510A Genetic and symbiotic diversity of rhizobia isolated from Ethiopian soils
Tulu Degefu Abdi1, Endalkachew Wolde-meskel2, Binbin Liu1, Anne Willems3, Ilse Cleenwick3, Åsa Frostegård1
1Norwegian University of Life Sciences (UMB), Norway, 2School of Plant and Horticultural Sciences, Hawassa University, Ethiopia, 3Ghent University, Belgium

Leguminous trees play an important role in agroforestry in Ethiopia partly due to their ability to establish a symbiosis with rhizobia for biological nitrogen-fixation (BNF). BNF plays a substantial role in linking the biological nitrogen cycle between the atmosphere and the earth and hence is a key factor in agricultural sector and in the rehabilitation of degraded soil by improving nutrient cycling and other soil physical conditions. By virtue of these, isolation and characterization of the legume associated rhizobia is crucial to develop inocula and thereby enable their use for sustainable agriculture. As the first step to such an approach, 240 rhizobial strains were isolated from root nodules of a large number of legumes species growing in diverse agro-ecological zones in southern Ethiopia and characterized based on multi locus sequence analyses of several housekeeping and symbiosis-related genes. Cross-inoculation experiments were also conducted on a range of legume species to determine the host ranges. The core gene sequence-based phylogenetic analyses showed that all test strains represent the branches designating the Rhizobium (6 genospecies), Mesorhizobium (3 genospecies), Ensifer (7 genospecies) and Bradyrhizobium (5 genospecies), with the majority of the test strains occupying positions distinct from any defined species in each of the genera. Further characterizations of selected strains belonging to the Mesorhizobium genus from the same collection using DNA-DNA hybridization, cellular fatty acid analyses and additional housekeeping genes revealed the presence of novel lineages, hence we described three new species within the genus. Our results while showing discordant phylogeny between housekeeping and symbiotic gene in some genera like Ensifer and Rhizobium also revealed similar patterns of grouping in some other genera like Mesorhizobium. The phylogenetic relationships based on the symbiosis-related genes were not similar to those shown by core genes for the strains representing Ensifer and Rhizobium, suggesting differences in the evolutionary history between the chromosomal and symbiotic genes. The cross-inoculation experiment, conducted to evaluate the symbiotic performance of the test strains on a range of legumes species, indicated the potential to improve N fixation through host selection. Our study confirms a high diversity of rhizobia in Ethiopia and, while contributing to the general knowledge of the biodiversity within the rhizobial genera, also highlights the need to focus on previously less explored bio-geographical regions to unravel as yet unidentified rhizobial resources.

511A Microbial ACC-deaminase biotechnology for improving the efficiency of Rhizobium inoculation in mung bean under salt affected conditions
Maqshoof Ahmad1, Zaher Ahmad Zahir2, Muhammad Arshad2, Muhammad Khalid2, Moazzam Jamil1, Sajid Mehmood Nadeem3
1University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, Pakistan, 2Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Pakistan, 3College of Agriculture, DG Khan, Pakistan

High ethylene concentration under different environmental stresses such as salinity is one of the contributing factors for premature senescence of different plant parts. Plants under salinity stress produce increased levels of ethylene which inhibit the plant growth. Microbial 1-aminocyclopropane-1-carboxylic acid (ACC)-deaminase is the enzyme which is present in some strains of plant growth promoting rhizobacteria (PGPR). These PGPR alone and also in combination with rhizobial strains promote plant growth by lowering the endogenous level of ethylene along with some other mechanisms. A number of rhizobacterial and Rhizobium strains were isolated from rhizosphere and nodules of mung bean, respectively. The rhizobacterial strains were screened on the basis of ACC metabolism assay. The selected rhizobacterial strains containing ACC-deaminase activity and Rhizobium were assessed for their ability to tolerate salt stress by conducting osmoadaptation assay. These strains were further screened for their ability to promote growth of mung bean seedlings under salt-stressed axenic conditions in growth pouch/jar trials. Three most effective strains of PGPR (Mk1, Pseudomonas syringae; Mk20, Pseudomonas fluorescens and Mk25, Pseudomonas fluorescens Biotype G), and Rhizobium phaseoli (M1, M6 and M9) were screened in co-inoculation for their growth promoting activity at original, 4 and 6 dS m⁻¹ under axenic conditions. Strains from three most effective combinations were tested alone and in combinations in pot and field trials for their potential to improve
growth, nodulation and yield of mung bean under salt-affected conditions. Results showed that salinity stress significantly reduced plant growth, some physiological parameters, nodulation and yield but inoculation with PGPR containing ACC-deaminase and rhizobia improved these, thus reducing the inhibitory effect of salinity. However, their combined application was more effective under salinity and the co-inoculation with PGPR strain Pseudomonas fluorescens and Rhizobium phaseoli was the most efficient for improving growth, nodulation, physiology and yield of mung bean. The effect of high ethylene concentrations on plant growth and performance of these strains for reducing this negative impact was also evaluated by conducting classical triple-response bioassay. Intensity of the classical triple response decreased due to inoculation with these strains as the root/shoot length of inoculated mung bean seedlings increased while stem diameter decreased which is typical indication of the dilution in classical triple response. Such bacteria could be very effective as co-inoculants to improve growth, physiology, nodulation and yield of mung bean under salt affected conditions. However, the degree to which these inoculants impart benefits to plant growth can vary with the conditions and PGPR strains. A PGPR strain with multiple traits could be more useful under diverse conditions compared to a strain containing single trait.

512A Honeybees govern bacterial communities composition in floral nectar
Yana Aizanberg-Gershtein*, Ido Izhaki, Malka Halpern
University of Haifa, Oranim, Israel

Floral nectar is considered the most important reward animal-pollinated plants offer to attract pollinators. Recently, we demonstrated for the first time that bacteria in nectar are abundant and diverse. Microbial communities in nectar may affect the nectar's chemical profile, thus directly controlling nectar consumption by flower visitors such as pollinators and nectar thieves. Consequently, they indirectly govern plant fitness. Our aim in this study was to explore whether honeybees, which act as pollinators of some plant species, transfer bacteria in and out of nectar, hence affecting the composition of microbial communities in the nectar. To gain a better understanding of the relationships between honeybees and nectar, we sampled nectar and honeybees from two plant species: Amygdalus communis and Citrus paradisi. To prevent contact of pollinators with some of the flowers, buds from each of the sampled plants were covered with net bags before blooming (covered flowers). Nectar samples were collected from uncovered as well as from covered flowers. Honeybees were also collected from the flowers' surroundings of each plant species. Using culture dependent and culture independent methods, we reinforced the conclusion of our previous study and demonstrated that bacteria in nectar are abundant and diverse and that the composition of bacterial communities was specific for each plant species. Comparative analysis of bacterial communities in nectar and honeybees was performed by 454-Pyrosequencing. The analysis was performed on five Amygdalus communis plants and three Citrus paradisi plants. In sum, 1,664 OTU's were obtained for 24 samples (with an average of 10,000 sequences per sample). The majority of sequences from all nectar and bee samples were classified as Gammaproteobacteria. The most dominant genus in the bee samples was Arsenophonus with the frequency of 43% in Amygdalus communis bees, and 88% in Citrus paradisi bees. The genus Arsenophonus is a group of symbiotic, mainly insect-associated bacteria with a rapidly increasing number of records. No significant differences were found between bacterial communities of the uncovered nectar flowers Citrus paradisi and Amygdalus communis and bacterial communities of honeybees collected from the same plant species. No significant differences were found between honeybees which were trapped on different plant species. Nevertheless, although no significant differences were found in the microbial communities among honeybees which were trapped on different plant species, each bee showed resemblance only to the nectar of the plant species from where it was trapped; similar results were obtained for the culture dependent method. It seems that apart from the endogenic symbiotic bacterial communities, honeybees possess bacterial communities that inhabit nectar of specific plant species they pollinate in a specific season. In conclusion, the honeybee pollinator may introduce bacteria into the nectar and/or may be contaminated by bacteria that were introduced to the nectar by other out-sources like nectar thieves. More research is needed to explore bacterial communities on other nectar visitors and their relationships with floral nectar and nectar pollinators.
512B  Molecular characterization of nitrogen-fixing bacteria and their colonization pattern in mangrove roots
Gabriela Alfaro-Espinoza*, Matthias Ullrich
Jacobs University Bremen, Germany

Nitrogen-fixing bacteria play a major role in re-mineralization processes in mangrove ecosystems. Anaerobic processes such as denitrification take place in the anoxic layers of mangrove sediments. Consequently, most of the nitrogen is lost and thus no longer available for metabolic processes in plants. Previous studies had shown that nitrogen-fixing bacteria interact with mangrove roots making nitrogen available for plants. Nitrogen fixation is a very important process in mangrove ecosystems, however, little is known about the colonization strategies, the physiological impacts, and the genes expressed by bacteria during the interaction with mangrove plants. Therefore, the establishment of a nitrogen-fixing bacteria-mangrove model system is necessary to study the molecular mechanisms of this interaction. Our aim was to first isolate and characterize nitrogen-fixing bacteria associated with root material of *Avicennia sp.* and *Rhizophora mangle*. Then, we investigate the colonization patterns of selected bacterial strains on mangrove roots. Nitrogen-free medium was used for the isolation of 9 bacterial strains assigned to two different phylogenetic classes. Isolates were characterized by their ability to fix atmospheric nitrogen, their phylogenetic affiliation using 16S rRNA gene sequencing, their genetic accessibility, and their ability to survive and colonize mangrove roots when inoculated with different other sediment-borne indigenous bacterial strains (fitness test). Two selected isolates, *Marinobacterium sp.* and *Vibrio sp.*, were labeled with the enhanced yellow fluorescent protein. We traced these isolates using confocal laser scanning microscopy. Our observations reveal that the labeled bacteria colonized mangrove roots. These isolates are promising candidates to establish a bacteria-mangrove model system to continue our investigation of the molecular mechanisms determining bacteria-mangrove interactions.

513A  Leafy vegetable - human pathogen interactions: a farm-to-fork perspective
Beatrix Alsanius*1, Lars Mogren2, Maria Grudén2, Christine Larsson3, Mehboob Alam2, Stephen Bürleigh2, Crișter Olsson2, Sofia Windstam2, Sofia Boqvist4, Ivar Vågholm4, Karin Söderqvist4, Siv Ahrné5, Göran Molin5, Lilly Kristensen2
1Swedish University of Agricultural Sciences, Sweden, 2SLU, Dept of Horticulture, Sweden, 3State University of New York, Biology Dept, USA, 4SLU, Dept of Veterinary Biomedicine and Health, Sweden, 5Lund University, Food Hygiene, Sweden

Fresh vegetables are highly perishable which becomes particularly critical in complex supply chains. An excellent example of a high-value product requiring a sophisticated production is ready-to-eat salads. Consumers increasingly demand fresh, tasty, and safe kitchen-ready salad mixtures. Horticultural production worldwide is alerted by an increasing number of outbreaks of food illnesses related to microbial contamination of fruits and vegetables. Leafy vegetables are a major vector for enteric contaminants which was emphasized by the German outbreak in May 2011. Horticultural production networks are particularly vulnerable as they are multinational. Contamination may occur during different stages of the farm-to-fork continuum; during preharvest, hygienic properties of irrigation water and organic manure are dominant factors for transmission. Failures occurring at an early stage of the production chain may not be counteracted in a later stage of the production chain, i.e. processing.

