healthy alfalfa. The strains were characterized by *in vitro* assays to determine biocontrol and plant growth promoting mechanisms. Bioassays performed under controlled conditions determined that *P. fluorescens*  $\alpha$ C119 has the ability to protect alfalfa seedlings from *P. debaryanum* damping-off. The strain was selected as a potential inoculant. It produces a biosurfactant compound with strong inhibitory activity against *P. debaryanum*. The compound was purified by HPLC and is being analyzed by mass spectrometry. To establish optimal growth conditions experimental designs (factorial design with a central point and central composite design assay) were performed. The selected conditions were: 15 g/l glycerol, 11 g/l yeast extract, pH8 and 31°C. Laboratory mid-scale fermentations were carried out to establish the parameters to be used in greater scale production. Field trials using inoculated peat were conducted to evaluate alfalfa growth promotion ability of strains. The results were very promising and an alfalfa biofertilizer based on *P. fluorescens* strains seems to be very feasible.

P13

**EFFECT OF *Azospirillum brasilense* ON GERMINATION VELOCITY IN DIFFERENT RACES OF *Eucalyptus globulus*****M. Puente<sup>1</sup>, J. García<sup>1</sup>, P. Pathauer<sup>2</sup>, and A. Peticari<sup>1</sup>**<sup>1</sup>Laboratorio de Bacterias Promotoras del Crecimiento Vegetal-IMYZA, INTA Castelar and <sup>2</sup>Bosques Cultivados-IRB, INTA Castelar. Los Reseros y Las Cabañas s/n, Villa Udaondo, Castelar, Buenos Aires, Argentina.

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*Azospirillum* is one of the most studied Plant Growth Promoting Rhizobacteria (PGPR) genera. It is well known that it is able to produce positive effects on a wide range of crops. The aim of the present study was to evaluate the effect of *A. brasilense* on the germination velocity of *Eucalyptus globulus*. The seeds of *E. globulus*, supplied by the germplasm bank of the Instituto de Recursos Biológicos- INTA, belonged to four races; three of them were obtained from different parts of Tasmania; 1- Northeast, 2- Southeast and 3- South and the fourth, came from King Island. The inoculant used was a liquid formulation based on *A. brasilense* strain Az 39, deposited at IMYZA INTA PGPB Collection. The concentration of bacterial inoculum was  $1.3 \cdot 10^9$  cfu mL<sup>-1</sup>. The treatments were organized in a factorial arrangement by the combination of two factors A\*B, where A is the race used (1, 2, 3 and 4) and B is the *Azospirillum* inoculant applied (a- Non-inoculated, b- Inoculated) in a randomized design with two replications. *Eucalyptus* seeds were submerged in the inoculants for a minute, and then fifty seeds per treatment were sown on towel paper in Petri plates and placed in a growth chamber at 25° C. The number of germinated seeds was recorded at 7, 8, 10, 11 and 14 days after sowing. Data were analyzed by ANOVA and mean comparison by using the Duncan's test (P<0.05). The percentage of germinated seeds at the above mentioned periods was considered as the dependent variable. Inoculation with *Azospirillum* increased the percentage of germinated seeds at 7 and 8 days after sowing in the treatments with all the races and it was significantly different in the treatment with the race from the South of Tasmania. This race was the only one showing a significant best response to the *Azospirillum* inoculation at 10, 11, and 14 days after sowing. For the four races, at 10 days after sowing, the non-inoculated treatments showed an average of germination percentage of 51% while in the inoculated treatments the average of germination percentage was 64%. The difference obtained was significant. From our results we concluded that inoculation with *Azospirillum* would increase the percentage of germination of *E. globulus* at 10 days after sowing. This effect would enhance the homogeneity of seedling stands obtained in the breeding ground, especially in the races with slower germination.

P14

**THE EFFECTS OF INOCULATION WITH DIFFERENT RHIZOBIA ON EUCALYPTUS SEEDLINGS****L.C. Qun, H. Bao Ling**

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Five strains of rhizobia (HM04, HM08, HJ06, ZG03 and ZG04), isolated from *Acacia melanoxylon*, *A. crassocarp* and straight-stem *A. auriculiformis*, were inoculated on seedling roots of *Eucalyptus grandis* × *Eucalyptus urophylla* (GL-9) to study the effect on seedling growth. The height, base trunk diameter, biomass of seedlings and nitrogen, phosphorus, and potassium content of soil were tested. The results showed that the height, base trunk diameter growth and biomass of seedlings treated with the rhizobia were obviously increased as compared with the control. The content of nitrogen in the soil also underwent significant enrichment. HJ06 treatment had the greatest effect on the growth index of *Eucalyptus*. The height, biomass and chlorophyll of seedlings treated with HJ06 increased by 100.2%, 155.9% and 57.1% respectively, compared with that of the control. The greatest effect of treatment with ZG03 was in the base trunk diameter growth, which increased by 43.3% as compared to the control. Treatment with HM08 maximally enriched the content of nitrogen in the soil, which increased up to 80.9%. The most significant increase with inoculation of ZG03 on soil phosphorus and potassium content was 100% and 39.7%, respectively. Therefore, HJ06 and ZG03 were selected as the best strains.

**P15****ENHANCED RICE PRODUCTION BY *Rhizobium leguminosarum* BV. *trifolii* IN EXTENSIVE FIELD INOCULATION TRIALS IN THE NILE DELTA OF EGYPT****Y.G. Yanni<sup>1</sup> & F. B. Dazzo<sup>2</sup>**<sup>1</sup>Dept. of Microbiology, Sakha Agric. Res. Station, Kafr El-Sheikh, Egypt; <sup>2</sup>Dept. of Microbiology & Molecular Genetics, Michigan State Univ., East Lansing, MI, USA

The Emergency Rice Initiative launched to assist 11 tropical African countries (<http://allafrica.com/stories/200806180893.html>) did not mention biofertilization to achieve agricultural sustainability, indicating that the benefits of PGPR to enhance crop productivity are still under-appreciated even after 110+ yrs of use. Earlier, we found that the clover root-nodule symbiont (*Rhizobium leguminosarum* bv. *trifolii*) also forms natural endophytic associations with rice, and certain strains promote rice growth under lab and field conditions. Here we report results of 24 additional field inoculation trials using 5 rice varieties inoculated with 7 indigenous rhizobial genotypes over 5 growing seasons in large farmer's fields in the Egypt Nile delta, performed at sites ranked for the last decade as the world's highest in rice productivity per unit area ([http://beta.irri.org/statistics/index.php?option=com\\_content&task=view&id=413&Itemid=192](http://beta.irri.org/statistics/index.php?option=com_content&task=view&id=413&Itemid=192)). Inoculation with certain single strains or multi-strain consortia significantly increased grain yield in 83.3% of the trials. Data on straw yield, harvest index (grain yield/total top biomass) and agronomic fertilizer N-use efficiency (kg grain yield/kg fertilizer-N) indicated positive agronomic benefits of inoculation. This extensive program indicates that our biofertilizer inoculants of selected rhizobial PGPR can enhance rice production in real-world agriculture in ways that can reduce the need for chemical N-fertilizer inputs to achieve high yields while maintaining agricultural sustainability and acceptable production economy.

**P16****ISOLATION OF MICROAEROPHILIC N<sub>2</sub>-FIXING BACTERIA FROM RICE CULTIVATED IN ENTRE RÍOS, ARGENTINA****L.P. Di Salvo, J.S. Escobar Ortega, I.E. García de Salamone**

Microbiología Agrícola y Ambiental, FAUBA. Buenos Aires, Argentina.

An inoculation experiment was conducted with rice (*Oryza sativa*) cultivated in Entre Ríos province, Argentina. Three treatments were considered: Control (seeds with both fungicide and micronutrient fertilizer); M1 (seeds as in control plus Inoculant); M2 (seeds with fungicide only plus Inoculant). Inoculant contained two strains of *Azospirillum brasilense* isolated from maize. At anthesis, the rhizospheres were sampled. Dilutions were made and inoculated into microplates to study carbon level physiological profiles. Seven wells, corresponding to different carbon sources and showing the highest microbial activity, were chosen for each sample. Aliquots from them were inoculated into vials with semisolid NFb medium (NFbss) in order to assess the ability to fix N<sub>2</sub>. Incubations were done at 28°C between ten and fifteen days. Samples of growth observed in NFbss were streaked into plates containing a general medium with Congo Red dye (CR). This isolation cycle was repeated twice. Eighteen isolates were inoculated in a general liquid medium at two pH: 6.0 and 6.8. After 120 h of shaking at 28°C, aliquots of these cultures were inoculated simultaneously into vials with semisolid media: NFb (NFbss), JNFb (JNFbss), LGI (LGIss) and NFb glucose (gNFbss). From initial 63 isolates, 48% were discarded because they did not form characteristic veil-like pellicles below surface in NFbss or their colonies on CR plates were not *A. brasilense* like. From the remaining 33 isolates, 61% showed ability to fix N<sub>2</sub> and the 90% of these showed different types of red colonies on CR. Only 12 isolates showed growth in general liquid medium at pH 6.0 and 6.8. However, in the pH 6.0 group only six isolates showed growth in NFbss and JNFbss, while seven showed growth in LGIss and gNFbss. In the pH 6.8 group, only nine of them showed growth in NFbss and LGIss, while seven and eight showed growth in gNFbss and JNFbss, respectively. Our data showed a gap of information to characterize N<sub>2</sub>-fixing microaerophilic bacteria.

P17

**ENDOPHYTIC BACTERIA FROM FIELD AND GREENHOUSE PLANTS WITH POTENTIAL FOR CONTROL OF TOMATO FOOT AND ROOT ROT****N. Malfanova<sup>1,2,3</sup>, F. Kamilova<sup>1</sup>, S. Validov<sup>1</sup>, V. Chebotar<sup>2</sup>, I. Tikhonovich<sup>2</sup>, and B. Lugtenberg<sup>1</sup>**<sup>1</sup>*Leiden University, Institute of Biology, Clusius Laboratory, Wassenaarseweg 64, 2333AL Leiden, The Netherlands;* <sup>2</sup>*All-Russia Research Institute for Agricultural Microbiology, Laboratory of Microbial Technology, Saint-Petersburg-Pushkin, Russia;* <sup>3</sup>*E-mail: N.Malfanova@biology.leidenuniv.nl*

This work was aimed at (i) building a collection of bacterial endophytes isolated from different plants and microenvironments, (ii) screening of these endophytic bacteria for a number of plant-beneficial traits, and (iii) selection of one or more bacterial isolates able to control tomato foot and root rot (TFRR) caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* (*Forl*) in soil. TFRR is an economically important disease of tomato. A total of 96 strains of endophytic bacteria were isolated from 7 field-grown and 3 greenhouse plants after chemical sterilization of plant surfaces. To eliminate siblings which were present among the bacterial isolates, the amplified ribosomal DNA restriction analysis (ARDRA) in combination with characterization of phenotypic traits was performed. ARDRA data were compared with those for production of cell-wall degrading enzymes, and 48 strains were deleted from the collection as probable siblings. The other 48 endophytic isolates were identified on the basis of their 16S rDNA sequences. Fifteen strains were excluded from the collection as potential human pathogens. The remaining 33 strains were tested for antibiosis *in vitro* against *Forl*. Isolates that possessed strong antifungal activity were checked for their ability to control TFRR in soil. This resulted in selection of one beneficial strain. The selected biocontrol strain is the gram-positive *Paenibacillus favisporus* bacterium isolated from stems of the field-grown fodder *Heracleum* sp.

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**STUDYING PGPR GENE EXPRESSION UNDER SOIL CONDITIONS USING TAQMAN QRT-PCR ASSAYS: COMPARISON OF QUANTIFICATION AND NORMALIZATION APPROACHES****N. DeCoste and M. Filion***Université de Moncton, Department of biology, Moncton, NB, Canada, E1A 3E9*

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**P19****PROBLEMS ASSOCIATED WITH BACTERIAL IDENTIFICATION BY 16S rDNA GENE SEQUENCING****J.A. McInroy<sup>1</sup>, J.W. Kloepper<sup>1</sup>, and M. R. Liles<sup>2</sup>**<sup>1</sup>*Department of Entomology and Plant Pathology, and* <sup>2</sup>*Department of Biological Sciences, Auburn University, Alabama, USA*

Correctly identifying bacterial PGPR strains is critical for their ultimate use in agriculture because regulatory approval depends, in part, on the species. With the spore-forming bacilli, strains identified as *Bacillus cereus*, *B. anthracis*, or *B. weihenstephanensis* are considered high risk strains by regulatory agencies in Canada and the U.S. Currently, the most standard method to identify bacterial strains is 16S rRNA gene sequencing followed by BLAST analysis. Our recent experience with this technique reveals that accurate identifications to species are dependent upon two key factors: adequate sequence length and database search parameters. Commercial identification services often use a sequence length of 400–600 base pairs. However, sequences of 600 bp or less frequently generate BLAST results with multiple species matching equally well among some non-speciated entries. Obtaining longer sequences (around 1,300 bp) typically changes the final species identification. After obtaining adequate sequence length, errors in identification will often result if databases without filters are used to compare the selected sequences. Ultimately, only type-strains (the strain chosen to typify each bacterial species when it is first described) have known identity, based on the international rules of systematic bacteriology. Limiting the database search to type-strain entries can increase identification accuracy, but databases containing all of the bacterial species are not available. We recommend sequencing the full >1300 bp and conducting BLAST analysis using a type-strain filter. It is then necessary to construct a phylogenetic tree with 16S sequences from type-strains of all known species in the suspect genus. The unknown bacterial sequence can then be inserted into the phylogenetic tree to determine identity based on the closest neighbor. Examples will be presented of how strain identification changes based on these principles.