The occurrence of *E. coli* O157:H7 may be decreased by increasing the interval between irrigation event and harvest. However the curve progression is depending on inoculum density. Furthermore, drought stress prior to harvest increases the level of bioactive compounds, such as carotenoids, flavonoids and ascorbic acid in leafy vegetables. Ascorbic acid is one of the major antioxidants in leafy vegetables. In in vitro assays, ascorbic acid promoted the proliferation of *E. coli* O157:H7 and synergistic effects were found when *E. coli* O157:H7 was exposed to both ascorbic acid and selected organic carbon or nitrogen sources. The response of *E. coli* O157:H7 to lysates of leafy vegetables as well as its performance on leafy vegetables exposed to preharvest drought stress are presented.
514A  Direct image-based enumeration of microbes decomposing biomass feedstocks
Jesus G. Alvelo-Maurosa*, Susan B. Leschine
University of Massachusetts, Amherst, United States

One of the greatest challenges for understanding the physiology and ecology of biomass-decomposing microbial communities is the measurement of cell growth on insoluble cellulosic substrates. Current methods such as colony count in agar plates, optical density, protein assay, product analysis via High Performance Liquid Chromatography, ELIZA provide only indirect measures of cell growth. Direct microbial cell enumeration has been suggested as a solution; however, quantification of microbes on plant substrates has proven to be difficult due to cell aggregation, position on and within the substrate, and cell-substrate differentiation. In order to solve this problem, we have developed a dual staining method that differentiates individual cells from each other and from the plant substrate. Clostridium phytofermentans, an anaerobic, mesophilic, cellulose-decomposing bacterium, was cultured with 30g/L Whatman #1 filter paper as growth substrate and stained using Fluorescent Brightener 28, a β-polysaccharide specific stain, and SYTO® 9, a nucleic acid stain. Cells were visualized by fluorescence microscopy and multiple images were obtained, focusing on ten different fields per slide. Open source software Image J and Adobe Photoshop CS5.1 Extended were used to quantify microbial cells. Growth curves, cell number as a function of time, were obtained and plotted every 24 hours. Cultures reached a density of 4.1x107 cells/ml at day 7 in a minimal defined medium. Furthermore, cell-cellulose interactions and sporulation morphologies were visualized in a three-dimensional image by capturing images at multiple focal planes. Autoquant 9.2 was used to deconvolve images and Imaris Bitplane 7.2.3 was used to perform visual 3D rendering. These results indicate that the dual staining method used in this study enables three-dimensional visualization and enumeration of microbial cells growing on insoluble cellulosic substrates.

515A  Isolation and characterization of Aurantimonas species responding to legume nodulation genotypes
Mizue Anda*, Seishi Ikeda, Shima Eda, Hisayuki Mitsui, Kiwamu Minamisawa
1Tohoku University, Japan, 2National Agricultural Research Center for Hokkaido Region, Japan

Various microbes reside in phyllosphere, however, ecological rules and driving forces underlying the relationship between microbes and host plants are largely unrevealed. Leguminous plants have established a symbiotic relationship with rhizobia, and regulate the degree of nodulation, known as the autoregulation of nodulation (AON). Our previous microbial community analysis demonstrated that nodulation phenotypes and nitrogen fertilization levels affected stem- and leaf-associated bacterial community of soybean. Among the communities, the relative abundance of Aurantimonas species, which belong to the order Rhizobiales, was especially sensitive to them. These results suggested that the populations of specific bacteria are controlled in a similar manner through the regulation systems of plant-rhizobia symbiosis. The ecology of Aurantimonas species in relation to plant association was largely unknown. The aim of this study was to isolate Aurantimonas species responding to AON, and examine whether the nodulation genotypes affect the colonization of the bacterium in legumes.

Firstly, PCR-assisted isolation was adopted for isolation of Aurantimonas species from enriched bacterial cells prepared from stems of field-grown, hypermodulating soybeans. Then, inoculation tests were conducted to evaluate the population levels of these bacteria in the wild type Lotus japonicus and its hypermodulating mutants, which are deficient in AON.

Thirteen of 768 isolates cultivated on Nutrient Agar medium were identified as Aurantimonas by colony PCR specific for Aurantimonas and 16S rRNA gene sequencing. A clustering analysis (>99% identity) of the 16S rRNA gene sequences for the combined datasets of the present and previous studies revealed 4 operational taxonomic units (OTUs) for Aurantimonas and showed the successful isolation of target bacteria for this group. ERIC- and BOX-PCR showed the genomic uniformity of the target isolates. In addition, phylogenetic analyses of Aurantimonas revealed a phyllosphere-specific cluster in the genus.

The representative isolate, Aurantimonas sp. strain AU20, was inoculated to the sterilized seeds of Lotus japonicus wild type Gifu and hypermodulating mutants (har1-4 or har1-5 mutants). Eleven days after sowing seeds to Leonard's jars, Mesorhizobium loti MAFF303099 was inoculated to the roots.
Then, thirteen and 30 days after inoculation (DAI) of *M. loti*, the shoots were grinded and subjected to CFU determination on NA plates. As a result, the CFU in the wild type 30 DAI was significantly lower than that in *har1* mutants (*p*<0.01). Number of nodules in wild type was significantly lower than *har1* mutants 13 and 30 DAI, indicating that the AON response of *L. japonicus* occurred at least 13 DAI.

These results showed that the population levels of AU20 were changed in the similar manner of the previous culture-independent community analysis in field-grown soybeans. We also present whether this decline of CFUs by *har1* is specific for *Aurantimonas* sp. AU20 as compared with *Enterobacter* sp. inoculation.

515B  Assessing the sources of biological nitrogen fixation in a rice-field incubation system using field 15N2-gas feeding
Qicheng Bei1, Georg Cadisch2, Zubin Xie1, Frank Rasche*2
1Chinese Academy of Sciences / Institute of Soil Science, China, 2University of Hohenheim / Institute of Plant Production and Agroecology in the Tropics and Subtropics, Germany

A range of different N2 fixing systems can contribute the N economy in flooded rice fields, but the direct evidence of N2 fixation and the proportional contribution of the heterotrophic and phototrophic N2 fixation is difficult to assess. The objectives of this study were thus to use a 15N2-gas feeding method based on exposing an intact paddy soil-rice system to a 15N2-enriched atmosphere to assess (i) the actual contribution and (ii) community structures of (active) phototrophic and heterotrophic N2-fixing bacterial populations under field conditions. Our developed system is an enclosed, automated and controlled growth chamber monitoring simultaneously humidity, temperature and carbon dioxide and continuously adjusts these according to ambient conditions. In this chamber, rice (*Oryza sativa* L.) growing in pots of flooded soil was exposed to a 15N2-enriched (7 atom-% 15N) atmosphere to assess BNF activities associated with rice. A non-enriched, 14N2-incubation system was included as control. To inhibit the activity of phototrophic N2-fixing bacteria, the surface of selected pots was covered by lightproof cotton. After 70 days incubation, surface soil samples (0-1 cm) were taken and used for 15N2-enrichment measurement and for studying community structures of the differently treated N2-fixing bacterial population. Incubation of soils with no rice cultivation revealed distinct differences between phototrophic and heterotrophic BNF (0.8 mg 15N pot-1 versus 0.4 mg 15N pot-1, respectively). In cultivated pots, BNF was higher than controls, but no significant difference was found between phototrophic (2.7 mg 15N pot-1) and heterotrophic (2.8 mg 15N pot-1) BNF. This effect was confirmed by *nifH* gene (nitrogenase) abundance which was higher in cultivated soils than those of uncultivated controls. A clear difference between treatments was found in the *nifH* gene community structures confirmed by T-RFLP profiles and substantiated by *nifH* gene libraries. To provide complementary information about those bacterial N2 fixers which were actively fixing N2, surface soil DNAs of the 15N and 14N chamber were subjected to 15N-DNA-SIP-based community fingerprinting. Although low enrichments were found in soil DNA and due to the drawback of the lower density gains with 15N-SIP, we separated “heavy” (enriched) from “light” DNA. The *nifH* gene-based T-RFLP analysis of density-resolved fractions indicated certain terminal fragments in the “heavy” fractions that were less dominant or absent in the “light” fraction. These phylogenetic differences are currently studied by *nifH* gene libraries. The presented study introduced the suitability of the 15N2-gas feeding technique to disentangle the importance and the specific contribution of phototrophic and heterotrophic BNF in paddy rice fields. Furthermore, the introduced 15N2-gas labelling technique combined with 15N-DNA-SIP showed, even with a low labelling success, its potential for future investigation of active BNF under natural field conditions.

516A  Investigation of the plant primary metabolism in response to *Verticillium* attack
Anja Buhtz*, Mandy Heinze, Rita Grosch
Leibniz-Institute of Vegetable and Ornamental Crops Großbeeren, Germany

Plant defence responses during pathogen infection are resource intensive and consequently lead to an increased demand for assimilates. Unlike the complex defence reactions including secondary metabolites, not much is known about how host plants react to higher energy or assimilate requests at the primary metabolic level. However, in contrast to existing studies with leaf pathogens invading assimilate-producing tissues, little is known about the influence of soil-borne pathogens on primary metabolism. Soil-borne fungi such as the wilt-inducing *Verticillium* spp. cause very high yield losses in crop production. Agriculturally important crops such as cauliflower, cotton, sunflower or several
Solanaceae (tomato, potato, eggplant, pepper) are often highly susceptible to *Verticillium* attack. This is mainly caused by *Verticillium dahliae* (Vd) and *Verticillium albo-atrum* (Va). These two agents affect several mono- and dicotyledonous plants given their broad host range of about 300 species. In addition, the production of microsclerotia during disease development makes plant protection and pathogen management even more complicated since they can last for more than 10 years in infected soil.

In the present study the Tomato-*Verticillium* pathosystem was used to monitor metabolic responses in sugar, amino- and organic acid content in leaf and root tissues of tomato plants infected with Vd. Preliminary data have shown the sensitivity of two tomato cultivars (Hildares and Moneymaker) to different Vd isolates –characteristic stunting symptoms accompanied with strong effects on plant biomass could be observed. Real-time PCR was used for the detection and quantification of fungal DNA in different plant tissues. To characterize the physiological status and plant responses upon Vd infection leaf photosynthesis measurements of infected plants were conducted at different days after inoculation (dpi). A significant decrease of both assimilation rate and stomatal conductance was observed between 14 and 21 dpi. Gas chromatography coupled with mass spectrometry was used to analyze different responses in amino acid and sugar content as well as organic acids of the TCA cycle. As a result 70 metabolites were identified in infected roots by comparing mass fragment spectral data with a reference compound library. Several primary metabolites have shown differential responses on fungal infection.

The characterization of plant physiological responses and analysis of metabolite changes upon soil-borne pathogen attack could contribute to understanding the high complexity of host response mechanisms, source-sink relationships or assimilate distribution and might help finding new strategies for vegetable gardening and limiting yield losses.

517A  **Wild and domesticated grapevines: endophyte community structure and differences**
Andrea Campisano¹, Sohail Yousaf¹, Michael Pancher¹, Barbara Sara Biagini²
¹Fondazione Edmund Mach, Italy, ²Università di Milano, Italy

Microbes found in asymptomatic plants are known as endophytes. Endophytic microbial communities associated with economically relevant crops are studied as they may provide tools for improving plant health, growth and overall quality. To test the hypothesis that in domesticated grapevines part of the microflora native to this plant in the wild was lost, we surveyed wild and domesticated grapevines.

We adopted a metagenomic approach to study grapevine endophytic communities. Both Automated Ribosomal Intergenic Spacer Analysis (ARISA) and Roche 454 technology were used to fingerprint and sequence (respectively) microbial DNA after PCR. To understand how synthetic fungicides introduced for pest control in modern agricultural practice may have shaped the grapevine associated microbiota, we also analysed the differences in microbial communities in plants from vineyards under organic or IPM management. Both bacterial and fungal endophytes were considered in this study.

518A  **Botryosphaeria associated with Acacia mangium and Pinus caribaea var hondurensis Venezuelan based on morphology and DNA sequence data**
Feraida Castro-Medina¹, Sari R. Mohali², Walter. D. Gubler³
¹Universidad Nacional Experimental de Guayana, Venezuela, ²Universidad de los Andes, Venezuela, ³University of California, United States

Botryosphaeria is a species-rich genus with a cosmopolitan distribution, commonly associated with dieback and cankers of woody plants. Species of the Botryosphaeriaceae are pathogens of many plantation trees, including species of Acacia and Pinus. The aim of this study was to identify species of the Botryosphaeriaceae family associated with Acacia mangium and Pinus caribaea var hondurensis plantations in Venezuelan region of Guayana. Four species of the Botryosphaeriaceae were identified based on combination of morphological characteristics and sequences from ITS, β-tubulin and EF-1α gene regions. One of these represent non described taxa as Diplodia guayanensis, the remaining three species collected include Lasiodiplodia theobromae, L. pseudechromobae and L. venezuelensis. Only the species of Botryosphaeriaceae inoculated in A. mangium were pathogenic, while in P. caribaea were not significantly different regarding the control. The Botryosphaeria spp showed no significant differences between them regarding the length size of the lesion produced in A.
mangiun, but a high virulence on this host. Lasiodiplodia venezuelensis and L. pseudotheobromae were the most pathogenic species in A. mangiun trees.

519A Genomic Investigation of Plant Growth-Promotion in Herbaspirillum sp. Strain GW103
Jong-Chan Chae*, Gun Woong Lee, Kui-Jae Lee
Chonbuk National University, South Korea

Plant growth-promoting bacterium, Herbaspirillum sp. strain GW103, was isolated from rhizosphere soil of the reed (Phragmites australis) in reclaimed land for the capability of utilizing 1-aminocyclopropan-1-carboxylate (ACC), a precursor of the plant hormone ethylene, as a sole nitrogen source. The isolate exhibited a growth promoting effect on Chinese cabbage (Brassica rapa ssp. pekinensis) under salinity stress. The genome sequencing was conducted in order for genome-wide investigation of plant growth-promoting activity of the strain. The obtained genome sequence had a total of 5,047,645 bp with a G + C content of 62.46% and 4,655 coding sequences. It did not have a plasmid. The genome annotation revealed several genes responsible for the adaptation of strain GW103 in plant niche. They included the genes encoding proteins involved in root adhesion, colonization/establishment, plant growth promoting compounds, and plant-microbe interaction. A gene encoding for ACC deaminase, an enzyme regulating the stress related hormone (ethylene) level in plants, was also found in the genome. Subsequently, the genome sequence analysis provides a better understanding of the synergistic interaction between the plant and Herbaspirillum sp. GW103.

520A Whitefly infestation elicits defense responses against bacterial pathogens on the leaf and root and belowground dynamic change of microflora in pepper
Hye Kyung Choi*,1, Soohyun Lee1, Hwe-Su Yi2, Boyoung Lee3, Choong-Min Ryu3
1Lee, South Korea, 2KRIIB, South Korea, 3University of Science and Technology / KRIIB, South Korea

Upon facing biotic stresses, plants orchestrate defence mechanisms via internal and external mechanisms that are mediated by signalling molecules such as salicylic acid, jasmonic acid, ethylene and various other volatile compounds. Although pathogen- and chemical-induced plant resistance has been studied extensively within the same plant compartment, the effects of aboveground (AG) insect-elicited plant defence on the resistance expression in roots and the belowground (BG) microbial community are not well understood. We assessed the effect of AG whitefly (Bemisia tabaci) attack on the elicitation of induced resistance against a leaf pathogen, Xanthomonas axonopodis pv. vesicatoria, a soil-borne pathogen, Ralstonia solanacearum, and on BG modifications of the rhizosphere microflora in peppers (Capsicum annuum). Symptom development caused by the two bacterial pathogens on leaves and roots was significantly reduced in whitefly-exposed plants as compared to controls. A combined treatment with benzothiadiazole (BTH) and whitefly caused an additive effect on induced resistance, indicating that whitefly-induced plant defence can utilize salicylic acid(SA)-dependent signalling. To obtain further genetic evidence of this phenomenon, we evaluated the gene expression of Capsicum annum pathogenesis-related protein (CaPRR) 1, CaPR4, CaPR10, and Ca protease inhibitor II, and observed increased expression after BTH and/or whitefly treatment indicating that AG whitefly infestation elicited SA and jasmonic acid signalling in AG and BG. Since the expression pattern of PR genes in the roots differed, we assessed microbial diversity in plants treated with BTH and/or whitefly. In addition to eliciting BG defence responses, a whitefly infestation of the leaves augmented the population of root-associated Gram-positive bacteria and fungi, which may have positively affected plant growth and induced systemic resistance. Whitefly feeding reduced plant size, which usually occurs as a consequence of the high costs of direct resistance induction. Our results demonstrate that whitefly-induced resistance against bacterial pathogens can cross the AG–BG border and may cause further indirect benefits on future plant development, because it can positively affect the association or plant roots with putatively beneficial microorganisms.

521A Seed defense priming by rhizobacteria
Hye Kyung Choi*, Geun Cheol Song, Choong-Min Ryu
University of Science and Technology / KRIIB, South Korea

Seed priming is a technique of controlled hydration and drying that result in more rapid germination when the seeds are re-imbibed. Defense priming that can be primarily induced by certain beneficial microbes and natural and synthetic compounds display more quickly or aggressively defense respond
to future biotic or abiotic stresses. In this study, the seed defense priming was newly developed by seed priming of root-associated Bacillus sp. and its supernatant. The optimization condition was determined by assessing different soaking and drying time using by a chemical inducer benzothiadiazole. Bacterial supernatants from the 515 bacilli strains isolated from various root and soil samples in Korea were tested induced systemic resistance against Pseudomonas syringae pv. lachrymans in cucumber seedling. Primed seeds in culture supernatants of strains PB69 and PB1502 were significantly reduced the symptom development as 40% and 28% respectively compared to control. Our results indicate that seed defense priming triggered by bacterial supernatants can be de novo method to trigger defense responses.

521B Cytokinin-elicited plant immunity by bacterial airborne signal
Hye Kyung Choi*, Hyo Bee Park, Soohyun Lee, Jaemyung Choi, Ilodo Hwang, Hitoshi Sakakibara, Choong-Min Ryu
1University of Science and Technology / KRIBB, South Korea, 2Pohang University of Science and Technology, South Korea, 3RIKEN, Japan

Plants need to be responded to challenges by insects, pathogens, and even saprophytes with extensive changes in gene expression that lead to systemic resistance. We previously discovered elicitation of induced systemic resistance (ISR) against plant pathogens and plant growth promotion by bacterial volatile emission to Arabidopsis. Plant seedlings exposed to bacterial volatile blends from a rhizobacterium Bacillus subtilis GB03, disease resistance against a soft rot pathogen Erwinia carotovora subsp. carotovora was significantly augmented through ethylene/jasmonic acid-dependent signaling. In this study, we evaluated cytokinin signaling that previously was an important player in bacterial volatile-elicited plant growth promotion as an important battery for ISR. To obtain direct evidences, the role of the cytokinin receptor AHK2, AHK3, and CRE1/AHK4 that activates a multistep phosphorelay at the plasma membrane was assessed. We also evaluated suppression of auxin signaling by antagonism elicited by upregulation of cytokinin signaling and direct binding of ARRR2 into promoter of a jasmonate maker gene PDF1.2 by ChiP assay. The ahk2 ahk3 null mutant plant was impaired bacterial volatile elicited-ISR indicating that the AKH2 and AHK3 receptor and its downstream can be involved. In contrast, the transcriptional expression of the Type A - ARRs including ARR6, ARR9, and ARR15 genes were down-regulated by the emission of bacterial volatiles. Further experiment revealed that the increased endogenous cytokinin contents by the increased expression of positive regulators Type-B ARRs and of cytokinin degrading enzymes CKX3 and CKX4 directly involved in ISR. The examining expression of PR1, PR2, PDF1.2, and VSP1 indicate that salicylic acid and jasmonate/ethylene signaling was implicated in this ISR machinery. Overall, our data indicate that bacterial volatile elicite ISR through orchestrating several plant hormone signaling mainly by cytokinin signaling and its cross-talking with major plant defense hormones.

522A Elicitation of induced resistance of Arabidopsis against Pseudomonas syringae by a C13 volatile organic compound produced by a rhizobacterium Paenibacillus polymyxa
Hye Kyung Choi*, Soohyun Lee, Boyoung Lee, Mohamed A. Farag, Hyo Bee Park, Joseph W. Kloepper, Choong-Min Ryu
1University of Science and Technology / KRIBB, South Korea, 2Cairo University, Egypt, 3Auburn University, United States

Some strains of plant growth-promoting rhizobacteria (PGPR) elicit induced systemic resistance (ISR) by emission of volatile organic compounds (VOCs) including the short hydrocarbons such as C4, acetoin and 2,3-butanediol. The efficacy of induction was strain-specific, with stronger protection against Pseudomonas syringae pv. maculicola ES4326 in plants exposed to VOCs released from Paenibacillus polymyxa E681 versus Arabidopsis plants exposed to a reference strain Bacillus subtilis GB03 which was previously shown to elicit ISR and plant growth promotion. VOC emissions released from E681 primed transcriptional expression of the salicylic acid, jasmonic acid, and ethylene signaling marker genes PR1, ChdB, and VSP2 respectively. Among more than thirty low molecular-weight VOCs, including methanethiol, isoprene, and an acetic acid-butyl ester, tridecane, a C13 hydrocarbon, was found to be released exclusively from strain E681 compared to strain GB03 which can induce PR1 and VSP2 genes. These results provide new insight into the existence of a long chain VOC signal molecule produced by P. polymyxa that serves as a bacterial trigger of growth promotion and protection in plants.
523A  Isolation Indigenous Acetic Acid Bacteria from Different Ecosystem
Noppawan Dachraksa*
Dept. of Biology, Thailand

The taxonomic studies on acetic acid bacteria on the several fruits, flowers and other natural resources in temperate area have been reported. The aim of this work is to isolate indigenous acetic acid bacteria from different ecosystems. Twenty kinds of Thai fruits, 10 sweet fermented rice and fermented fruits were collected as indigenous sources of acetic acid bacteria. The samples were enriched in distilled water supplemented with 4% ethanol (v/v) at 30°C for 5 days. The soaking solution were spread on bromocresol purple ethanol agar plate for isolation of acetic acid bacteria. The colonies with yellow zone were collected as acid producer. Fifty-nine isolates of Gram-negative and rod-shaped bacteria were obtained. The isolates were examined for the ability to oxidize acetate on bromocresol green ethanol agar plate and tested for acetic acid production. The numbers of acetic acid bacteria were found to be high in rambutan, Thai sweet plum, mango and lychee respectively. The sweet fermented rice and fermented fruits show lower number of indigenous acetic acid bacteria. The isolates from rambutan produced high acetic acid with good flavor. This indicated that Thai fruits were good source of acetic acid bacteria.