**P20****SYNERGISTIC INTERACTIONS BETWEEN POPLAR AND BACTERIA TO IMPROVE PLANT ESTABLISHMENT, PHYTOREMEDIATION AND SUSTAINABLE FEEDSTOCK PRODUCTION ON MARGINAL SOILS****D. van der Lelie<sup>1</sup>, S. Taghavi<sup>1</sup>, S. Monchy<sup>1</sup>, N. Weyens<sup>2</sup>, L. Odom<sup>3</sup>, A. Hoffman<sup>3</sup>, J. Vangronsveld<sup>2</sup>, and L.A. Newman<sup>1</sup>**<sup>1</sup>*Brookhaven National Laboratory, Upton, NY,* <sup>2</sup>*University of South Carolina, Columbia, SC* and <sup>3</sup>*Hasselt University, Diepenbeek Belgium*

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**USE OF PLANT GROWTH-PROMOTING RHIZOBACTERIA IN THE RECOLTIVATION OF WASTE DUMPS OF THE POTASH INDUSTRY****S. Koch<sup>1</sup>, H. Schmeisky<sup>2</sup>, F. R. J. Schmidt<sup>1</sup>**<sup>1</sup>Department of Microbiology and <sup>2</sup>Department of Landscape Ecology and Nature Conservation, University of Kassel, Germany

Large amounts of residues from potash mining have to be piled up aboveground annually. Precipitation on these tailings piles produces salt water which partly has to be led into groundwater or rivers. One way to cope with this heavy ecological burden is to cover the piles with vegetation resulting in improved water holding capacity and evapotranspiration. One dump in Germany had already been partially covered for eleven years with plantable residues from the aluminium recycling (REKAL/SAV), but the recultivation still has to be successfully established: despite continuous fertilization the sown plants show deficiency symptoms, which might be caused by fixation of applied phosphate fertilizer due to the properties of the cover substrate. To cope with these problems, the use of plant growth-promoting rhizobacteria (PGPR) is intended. An *in situ* survey in the rhizosphere of the pioneer plant perennial ryegrass (*Lolium perenne*) resulted in a collection of bacterial strains, all of which solubilized inorganically fixed phosphate *in vitro*. Further studies revealed eight of the strains to show ACC deaminase activity as well as production of siderophores and the phytohormone auxin. In first greenhouse trails with perennial ryegrass grown on REKAL/SAV and inoculated by alginate beads, the strain *Pseudomonas fluorescens* 2.2 led to a significant increase in dry weight of over 50% compared to the uninoculated control. This promising result is further to be validated and the exact mechanism of plant growth promotion to be revealed before the isolate finally can be applied to aid in the recultivation.

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**ECOLOGICAL EFFECTS OF CRUDE OIL RESIDUES ON THE FUNCTIONAL DIVERSITY OF SOIL MICROORGANISMS IN THREE WEED RHIZOSPHERES****Q. R. Zhang<sup>1</sup>, Q. X. Zhou<sup>1,2</sup>**<sup>1</sup>Key Laboratory of Terrestrial Ecological Process, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, P. R. China and <sup>2</sup>College of Environmental Science and Engineering, Nankai University, Tianjin, P. R. China

Ecological effects of crude oil residues on weed rhizospheres are still vague. The quantitative and diversity changes and metabolic responses of soil-bacterial communities in common dandelion (*Taraxacum officinale*), jerusalem artichoke (*Silphium perfoliatum* L) and evening primrose (*Acalypha australis* L) rhizospheric soils were thus examined using the method of carbon source utilization. The results indicated that there were various toxic effects of crude oil residues on the growth and reproduction of soil bacteria, but the weed rhizospheres could mitigate the toxic effects. Total heterotrophic counting colony-forming units (CFUs) in the rhizospheric soils were significantly higher than those in the non-rhizospheric soils. The culturable soil-bacterial CFUs in the jerusalem artichoke (*Silphium perfoliatum* L) rhizosphere polluted with 0.50 kg/pot of crude oil residues were almost twice as much as those with 0.25 kg/pot and without the addition of crude oil residues. The addition of crude oil residues increased the difference in substrate evenness, substrate richness, and substrate diversity between non-rhizospheric and rhizospheric soils of common dandelion (*Taraxacum officinale*) and evening primrose (*Acalypha australis* L), but there was no significant ( $p > 0.05$ ) difference in the Shannon's diversity index between non-rhizospheric and rhizospheric soils of jerusalem artichoke (*Silphium perfoliatum* L). The rhizospheric response of weed species to crude oil residues suggested that jerusalem artichoke (*Silphium perfoliatum* L) may be a potential weed species for the effective plant-microorganism bioremediation of contaminated soils by crude oil residues.

**P23****THE MULTIPLE PERSONALITIES OF *Streptomyces* SPP. FROM THE RHIZOSPHERE OF APPLE CULTURED IN BRASSICA SEED MEAL AMENDED SOILS****M. Mazzola<sup>1</sup>, X. Zhao<sup>1</sup>, Y. Tewoldemedhin<sup>2</sup>, and A. Mcleod<sup>2 1</sup>***USDA-ARS, Tree Fruit Research Lab, Wenatchee, WA; <sup>2</sup>Dept Plant Pathology, Stellenbosch University, Stellenbosch, SA*

Brassicaceae seed meal soil amendments provide control of Rhizoctonia root rot, in part, through the proliferation of indigenous rhizosphere colonizing *Streptomyces* spp. Studies were conducted to assess the relative role of antibiosis and nitric oxide (NO) production in the capacity of *Streptomyces* strains to control *R. solani* AG-5. Among the four dozen isolates tested, there existed no clear association between capacity to suppress in vitro growth or produce NO and ability to suppress apple root infection by *R. solani* AG-5. Among those providing disease suppression, the NO-producing population provided greater disease control than the non-producing population, however several exceptions were observed. Isolates of *S. atratus* (Antibiosis (Ab)-minus, NO-low), *S. avidinii* (Ab-minus, NO-high), and *S. cirratus* (Ab-low, NO-high) consistently suppressed apple root infection by *R. solani* AG-5. Surprisingly, *R. solani* root infection was significantly elevated in the presence of *S. vinaceus* (Ab-moderate, NO-high). When co-inoculated with certain isolates of *S. herbaricolor* (Ab-high, NO-low) root infection was significantly elevated resulting in elicitation of novel leaf symptoms that were not observed on plants grown in soils infested with the pathogen alone. When examined individually, these same *S. herbaricolor* isolates induced leaf necrosis and inhibited apple seedling root development. Knowledge of these diverse interactions between the seed meal-modified *Streptomyces* population and apple roots will be of value in managing this population for the suppression of *R. solani* AG-5.

**P24****IMPACT OF GENETICALLY MODIFIED WHEAT ON THE FREQUENCY AND GENETIC DIVERSITY OF ROOT-COLONIZING PSEUDOMONADS ASSOCIATED WITH SOIL FERTILITY****J. Meyer<sup>1</sup>, C. Keel<sup>2</sup>, M. Maurhofer<sup>1</sup>***<sup>1</sup>Plant Pathology, Institute of Integrative Biology, ETH Zurich, and <sup>1</sup>Department of Fundamental Microbiology, University of Lausanne, Switzerland*

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**PLANT GROWTH-PROMOTING BACTERIA IN SOILS UNDER CROP ROTATION OR CONTINUOUS CROPPING****N. Bajsa<sup>1,2\*</sup>, G. Azziz<sup>1</sup>, H.C. Coutinho<sup>3</sup>, A.S. Rosado<sup>4</sup>, A. Arias<sup>1</sup>**<sup>1</sup>*Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay.* <sup>2</sup>*Facultad de Ciencias, UdelaR, Montevideo, Uruguay.* <sup>3</sup>*Embrapa Solos, Rio do Janeiro, Brasil.* <sup>4</sup>*Universidade Federal do Rio de Janeiro, Brasil.*

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**STRUCTURE AND DIVERSITY OF RHIZOSPHERE BACTERIAL COMMUNITIES IN WILD AND CULTIVATED OLIVES****B.B. Landa, S. Aranda, and J.A. Navas***Institute of Sustainable Agriculture (IAS), CSIC, P.O. Box 4084, 14080 Córdoba, Spain.*

The olive tree, derived from the domestication of wild olive, is the main oleaginous crop of the Mediterranean Basin. Spain is the world leading country for olive production, with more than 2.4 million ha, of which 63% are located in Andalusia. Olive cropping systems include agroforestry stands (in marginal soils and hills), traditional groves and new intensive orchards. This study has assessed the effect of soil management systems [SMS: conventional light tillage (LT) or normal tillage (T), vegetative ground cover controlled by grazing (G), intensive grazing (IG) or mowing (M)] on bacterial population structure using terminal restriction fragment length polymorphism (T-RFLP) analyses of amplified 16S rDNA sequences in organic olive orchards. For this purpose, 46 olive orchards were sampled in ‘Sierra Morena’ region (mountainous landscape) and in ‘Campiña’ region (rolling landscape), and in 12 soils with natural vegetation near the orchards to serve as benchmarks of undisturbed soils. Additionally, 10 wild-olive havens were evaluated for comparison. Principal Component Analysis of T-RFLP revealed main changes in the relative abundance of bacterial terminal restriction fragments (TRF) in relation to the landscape of origin (mountainous *versus* rolling), with wild-olive havens being a unique reservoir of bacterial diversity. Also, a transition strategy was observed from ‘Sierra Morena’ soils to ‘Campiña’ soils and from Natural areas to cultivated ones. In the mountainous landscape no clear distinction was observed between the SMS, whereas in the rolling landscape this transition was evident for T *versus* M. Finally, in each landscape and SMS, a different subset of TRFs that substantially contributed to the variation along the first two principal components or discriminate among SMS in discriminant Function Analysis was identified. In silico analysis of the bacterial 16S rDNA sequence database is being performed to identify well-characterized genera of bacteria that may be associated with and be an indicator of different SMS.

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**ALTITUDE-DEPENDENT DIVERSITY OF *Trichoderma* COMMUNITIES ON TENERIFE ISLAND****C. Zachow<sup>1</sup>, C. Berg<sup>2</sup>, H. Müller<sup>1</sup>, I.S. Druzhinina<sup>3</sup>, C.P. Kubicek<sup>3</sup> and G. Berg<sup>1</sup>**<sup>1</sup>Institute of Environmental Biotechnology, Graz University of Technology, Graz, Austria; <sup>2</sup>Institute of Plant Sciences, Karl-Franzens-University of Graz, Graz, Austria; <sup>3</sup>Institute of Chemical Engineering, Research Area Gene Technology and Applied Biochemistry, Vienna University of Technology, Vienna, Austria

Knowledge about fungal diversity scaling relationships relative to that of plants is important to understand ecosystem functioning. Tenerife Island is represented by six different vegetation zones characterized by specific abiotic conditions and plant communities with a high proportion of endemic plants. This natural laboratory was used to study terrestrial biodiversity with a special focus on the genus *Trichoderma* in different altitudes. From 12 sampling points dispersed on the whole island and from different altitudes (10 to 3500 m), *Trichoderma* communities were analysed by selective isolation and by single-strand conformation polymorphism (SSCP) analysis. In contrast to results regarding the fungal community at all, *Trichoderma* specific SSCP resulted in low diversity of mainly cosmopolitan species, for example *Hypocrea lixii*/ *T. harzianum* [1]. The dominance of *T. harzianum* was confirmed by cultivation; the species was found mainly up to an altitude of 1000 m above sea level. The highest diversity index were found in high areas, the mountain desert and –semi desert ( $H' = 1.4$  and  $H' = 1.6$ ; 3500 m and 2000 m), whereas the lowest diversity were found in the desert ( $H' = 0.1$ ; 10 m). On *Trichoderma*-specific media colony forming numbers were in the range of  $\log_{10}$  4.0 to 5.7 CFU per g fresh weight. All *Trichoderma* isolates exhibited an extraordinarily high *in vitro* antagonistic potential towards different plant pathogens. All strains were highly active against *B. cinerea*, *G. bidwellii*, *R. solani* AG2-2IIIB and AG4, *S. rolfsii*, and *V. dahliae*. The antagonistic spectrum and competitiveness of the *Trichoderma* isolates of Tenerife demonstrated a high potential for biological control purposes.

[1] Zachow *et al.* (2008) The ISME journal 3:79-92.

P28

**PHENAZINE-PRODUCING FLUORESCENT *Pseudomonas* SPP.: DIVERSITY AND BIOGEOGRAPHY IN CENTRAL WASHINGTON STATE****J.A. Parejko<sup>1</sup>, D.V. Mavrodi<sup>2</sup>, O.V. Mavrodi<sup>2</sup>, D.M. Weller<sup>2</sup>, L.S. Thomashow<sup>2</sup>**<sup>1</sup>School of Molecular Biosciences, Washington State University and <sup>2</sup>USDA-ARS, Root Disease and Biological Control Research Unit, Pullman, Washington, USA

Strains of the rhizosphere bacterium *Pseudomonas fluorescens* produce redox-active phenazine antibiotics that suppress a wide variety of soilborne plant pathogens. Our laboratory recently detected these bacteria at population levels up to  $10^6$  colony-forming units (cfu) per gram of root (fresh weight) on dryland wheat and barley from commercial fields in central Washington State. A regional survey has indicated the high populations are limited to non-irrigated cereals grown within an area roughly bounded by 46.3° and 47.9° N and 117.5° to 119° W (ca three million acres). An average yearly precipitation level below 15 inches and lack of irrigation appear to be major population level determinants. In 2007 and 2008 we recovered 396 phenazine-producing *Pseudomonas* isolates from wheat and barley roots grown in this area. These isolates comprise 30 genotypes based on DNA fingerprinting, with an additional genotype formed by *P. fluorescens* 2-79 isolated from the same area in 1979. According to 16S rDNA and housekeeping gene sequence analysis, the majority of our isolates form a unique group separate from previously characterized pathogen-suppressing phenazine-producers. However, the isolates show close association to a different fluorescent *Pseudomonas* species, *P. orientalis* CIP 105540 (unknown as to its phenazine-producing ability) as well as *P. gessardii* CIP 105469 and *P. synxantha* IAM 2356. The specific biogeographic limits and robust diversity indicate a wide distribution of phenazine production within strains of different fluorescent *Pseudomonas* species limited to non-irrigated dryland wheat.

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**DIVERSITY IN N-ACYL-HOMOSERINE LACTONE PRODUCTION OF PLANT GROWTH PROMOTING RHIZOBACTERIA****D. Li<sup>1</sup>, A. Fekete<sup>2</sup>, M. Rothballer<sup>1</sup>, M. Schmid<sup>1</sup>, P. Schmitt-Kopplin<sup>2</sup> and A. Hartmann<sup>1</sup>**<sup>1</sup> Department Microbe-Plant Interactions and <sup>2</sup> Institute of Ecological Chemistry, Helmholtz Zentrum München, German Research Center for Environmental Health, D-85764 Neuherberg, Germany

N-acyl-homoserine lactones (AHLs) are a group of small signalling molecules used by Gram-negative bacteria to optimize physiological adaptation to changing environmental conditions via quorum sensing. In addition, plants can also respond to these molecules with plant growth promotion or increased pathogen resistance.

Isolates of the alpha proteobacterium *Rhizobium radiobacter*, recently identified as endofungal bacterium in the plant growth promoting fungus *Piriformospora indica*, are able to synthesize a variety of oxo- and hydroxy-C8- to C10- HSL-compounds as identified by FTICR/MS- and UPLC-analysis. Most interestingly, *Rhizobium radiobacter* also produced coumaroyl HSL-compounds when coumaric acid is supplied to the medium. Since coumaric acid is a natural compound of plant origin in the rhizosphere, this may provide a new group of signaling compounds with yet unknown function in plant / microbe interaction.

*Acidovorax* sp. N35 is a  $\beta$ -proteobacterium, which was isolated from the surface sterilized wheat roots. This bacterium undergoes phase variation, with one phase type showing characteristic rough colony shapes on agar plates and forming flocks in liquid medium (N35e), while the other type grows in smooth colonies and without flocculation in liquid medium (N35r). Among others, a rare AHL-molecule structure (3-hydroxy-decanoyl homoserine lactone) was identified as the dominant signalling substance of the flocculating type N35e which produced higher amounts of AHLs as compared to the N35r type. Thus, AHL-signalling may be involved in the complex phase variation process of rhizosphere bacteria.

P30

**PERCEPTION OF BACTERIVOROUS PROTOZOA BY *Pseudomonas fluorescens* MEDIATES REGULATION OF CYCLIC LIPOPEPTIDE SURFACTANTS AS AN EFFECTIVE DEFENSE AGAINST PREDATION****M. Mazzola<sup>1</sup>, I. deBruijn<sup>2</sup>, M.F. Cohen<sup>3</sup> and J. M. Raaijmakers<sup>2</sup>**<sup>1</sup> USDA-ARS, Wenatchee, WA, USA; <sup>2</sup> Wageningen University, Wageningen, the Netherlands; <sup>3</sup> Sonoma State University, Rohnert Park, CA, USA

Cyclic lipopeptides (CLPs) are produced by a diversity of bacterial genera, possess biosurfactant activity, and play a key role in a multiplicity of natural functions for the producing bacteria, including swarming motility and biofilm formation. CLPs have the capacity to disrupt membrane integrity leading to the cell lysis of certain microbial life stages, including oomycete zoospores. This study shows that the CLPs massetolide A and viscosin produced by the bacteria *Pseudomonas fluorescens* strains SS101 and SBW25, respectively, lead to lysis of protozoan trophozoites and confer protection from predation by the amoeba-flagellate *Naegleria americana*. In vitro, massetolide A-producing SS101 and viscosin-producing SBW25 were significantly less susceptible to grazing by *N. americana* than the corresponding CLP-deficient mutants 10.24- $\Delta$  *massA* 17A8- $\Delta$  *viscA*. Genetic complementation of the *massA* mutation in 10.24- $\Delta$  *massA* restored massetolide A biosynthesis and protection from *N. americana* predation. Exposure of SS101 or SBW25 to *N. americana* resulted in the up-regulation of CLP biosynthesis in both strains. In the presence, but not absence, of *N. americana*, populations of 10.24- $\Delta$  *massA* and 17A8- $\Delta$  *viscA* exhibited a more rapid decline in soil relative to the parental strains. Collectively these results show, for the first time, that CLPs produced by *Pseudomonas* contribute to survival in soil and are potent metabolites in the bacterial defense against protozoan predation.

**P31****INTERACTIONS BETWEEN A MYCORRHIZA HELPER BACTERIUM AND ARBUSCULAR MYCORRHIZAS****B. Pivato<sup>1,2</sup>, E. Gamalero<sup>1</sup>, B. Barbonaglia<sup>1</sup>, G. Berta<sup>1</sup>, Ph. Lemanceau<sup>2</sup>**<sup>1</sup>DISAV, University of Eastern Piedmont, Alessandria, Italy and <sup>2</sup>INRA, UMR MSE, Dijon, France

*Pseudomonas fluorescens* C7R12 was shown to promote arbuscular mycorrhization and to act as a ‘Mycorrhiza Helper Bacteria’ (MHB). The complex interactions between this model strain, Arbuscular Mycorrhizal (AM) fungi and host-plants were analysed by assessing the specificity of these interactions and by characterizing bacterial cell organization on mycorrhizal roots.

Evaluation of the interaction specificity relied on the comparison of the bacterial effect on (i) the *in vitro* saprophytic growth of *Glomus mosseae* and *Gigaspora rosea* and (ii) the root colonization of two different plant species (*Medicago truncatula* and *Lycopersicon esculentum*) by the two AM fungal species. Characterization of bacterial cell organisation relied on microscopic observations (immunofluorescence technique and confocal laser scanning microscopy) made on mycorrhizal (myc<sup>+</sup>, *G. mosseae*) and non-mycorrhizal (myc<sup>-</sup>) roots. The results obtained showed that *P. fluorescens* C7R12 promoted the *in vitro* saprophytic growth of *G. mosseae* but not that of *Gi. rosea*. This bacterial strain also promoted mycorrhization of medic and tomato with *G. mosseae*, but not that with *Gi. rosea* for any of the plants tested. Microscopic observations allowed the identification of five types of cell organization (Organization Types OT): small single cells, large single cells, cells by pairs, cells in micro-colonies, and cells in strings. The frequencies of each OT differed significantly on myc<sup>+</sup> and myc<sup>-</sup> roots. Bacterial cells were more frequently single on myc<sup>+</sup> roots, and in micro-colonies and strings on myc<sup>-</sup> roots, suggesting that they were dividing more frequently on myc<sup>-</sup> than on myc<sup>+</sup> roots. Indeed, the root area covered by bacterial cells was significantly higher on myc<sup>-</sup> than on myc<sup>+</sup> roots. Taken together, these results indicate that promotion of AM was fungal but not plant specific and suggest that C7R12 cells were more stressed on myc<sup>+</sup> than on myc<sup>-</sup> roots.

**P32****A STUDY OF *Bacillus subtilis* INVADING *Robinia pseudoacacia* ROOTS****B. Huang<sup>1</sup>, C. Lü<sup>1</sup>, B. Wu<sup>2</sup>, P. Zhuang<sup>1</sup>, X. Yu<sup>1</sup>**<sup>1</sup>College of Forestry, Guangxi University and <sup>2</sup>Guangxi Key Laboratory of Subtropical Bioresources Conservation and Utilization, Nanning, Guangxi, P.R. CHINA

*Bacillus subtilis* strains GXXI07 and GXMO82 were isolated from *Podocarpus imbricatus* and *Podocarpus macrophyllus* var. *maki* root nodules. 16S rDNA was sequenced and identified. When inoculated to roots of the legume *Robinia pseudoacacia*, strains GXXI07 and GXMO82 had the same ability to invade the roots. The result of the ultrastructure observations indicate that strains GXXI07 and GXMO82 invading *R. pseudoacacia* act like *Rhizobium*. They invaded *R. pseudoacacia* via root hairs and led to root nodules. The infection thread structures were similar to those of *Rhizobium* too. But the structures of infected cells are not exactly the same. The number of the thalli was fewer and generally distributed. The host cytoarchitecture did not change at the same time. The formation of the *B. subtilis* peribacteroid membrane was dissimilar. The manner of the thalli penetrating the host cell wall is also different from that of *Rhizobium*. We discovered that the manner and the behavior of the *B. subtilis* strains are not only different from those of *Rhizobium* but also different from those of plant pathogens. The thallus first loosens the host cell wall and then traverses the wall to the next cell without destroying it.

**P33****OCCURRENCE AND DIVERSITY OF THE FIT INSECT TOXIN LOCUS IN PLANT-BENEFICIAL PSEUDOMONADS****B. Ruffner<sup>1</sup>, M. Péchy-Tarr<sup>2</sup>, C. Keel<sup>2</sup>, M. Maurhofer<sup>1</sup>**<sup>1</sup>Plant Pathology, Institute of Integrative Biology, ETH Zurich, and <sup>2</sup>Department of Fundamental Microbiology, University of Lausanne, Switzerland

The application of plant-beneficial pseudomonads provides a promising alternative to chemical pest management in agriculture. The fact that *Pseudomonas fluorescens* CHA0 and Pf-5, both well-known biocontrol agents of fungal root diseases, exhibit also potent insecticidal activity is of particular interest, as these plant-beneficial bacteria naturally colonize the rhizosphere of important crop plants. Insecticidal activity in these strains depends on a novel locus encoding the production of a protein toxin termed Fit (for *P. fluorescens* insecticidal toxin). To gain a better understanding of the ecological relevance of the *Pseudomonas* anti-insect activity, we have begun to investigate the occurrence and molecular diversity of the Fit toxin genes among root-associated pseudomonads. To this end, we have screened a large world-wide collection of fluorescent *Pseudomonas* sp. isolated from the roots of different plant species using molecular fingerprinting techniques. The strains are already well characterized for exoproduct patterns and disease-suppressive ability and are currently being tested for insecticidal activity in a wax moth larvae assay system.