524A  Do we understand AM fungal dynamics sufficiently to utilise the symbiosis to maintain crop yield in the future?
Tim Daniell*, Jane Davidson, Sandra Caul, Alexander van den Bos, Katherine Becker, Alison Bennett
The James Hutton Institute, United Kingdom

Modern agricultural systems typically rely on high inputs to maintain or improve yields. This has been made possible by ready access to cheap fertiliser and agrochemicals in concert with breeding of varieties that grow well under high input agriculture. This time of plenty is coming to an end with rising energy prices impacting the provision of cheap N fertilisation through, for example, the Haber Bosch process and limitation of phosphate bearing rock. This issue is further compounded through a continually increasing world population. If we are to maintain agricultural production with decreasing inputs we need to alter the way we grow crops so that we use resources more efficiently. The benefits of the arbuscular mycorrhizal symbiosis include improved plant Phosphorus nutrition and water relations, resistance to pathogens, and carbon supply to the fungal partner. The symbiosis is present in the majority of plant species, including crops plants with the natural status of most field grown plants being mycorrhizal. However the relationship is complex traditionally around 150 fungal species are recognised but this is likely a gross underestimation. Second, there is variability in the performance of the relationship between plant and fungal partners and variation in the performance of the symbiosis exists between host species and fungal isolates of a single species, leading to an infinite number of outcomes when different AM fungi and plant species are combined.

Our ability to utilise this symbiosis to aid crop growth in the future is hampered by a lack of knowledge relating to the biology and ecology of the interaction. It is known, for example, that the diversity of this group is reduced under conventional agriculture. The mechanisms that drive this remain obscure although reduced tillage and low input systems appear to support a higher richness of fungi. Any low diversity of AM fungi in agriculture may further be compounded by host preference and/or temporal dynamics both of which have been observed in the symbiosis. Also breeding of crop cultivars under high fertilisation conditions may reduce any positive response of released crop cultivars to colonisation.

Here we present results from a range of studies that explore these factors to test if:

1. Physical disturbance (through tillage) affects AM fungal communities colonising plants) where we demonstrate that tillage affects AM fungal community dynamics with shallow and deep structures developing differently under conventional ploughing and minimum tillage regimes

2. The AM symbiosis under conventional agriculture displays clear temporal dynamics or host preference between plant host and fungal partners where we show that both time and host plant affect the fungal communities developing in root of crop plants.
3. The response of barley (Hordeum vulgare) to colonisation has been affected by breeding under high nutrient conditions. Here we see that although there is significant variation in the response of barley to colonisation this does not appear to be linked to cultivar age and that response of the species in general is weak.

525A  Nitrogen fixation efficiency of indigenous rhizobial strains isolated from soils of southwest Nigeria

Michael Olajire Dare*1, Abisoye Ojo2
1Federal University of Agriculture, Abeokuta, Nigeria, 2University of Ibadan, Nigeria

Legume-rhizobia symbiosis is an efficient source of soil nitrogen (N) for sustainable agriculture. Sourcing for efficient local/indigenous rhizobial strains for legume inoculation can help smallholder agriculture to reduce cost and increase crop yield. Indigenous rhizobial strains in soils of southwest Nigeria were evaluated for N fixation efficiency. Infectivity assay was conducted on 300 indigenous rhizobial strains isolated from three sites: Idi-Ayunre located in the rainforest, Orile-Iluugun and University of Ibadan Teaching and Research Farm (UITRF) located in derived savanna regions. The best three indigenous rhizobial isolates in infectivity assay: IDC8, TRC2 and O1Sa-6e, with two exotic strains R25B and IRj2180A were compared for N fixing efficiency in field experiments conducted in the three locations using three soybean (TGx1448-2E, TGx1908-1F, TGx1910-2F), two cowpea (IT89KD-288, IT90K-277-2) and one lablab (NAFR14) varieties. All the field experiments were in randomized complete block design with three replicates. Nitrogen fixation was significantly affected by rhizobial strains and legume varieties. In soyabean field at UITRF, isolates O1Sa-6e and TRC2 fixed 75.5 and 70.1 kg N/ha respectively, which were significantly higher than those of other strains. In cowpea fields, N fixed was significantly higher under O1Sa-6e inoculated plot (44.0 kg N/ha) at UITRF and IDC8 inoculated plot (43.6 kg N/ha) at Idi-Ayunre. The three indigenous rhizobial strains have high potentials for increased N fixation in southwest Nigeria and can successfully be used in place of exotic rhizobial strains.

526A  Genomics and ecology of Lysobacter species

Irene de Bruijn*1, Joeke Postma2, Jos Raaijmakers1
1Wageningen UR, Netherlands, 2Plant Research International, Netherlands

Lysobacter species are Gram-negative bacteria that are widely distributed in diverse ecosystems, including soil, rhizosphere and freshwater habitats. Various members of this bacterial genus have activity against a range of other (micro)organisms, including bacteria, fungi, oomycetes and nematodes. They produce a variety of extracellular enzymes and antibiotic compounds, most of which have not been structurally identified yet. Lysobacter species were shown to be more abundant in soils suppressive to Rhizoctonia solani, a devastating fungal pathogen of numerous economically important crops such as sugar beet, potato and rice. Isolation and subsequent phylogenetic analyses revealed that three Lysobacter species are present in the suppressive soil: L. antibioticus, L. capsici and L. gummosus. To date, the mechanisms involved in pathogen control by these Lysobacter species have not been resolved and also the bioactive compounds and corresponding genes remain elusive. Moreover, little is known about the ecology and population dynamics of these Lysobacter species in soil and rhizosphere environments. Results will be presented on several phenotypic and genotypic traits of these Lysobacter species, including antimicrobial activity, gliding motility, biofilm formation, plant growth promotion and phylogeny. Several isolates representing different species were selected for genome sequencing. Comparative genomics is currently performed to find differences between the species, and to explore these genomes for genes or gene clusters involved in the biosynthesis of antimicrobial compounds and plant growth promotion.
Development of models for studying bacteria-plant interaction has been a long-standing effort in the literature. The application of whole genome transcriptome analysis by RNA sequencing technology has been elucidating specific physiological traits as well as the underlying regulatory mechanisms for bacterial plant-root colonization. The purposes of this study were to develop and apply an in vitro system to study the interaction between a facultative methylotrophic bacteria Methylobacterium mesophilicum strain SR1.6/6 during the interaction with soybean seedlings roots. The colonization of the roots was regularly monitored by scanning electron microscopy 24, 48 and 72 hours after bacterium inoculation. Root-adhered bacterial cells forming biofilm were efficiently removed from the surface of the roots, and analyzed in comparison to free living cells under the influence of plant molecules exudates (planktonic cells) and bacterial cell in the same conditions, except by the absence of the seedlings (control cells). The course of colonization followed a chronological order along time-sampling: 24 hours - single-cells wide-spread adhered on the surface of the roots, 48 hours - formation of microcolonies (‘effectiv colonization’), and 72 hours - increase of fibrillar materials (i.e. extracellular polymeric substances), and consequently establishment of macrocolonies. The application of whole genome transcriptome analysis resulted in a broad profile of gene expression. Reads were annotated by BLASTX (threshold e-value of 10^-6), assigned to gene ontology categories and read counts per protein were tabulated and normalized as a proportion of the total number of annotated reads in each sample. The cross-comparison of transcriptional profiles resulted in a total of 280 differential expressed genes. The expression of genes related to methanol/etanol metabolism, adaptation to microaerophilic growth conditions, oxidative stress response, siderophore production, peptidoglycan and hohanoid biosynthesis were mainly induced in biofilm bacterial cells, while genes related to essential cell metabolism and division were mostly observed in planktonic and control cells. Our results provide deep insights into the mechanisms modulating bacteria-plant interaction, significantly distinguishing biofilm to planktonic treatment, showing that the physical contact is a crucial step on bacteria-plant association. In addition, these results pinpoint specific bacteria gene expression patterns in response to molecular signals secreted by plant and highlight metabolic process that support this associated bacteria-plant lifestyle and behavior in the rhizosphere.

527B  Role of climate change in terrestrial microbial feedbacks and disease spread
Barbara Drigo*, Ian C. Anderson, Brajesh K. Singh, John W.G. Cairney
Hawkesbury Institute for the Environment, University of Western Sydney, Australia

Soil microbial processes have a central role in the global fluxes of the key biogenic greenhouse gases and are likely to respond rapidly to climate change. Terrestrial ecosystems survival and adaptation are dependent on soil microbial communities. Whether climate change effects on microbial processes lead to a positive or negative feedback for the terrestrial ecosystems resilience is unclear. This study examines the direct and indirect effects of elevated atmospheric CO2 concentrations, temperature rising and changes in rainfall patterns on soil microbial communities associated with Australian forest ecosystems. *Eucalyptus spp.* trees were grown in the field for 6 years under ambient (circa 350 ppm) or elevated (circa 700 ppm) CO2 conditions, different watering and temperature regimes in 12 whole tree chambers. Analysis involving a suite of genetic approaches allowed us to characterize the size and structure of the affected soil-borne microorganisms. Multivariate analysis of 454 pyrosequencing, neutral/phosphate lipid fatty acids and real-time PCR approaches revealed that elevated CO2 intensified the effect of warming and drought by significantly altering the bacterial and fungal communities composition associated with *Eucalyptus spp*. rhizosphere and bulk soil. As opposed to simply increasing the biomass of soil-borne microbes at ambient CO2 under changes in rainfall patterns and temperature, elevated atmospheric CO2 strongly selected for opportunistic plant-associated microbial communities and effected shifts in microbial community composition. Moreover, illumina’s sequencing by synthesis (SBS) technology and real-time PCR analysis showed that alterations in temperature and rainfall patterns were important factors in making new territory hospitable to the proliferation of soil-borne human pathogens, such as *Clostridium perffigens*, *Cryptococcus neoformans*, *Pseudomonas aeruginosa* and *Burkholderia cepacia*. Collectively, our data...
PS27 – Plant-Microbe Interactions

Thursday 23 August

demonstrate that alterations to atmospheric CO₂ concentrations combined with changes in temperature and rainfall patterns drastically modify the structure of soil bacterial and fungal communities and contribute to the proliferation of soil-borne human pathogens. These responses are expected to impact humans, biodiversity and soil food-web interactions, and may influence the direction and magnitude of terrestrial ecosystem/atmosphere feedbacks that regulate global change in forest ecosystems.

528A  Evolution of cooperation in arbuscular mycorrhizal fungi
Marie Duhamel*1, E. Toby Kiers2, Philippe Vandenkoornhuyse1
1Université de Rennes1 UMR 6553, France, 2Vrije Universiteit of Amsterdam - Animal Ecology, Netherlands

The 450-million-year-old arbuscular mycorrhizal symbiosis is likely the world’s most abundant mutualism, more than 80 % of plants are colonised by these fungi. This symbiosis involves nutritional exchanges: plants give carbohydrates to fungi and fungi give minerals to the plants. Plants are colonized by diverse communities of unrelated arbuscular mycorrhizal fungi. These strains vary in the benefits they provide to the host. Some are very high-quality giving high amounts of phosphorus and others are low-quality and provide little benefit. These different fungi intermingle on a small spatial scale (for example a 1mm section of a single host root), making mechanisms to reward and to select beneficial fungi difficult to envisage from an anatomical and physiological perspective. Therefore, it is necessary to test if hosts can preferentially allocate resources when arbuscular mycorrhizal fungi compete within a single root system.

We employed stable isotope probing to determine patterns of carbon allocation from the host plant to the fungus. The technique is based on the integration of 13C labeled growth substrates into nucleic acids, then an ultracentrifugation using a Cesium TriFluoro Acetate gradient. This technique allows researchers to successfully distinguish carbon allocation patterns between arbuscular fungal strains. Here we want to know if the host plant allocates more carbon to the high-quality strain when inoculated with multiple strains varying in cooperation level.

We used three arbuscular mycorrhizal fungi strains: Glomus intraradices, Glomus custos and Glomus aggregatum. These strains vary in the benefits (promotion of plant growth, phosphorus transfer) that they provide to the host plant (Medicago truncatula in our experiment).

Under greenhouse conditions, Medicago truncatula seedlings were inoculated with equal proportion of the three arbuscular mycorrhizal fungi strains which were allowed to directly compete for host carbon on the root system.