**P34****THREE STRAINS OF *Pseudomonas fluorescens* EXHIBIT DIFFERENTIAL TOXICITY AGAINST *Drosophila melanogaster*****M. Olcott<sup>1</sup>, M. Henkels<sup>2</sup>, K. Rosen<sup>1</sup>, F. Walker<sup>1</sup>, B. Sneh<sup>3</sup>, J. Loper<sup>2</sup>, B. Taylor<sup>1</sup>**<sup>1</sup>Department of Zoology, Oregon State University; <sup>2</sup>USDA-ARS Horticultural Crops Research Laboratory, Corvallis, OR, USA; <sup>3</sup>Department of Plant Sciences, Tel Aviv University, Israel

Three strains of *Pseudomonas fluorescens* were tested for toxicity to *Drosophila melanogaster* in an insect feeding assay. Insect eggs were placed on the surface of a non-nutritive agar plate supplemented with a food source that was non-inoculated or inoculated with *P. fluorescens* Pf0-1, SBW25, or Pf-5; and insect development and survival were evaluated over time. Strain Pf0-1 had no significant effect on insect survival and development, whereas strain Pf-5 caused dose-dependent lethality and morphological defects in eyes and wings in the surviving adult flies. In addition, Pf-5 caused a dose-dependent delay in the onset of metamorphosis relative to non-inoculated controls. A *gacA* mutant of Pf-5 caused no significant mortality, morphological defects, or developmental delay, indicating that factors responsible for these effects are controlled by the GacS/GacA global regulatory system. Strain SBW25 also caused insect mortality, but to a lower level than caused by Pf-5, and surviving larvae did not exhibit developmental delays. Strain SBW25 ingestion caused some insects to exhibit a profound, fatal systemic melanization reaction at larval, pupal or adult stages. These experiments demonstrate that *P. fluorescens* Pf-5 and SBW25, when introduced through natural routes of infection, can cause mortality of *D. melanogaster*. Furthermore, strain Pf-5 causes delays in the onset of metamorphosis and produces morphological defects in surviving adult flies that are independent of host survival.

P35

### PHOSPHATE SOLUBILIZING BACTERIA: CHARACTERIZATION FOR THEIR ABILITY TO PRODUCE ORGANIC ACIDS AND SOLUBILIZE INORGANIC PHOSPHATES

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The main objective of this study was to characterize organic acids produced by rhizospheric bacteria with growth promoting activity (PGPR) that had shown *in vitro* ability to solubilize insoluble inorganic phosphates present in acid soils. This was related to their ability to mobilize sparingly soluble phosphates and their potential as biofertilizers in tropical soils. High-performance liquid chromatography (HPLC) was the analytical technique applied. Bacteria were isolated from maize rhizosphere developed in Venezuelan agricultural systems, and were biochemically characterized and identified as: *Bacillus pumilus*, *Pseudomonas fluorescens*, *Burkholderia cepacia* and *Bacillus circulans*. Inorganic phosphates tested in liquid cultures were: iron (FePO<sub>4</sub>) and aluminum (AlPO<sub>4</sub>) phosphates, calcium phosphate Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> and a Venezuelan rock phosphate Riecito (RP). Tested bacteria were able to reduce pH *in vitro*, suggesting they are capable of producing organic acids. The most readily solubilized phosphate was FePO<sub>4</sub>, followed by AlPO<sub>4</sub>, Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> and RP. Bacterial phosphate solubilizing ability showed the following order: *B. cepacia* > *B. circulans* > *B. pumilus* > *P. fluorescens*. HPLC analysis indicated that *B. cepacia* produced tartaric acid, oxalic and malic acids, which gave it the best phosphate solubilizing ability of the bacteria tested in this work. *B. cepacia* appears to be a promising species for application as a biofertilizer in Venezuelan acid soils that have high phosphate fixing capacity and are rich in iron and aluminum phosphates.

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### ISOLATION AND CHARACTERISATION OF NOVEL PGPR FROM WESTERN AUSTRALIAN SOILS FOR IMPROVED GRAIN PRODUCTION

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The Western Australian ‘wheat belt’ is an area that is derived from an ancient land surface that has been heavily eroded and more than half of the land with the right climate for growing wheat is infertile sandy and iron-stone gravel soils (Henzell 2007). The development of these soils has required a heavy input of trace elements, superphosphates, nitrogenous fertilizers and weed and pest control chemicals. However the sustainability of the grain industries’ dependence on these agrichemicals is in dispute. As biological nitrogen fixation (BNF) by rhizobia is a major input of nitrogen to Western Australian agricultural soils it was hypothesised that other biological agents may decrease the industries dependence on these chemicals. To this end, 179 potential PGPR were isolated from the rhizosphere of native and crop species and screened for the ability to produce auxins and/or ACC deaminase. Selected isolates were screened for plant growth promotion using growth pouch assays and field trials and a subset of these selected for further study. These were further screened for PGPR traits such as siderophore production, antifungal activity and phosphorous solubilisation. Six isolates showing strong phosphorus solubilisation *in vitro* have been tested in the glasshouse on wheat plants for their ability to solubilise tri-calcium (insoluble) phosphorous.

Henzell, T. (2007). Australian Agriculture: Its History and Challenges. CSIRO Publishing. Melbourne.

P37

**PHOSPHATE SOLUBILIZATION BY RHIZOSPHERE FUNGI (*Aspergillus* AND *Penicillium*) ISOLATED FROM CAMEROONIAN SOILS AND THEIR EFFECT ON GROWTH AND NUTRIENT UPTAKE OF BARLEY**

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Fungal isolates representing two genera (*Aspergillus* and *Penicillium*) were isolated from soil samples collected from three different land use types (forest, fallow and farm) in each of the five agro ecological zones of Cameroon, which represent a wide range of soil acidity, aluminum and iron toxicity. They were tested qualitatively for phosphate solubilizing activity on agar plates as well as quantitatively in liquid media containing different sparingly soluble phosphate sources ( $\text{Ca}_3(\text{PO}_4)_2$  or  $\text{AlPO}_4 \cdot \text{H}_2\text{O}$  or  $\text{FePO}_4 \cdot 2\text{H}_2\text{O}$ ). Subsequently a greenhouse test with the most efficient isolates was conducted to assess their efficacy *in vivo* to promote the growth of barley (cv Optic) plants grown in soils amended with the different phosphate types. The isolates tested showed high solubilizing index on agar plates through the formation of halos around the fungal colonies. Also, they effectively solubilized the different sparingly phosphates in liquid media and released considerable amounts of P into the medium. We will present results which demonstrate whether these isolates are useful for promoting plant growth via phosphate-solubilization

Effective isolates of phosphate-solubilizing fungi identified in this study will be used to improve the sustainability of subsistence agriculture in Cameroon, reducing the need for inorganic inputs.

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**PLANT GROWTH PROMOTION BY *Bacillus amyloliquefaciens* FZB45 DEPENDS ON INOCULUM CONCENTRATION AND P-RELATED SOIL PROPERTIES**

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See oral presentation abstract 48.

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**BIOLOGICAL CONTROL OF WHEAT FUNGAL PATHOGENS BY BACTERIA OF THE GENUS *Pseudomonas*****N. Walter<sup>1</sup>, H. Aly<sup>2</sup>, C. Taylor<sup>2</sup> and P. A. Okubara<sup>1</sup>**<sup>1</sup>USDA ARS Root Disease & Biological Control Research Unit, Pullman, WA<sup>2</sup>Donald Danforth Plant Science Center, St. Louis, MO

Wheat, the main cereal grown in the US, is subject to more diseases than any other grains and wheat production lost to diseases is estimated to reach 10-30% in the Pacific Northwest annually. Currently, fungicides, crop rotation and tillage are the most widely used control measures to suppress soilborne diseases. However, these practices have met with limited success because they fail to control disease consistently. Biological control is thus being considered as an alternative to reducing the use of chemicals in cereal production systems. Strains of *Pseudomonas* spp. have been shown to suppress a wide variety of fungal root pathogens in various crops (Haas and Keel 2003), leading to an increased interest in developing antagonistic microorganisms for use as biological control agents. Sixty-two *Pseudomonas* isolates shown to interfere with *C. elegans* growth and viability were tested in vitro against five fungal root pathogens of wheat. Fifty-four strains (85%) had activity against at least one fungal pathogen in plate assays, and 11 strains had activity against all five pathogens. The isolates have been characterized for motility, exoprotease activity, production of siderophores, hydrogen cyanide (HCN), polysaccharides and fluorescence, and production of known antibiotics but no single commonality exists among nematode lethal and/or fungal growth inhibitory strains. Progress in quantifying the ability of the strains to inhibit the pathogen damage in greenhouse assays will be reported. Selected strains will be studied further for production of novel antibiotics and to shed light on the mechanisms of biocontrol.

P40

**PRODUCTION OF ANTIMICROBIAL COMPOUNDS BY *Bacillus subtilis* UA321 AGAINST *Mycosphaerella fijiensis*****V.V. Escobar<sup>1</sup>, L.S. Zapata<sup>1</sup>, R.N. Moncada Ossa<sup>1</sup>, S. Mosquera López<sup>1</sup>, M. Ramírez Correa<sup>2</sup>, J. Jairo Mira Castillo<sup>3</sup>**<sup>1</sup>Laboratorio de Biotecnología, Universidad EAFIT, Medellín, Colombia. <sup>2</sup>Laboratorio de Control Microbiológico, Universidad de Antioquia, Medellín, Colombia. <sup>3</sup>Centro de Investigaciones del Banano “Cenibanano”, Carepa (Antioquia), Colombia.

*Mycosphaerella fijiensis* Morelet, the causal agent of the foliar fungal disease black Sigatoka, is the major worldwide constraint for banana and plantain (*Musa* spp) production. The main control of this pathogen is based on fungicide application, which causes environment and human health concerns and generates high production costs. For these reasons, new control strategies are required and among them, biological control is a promising alternative. The *Bacillus subtilis* strain UA321, originally isolated from the chrysanthemum rhizosphere, was tested *in vitro* against four fungal phytopathogens (*Fusarium oxysporum*, *Fusarium solani*, *Botrytis cinerea* and *M. fijiensis*) and showed high antifungal activity. To identify differences in bacterial growth and production of secondary metabolites against *M. fijiensis*, the release of antimicrobial compounds was evaluated in three different culture media: tryptic soy broth (TSB), nutrient broth (NB) and medium optimized for lipopeptide production (MOLP). Cell free supernatants of MOLP medium showed higher antifungal activities against *M. fijiensis* than TSB and NB extracts. On average, 70% inhibition was observed with MOLP, 53% with TSB, and 28% with NB cell free extracts. The antifungal activity of cell-free filtrates of MOLP medium was subjected to stability tests in order to gain insight into the chemical nature of the responsible compounds. The antifungal activity of the MOLP supernatants was stable at high temperatures (30°C, 50°C and 70°C) and to different pH (3, 7 and 13). Our results indicate that these metabolites produced by *B. subtilis* UA 321 in MOLP medium are not proteins as shown by their resistance to these factors.

**P41****A MAJOR CHITINASE PRODUCED BY *Chromobacterium* SP. C-61 IS NOT A MAJOR DETERMINANT IN INHIBITION OF PHYTOPATHOGENIC FUNGI****H. S. Choi<sup>1</sup>, S. H. Park<sup>1</sup>, J. H. Lee<sup>1</sup>, S. K. Park<sup>2</sup>, Y. C. Kim<sup>1</sup>**<sup>1</sup>Department of Plant Biotechnology, Chonnam National University and <sup>2</sup> Department Agricultural Biology, Suncheon National University, South Korea

A biological control rhizobacterium, *Chromobacterium* sp. C-61, possess strong chitinolytic and antifungal activity *in vitro*. However, little is known about the regulation and role of chitinase of *Chromobacterium* sp. C-61 in its antimicrobial and biocontrol activity. To investigate the functional role of extracellular chitinase encoded by the *chi54* gene of *Chromobacterium* sp. C-61, we constructed a chitinase knock-out mutant through marker exchange mutagenesis by insertion of an *nptII* cassette into the open reading frame of *chi54*. To obtain enhanced activity of chitinase in strain C-61, we constructed a mutation that replaced the mutated *chi54* gene with the complete *chi54* gene containing the T218S mutation of *Chromobacterium* sp. C-61. The chitinase activity of the T218S mutant was 1.5-fold higher than that of wild-type strain. No chitinase activity was detected in the *chi54* mutant in various kinds of chitinase assays. In addition, antifungal activity against various phytopathogenic fungi was similar among the wild-type strain, the Chi54 mutant and the T218S mutant. These results demonstrated that production of chitinase by *Chromobacterium* sp. C-61 may not play a major role in inhibition of phytopathogenic fungi, but growth inhibition of phytopathogenic fungi could be the result of a combination of other secondary metabolites and extracellular enzymes produced by this strain.

**P42****PRESENCE AND CHARACTERIZATION OF A PHYTASE-CODING GENE IN IMPORTANT *Bacillus*-PGPR STRAINS****C.A. Ramírez<sup>1,2</sup> and J.W. Kloepper<sup>1</sup>**<sup>1</sup>Department of Entomology and Plant Pathology, Auburn University, Auburn, Alabama, USA and <sup>2</sup>Instituto de Biología, Universidad de Antioquia, Medellín, Colombia

Phytase activity is important for cycling of P and plant nutrition. However, plants produce low levels of phytase and have poor access to P from phytate. In contrast, several phytase-producing soil microorganisms have been isolated, suggesting their role in P uptake by plants. Previously, phytase activity of *Bacillus amyloliquefaciens* FZB45 was associated with plant growth promotion. The FZB45 phytase *phyC* gene is highly similar to phytase genes of other *B. subtilis* and *B. amyloliquefaciens* strains but not of other microorganisms. However, the promoter of FZB45 *phyC* is considerably different from that of the silent *B. subtilis* 168 *phyC* gene. The objectives of the current study were to determine if the presence of the *phyC* gene is common among important PGPR bacilli and to compare the structure of this gene among them. Primers that specifically target internal sequences of *phyC* were used for screening the strains *B. amyloliquefaciens* IN-937a, *B. pumilus* INR-7, *B. subtilis* GB03, *B. subtilis* MBI600, and *B. subtilis* UA321. Amplification was obtained from GB03 and MBI600, along with the phytase-producing strains *B. amyloliquefaciens* FZB24, FZB42, and FZB45 as positive controls. Amplicons were sequenced and new primers were designed to amplify the promoter regions. Cluster analysis of the open reading frames (ORF) showed that the *phyC* sequences of GB03 and MBI600 were very similar, and that these were closer to FZB24 and FZB42 than to FZB45; nevertheless, the ORF sequences of all the strains were more than 98% similar to the *phyC* of FZB45. Promoters were well conserved among all the strains with similar -10 and -35 sequences separated by 21 bp and PhoP binding sites. However, some strains possess a transition in the transcription initiation site and a transversion in the ribosomal binding site. Current work is being conducted to establish taxonomical relationships among the strains and compare their impact on plant P-uptake.

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### REPRESSION OF THAXTOMIN BIOSYNTHESIS GENE EXPRESSION AND GROWTH INHIBITION OF COMMON SCAB-INDUCING *Streptomyces* SPP. BY ANTAGONISTIC *Pseudomonas* SPP.

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Common scab is an economically important disease of potato caused by species belonging to the bacterial genus *Streptomyces*. Pathogenic *Streptomyces* spp. rely on pathogenicity- and virulence-related factors, including phytotoxins thaxtomins and, to a lesser extent, the necrogenic protein Nec1 and the tomatinase TomA. Biological control of common scab using antagonistic *Pseudomonas* spp. is currently being explored as an alternative control strategy. To our knowledge, *Pseudomonas* spp. have never been used as biocontrol agents (BCAs) of common scab of potato and little is known about the pathogen's responses to BCAs. In this study, we investigated the ability of three antagonistic *Pseudomonas* spp. isolates (LBUM 223, LBUM 300 and LBUM 647) to (a) inhibit growth of genetically diverse scab-inducing *Streptomyces* spp. isolates, and to (b) repress *txtA* and *txtC* (thaxtomin biosynthesis genes), *nec1*, and *tomA* gene expression in *Streptomyces* spp. isolates. *Pseudomonas* spp. isolates LBUM 223 and LBUM 300 significantly inhibited growth of the pathogens in plate inhibition assays, whereas LBUM 647 did not. Effects of the BCAs on gene expression were studied using TaqMan-based quantitative reverse transcriptase polymerase chain reactions. None of the *Pseudomonas* spp. isolates significantly altered transcription of the *nec1* and *tomA* genes. However, gene expression of *txtA* and *txtC* was significantly repressed by *Pseudomonas* spp. LBUM 223 in all *Streptomyces* spp. isolates under study. By inhibiting growth and repressing thaxtomin biosynthesis gene expression, *Pseudomonas* spp. LBUM 223 is a promising candidate in controlling common scab of potato.

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### ANALYSIS OF THE TYPE III SECRETION SYSTEM FROM *Pseudomonas fluorescens* Q8r1-96

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We have shown that near-identical strains of *Pseudomonas fluorescens* colonize the roots of wheat at levels that differ markedly, ranging from simple commensalism to a more sophisticated relationship better described as a mutualistic symbiosis. In many biological systems, such interactions are mediated by type III secretion systems (TTSS). Using DNA probes based on sequences spanning different regions of TTSS loci from other plant-associated bacteria, we screened a collection of 32 different genotypes of 2,4-diacetylphloroglucinol-producing *P. fluorescens* and detected the TTSS apparatus in all of them except for Pf-5 and the closely related strain CHA0. We then sequenced the TTSS locus from strain Q8r1-96 and demonstrated that it carries full-length copies of the structural inner membrane protein HrcV, the ATPase HrcN, and a dedicated sigma factor HrpL, and has overall organization similar to that of the *hrp/hrc* locus of *P. syringae* pv. *phaseolicola*. We created a mutation in the TTSS gene cluster of Q8r1-96 by deleting the region spanning the *hrpOP* and *hrcQR* genes. The resultant mutant did not differ from the parental strain under *in vitro* conditions in colony morphology, growth kinetics, biofilm formation and BIOLOG carbon source utilization profiles, nor in production of DAPG, siderophores, and exoprotease. However, the TTSS mutant was less competitive than Q8r1-96 in the wheat rhizosphere when introduced into raw soil alone or in mixed inoculation (1:1 ratio) with the parental strain, and was impaired in the ability to control the take-all pathogen of wheat.

P45

**METABOLITES FROM *Pseudomonas chlororaphis* O6 DIFFERENTIALLY INHIBIT GROWTH OF PHYTOPATHOGENIC FUNGI**S. H. Han<sup>1</sup>, J. Y. Park<sup>1</sup>, H. H. Im<sup>1</sup>, A. J. Anderson<sup>2</sup>, Y. C. Kim<sup>1</sup><sup>1</sup>Department of Plant Biotechnology, Chonnam National University, Gwangju, Korea, <sup>2</sup> Department of Biology, Utah State University, Logan UT84322, UT, USA.

*Pseudomonas chlororaphis* O6 suppresses growth of the wilt pathogen *Fusarium oxysporum* f. sp. *radicis-lycopersici* and the fungus causing wheat and barley scab, *F. graminearum*. Metabolites produced by *P. chlororaphis* O6 that could be involved in fungal suppression include phenazines and pyrrolnitrin. To determine the effective compounds of *P. chlororaphis* O6, we used a GacS mutant, a RpoS mutant, a Phz mutant, and a PrnA mutant with two different growth media, mung bean agar and potato dextrose agar, against various phytopathogenic fungi. The *P. chlororaphis* GacS, RpoS, and PrnA mutants lost antifungal activities on mung bean agar medium, and the GacS and Phz mutants lost antifungal activities on potato dextrose medium against various plant pathogenic fungi. Biochemical and transcriptional analyses of *P. chlororaphis* O6 indicated that the strain produced pyrrolnitrin as a major antibiotic metabolite, while phenazine was produced mainly on potato dextrose medium. These results indicated that production of these antimicrobial agents was sensitive to the nutrients supplied to the bacterium and required functional GacS and RpoS regulation. These factors may in part account for the variability of biocontrol under field conditions.

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**INVOLVEMENT OF PHENAZINES AND BIOSURFACTANTS IN BIOCONTROL OF *Pythium myriotylum* ROOT ROT ON COCOYAM BY *Pseudomonas* SP. CMR12A**J. D'aes<sup>1</sup>, K. De Maeyer<sup>1</sup>, T. Leys<sup>1</sup>, D.M. Mavrodi<sup>2</sup>, L.S. Thomashow<sup>2</sup> and M. Höfte<sup>1</sup><sup>1</sup>Laboratory for Phytopathology, Faculty of Bioscience Engineering, Ghent University, Belgium and <sup>2</sup>USDA-ARS, Root Disease and Biological Control Research Unit, Pullman, Washington, USA

*Pseudomonas* sp. CMR12a was isolated from the rhizosphere of the tropical tuber crop cocoyam and produces both phenazines and cyclic lipopeptide (CLP) biosurfactants. CMR12a was shown to be an efficient biocontrol agent of *P. myriotylum* on cocoyam. To assess the importance of phenazine and biosurfactant production for its antagonism, biosynthesis mutants were constructed in CMR12a by site specific mutagenesis. The phenazine mutant, CMR12a-Δphz, no longer produced phenazines, whereas other characteristics were comparable to that of the wild type. The most outstanding features of CMR12a-CLP, the biosurfactant mutant, were deficiency for CLP production, an altered swarming morphology and, remarkably, an increased production of phenazines. *In vitro*, spent culture supernatant of CMR12a and CMR12a-Δphz strongly inhibited growth of *P. myriotylum*, whereas the supernatant of CMR12a-CLP could not visibly reduce the growth of the pathogen. Infection experiments showed that CMR12a-Δphz still provided biocontrol towards *P. myriotylum*, while CMR12a-CLP lost most of its biocontrol capacity, although it was still able to colonize the cocoyam roots. These observations indicate that the ability of CMR12a to suppress *P. myriotylum* on cocoyam can mainly be attributed to its biosurfactant production. Consequently, the biocontrol mechanism appears to be different from that of *P. aeruginosa* PNA1, for which it was shown that phenazines and biosurfactants act synergistically in the biocontrol of *P. myriotylum* on cocoyam.

**P47****TEMPERATURE- DEPENDENT EXPRESSION OF *phzM* WITH ITS REGULATORY GENES *lasI* and *ptsP* in *Pseudomonas* sp. M18****J. F. Huang, H. Y Zhang, Y.Q. Li, X. Q Huang and Y.Q. Xu***Laboratory of Microbial Metabolism (Ministry of Education), School of Life Science and Biotechnology, Shanghai Jiao Tong University, 800 Dong-Chuan Road, Shanghai China. 200240*

*Pseudomonas* sp. strain M18, an effective biocontrol agent isolated from the melon rhizosphere, shares a genetic background similar to that of the opportunistic human pathogen *P. aeruginosa* PAO1. However, the predominant phenazine produced by strain M18 is phenazine-1-carboxylic acid (PCA) rather than pyocyanin (PYO): the quantitative ratio between PCA and PYO is 105 to 1 at 28°C in strain M18, while the ratio is 1 to 60 at 37°C in strain PAO1. Here, we provide evidence that the differential production of the two phenazines in strains M18 and PAO1 is related to temperature-dependent and strain-specific expression patterns of *phzM*, a gene involved in the conversion of PCA to PYO. Transcriptional levels of *phzM* were measured by qRT-PCR and by determining the activity of the transcriptional and translational fusion *phzMP'*-*lacZ* in strains M18 and PAO1, respectively. Further, using *lasI*- and *ptsP*-inactivated mutants of M18 and PAO1, respectively, we show that expression of *phzM* is positively regulated by the quorum sensing protein LasI and negatively regulated by the phosphoenolpyruvate phosphotransferase protein PtsP, also in a temperature-dependent and strain-specific manner. The differential production of the phenazines PYO and PCA by PAO1 and M18 might be a consequence of selective pressures imposed on *P. aeruginosa* PAO1 and its relative, M18, in two different ecological niches over a long evolutionary process.

**P48****DEVELOPMENT AND TESTING OF SECONDARY METABOLITE MUTANTS OF *Pseudomonas fluorescens* Pf-5****T.A. Kidarsa, M.D. Henkels, D. Lui, and J.E. Loper***USDA-ARS, Horticultural Crops Research Unit, Corvallis, Oregon*

*Pseudomonas fluorescens* Pf-5, a biological control agent of soil-borne plant diseases, produces at least ten secondary metabolites. Several of these metabolites, including hydrogen cyanide, pyrrolnitrin, pyoluteorin and 2,4-diacetylphloroglucinol have well-characterized roles in biological control. Functions of other metabolites, such as the newly characterized rhizoxin analogs and the lipopeptide, orfamide A, also display antagonistic biological activity. We have modified a system developed in *P. aeruginosa* for the creation of unmarked deletion mutants for use in Pf-5. This has allowed the creation of mutations in each of the known secondary metabolite biosynthetic gene clusters. These mutations have been sequentially introduced into Pf-5 resulting in strains containing multiple numbers and combinations of deletions. Biological assays utilizing these mutants have been developed to identify the relative contribution of identified secondary metabolites to the phenotype expressed by wild type Pf-5. Wild type Pf-5 shows phytotoxic effects on seed germination and root elongation when applied to rice seed. From assays testing a series of mutants applied to rice seed we found major contributing factors of phytotoxicity to include 2,4-diacetylphloroglucinol and rhizoxin analogs. In antagonism tests against *Pseudomonas syringae* on King's Medium B containing iron, a novel compound produced from an orphan gene cluster in the Pf-5 genome was found to be inhibitory to the pathogen.

P49

**EFFECTS OF THE METABOLITES PRODUCED BY COEXISTENT MICROORGANISMS ON THE ANTIBIOTIC PRODUCTION BY FLUORESCENT PSEUDOMONADS****N. Someya<sup>1</sup>, T. Yoshida<sup>2</sup>, M.T. Noguchi<sup>2</sup>, H. Sawada<sup>3</sup>, K. Tsuchiya<sup>4</sup>**<sup>1</sup>National Agricultural Research Center for Hokkaido Region, Memuro, Hokkaido, <sup>2</sup>National Institute for Agro-Environmental Sciences, Tsukuba, Ibaraki, <sup>3</sup>National Institute of Agrobiological Sciences, Tsukuba, Ibaraki and <sup>4</sup>Faculty of Agriculture, Kyushu University, Hakozaki, Fukuoka, Japan

See oral presentation abstract 28.