After ten weeks of growth, we pulse labelled the plants with 13CO2 and harvested the roots at three different time points. Total RNA was extracted from roots. The RNA containing the “heavy” carbon isotope was separated from the “light” RNAs, after which the identity of the RNAs was revealed after a reverse transcription and a quantitative PCR.

We found that the most cooperative fungi, Glomus intraradices, had the most enriched RNA, suggesting it received more carbon from the plant. The two other less cooperative mycorrhizal fungi received less carbohydrates. We argue that the plant is able to discriminate among strains, and preferentially allocate carbohydrates to the more cooperative fungi. This mechanism can help to explain the stability of the arbuscular mycorrhizal symbiosis.
529A  Bacterial rhizosphere communities of eight Salix lines with different potential to accumulate Cd and Zn
Katharina Fallmann*1, Melanie Kuffner1, Brigitte Hai2, Michael Schloter2, Thomas Rattei3, Dmitrij Turaev3, Markus Puschenreiter4, Angela Sessitsch1
1AIT Austrian Institute of Technology GmbH, Health & Environment Department, Austria, 2Helmholtz Zentrum München, German Research Center for Environmental Health, Germany, 3University of Vienna, Department of Computational Systems Biology, Austria, 4BOKU-University of Natural Resources and Life Sciences, Department of Forest and Soil Sciences, Austria

Heavy metal accumulating plants are studied due to their application potential for the gentle remediation of polluted soils. In phytoextraction, contaminants are removed from a site by harvesting the shoots of suitable plants, such as fast-growing willows, which accumulate heavy metals like Cd and Zn in their above-ground tissues. Currently the time required is the most problematic limitation of phytoextraction. However, it is known that certain bacteria facilitate the uptake of heavy metals by plants. Understanding the underlying mechanisms could help to exploit the plant-microbe interaction for the development of more efficient phytoextraction technologies.

In this work, rhizosphere microbial communities of Salix species with different heavy metal uptake potential were studied, including S. babylonica, S. fragilis, S. matsudana x alba, S. purpurea and four different lines of S. smithiana, a species particularly promising for phytoextraction. In a pot experiment six replicate cuttings were grown in soil from a site contaminated with Cd and Zn. The metal concentration in leaves was measured by ICP-MS, and rhizosphere samples were subjected to 454 sequencing of the V13 region of the bacterial 16s rRNA gene.

Foliar Cd concentrations, which ranged from 14 to 84 mg kg-1 dry matter, were inversely correlated with OTU richness in the rhizosphere. Differences in the community structure were detected between rhizosphere samples of the four S. smithiana lines on the one hand and the four other Salix species on the other hand. Taxa that preferentially occurred in the rhizosphere of plants with high foliar heavy metal concentrations or in specific association with S. smithiana were identified. The observed alpha- and beta-diversity effects occurred at high and medium taxonomic resolutions (genetic distances 0.03, 0.05 or 0.10), whereas at distances of 0.20 or 0.25, no significant correlations between the rhizosphere community structure and the plant genotype or foliar heavy metal concentration were found.

Bacterial rhizophere communities of the experimental plants were composed similarly in terms of high-rank taxa (like phyla), but at the fine level they differed in alpha- and beta-diversity. The approach to use a pot experiment, in which all plants were grown in the same soil, might have enhanced the detection of plant-specific effects on rhizosphere community structures, because the effect of different soil pH, which has often been described as the dominant factor shaping bacterial communities, was circumvented by the experimental design in the present survey.

530A  Biostimulation of the native and introduced quorum-quenching Rhodococcus erythropolis populations for protecting potato plants against the soft-rot bacterial pathogens
Denis Faure*, Amélie Cirou, Nicolas Mothe, Samuel Mondy, Mélanie Tannières, Anthony Kwasiiborski, Yves Dessaux
CNRS, France

Degradation of quorum-sensing (QS) signals N-acylhomoserine lactones (AHL) by soil bacteria is proposed as a beneficial trait for protecting crops, such as potato plants, against the worldwide pathogen Pectobacterium. In this work, analytical chemistry and microbial and molecular approaches, including rs-454, have been combined to explore biostimulation of the native and introduced AHL-degrading Rhodococcus erythropolis bacterial populations in the rhizosphere of potato plants which were cultivated in farm greenhouses under hydroponic condition, as well as in soil macrocosms and field condition.

We first characterized an AHL-degrading R. erythropolis isolate R138, which was introduced in the potato rhizosphere (Cirou et al. 2011 Res Microbiol.). We also identified gamma-heptalactone (GHL)

Different combinations of GHL- and R138-treatments were compared in root colonization by AHL-degrading bacteria using a cultivation-based approach (percentage of AHL-degrading bacteria), pyrosequencing of PCR amplified rrs loci (total bacterial community) and qPCR of the qsdA gene that encodes an AHL-lactonase in R. erythropolis. The experiments were performed in an industrial, hydroponic condition, as well as greenhouse assays. The higher densities of AHL-degrading R. erythropolis population in rhizosphere were observed when GHL- and R138-treatments were associated. Under this condition, the introduced R. erythropolis R138 displaced the native R. erythropolis population. Moreover, chemical analyses revealed that GHL and GCL, and their by-products, gamma-hydroxyheptanoic acid and gamma-hydroxycaproic acid, rapidly disappeared from the rhizosphere and did not accumulate in plant tissues (Cirou et al. 2012 Appl. Env. Microbiol. and unpublished data).

Finally, the protecting effect of this strategy was evaluated in a field experiment in which both the biocontrol agent R. erythropolis R138 and the plant pathogen P. atrosepticum CFBP6276 were introduced. This integrative study highlights biostimulation of quorum-quenching R. erythropolis populations as a potential approach for targeting virulence of plant pathogen in which virulence is regulated by QS.

531A  **Microbispora** spp. are the dominant actinobacterial endophytes of Australian rice plants
 Christopher Franco*, Fitri Widiantini
  *Flinders University, Australia

Actinobacteria are present within most plants and diverse endophytic populations are found in a variety of crop plants. Our aim is to utilize members of this group of microorganisms as inoculants due to their ability to colonise internal tissues of host plants, coupled with production of antimicrobial agents and plant growth hormones, and the elicitation of induced systemic resistance. In this study, the actinobacterial endophyte populations of rice plants grown at Yanco along the Murray/Murrumbidgee river irrigation site in Australia were evaluated using culture-independent and culture-based methods. The dominant population that was isolated from rice plants, collected at different growth stages, at 27°C and 37°C were found to belong predominantly (93%) to the genus Microbispora, with an increase in number with the age of plants. As it is not usual to find such a high proportion of members of one genus, other than the ubiquitous Streptomyces, in most terrestrial environments including endophytic ones, the isolation and characterisation was repeated the following year yielding similar results. A total of 264 isolates were cultured from various parts of the rice plants - leaf sheath (33.7%); roots (29.9%); leaf blade (24.6%) and stem (11.8%). The other genera represented were Streptomyces, Kitasatospora, Saccharothrix, Saccharopolyspora, Pseudonocardia and Actinoallolurus. Isolation of actinobacteria from rice field soil samples using the same conditions as for endophytes yielded a population that was predominantly Streptomyces.

The preponderance of Microbispora spp. in plants and streptomycetes in soils was confirmed by T-RFLP analysis of the DNA extracted from soils and rice plants at different stages of growth. To confirm these unexpected observations, 4 different rice cultivars were grown in 4 soils, including soils from the original rice fields. Greater than 85% of isolates from the 16 combinations of cultivars and soils were Microbispora species confirming their status as the natural microflora of Australian rice plants. In addition, it was noted that rice plants of all 4 cultivars of grown in one of the soils, from a wheat pasture, yielded low numbers of cultivable actinobacteria, even though actinobacteria were isolated from that soil. TRFLP analyses of these systems, confirmed the results of the cultivation method. Therefore, soil type, or the intrinsic microbial population has a strong affect on he endophytic population.
Analysis of the diversity within the Microbispora isolates by BOX-PCR fingerprinting revealed a high genetic variation, even though phylogenetic analyses of the almost complete 16S rRNA gene sequences showed sequence similarity ranging from 98.2 to 99.6% to *M. karnatakensis*, *M. amethystogenes*, *M. diastatica*, *M. chromogenes* or *M. parva*. However, as there were clear differences with the type strains, further detailed phylogenetic characterization including sequence analysis of the gyrB gene, and DNA-DNA hybridisation showed that at least 12 Microbispora strains were distinct from the type strains and each other. These results reveal ecological interactions that may have a significant impact on rice crop production and point to further studies to reveal the richness of these interactions.

532A  Potential for increased N₂O emissions from denitrification in vegetated areas in a free water surface constructed wetland

Arantzazu García Lledó∗1, Ariadna Vilar-Sanz1, Rosalia Trias1, Sara Hallin2, Lluís Bañeras1

1University of Girona, Spain, 2Swedish University of Agricultural Sciences, Sweden

Constructed wetlands are attractive low-cost alternatives to nitrogen removal from wastewater. Vegetation plays an important role in this process, mainly by the stimulation of the plant associated microbiota. Net nitrogen loss is due to coupled nitrification and denitrification, but incomplete denitrification can occur, resulting in emission of greenhouse gases, such as nitrous oxide. If this occurs, a man-made system devoted to nutrient removal may turn into an environmentally unfriendly facility. To what degree this occurs in constructed wetlands is still a matter of debate. We determined in situ nitrate reduction activities and genetic potentials for N₂O emissions in relation to vegetated and non-vegetated areas in a free water surface constructed wetland at different water discharge and influent characteristics.

Potential denitrification activities (PDA) were determined as the reduction of amended nitrate in closed stainless steel chambers. Genetic potential for N₂O emissions was estimated through quantitative real-time PCR analyses of functional genetic markers for denitrification (nitrate reductases narG and napA, nitrite reductases nirS and nirK and nitrous oxide reductase nosZ) and their abundance in relation the 16S rRNA gene abundance.

Significantly higher PDA rates were found in sediments covered with Typha (39.0-69.4 mg/m²/h). Additionally, nitrogen removal rates showed that higher efficiencies were measured in wetlands where Typha was the dominant vegetation. The abundance of denitrification genes showed that the abundance of the nosZ was lower (down to 100 fold) than the other genes in the denitrification pathway. These differences were more pronounced during periods of high nitrate loadings, coinciding with lower nitrogen removal rates in the wetland. Gene abundances were significantly affected by sampling period (differing in water discharge and influent composition) and the type of sediment. The presence of vegetation, especially Phragmites, increased all genes but napA and nirS. For all genes, sediment carbon and nitrogen content correlated positively and significantly with abundance. The relative abundance of denitrification genes was used to assess removal efficiencies in terms of potential accumulation of intermediates in the denitrification pathway. The effect of vegetation on relative gene abundances were only detected in combination with the sampling period and resulted in an increase of the potential for nitrous oxide emissions in vegetated areas. Almost two orders of magnitude in abundance of nitrite reductases (qnirS + qnirK) and nitrous oxide reductase (qnosZ) were found during periods of high nitrate concentration at the inflow and total carbon in sediment.

In conclusion, denitrification gene abundances suggest that the denitrifying community changed according to wetland nutrient concentration and the type of vegetation. Thus, wetland plant species may affect the denitrifier community to harbor a lower nosZ to nir gene ratio, which potentially can result in increased nitrous oxide end-product.
Future sources of energy need to be both sustainable and economic. Bioenergy crops such as switchgrass have the potential to fulfill these requirements, which can be aided by microbial communities associated with plants providing beneficial services such as protection from pathogens and acquisition of limiting nutrients. We explored potential services provided by plant-associated microbes using cultivation-based techniques and metagenomics. Isolates from switchgrass vegetation and roots were screened for plant beneficial properties, and metagenomic sequencing of the rhizosphere was used to access functional and taxonomic diversity to gain information not accessible by cultivation.