P50

***Saccharomyces cerevisiae* GENOME-WIDE MUTANT SCREEN FOR SENSITIVITY TO 2,4-DIACETYLPHLOROGLUCINOL, A BIOCONTROL ANTIBIOTIC PRODUCED BY *Pseudomonas fluorescens*****Y. – S. Kwak<sup>1</sup>, S.J. Han<sup>2</sup>, L.S. Thomashow<sup>3</sup>, J.T. Rice<sup>1</sup>, T.C. Paulitz<sup>3</sup>, D. Kim<sup>2</sup> and D.M. Weller<sup>3</sup>**<sup>1</sup>Dept. of Plant Pathology, Washington State University, Pullman, WA USA; <sup>2</sup>Dept. of Bio and Brain Engineering, KAIST, Daejeon, South Korea; <sup>3</sup>USDA-ARS, Pullman, WA USA

See oral presentation abstract 14.

P51

**NEW INSIGHTS INTO THE MODE OF ACTION OF *Stenotrophomonas* -PGPR AND OPPORTUNISTIC HUMAN PATHOGEN****H. Müller<sup>1</sup>; C. Schmidt<sup>1</sup>, D. Egamberdieva<sup>2</sup>, B. Lugtenberg<sup>3</sup>, G. Berg<sup>1</sup>**<sup>1</sup>TU Graz, Environmental Biotechnology, Graz, Austria, <sup>2</sup>National University of Uzbekistan, Tashkent, Uzbekistan and <sup>3</sup>Leiden University, Leiden, The Netherlands

The genus *Stenotrophomonas* is of high medical, ecological and biotechnological interest due to the versatility of the different species. The ability of *Stenotrophomonas* to associate with eukaryotic cells results in pathogenicity for humans, as in case of *S. maltophilia*, or in promotion of plant growth and antagonistic behaviour against fungal plant pathogens as in case of *S. maltophilia* and *S. rhizophila*. Both strains are plant colonizers and actively multiply in the rhizosphere, interestingly also the clinical isolate. Strains of *Stenotrophomonas* enhance plant productivity by several mechanisms, e.g., i) the production of the phytohormone indole-3-acetic acid, ii) nitrogen-fixation, and iii) oxidation of elemental sulphur which in turn provides sulphate for the plants. *In vitro*, both *Stenotrophomonas* isolates show an antifungal activity and they are also highly resistant to several antibiotics, which may help to compete in the rhizosphere. Furthermore, *Stenotrophomonas* strains possess extraordinary high hydrolytic activity and production of volatile organic compounds, which inhibit mycelial growth of the soil-borne pathogen *Rhizoctonia solani*. Interestingly, *S. rhizophila* synthesises the compatible solutes trehalose and glucosylglycerol after salt shock, which may explain the high effect, which was found for *Stenotrophomonas* treated plants in saline soils in Uzbekistan. However, due to the opportunistic character of *S. maltophilia* only the non-pathogenic *S. rhizophila* is a promising candidate for biocontrol and stress protection on plants.

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**PLANT-ASSOCIATED BACTERIA AND THEIR IMPACT ON FRUIT FLAVOR****G. Berg<sup>1</sup>, M. Verginer<sup>1</sup>, B. Siegmund<sup>2</sup>, E. Leitner<sup>2</sup>***Graz University of Technology, <sup>1</sup>Institute of Environmental Biotechnology, <sup>2</sup>Institute of Food Chemistry and Technology, Petersgasse 12, A-8010 Graz, Austria*

Plant-associated microorganisms fulfill important functions for their host. Whereas promotion of plant growth and health are well-studied, little is known about the impact of microorganisms on plant flavor. Two studies will be presented, which show the impact of plant-associated bacteria on flavor of grape and strawberry fruits.

To analyze the production of sensory-active volatiles of grape berry-associated microorganisms, from four different vineyards in Burgenland (Austria), samples of fruits of the red cultivar 'Blaufränkisch' were taken during harvest time. The production of volatiles was analysed for the microbial community (bacteria, yeasts, fungi) in the carposphere of grapes as well as for single isolates. The microbial communities produced clearly distinct aroma profiles. Furthermore, half of grape-associated microorganisms produced a broad spectrum of aroma compounds. Interestingly, well-known and typical flavour components of red wine were detected to be produced by microbes; for example, 2-methylbutanoic acid, 3-methyl-1-butanol (isoamyl alcohol) and ethyl octanoate.

Although over 350 different volatiles have been identified in the flavour of strawberries, only a limited number of compounds have been made responsible for the formation of the typical strawberry flavour. Special emphasis has to be put on two furanoid compounds 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furanol<sup>®</sup>, DMHF) and 2,5-dimethyl-4-methoxy-3(2H)-furanone (mesifurane, DMMF). Investigations within the last decade showed that methylotrophic bacteria in symbiosis with the strawberry plant are responsible for the biosynthesis of the furanoid compounds. To enhance the flavour quality of strawberries on a natural way, two selected strains of the genus *Methylobacterium* were grown in laboratory scale and applied on strawberry plants in greenhouse and field studies. *Methylobacterium* treatments resulted in statistically significant enhancement of strawberry flavour, which was analysed by sensory analyses as well as analytical techniques. Methylobacteria were able to colonise strawberry leaves in high abundances: using a specific-designed probe and qRT-PCR cell numbers up to 10<sup>7</sup> cells per gram of leaves were measured five weeks after treatment. Using a *gfp*-labeled strain, colonization behaviour of methylobacteria on the surface of strawberry leaves was observed with a confocal laser scanning microscope. They showed a typical colonisation pattern because they prefer the surrounding of stomata as well as trichomes.

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### DEVELOPMENT OF EFFECTIVE AGENTS ENHANCING PLANT QUALITY AND HEALTH BASED ON ECOLOGICAL BACKGROUNDS AND MOLECULAR MODE OF ACTION

**H. Müller, M. Verginer, C. Zachow, G. Berg**

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Problem-oriented development based on ecological knowledge and molecular tools resulted in a selection of microbial agents intended to protect plants against phytopathogenic fungi and/or to promote plant growth and quality. On the one hand, pathogen-specific antagonists were selected and promoted which effectively colonize the plant roots and suppress disease development. Consequently, two products named Rhizostar<sup>®</sup> and Salavida<sup>®</sup> based on *Serratia plymuthica* HRO-C48 to control *Verticillium dahliae* and *Pseudomonas trivialis* 3Re2-7 to suppress *Rhizoctonia solani*, respectively, were developed for affected crops. Another strategy based on complementary set of biological control agents consisting of *P. trivialis* RE\*1-1-14, *P. fluorescens* L13-6-12, *S. plymuthica* 3Re4-18 and *Trichoderma reesei* G1/8 which is applied to sugar beets threatened by *R. solani*. Secondly, in the recent past, special attention has been paid to the desertification and salinization of agricultural areas in arid and semiarid regions. We demonstrated the potential of salt tolerant plant growth promoting bacteria (*viz.* *Stenotrophomonas rhizophila* P69) to significantly improve growth of crop plants stressed by drought and elevated salinity. Besides the quantity of yield, the quality of agricultural goods is of public relevance. We have shown that *Methylobacterium* strains applied during flowering stage positively influence the flavour of strawberry fruits. To facilitate product development, our research relies upon comprehensive studies about the mode of action and the regulation thereof, interaction with the plant, and the ecological impact.

P54

### CHARACTERIZATION AND APPLICATION OF PGPR FOR CONTROL OF POST-HARVEST FUNGAL DISEASES OF POME FRUIT

**D.L. Mantyka<sup>1</sup>, C. Nagel<sup>1</sup>, D. Hirkala<sup>1</sup>, P. Sholberg<sup>2</sup>, L.M. Nelson<sup>1</sup>**

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Fungal decay during post-harvest storage of fruit leads to losses of 10-20% of fruit in North America. Control by synthetic fungicides is becoming less effective as the pathogens become resistant and there is interest in the use of biologicals, but no biological control agents have been registered for post-harvest use in Canada. Plant growth-promoting rhizobacteria (PGPR) adapted to cold climates may be a potential source of control agents. Putative PGPR isolated from the rhizosphere of legumes grown in Saskatchewan soils were investigated for their efficacy in the suppression of three major post-harvest pathogens of pome fruit, *Penicillium expansum*, *Botrytis cinerea* and *Mucor piriformis* during cold storage. Four isolates identified by sequencing their 16S rRNA, *Pseudomonas fluorescens* (isolates 1-112, 2-28 and 4-6) and *Serratia plymuthica* (isolate 6-25) were selected for their ability to suppress fungal decay *in vitro* on plates and on wounded apples under cold storage conditions. The isolates were grown on apple extract medium at a range of temperatures and pH. The bacteria used glucose in the apple extract medium, grew well at temperatures from 1 to 28°C and at pH 5-7. A green fluorescent protein marker (GFP) was introduced into the bacterial isolates and their survival and colonization patterns on wounded apples are being monitored via confocal and epi-fluorescent microscopy. These data demonstrate the potential for application of PGPR for control of non-rhizosphere fungal pathogens such as those responsible for post-harvest decay of pome fruit.

P55

**CULTIVAR-DEPENDENT RHIZOSPHERE COLONIZATION, ANTIFUNGAL METABOLITE ACCUMULATION AND GENE EXPRESSION IN THE WHEAT-*Pseudomonas* INTERACTION****P.A. Okubara<sup>1</sup>, N. Walter<sup>1</sup>, R.F. Bonsall<sup>1</sup>, D.R. Call<sup>2</sup>, D.Z. Skinner<sup>3</sup>**<sup>1</sup>USDA-ARS, Root Disease and Biological Control Research Unit, Pullman, Washington, USA; <sup>2</sup>Animal Disease Biotechnology Facility, Washington State University, Pullman; <sup>3</sup>USDA-ARS, Wheat Genetics, Quality, Physiology and Disease Research Unit, Pullman

We explored the role of host genotype in three aspects of the wheat-*Pseudomonas* biocontrol interaction: rhizosphere population density, accumulation of rhizosphere 2,4-diacetylphloroglucinol (DAPG), and *Pseudomonas*-mediated changes in root gene expression. Wheat cultivars varied in ability to support *P. fluorescens* strain Q8r1-96, an aggressive and persistent colonizer, compared to strain Q2-87, a moderately aggressive, less persistent colonizer. Of 27 cultivars tested, Finley and six others supported significantly ( $P < 0.05$ ) higher rhizosphere populations of Q8r1-96 than Q2-87 after 14 d in a non-pasteurized, non-agricultural soil. Cultivar Tara supported relatively high population densities of both bacterial strains, whereas Buchanan supported low population densities of both strains. In a soil-free system, roots of cultivars Tara and Finley accumulated more DAPG when colonized by Q8r1-96 compared to Q2-87. In contrast, Buchanan accumulated the same amounts of DAPG during colonization by both strains, even though Q8r1-96 produced about 100-fold more metabolite than Q2-87 in King's Medium B. These findings demonstrated that rhizoplane DAPG accumulation, like rhizosphere population density, is dependent upon a cultivar-bacterial strain interaction. Cultivar-dependent responses to rhizobacteria were also noted at the gene expression level. In microarray experiments, genes were differentially up- or down-regulated in wheat near-isogenic lines 442, 443 and in cv. Finley in response to root colonization by Q8r1-96. Progress on transcriptional changes correlated with Q8r1-96 colonization in roots of adapted cultivars Finley, Tara and Buchanan will be discussed.

P56

**COLONISATION PATTERN OF *Pseudomonas fluorescens* 1N ON RICE SEEDLING ROOTS****K. Amprayn<sup>1,3</sup>, M. Kecskés<sup>1</sup>, G. Krishnen<sup>1</sup>, L. Pereg-Gerk<sup>2</sup>, I.R. Kennedy<sup>1</sup>**<sup>1</sup>Faculty of Agriculture, Food and Natural Resources, The University of Sydney, <sup>2</sup>School of Biological Sciences, University of New England, NSW, Australia and <sup>3</sup>TISTR, Department of Agricultural Technology, Khlong 5, Patumthani, Thailand

Colonisation patterns of *Pseudomonas fluorescens* strain 1N on three Australian rice (Amaroo, Reiziq and Kyeema) roots were studied under gnotobiotic conditions using *lacZ*-labeled strains. Four-day-old hydroponically grown seedlings were inoculated with bacterial suspensions. Cell distribution and time-course colonisation were monitored by microscopic observation of X-gal stained roots. Colony-forming units (CFU) of bacterial cells on the roots was determined by dilution plating assay. The microscopic observations showed that bacterial cells rapidly attached to the root surfaces of seedlings and three hours after inoculation they had spread along the primary roots including all seminal roots. Bacterial cells were not or rarely recorded on the root tip, but they densely colonized the intercellular spaces and lateral root junctions in all varieties. Abundant single and dividing cells distributed evenly on root surface of Amaroo rice variety while the roots of Reiziq and Kyeema varieties were massively covered by microcolonies. The plate assays showed that cell number was reached the peak at 2, 3 and 1 day after inoculation (DAI) in Amaroo, Reiziq and Kyeema respectively. The decline in bacterial population of all rice cultivars were observed at 3 DAI. Significant differences in the total length of roots of Reiziq were recorded by comparing control and inoculated plants.