We assayed isolates for nitrogen fixation via growth on nitrogen-free media and for the presence of the nifH gene. We also assayed isolates for production of the plant hormone indole-3-acetic-acid, solubilization of phosphorus, the production of iron binding siderophores and inhibitory effects on several root pathogens. We used next generation sequencing to produce 16S rRNA gene pyrotag sequences and 125 Gb of shotgun metagenome libraries of switchgrass associated soils. These libraries were constructed from three samples, two replicate rhizosphere samples and one bulk soil sample. RDP was used to examine the pyrotag sequences and MG-RAST was used to examine the taxonomic diversity of microbial ecosystem services.

Of the 110 isolates assayed, 74 were chosen for full-length sequencing of the 16S rRNA gene to obtain the nearest reference relatives. These isolates included members of the orders Burkholderiales, Methanomicrobiales, Flavobacteriales, Actinomycetales, Bacillales, Clostridiales, Xanomonadales, with the Pseudomonadales the largest group. These isolates are a small fraction of the diversity found within the shotgun and 16S pyrotag data sets. Clustering of 16S rRNA gene sequences at 3% dissimilarity yielded 4,996 clusters.

Of the isolates assayed, 53 tested positive for one or more of the ecosystem service functions. The majority of these belong to the Xanomonadales or the Pseudomonadales. The metagenomic data was searched for the presence of these 53 isolates; sequences of four isolates (Bacillus cereus, Pseudomonas fluorescens, Pseudomonas syringae, and Stenotrophomonas maltophilia) were found. For each of the four isolates, proteins corresponding to the isolates included the assayed functions for which these organisms tested positive. For example, Bacillus cereus tested positive for production indole-3-acetic-acid and iron binding siderophores. The subset of the metagenome with sequences matching those of B. cereus included sequences from the detected auxin biosynthesis subsystem and the Fe-bacillibactin uptake subsystem. Much of the diversity found in subsystems related to plant beneficial function, however, is not represented in the isolates.

Cultivation-based studies of microbial communities detected several plant beneficial functions and remain an important approach for functional validation while metagenomics is more comprehensive in detecting taxa and potential function. Hence, they are complementary and their information can be used in concert to guide future research for a more comprehensive functional understanding.

Phosphorus commonly limits crop yield and is frequently applied as fertilizer, however, supplies of rock phosphate are diminishing. Plants have evolved many mechanisms to improve their P acquisition and understanding of these traits will improve the long-term sustainability of agriculture. Here we utilise a mutant population of barley (Hordeum vulgare L.) to assess the impact of root hair length on P acquisition.
acquisition and yield and focus on the impact of interactions between root hairs and soil microorganisms including mycorrhizae and free-living nematodes under P-deficient conditions.

Mutants with various root hair phenotypes were grown in the glasshouse in a range of experiments aimed at understanding plant responses to P-deficit and interactions with soil microorganisms. Plants were harvested at a range of growth stages from seedling through to maturity and variables including root length, root hair length, rhizosheath weight, mycorrhizal infection, nematode numbers, nematode damage, microbial community structure, P-accumulation and yield were measured. In addition, material was taken for transcriptional studies to identify candidate genes involved in the tolerance mechanisms in the various germplasm.

The results confirmed that root hair length was related to rhizosheath production and was important for biomass and P accumulation in barley. In contrast, only the presence of root hairs was critical for yield production under P-deficient conditions. Results also demonstrate that barley relies on root hairs rather than mycorrhizae as a P-deficit tolerance mechanism. Despite enhanced mycorrhizal symbiosis in phenotypes lacking root hairs these phenotypes were still disadvantaged under P-deficient conditions. In addition it was apparent that genotypes without root hairs suffered the greatest stress under conditions of extreme P-deficit and this was only ameliorated by the presence of root hairs and not by presence of mycorrhizae. Significant interactions with free living nematodes were also noted. The results from the transcriptomics analysis identified 248 genes differentially regulated under extreme combined P and water stress specific to no root hair genotypes. A number of genes involved in P-deficit response, biological stress tolerance, water-stress tolerance, oxidative stress, tip growth and membrane restructuring were identified as being upregulated in phenotypes lacking root hairs.

Overall this research suggests that, although root hair length is not important for maintaining yield, the presence of root hairs is implicit to sustainable yield of barley under P-deficient conditions. Therefore potential interactions between root hairs and soil microorganisms might have a large impact on the ability of barley to cope with P-deficiency in the field. The results also suggest that the different root hair phenotypes have different transcriptomic responses to P-deficit and the responses in phenotypes lacking root hairs are more severe than in phenotypes with root hairs. Interactions between these responses, mycorrhizae and nematodes will be discussed. This research indicates that, in barley, future breeding programmes should concentrate on improving or maintaining root hair phenotypes. Root hairs are a vital phenotype for the future sustainability of barley production and negative interactions with soil microorganisms should be minimised.

534A  Bacterial diversity and plant growth-promotion potential in Andean potato rhizosphere soils
Jonas Ghyselinck*1, Eileen O’herlihy2, Barbara Doyle Prestwich2, Siva Velivelli2, Koenraad Van Hoorde1, Hoste Bart1, Kim Heylen1, Paul De Vos1
1Ghent University, Belgium, 2University College Cork, Ireland

The potato plant (Solanum tuberosum) is an important crop worldwide with a key role in the world’s global food system. The Central Andean Highlands are the center of origin of the potato plant and form an interesting location for bacterial diversity studies. Ages of mutualism between potato plants and soil bacteria in this region support the hypothesis that Andean soils contain interesting plant growth-promoting bacteria. Moreover, due to the limited anthropogenic influences and a low chemical input, these soils are expected to harbour a rich bacterial diversity. Nevertheless, the Andean potato rhizosphere ecosystem remains underexplored. The European FP7 project VALORAM aims at promoting sustainable agriculture by applying natural microbial resources. With the application of biofertilizer- and biocontrol agents, the need for pesticides and chemical fertilizers may significantly be reduced. Therefore, we aimed at isolating and identifying bacteria obtained from the potato rhizosphere in Andean soils, which were subsequently screened for plant growth-promotion characteristics.

A total of 585 bacterial isolates were obtained from the rhizosphere of potato plants in eight fields in Bolivia and Peru. Media used for bacterial cultivation were ten-fold diluted Trypticase Soy Agar and a standard mineral base supplemented with γ-caprolactone, a compound structurally related to N-acylhomoserine lactones, as the sole carbon source. All isolates were screened for broad spectrum pathogen suppression by analyzing antagonistic properties against Phytophthora infestans and
Rhizoctonia solani in dual-culture assays. If positive, mechanisms of antagonism were determined and isolates were further tested for phosphate solubilisation, 1-aminocyclopropane-1-carboxylate deaminase activity and indole-3-acetic acid production. Dereplication was performed with matrix assisted laser desorption/ionisation time-of-flight mass spectrometry. Subsequent identification was based on 16S rRNA gene sequencing and multi locus sequence analysis.

Ten percent of the 585 isolates (n=58) were effective pathogen suppressors in vitro with 56 isolates showing potential for broad spectrum biocontrol. Further testing indicated phosphate solubilization activity (n=48), and production of the plant growth-promoting agents indole-3-acetic acid (n=12) and 1-aminocyclopropane-1-carboxylate deaminase (n=32). Identification results were used to evaluate the pathogenicity of the antagonists. Results were processed in a scoring system, which allowed to highlight the bacterial strains most interesting for field trials.

Our approach enabled an optimal selection of bacterial strains for use in field trials. The approach was based on a scoring system using results from in vitro plant growth-promotion experiments. Two isolates were selected from an initial population of 585, which are ready to be applied for field trials.

The research leading to these results has received funding from the European Community's Seventh Framework Programme FP7/2007-2013 under grant agreement N° 227522.

535A Effect of supplementation of mixed ruminal microflora on bioconversion of waste in biogas using cow dung as co-substrate
Gunjan Goel*, Srikant Kaushik
Maharishi Markandeshwar University, India

The need for more research into biogas production as a renewable energy source alongside the added benefit of solving major environmental problems posed by the wastes used as substrates is well established. In this investigation, a series of batch experiment was conducted for 21 days for production of biogas using rumen fluid of ruminants as inoculum. Vegetable waste (T1) and agricultural waste (T2) were evaluated taking cattle dung as control substrate. A 100 grams of substrate was fed to each biodigester (1:1, cattle dung:waste) and mixed with rumen fluid in ratio of 10:1. One set of substrate without rumen fluid was used to examine the effect of rumen fluid on gas production. The operating temperature varied from 35±2°C. At every 2 days interval, total gas, methane and loss in total solids were measured. The cumulative volume of biogas produced and partitioning of carbon to total gases was used as a marker for biodigester performance. The total gas production reached its maxima at day 2 with 30ml in T1 giving a cumulative biogas production of 150 ml at the end of the 21st day of the experiment followed by T2 (90ml) and control (60ml). The maximum methane production per unit of total gas (0.70) as well as per unit substrate degraded (0.42) was also observed for T1. Rumen fluid inoculum resulted in increase in biogas production rate and efficiency as the substrate without rumen fluid gave the kinetic parameters total gas (90, 65) and methane per unit total gas (0.5, 0.4) for T1 and T2 respectively. The higher partitioning of substrate carbon to methane indicates that the wastes can be managed through supplementation of mixed ruminal microflora to the wastes.

536A Effect of nitrogen input on carbon assimilation and photosynthate partitioning within the plant-microbe-soil system in the rhizosphere of beech
Silvia Gschwendtner*, Tillman Lueders*, Franz Buegger, Michael Schloter
1Technische Universität München, Germany, 2Helmholtz Zentrum München, Germany

Photosynthesis by plants is the driving force of carbon cycling between soil and the atmosphere. It is considered that the potential accumulation of carbon in soil could help mitigate the continuous increase of atmospheric CO₂ because of its large capacity for C sequestration. Due to the close interaction between carbon and nitrogen cycles in forests, increased nitrogen input into forests soils can increase foliar biomass and the concentration of the photosynthesizing enzyme RUBISCO, leading to enhanced photosynthesis rates and consequently a larger amount of assimilates transferred into the rhizosphere.

To investigate the influence of nitrogen input (10 kg ha⁻¹) on carbon partitioning within the plant-rhizosphere-soil system of beech, a ¹³C-CO₂ (99 atom% ¹³C) pulse labelling greenhouse experiment
was performed using 3 months old beech seedlings. The labelling was performed for 6 hours per day over a period of two weeks, whereas CO₂ concentration was kept between 300 – 400 µmol mol⁻¹. In order to follow carbon partitioning within the plant and to identify the primarily degraders of root exudates as well as to get insight into soil food webs, samples (plant, rhizosphere and bulk soil) were taken five times during the labelling period. ¹³C allocation in plant biomass, water-extractable carbon and microbial biomass carbon was determined by isotopic ratio mass spectrometry. The fractions of microorganisms receiving ¹³C from the plant were separated from the non-consumers via DNA- and RNA-SIP, followed by quantitative real-time PCR and T-RFLP analysis. The presentation will give detailed results on the allocation pattern.

537A  Attenuated immune response to endophytic bacteria favours successful interaction with host plant
Garima Gupta*, Abhijit Das, Prabhat Jha
Birla Institute of Technology and Sciences, Pilani, India

Endophytic bacteria are beneficial and reside inside the plants without exerting any deleterious effects on their host. Most of them gain entry into the plant roots from rhizosphere mainly through wounds, cracks and the emergence point of lateral roots. Despite the fact that most of the endophytic bacteria benefit plants, they are still alien to the host. Plants respond the invading microorganisms through enhancing level of defence molecules, which counteract to the attack of invaders. Therefore, it is intriguing to know immune response of the plants towards endophytic bacteria which differentiate them from their pathogenic counterpart. Therefore, to elucidate mechanism for successful association of the endophytic bacteria with host plant, aim of the present study was to compare immune response of endophytic bacteria Pseudomonas aeruginosa PM389 and pathogenic bacteria Erwinia carotovora in wheat plants (Triticum aestivum var.GW322).