P57

**INSIGHT INTO THE EFFECTS OF BACTERIAL VOLATILES ON RHIZOSPHERE COMPETENCE****H. Yi<sup>1,2</sup>, Y. Ahn<sup>1,3</sup>, S. Ghim<sup>2</sup> and C. Ryu<sup>1</sup>**<sup>1</sup>Bio-industry and Biochemistry Research Center, KRIBB, Daejeon 305-600, <sup>2</sup>School of Life Science, Kyungpook National University, Daegu 702-701 <sup>3</sup>Dept. of Biological Science, KAIST, Daejeon 305-701, Korea.

*Bacillus subtilis* is a member of a Gram-positive bacterial group that thrives everywhere in soil and on plants. Certain strains of root-colonizing *B. subtilis* have been reported to increase plant productivity and are referred to as plant growth-promoting rhizobacteria. Among bacterial determinants of the plant growth promotion, the bacterial volatiles 2,3-butanediol and acetoin from *B. subtilis* were shown to elicit both enhancement of plant growth and induced resistance. However, little is known about the function of bacterial volatiles on the bacteria themselves *in situ*. Here we compared the bacterial root competence of a *B. subtilis* 2,3-butanediol null mutant strain BSIP1174 and an overexpressing mutant strain BSIP1171 to the wild type strain 168 on the pepper roots. At 14 days after bacterial inoculation, the population density of strains BSIP1171 and 168 were significantly higher than that of strain BSIP1174. Unexpectedly, fungal numbers were reduced on pepper roots treated with strains BSIP1171 and 168, compared with strain BSIP1174 treatment. To test the direct effect of 2,3-butanediol against fungi, pharmacological application of 2,3-butanediol was carried out. 2,3-butanediol did not affect the inhibition of fungal growth. However fewer fungal colonies were detected from 2,3-butanediol treated roots indicating that 2,3-butanediol induced *de novo* production of root exudates containing antifungal compounds. More interestingly, the 2,3-butanediol-induced root exudates did not inhibit bacterial growth of strains 168 and BSIP1171 but did inhibit the null mutant strain BSIP1174. Taken together, our results suggest that a bacterial volatile compound acts with dual functions: elicitation of plant basal immunity against indigenous fungi and enhancement of the bacterial capacity to maintain rhizosphere competence.

P58

**DYNAMICS OF SPORE GERMINATION AND COLONIZATION OF GERMINATING SEEDS WITH A *BACILLUS PUMILUS* STRAIN****J.W. Kloepper<sup>1</sup>, L. De La Fuente<sup>1</sup>, C. Ramírez<sup>1</sup>, M. Burkett-Cadena<sup>1</sup>, J.A. McInroy<sup>1</sup>, I.B. Sorokulova<sup>2</sup>, O. Pustovyy<sup>2</sup>, and V. Vodyanoy<sup>2</sup>**<sup>1</sup>Department of Entomology and Plant Pathology; <sup>2</sup>College of Veterinary Medicine Auburn University, Auburn, Alabama, USA.

See oral presentation abstract 30.

**P59****DEVELOPING A COMBINED GENOMICS AND NATURAL PRODUCT CHEMISTRY PLATFORM FOR THE COMMERCIAL BIOFUNGICIDE SERENADE® (*Bacillus subtilis* QST713)****M. Guilhabert-Goya<sup>1</sup>, E. Holtzapple<sup>1</sup>, J. Jimenez<sup>1</sup>, D. Joo<sup>1</sup>, S. Lego<sup>1</sup>, D. Lindhard<sup>1</sup>, S. Mills<sup>1</sup>.**<sup>1</sup>Research and Development Department, AgraQuest, Inc., Davis, USA.

SERENADE® is the most widely used foliar biofungicide globally, with registrations in 23 countries. The active ingredient QST713 is a unique, genetically distinct, proprietary strain of *Bacillus subtilis*. QST713 synthesizes high levels of three classes of lipopeptides that work synergistically to control fungal pathogens and provide superior antifungal activity. In addition, QST713 produces a range of other antifungal and antibacterial compounds and stimulates plant systemic resistance and overall growth. As part of our commitment to improve SERENADE® and provide solutions for growers, we are dedicated to basic research on QST713 to understand how a PGPR can be such an effective foliar disease control agent. To this end, we are developing a Molecular Genetics platform to guide our analysis and enhance our product support. The Molecular Genetics platform will also direct Natural Product Chemistry to identify additional antimicrobial compounds produced by QST713. The *B. subtilis* QST713 draft genome sequence reveals an unexpected capacity to produce secondary metabolites with more than 9% of the genome devoted to non-ribosomal synthesis of antimicrobial compounds. Additionally, molecular genetics in “wild” *Bacillus* strains has historically been limited by their recalcitrance to transformation. We evaluated the ability of eleven native, shuttle or integrative plasmids to transform QST713. While all plasmids failed to transform QST713 by natural competence, electroporation and protoplast transformation of pUB110 yielded 1 and 10<sup>3</sup> transformant/ug of DNA in QST713, respectively. We were also successful in transforming QST713 with the Theta origin of replication plasmids pHCMC04 and pHCMC05. We have also initiated a range of academic collaborations to explore various aspects of the chemistry and biology of QST713 activity.

**P60****THE TRANSCRIPTOMIC FINGERPRINT OF THE *Pseudomonas fluorescens* Pf-5 GacS/GacA SIGNAL TRANSDUCTION SYSTEM****K. Hassan<sup>1</sup>, I. Paulsen<sup>1</sup>, A. Johnson<sup>2</sup>, Q. Ren<sup>2</sup>, B.T. Shaffer<sup>3</sup>, and J. Loper<sup>3</sup>**<sup>1</sup>Department of Chemistry & Biomolecular Sciences, Macquarie University, Sydney, Australia; <sup>2</sup>The J. Craig Venter Institute, Rockville, MD; and <sup>3</sup>USDA-ARS, Horticultural Crops Research Laboratory, Corvallis, OR, USA

A whole genome oligonucleotide microarray was used to assess the global transcriptomic consequences of a *gacA* knock-out mutation in *P. fluorescens* Pf-5. Modest changes to the *P. fluorescens* Pf-5 transcriptome were observed during early exponential growth phase in the *gacA* null mutant. In contrast, *gacA* inactivation resulted in profound changes in the transcriptome during early stationary growth phase. For example, transcription of genes involved in the production of hydrogen cyanide, pyoluteorin, and the extracellular protease AprA were underexpressed in the *gacA* knockout mutant, whereas the transcription of genes functioning in iron uptake and iron uptake regulation, including a large number of related extra-cytoplasmic function sigma factors, were significantly higher in the *gacA* mutant than in wild-type *P. fluorescens*. Further notable effects of *gacA* inactivation were observed in the transcription of genes encoding components of a type VI secretion system and cytochrome C oxidase subunits. Additionally, the transcription of genes within several previously undescribed putative biosynthetic operons was highly modulated by *gacA* inactivation. These results provide insight into characterized GacS/GacA-controlled phenotypes and highlight a range of novel genes and gene clusters in the genome of *P. fluorescens* Pf-5.

**P61****IDENTIFICATION AND CHARACTERIZATION OF GACS-REGULATED PROTEINS IN A BIOCONTROL BACTERIUM, *Pseudomonas chlororaphis* O6**J. Y. Park<sup>1</sup>, C. H. Kim<sup>1</sup>, B. R. Kang<sup>2</sup>, S. H. Han<sup>1</sup>, A. J. Anderson<sup>3</sup>, Y. C. Kim<sup>1</sup><sup>1</sup>Department of Plant Biotechnology, Chonnam National University, Gwangju, Korea, <sup>2</sup> Jeonnam Agricultural Extension Service Center, Naju 520-715, Jeonnam, Korea, and <sup>3</sup> Department of Biology, Utah State University, Logan UT84322, UT, USA.

The GacS/GacA two component system in Gram-negative bacteria is a key global regulator involved in many biological processes. Even though many intermediate signal pathways of the GacS/GacA system have been identified and proposed, the downstream genes and proteins of the GacS/GacA regulon have not yet been fully elucidated. In order to identify the proteins regulated by the GacS sensor kinase, 2-D gel electrophoresis analysis of total proteins from the wild-type, the GacS mutant and the complemented GacS mutant were performed. A total of 14 down-regulated protein spots were characterized by MALDI-TOF and Q-TOF analysis. Genes encoding down-regulated protein spots were cloned by PCR and transcriptional expression of each gene was examined in the wild-type, the GacS mutant, and the complemented GacS mutant by RT-PCR analysis. Real-time RT-PCR analysis indicated that the selected 14 genes were regulated by the GacS sensor kinase at the transcriptional level. To determine the roles of the GacS-regulated proteins, a *P. chlororaphis* O6 mutant lacking an anthranilate para-aminobenzoate synthesis component I gene (*ascI*), which is involved in biosynthesis of tryptophan from chorismate, or a tryptophan halogenase (*prnA*) gene, involved in biosynthesis of pyrrolnitrin, was constructed throughout marker exchange mutagenesis. The AscI and PrnA mutants of *P. chlororaphis* O6 lost induced systemic resistance against *Pectobacterium carotovorum* SCCI. Root colonization and scanning electron microscopic observation indicated that *P. chlororaphis* O6 did not colonize tobacco roots. We are able to isolate unidentified GacS-regulated proteins and this information will open opportunities to investigate roles and phenotypes of GacS-regulated proteins in biocontrol rhizobacteria.

**P62****PROTEOMIC ANALYSIS TO IDENTIFY RPO-S-REGULATED PROTEINS IN A BIOCONTROL RHIZOBACTERIUM, *pseudomonas chlororaphis* O6**S. A. Oh<sup>1</sup>, J. K. Park<sup>1</sup>, S. H. Han<sup>1</sup>, J. Y. Park<sup>1</sup>, A. J. Anderson<sup>2</sup>, Y. C. Kim<sup>1</sup><sup>1</sup>Department of Plant Biotechnology, Chonnam National University, Gwangju, Korea, and <sup>2</sup> Department of Biology, Utah State University, Logan UT84322, UT, USA.

*Pseudomonas chlororaphis* O6 produces secondary metabolites such as phenazines, pyrrolnitrin, siderophore, protease, and hydrogen cyanide (HCN) that are involved in suppression of the growth of plant pathogens, induction of systemic resistance to various plant diseases, and resistance to oxidative stress. In this study, we performed proteomic analysis to identify RpoS-regulated proteins and elucidate the RpoS-mediated signal pathway. Compared with the proteome of the *P. chlororaphis* O6 wild-type, 11 protein spots were severely diminished in *rpoS* mutant cells. Furthermore, RpoS appeared to have a negative effect on the regulation of some *P. chlororaphis* O6 genes, as the intensities of three polypeptides were significantly increased in the mutant cells relative to the wild type. The proteins differentially expressed in the wild-type strain versus the *rpoS* mutant were identified based on their tryptic peptide masses. RpoS-regulated proteins are related to tryptophan metabolism, such as tryptophan halogenase (FixC) and tryptophan monooxygenase; oxidative stress, such as peroxidase (AhpC) and glutathione peroxidase (Gpx); secretion, such as the polyamine ABC transporter (PotA), the TonB-dependent outer membrane heme receptor (CirA), and the type I secretion outer membrane protein (TolC); a global regulator, putative serine protein kinase (PrkA); and general metabolism, such as heme oxygenase (HemeO) and S-adenosyl-methyltransferase (MraW). We are currently investigating the roles of the RpoS-regulated proteins in *P. chlororaphis* O6.

**P63****RpoS SIGMA FACTOR IN A BIOCONTROL RHIZOBACTERIUM, *Pseudomonas chlororaphis* O6, REGULATES EXPRESSION OF PYRROLNITRIN, BUT IS NOT INVOLVED IN INDUCTION OF SYSTEMIC RESISTANCE**S. A. Oh<sup>1</sup>, J. K. Park<sup>1</sup>, S. H. Han<sup>1</sup>, A. J. Anderson<sup>2</sup>, Y. C. Kim<sup>1</sup><sup>1</sup>Department of Plant Biotechnology, Chonnam National University, Gwangju, Korea, and <sup>2</sup> Department of Biology, Utah State University, Logan UT84322, UT, USA.