Seedlings of wheat plants were grown in Hoagland media under hydroponic condition [14 h-light ⁄ 10 h-dark cycle at 27°C and 25°C (day–night, respectively)]. Plants were challenged with endophytic and pathogenic bacteria with a population of 10⁷-10⁸ cells/ml separately and harvested at a regular interval upto six days after inoculation (DAI). Un-inoculated plants were used as control. Plant samples were crushed in liquid nitrogen for analysis of defence enzymes namely peroxidase, polyphenol oxidase, phenylalanine ammonia lyase and β-glucanase using standard methods. In a separate experiment with similar experimental set-up, population of endophytic and pathogenic bacteria was estimated at various time intervals after bacterial inoculation to plants.

Level of all the defense enzymes produced in response to endophytic bacteria was significantly lower than that of pathogen. Stimulated level of peroxidase was recorded upto 2⁴ day of pathogenic bacteria, where as reduced level of the same was observed in response to endophytic bacteria. Similarly, increase in level of polyphenol oxydase was observed in response to only pathogenic bacteria after 2⁴ day. On the otherhand, both endophyte and pathogen showed a significantly higher activity of phenylalanine ammonia lyase on the 1⁴ day than control, but a sustained higher level of activity was observed in case of pathogen on 3⁴ and 4⁴ day when compared to both endophyte and control. A significantly higher enzymatic activity of β-glucanase was observed in case of the pathogen on 1⁴ day than both control and endophyte, but the activities in all the three cases showed no significant difference in the days that followed. In order to correlate level of defence enzyme with bacterial colonization in root, bacterial population was estimated at various time intervals after inoculation. Endophytic bacterial population was initially lower, but the size of population increased after 1⁴ day and remained almost constant for rest of the study period. On the otherhand, population of pathogenic bacteria was higher initially but the population size kept fluctuating for rest of the study period from 1⁴ day.

Results of the comparative analysis indicates attenuated defense response in challenged host plants towards endophytic bacteria in comparison to that of pathogenic bacteria, which help them establish inside endosphere of roots.
538A  Seasonal variation in protease enzyme production in ectomycorrhizas and soil solution in boreal Scots pine forest
Jussi Heinonsalo*, Kirsi Bäcklund, Annele Hatakka, Timo Vesala, Jukka Pumpanen
University of Helsinki, Finland

Nitrogen (N) is typically growth-limiting nutrient in boreal forests despite large N pool. Major part of soil N is in organic form, either as inactive and immobilized proteins or part of recalcitrant humic matter. Proteases are hydrolytic enzymes that degrade proteins to amino acids that can be directly used by many soil organisms.

Boreal forest trees form symbiosis with ectomycorrhizal fungi. Understorey vegetation in boreal forests is dominated by shrubs like Vaccinium sp. and Calluna sp. that form ericoid mycorrhizal symbiosis. Both ectomycorrhizal and ericoid fungi may produce proteases. Recently, it has been shown that plant photosynthesis, belowground carbon allocation and soil (organic) N uptake are tightly bound and that ectomycorrhizal fungi are of key importance in this process. Increased N uptake stimulated by root-associated microbes may have positive feedback on ecosystem’s net primary production.

The aim of the study was to investigate whether the ectomycorrhizal root tips are active in producing proteolytic enzymes in the field and is there any seasonal variation in the enzyme production. We also analyzed proteases from soil solutions.

We sampled 15 soil cores each month from March until October in the intensive monitoring site SMEAR II (www.icos-infrastructure.fi) with pine forest type vegetation in central Finland. Roots were separated from humus layer soil. From each core, eight randomly selected Scots pine ectomycorrhizal root tips were analyzed for their proteolytic enzyme production using FITC-casein as the substrate. Each month approximately 100 mycorrhiza was measured. From homogenized bulk soil, soil solution was collected and proteases quantified. Results were compared with temperature, soil moisture, photosynthesis and soil respiration that were measured continuously in the site. Most common mycorrhizal morphotypes found in the site were isolated as pure cultures, sequence-identified and their protease production measured.

Approximately 10% of all ectomycorrhizal root tips produced proteolytic enzymes. The production ranged from 0-12 µg of protease per m^2 of soil. The production had statistically non-significant peaks in the spring and in the autumn indicating that they are not positively correlated with temperature, photosynthesis or soil respiration. Protease production per m^2 soil (assayed from soil solution) were generally 10-20 times higher than in mycorrhizas highlighting the significant role of fungal external hyphae and other heterotrophic organisms in soil proteolytic capacity. Piloderma sp., Suillus variegatus and S. bovinus were isolated and all of them showed significant protease production in pure cultures.

Our results show clear evidence that ectomycorrhizal fungi play an important role in soil organic nitrogen mobilization. The results will be discussed in the context of forest ecosystem’s response to elevated CO₂ levels and soil carbon pool.

539A  The genus Burkholderia: the good, the bad, and the ugly
Ann M. Hirsch*, Paulina Estrada-de los Santos, George Weinstock, Annette Angus
1UCLA, USA, 2Instituto Politécnico Nacional, Mexico, 3Washington University, USA

Burkholderia is a genus composed of bacteria formerly in the RNA homology Pseudomonas group II that have commensal, pathogenic, or symbiotic lifestyles. Recently, more attention has been paid to the symbiotic Burkholderia species, especially those that establish nitrogen-fixing nodules on legumes. Genomic analysis of certain species has revealed the presence of nif (and nod in Burkholderia tuberum and B. phymatum) genes, which are required for symbiotic nitrogen fixation and effective nodulation. The nod genes were until recently thought only to exist in the genomes of the Alphaproteobacteria (Rhizobiaceae), but the discovery of the beta-rhizobia changed this perception. It also has led to the desire to use these arid environment-adapted species as inocula for crop legumes in the Southern Hemisphere. The nodulating species are native to South Africa, Australia, and Brazil. However, because many Burkholderia are serious pathogens on humans and domestic animals, concerns have been expressed about using the symbiotic species for inoculating agricultural crops.
Because of their potential for promoting plant growth and health, especially in marginal soils, we utilized Ceanorhaditis elegans and HeLa cell assays to demonstrate unequivocally that plant-associated Burkholderia species are not pathogenic. We also searched for genes that are hallmarks of pathogenicity such as various secretion systems. In addition, the distinct differences in the G+C content of the plant-associated versus the pathogenic strains, and the use of 5 concatenated housekeeping gene sequences clearly shows the phylogenetic separateness of the plant-beneficial species and those harmful to humans.

539B Construction of a phytoremediation system using vetiver grass and endophytic bacteria, Achromobacter xylosoxidans F3B for the remediation of phenolic pollutants
Ying-Ning Ho*, Dony Chacko Mathew, Hsing-Mei Chiang, Zhang-Gong Hao, Chieh-Chen Huang
National Chung-Hsing University, Taiwan

Phytoremediation of water soluble and volatile organic compounds is often inefficient because plants do not completely degrade these compounds through their root systems. The application of functional endophytic bacteria within plants is one of a potential strategy that could enhance the plant’s efficiency in phytoremediation. In this study, endophytic bacteria Achromobacter xylosoxidans F3B that could utilize aromatic compounds as sole carbon source was chosen and inoculated into vetiver grass. Vetiver grass is a fast-growing, perennial grass, with a long, massive root system, which can penetrate to the deeper layers of the soil, because of these advantages; we inoculated F3B into vetiver grass. Achromobacter xylosoxidans F3B can use benzene, toluene, ethylbenzene, xylene, catechol, and phenol as sole carbon source and has resistance to heavy metals such as Mercury, Cadmium and Lead. We used a specific real-time PCR detection method for confirming the stability of F3B and denaturing gradient gel electrophoresis (DGGE) for endophytes diversity after inoculation. We also isolated and screen endophytes from vetiver. The results showed that the endophytic bacteria F3B could maintain stable population in plant roots and change endophytes diversity. We demonstrate that endophytic bacteria Achromobacter xylosoxidans F3B, a natural endophyte of reed improve the in plant degradation of toluene in vetiver, resulting in a decrease in its phytotoxicity, and a 30% reduction of its evapotranspiration through the leaves. Achromobacter xylosoxidans F3B can help plant to tolerate stress from aromatic compounds and to improve phytoremediation of phenolic pollutants.

540A Will selection conditions alter root-microbial relationships and influence carrot productivity in organically managed production systems?
Lori Hoagland*, Jessica Brazelton¹, Christopher Zelaya², Micaela Colley³, John Navazio³, Phillip Simon⁴
¹Purdue University, USA, ²National Agricultural University of Honduras, Honduras, ³Organic Seed Alliance, USA, ⁴USDA-ARS Vegetable Crops Research Unit, USA

Crop varieties selected in conventionally managed production systems are not always the most productive varieties when grown in organically managed systems. It is well known that microbial species inhabiting the plant rhizosphere can positively influence crop productivity. Benefits of positive root-microbial relationships include plant growth promotion, increased nutrient uptake and reduced pathogen infection. Because functional root-microbial relationships often represent a cost to the plant, selecting for high yield under conventional systems managed using abundant nutrient and pesticide inputs may inadvertently result in selection of varieties that lack the ability to benefit from beneficial root-microbial relationships. In contrast, selecting in organically managed systems where plants are more dependent on these relationships to sustain high productivity and quality may result in crop varieties with greater ability to form these beneficial associations, and result in greater performance when grown in organically managed systems. To test this hypothesis, we are selecting new novel colored and nutritious carrots in paired organic-conventional research trials at four locations in the U.S. Preliminary results indicate that soil microbial activity and abundance are greater in the organically managed systems. The composition and abundance of microbial species inhibiting the rhizosphere of a subset of carrot breeding accessions in each system is underway. We expect this research to result in identification of new productive carrot varieties adapted to organic systems, and increase our understanding of the impact of varietal selection conditions on root-microbial relationships and crop productivity.
541A Rhizobia as plant growth promoter for maize under reduced water conditions
Muhammad Baqir Hussain*, Zahir Ahmad Zahir, Hafiz Naeem Asghar
University of Agriculture, Faisalabad, Pakistan

Maize, an input intensive cash crop, needs biotechnologies for its production sustainability during water crisis. For the reason, thirty rhizobia were isolated from the nodules of legumes (lentil, mung bean and chickpea) and tested for drought tolerance capability with PEG-6000 induced water stress. Proficient drought sustaining rhizobial isolates (RS-1, RS-2, RS-3, RS-6, RS-7, RS-8, RS-10, RS-12 and RS-13) were assessed for their plant growth promoting activity on maize seedlings under drought in gnotobiotic conditions. From the results of the plant growth promotion activity trial, four strains (RS-1, RS-3, RS-8 and RS-12) were selected for evaluation in pot conditions. Findings of the pot trial demonstrated significant improvement in photosynthesis system and water use efficiency of maize crop due to inoculated rhizobia against un-inoculated control. Chlorophyll contents were also significantly improved with rhizobial inoculation compared to un-inoculated control at different growth stages (five leaf and silking) under drought. Selected strains were tested for possible mechanisms of action for plant growth promotion including phosphorus solubilization, indole acetate acid, siderophores, chitinase, oxidase, organic acids and exopolysaccharides production. Moreover, starvation and drought survivability tests of these isolates supported their capability to stay alive under low organic matter and less moisture conditions, respectively. Outcomes of these experiments reveal the immense potential of rhizobia to improve growth and ameliorate the impact of drought in maize under water deficit conditions and expose the interest of the researchers to further explore rhizobial inoculation biotechnology in non-legumes under drought.