*Pseudomonas chlororaphis* O6 produces secondary metabolites that are involved in suppression of the growth of plant pathogens and induction of systemic resistance against various plant diseases. In this study, we determined the role of the *rpoS* gene in production of secondary metabolites and induction of systemic resistance by *P. chlororaphis* O6. Phenazine and pyrrolnitrin were not produced by a *P. chlororaphis* O6 GacS mutant, but a mutation in *rpoS* did not affect production of phenazine, and diminished production of pyrrolnitrin. Real time RT-PCR and biochemical analysis indicated that *phz* and *prnA* genes encoding for phenazine and pyrrolnitrin biosynthesis, respectively, are under control of the sensor kinase GacS, but only expression of the *prnA* transcript was regulated by *rpoS* through GacS activation. Inhibition of plant fungal pathogens was greatly reduced in the GacS mutant, but no reduction of fungal inhibition was observed in the RpoS mutant. Tobacco root colonization by the RpoS mutant was less than that of the wild type, but this mutant was able to induce systemic resistance in tobacco to a soft-rot pathogen at wild-type level. This study indicated that *rpoS* regulated expression of pyrrolnitrin and is important in root colonization.

**P64****DROUGHT STRESS RESISTANCE INDUCTION AND PLANT GROWTH PROMOTION BY THE MULTI-FUNCTIONAL PGPR *Bacillus licheniformis* K11 IN PEPPER PLANTS**J. Lim and S. – D. Kim\*

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See oral presentation abstract 44.

**P65****BACTERIAL DETERMINANTS AND HOST IMMUNE MECHANISMS UNDERPINNING *Pseudomonas fluorescens*-INDUCED SYSTEMIC RESISTANCE IN RICE**D. De Vleeschauwer<sup>1</sup>, P.A.H.M. Bakker<sup>2</sup>, M. Höfte<sup>1</sup><sup>1</sup>Laboratory of Phytopathology, Ghent University, Belgium and <sup>2</sup>Plant-Microbe Interactions, Utrecht University, The Netherlands

See oral presentation abstract 43.

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**DIFFERENT DETERMINANTS OF *Pseudomonas fluorescens* WCS374R ELICIT INDUCED SYSTEMIC RESISTANCE IN *Arabidopsis thaliana* AGAINST A BACTERIAL AND A VIRAL DISEASE****M. Djavaheri<sup>1</sup>, J. Mercado-Blanco<sup>2</sup>, L.C. Van Loon<sup>1</sup>, P.A.H.M. Bakker<sup>1</sup>**<sup>1</sup>Plant-Microbe Interactions, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands e-mail:P.A.H.M.Bakker@uu.nl; <sup>2</sup>CSIC, 14080 Cordoba, Spain

*Pseudomonas fluorescens* WCS374r is able to induce resistance in *Arabidopsis thaliana* against *Alternaria brassicicola*, *Botrytis cinerea*, *Hyaloperonospora arabidopsidis*, Turnip Crinkle Virus (TCV) and *Pseudomonas syringae* pv. *tomato* DC3000 (Pst). WCS374r produces salicylic acid (SA) and other iron-regulated metabolites, including pseudobactin (psb) and pseudomonine (psm), a siderophore with a SA moiety. Mutants affected in the production of one or more of these metabolites were used to unravel their role in induced systemic resistance (ISR) by WCS374r against Pst and TCV. SA and/or psm biosynthesis, and simultaneous biosynthesis of psb by WCS374r are required to effectively trigger ISR against TCV. WCS374r-mediated ISR against Pst, however, does not rely on the production of any of the iron-regulated metabolites, because all mutants impaired in the biosynthesis of one or several of these metabolites triggered ISR. Against Pst the flagella of WCS374r appear to be involved, since non motile mutants of WCS374r no longer elicit ISR and flagella preparations of the wild type strains did trigger induced resistance. These results have to be interpreted with care though because the non motile mutants colonized the roots significantly less compared to the wild type. Our data suggest that different determinants of WCS374r are required for ISR against different pathogens.

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**EXOPOLYSACCHARIDE OF *Pseudomonas chlororaphis* O6 IS A MICROBIAL DETERMINANT FOR INDUCED SYSTEMIC RESISTANCE AGAINST PLANT DISEASES AND ABIOTIC STRESSES****J. Y. Park<sup>1</sup>, S. M. Cho<sup>2</sup>, K. Y. Yang<sup>1</sup>, B. H. Cho<sup>1</sup>, A. J. Anderson<sup>3</sup>, Y. C. Kim<sup>1</sup>**<sup>1</sup>Department of Plant Biotechnology, Chonnam National University, Gwangju, Korea, <sup>2</sup> Department of Floriculture, Chunnam Techno College, Jeonnam 516-911, Korea, and <sup>3</sup> Department of Biology, Utah State University, Logan UT84322, UT, USA.

A plant growth promoting rhizobacterium, *Pseudomonas chlororaphis* O6, is able to induce systemic resistance against *Pectobacterium carotovorum* subsp. *carotovorum* SCC1 and *Pseudomonas syringae* pv. *tabaci*, and against abiotic stresses such as salt and drought stresses. Previous studies indicated that 2R, 3R-butanediol produced by *P. chlororaphis* O6 was a key compound for induced resistance against *P. carotovorum* subsp. *carotovorum* SCC1 and drought stress, and 4-carbamoylphenyl acetate from *P. chlororaphis* O6 was involved in induction of resistance against *Pseudomonas syringae* pv. *tabaci*. In this study, we identified exopolysaccharide (EPS) produced by *P. chlororaphis* O6 as also able to elicit systemic resistance against *P. carotovorum* subsp. *carotovorum* SCC1 and drought stress. Systemic or local application of EPS (100ng/plant) on tobacco roots or leaves caused great enhancement of drought tolerance. Tolerance to drought was correlated with reduced water loss in EPS-treated plants and with stomatal closure, indicated by the size of stomatal aperture and percentage of closed stomata. Stomatal closure and drought resistance were mediated by EPS of *P. chlororaphis* O6. We conclude that the bacterial EPS from *P. chlororaphis* O6 is a major determinant in inducing resistance to drought and plant disease, and are currently under investigating molecular mechanisms involved EPS-mediated systemic resistance against drought and plant disease.

**P68****INDUCED SYSTEMIC RESISTANCE ELICITED BY BACTERIAL GENETIC MATERIALS****B. Lee<sup>1,2</sup>, S. Lee<sup>2</sup>, and C. Ryu<sup>1,2</sup>**<sup>1</sup>*Field of Functional Genomics, University of Science and Technology, Korea*<sup>2</sup>*Industrial Biochemistry and Bioenergy Research Center, KRIBB, Daejeon, Korea*

Plants need to protect themselves against multiple pathogens at the infection site as well as at distal parts. Recent reports have indicated that plants perceive general cues from microbes that are referred to as microbe-associated molecular patterns (MAMPs). The plant innate immunity elicited by recognition of MAMPs such as flagellin and elongation factor and structural materials have been intensively studied. Here, we provide evidence that bacterial genetic materials can be MAMPs to increase plant defense responses against a bacterial pathogen. Infiltration of *Arabidopsis thaliana* with total RNA from the rhizobacterium *Paenibacillus polymyxa* or a pathogenic bacterium, *Pseudomonas syringae* pv. tomato, resulted in enhanced resistance to subsequent infection by *P. syringae* pv. tomato as compared infiltration with a water control. A 50K *Arabidopsis* microarray was employed to analyze genome-wide transcription in *Arabidopsis* plants following treatment with bacterial RNA. The transcriptional expression of plant defense-related genes and its transcription factor were up-regulated. Our results indicate that bacterial genetic material can be a potential MAMP and a bacterial determinant for induced resistance.

**P69****GB03 VOLATILES INDUCE GROWTH PROMOTION AND SALT TOLERANCE IN AGRICULTURAL SPECIES *Eruca sativa* (ARUGULA) CLOSELY RELATED TO ARABIDOPSIS****M.A. Aziz, H. Zhang, X. Cao, and P.W. Paré***Department of Chemistry and Biochemistry, Texas Tech University; Lubbock, Texas, 79409*

Volatile elicitors from the beneficial soil bacterium *Bacillus subtilis* (GB03) induce growth promotion and confer salt tolerance in the model plant *Arabidopsis*. While such bacterial signals are known to trigger these plant responses through gene specific regulation, whether and how growth promotion and salt tolerance are affected in other plant systems have yet to be investigated. Here, we provide biochemical evidence that the salad species *Eruca sativa* (arugula) increases growth and salt tolerance with exposure to GB03 volatile signals. We will present growth promotion, salt tolerance, gene expression, and endogenous sodium ion (Na<sup>+</sup>) data from arugula with plant exposure to GB03 treatment.

**P70****IDENTIFICATION OF INDUCED DISCRETE PLANT GENES BY ROOT COLONIZATION WITH *Pseudomonas chlororaphis* O6, WHICH INDUCES PLANT TOLERANCE TO DROUGHT AND PATHOGEN CHALLENGE**S. A. Oh<sup>1</sup>, S. M. Cho<sup>2</sup>, K. Y. Yang<sup>1</sup>, B. H. Cho<sup>1</sup>, A. J. Anderson<sup>3</sup>, Y. C. Kim<sup>1</sup><sup>1</sup>Department of Plant Biotechnology, Chonnam National University, Gwangju, Korea, <sup>2</sup>Department of Floriculture, Chunnam Techno College, Jeonnam 516-911, Korea, and <sup>3</sup>Department of Biology, Utah State University, Logan UT84322, UT, USA.

Root colonization of *Arabidopsis thaliana* by *Pseudomonas chlororaphis* O6 induces systemic tolerance against pathogenic challenge, and drought and salt stresses. Annealing primer controlled-PCR revealed eleven genes that were up-regulated and five genes that were down regulated in leaves upon root colonization by *P. chlororaphis* O6. Up-regulated genes included: NIT1 involved in IAA metabolism, a cold responsive gene *cor15a*, and a NO-responsive cysteine protease RD21a, as well as genes with products involved in cell signaling, transcription, protein synthesis and degradation. Transcripts of the jasmonic acid-marker genes, VSP-1 and pdf-1.2, the salicylic acid regulated gene, PR-1, and the ethylene-response gene, HEL, also were up-regulated by root colonization with *P. chlororaphis* O6, but differed in their responsiveness to drought stress. Priming for greater transcript accumulation was noted for some of these genes in *P. chlororaphis* O6-inoculated plants upon drought stress. Consequently, we identified several priming genes upon root colonization, and these genes regulated by jasmonic acid, ethylene and salicylic acid could play an important role in the systemic induction of both abiotic and biotic stress by root colonization of *P. chlororaphis* O6.

**P71****EVALUATION OF INDIGENOUS *Pseudomonas fluorescens* ISOLATES FOR PLANT GROWTH PROMOTION AND BIOCONTROL EFFICACY**M. Anand<sup>1</sup>, M. K. Naik<sup>1</sup> and S. R. Niranjana<sup>2</sup><sup>1</sup>Department of Plant Pathology, University of Agriculture Sciences, College of Agriculture Raichur, <sup>2</sup>Department of Applied Botany and Biotechnology Mysore University, Mysore India

The mechanism by which PGPR affect plants involves production of diverse metabolites that function in plant growth promotion (PGP). Fluorescent pseudomonads dominate in the rhizosphere and possess several properties that have made them biocontrol agents of choice, and their efficacy is prominent among indigenous isolates due to increased adaptation to local conditions. Therefore, the efficacy of eight indigenous isolates of *Pseudomonas fluorescens* (Pf) obtained from the rhizosphere of crops was evaluated for biocontrol and PGP activity. Among them, Pf4 isolate recorded good biocontrol activity on a wide spectrum of plant pathogens. Further, the isolates were tested for inducing systemic resistance against a hot pepper–*Fusarium solani* system. Pf4 was proven to be the best in induction of defense related enzymes, peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, total phenol and  $\beta$ -1,3 glucanase. A high vigour index including germination root and shoot lengths was observed in hot pepper treated with Pf4 challenged with *F. solani*. Pf4 also produced the maximum amount of antibiotic such as phenazine (7.92 absorbance at 367 nm) compared to other isolates. Six of the *P. fluorescens* isolates produced antimicrobial metabolites such as hydrogen cyanide (HCN), with Pf3, Pf4, and Pf8 noted as high HCN producers whereas Pf2, Pf5 and Pf6 were moderate HCN producers. Four of the *P. fluorescens* isolates produced salicylic acid (SA) with Pf4 recording maximum SA (24.46 absorbance at 527 nm). Thus indigenous Pf4 emerged as the most potential bioagent and PGPR.

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**TONB DEPENDENT RECEPTORS OF *Pseudomonas fluorescens* PF-5 AND  
SIDEROPHORE UPTAKE**

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See oral presentation abstract 25.

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