542A Endophytic bacterial diversity of balloon flower (Platycodon grandiflorum) root and their antimicrobial activities based on plant age
Shah Md. Asraful Islam*
Patuakhali Science and Technology University, Bangladesh

Balloon flower (Platycodon grandiflorum) is widely cultivated vegetable and used as a remedy for asthma in East Asia. Experiments were conducted to isolate endophytic bacteria from one, three and six year old balloon flower roots and to analyze the enzymatic, antifungal and anti-human pathogenic activities of the potential endophytic biocontrol agents obtained. Endophytic bacteria were isolated from one year, three years and six years old balloon flower (Platycodon grandiflorum) roots and sequenced based on 16S rDNA. Phylogenetic analysis was performed using neighbor-joining methods. Bootstrap analysis was performed using data resampled 1,000 times using the DNAMAN analysis system. Agar diffusion method was used for the detection of extracellular hydrolytic enzyme activity of the isolated balloon flower endophytic bacteria. An in vitro bioassay was conducted to evaluate antagonistic properties and anti-human food-borne pathogenic activity of isolated endophytic bacteria. Total 120 bacterial colonies were isolated from the interior of all balloon flower roots samples. Phylogenetic analysis based on 16S rRNA gene sequences showed that the population of 'low G+C gram positive bacteria' (LGCGPB) gradually increased 60.0% to 80.0% from one year to six years balloon flower sample. On the other hand, maximum hydrolytic enzyme activity showing endophytic bacteria was under LGCGPB, among the bacterial strains, Bacillus sp. (BF1-1 and BF3-8), Bacillus sp. (BF1-2 and BF3-5) and Bacillus sp. (BF1-3, BF3-6 and BF6-4) showed maximum enzyme activities. Besides, Bacillus licheniformis (BF3-5 and BF6-6) and Bacillus pumilus (BF6-1) showed maximum antifungal activity against Phytophthora capsici, Fusarium oxysporum, Rhizoctonia solani and Pythium ultimum. Moreover, Bacillus licheniformis was found in three years and six years balloon flower roots, but Bacillus pumilus was found only in six years sample. It is presumed that older balloon flower plants invite more potential antifungal endophytes for their protection from plant diseases. In addition, Bacillus sp. (BF1-2 and BF3-5) showed maximum anti-human pathogenic activity. So plant age is presumed to influence diversity of balloon flower endophytic bacteria.

543A Multiplex-PCR for quick strain-identification of plant-pathogenic bacteria, phytoplasma
Shigeyuki Kakizawa*, Yoichi Kamagata
National Institute of Advanced Industrial Science and Technology (AIST), Japan

Phytoplasmas are plant-pathogenic bacteria which cause diseases in several hundred plant species and significant yield loss in crop production. They are transmitted by insects, and plants infected with phytoplasmas show characteristic symptoms, such as yellowing, proliferation and stunting.
Phytoplasmas usually do not kill the host plants in short time, and phytoplasma-infected plants result also more susceptible to infection by other pathogens such as fungi and viruses, thus the possibility of epidemic spreading of phytoplasmas and other pathogens become to be serious. Despite their economic importance, phytoplasmas remain the most poorly characterized plant pathogens, primarily because efforts at in vitro culture and mutagenesis have been unsuccessful. More than several hundreds of phytoplasma strains were reported worldwide so far. Detection and strain-identification of phytoplasmas in an early stage of infection are important to prevent the spread of phytoplasma diseases, thus rapid detection techniques are necessary for the pest control.

Multiplex-PCR is a PCR method to amplify multiple DNA fragments in a tube, so it could easily detect the existence of several genomic DNA regions. Here, we developed a multiplex-PCR system to amplify several phytoplasma genes. Based on the genome sequence of ‘Candidatus Phytoplasma asteris’, 18 primer pairs were designed to amplify 18 single copy genes, covering wide regions of the phytoplasma genome. Nine genes could be amplified in one PCR tube, so the existence of 18 genes could be analyzed in two PCR reactions. This multiplex-PCR was performed using DNAs form several phytoplasma strains, and different amplification patterns were obtained between strains. Thus, this method would be useful for the quick identification of phytoplasma strains. Direct sequencing was also possible after the multiplex-PCR amplification.

543B Competition between subalpine grassland plant species and microbes for nitrogen in the rhizosphere under different redox conditions - a greenhouse approach
Eva-Maria Kastl*1, Silvia Gschwendtner2, Michael Schloter1
1Helmholtz Zentrum München, Environmental Genomics, Germany, 2Technische Universität München, Institute of soil ecology, Germany

Natural grasslands are important hotspots for biodiversity and other ecosystem services of soils. Gramineous plants are the major part of above ground biodiversity. These plants differ greatly in nitrogen uptake strategies depending on the respective species. Whereas exploitative plants need high amounts of nitrogen compounds mainly ammonia for growth, conservative plants require lower amounts and can use also selected amino acids for growth. So far, the influence of plant nitrogen uptake strategies on microbial communities in soil and rhizosphere is largely unknown. However, it can be hypothesized that the microbial rhizosphere community of exploitative plants differs from that of conservative plants due to high competition between exploitative plants and microbes for available nitrogen. Therefore the aim of this study was to investigate the microbial rhizosphere community structure of subalpine gramineous plants with different nitrogen uptake strategies under different redox conditions in soil focusing on microbes involved in nitrogen transformation.

We performed a greenhouse study using Achillea millefolium (exploitative), Bromus erectus (intermediary) and Briza media (conservative) in a sandy, nutrient poor soil at two different water contents (50% and 80% of the max water holding capacity, respectively). Plants received 40 kg NH₄NO₃ ha⁻¹ after 7 days and 60 kg NH₄NO₃ ha⁻¹ after 21 days of growing. After 28 days the rhizosphere of the plants was sampled. The microbial rhizosphere community was investigated by quantification of functional genes involved in nitrification (bacterial and archaeal amoA) and denitrification (nirK, nirS and nosZ) by real-time PCR. Soil ammonium and nitrate concentrations as well as total carbon and nitrogen contents were determined. Furthermore potential enzyme activities of nitrification and denitrification were analyzed.

Detailed results and correlations between growth strategies of plants, microbial communities in the rhizosphere and nitrogen allocation pattern will be presented in the poster.

544A Horticultural cropping systems in the shadow of environmental demands
Sammar Khalil*1, Anna Karin Rosberg1, Samareh Gharraie1, Malin Hultberg1, Karl-Johan Bergstrand1, Justine Sylla2, Walter Wohanka1, Nicola Gruyer3, Beatrix Alsanius1
1Department of Horticulture- Microbial Horticulture Group, Swedish University of Agricultural Sciences, Sweden, 2Geisenheim Research Center, Germany, 3Centre de recherche en Horticulture, Pavillon Envirotron, Université Laval, Canada

Within the Swedish food production, horticulture is a major player with an annual turnover of around 5 billion. Within this sector production of fresh produce such as fruits, berries and vegetables both
indoors and outdoors is performed. The need of cultivation systems for intensive horticultural crop production that meets environmental objectives in addition to economic ones has become a demand not only by legislation but also from consumers and growers. Aspects regarding eutrophication, plant pathogens as well as production of environmentally friendly and healthy food are matters of concern and have to be solved.

A reduction of eutrophication could be achieved by using closed cultivation system where the irrigation effluent is reused which allows a more efficient use of nutrients compared to run-to-waste systems. The use of microalgae and wetland as strategies to reduce the leach of nutrients is presented. Biological control is a promising and environmentally friendly tool to control plant pathogens. Effective use of biocontrol agents can lead to a reduction in the use of chemical protection agents which are harmful to the environment and health. Different biocontrol agents have shown the ability to control root diseases on tomato grown in closed cultivation system or leaf pathogens on strawberry. In addition to biological control, the environmental profile of horticultural cropping systems, indoors, could be enhanced by the use of LED light as energy efficient systems. The effect of these strategies on the resident microflora on roots or leaf is also presented.

545A Diversity of Acetic Acid Bacteria Isolated from Tropical Thai Fruits
Tharinee Klawpiyapamornkun*1, Sakunnee Bovonsombut1, Sittisin Bovonsombut2
1ChiangMai University, Thailand, 2Maejo University, Thailand

Acetic acid bacteria are Gram negative aerobic bacteria capable of oxidize ethanol to acetic acid. They are used in the industrial production of vinegar. The present study aims at isolation of acetic acid bacteria from various kinds of Thai fruits and fermented fruit juices. Sixteen kinds of Thai fruits and 4 fermented fruit juices were collected from the northern area. The fruit samples were enriched in distilled water supplemented with 4% ethanol (v/v) and incubated at 30°C for 5 days. The soaking solution and the fruit juice were spread on bromocresol purple ethanol agar plate for isolation of acetic acid bacteria. The colonies with yellow zone were collected as acid producer. Ninety two isolates of Gram-negative and rod-shaped bacteria were obtained. The isolates were examined for the ability to oxidize acetate on bromocresol green ethanol agar plate and tested for acetic acid production. Acetic acid bacteria were found to be high in cantaloupe, papaya, long-kong and pine apple fermented juice respectively. Eighty five isolates were Acetobacter, seven isolates were Gluconobacter which was found only from long-kong. The diversity of acetic acid bacteria in Thai fruit is therefore depended on the type of fruit which are good source of acetic acid bacteria.

546A The mycobiome of wheat
Kamilla Knorr*1, Annemarie F. Justesen1, Lise N. Jørgensen1, Hans Pinnschmidt2, Jiaoyu Wang1, Mogens Nicolsen1
1Department of Agroecology, Aarhus University, Denmark, 2Department of Medical Biometry and Epidemiology, University Medical Center Hamburg-Eppendorf, Germany

Wheat (Triticum aestivum) is the most important cereal crop in Europe and the third most important worldwide. Grain yield and quality is often affected by pathogenic fungi. ”One microbe, one disease” studies have for many years led to a better understanding of wheat diseases and their associated pathogens. In recent years, 2nd generation sequencing techniques have enabled studies on entire fungal communities, including the unculturable and rare fungi.

The aim of our studies was to explore potential poly-microbial interactions and effects in above-ground plant parts of wheat in relation to a diverse set of factors in the agroecosystem. Our studies used deep amplicon 454 pyrosequencing targeting the internal transcribed spacer-1 (ITS1) region of the ribosomal DNA for fungal identification. Pooled ITS1-amplicons were pyrosequenced and analysed using a CLOTU-based bioinformatics pipeline. Pyrosequencing data was supplemented with qPCR quantification of selected fungi and total fungal biomass. The molecular data was compared to results from fungal culturing and identification as well as comprehensive amounts of field data.

In three studies we have explored 1) the fungal community composition in wheat grain from field-collected samples, 2) the effects on the endophytic fungal community in stems and leaf sheaths of organically managed versus conventionally managed wheat, and 3) the community composition in the phyllosphere of target versus non-target fungi in yellow rust (Puccinia striiformis) infected wheat as
affected by three different fungicides, two dose rates and three treatment timings. In all studies we found that fungal communities are affected by the variables in the studied systems.

Studies like ours will provide insight into the diversity, stability, and complexity of wheat fungal communities and thereby add to a better understanding of poly-microbial interactions and effects in wheat.

546B  Innate immunity, salicylic acid, and Arabidopsis phyllosphere microbiome
James M Kremer1,2, Sheng Yang He2, James Tiedje1
1Michigan State University, USA, 2GBMF/HHMI, Michigan State University, USA

Most vascular plants are rooted in soil, a highly diverse and complex microbial ecosystem rich with potential pathogens and symbionts. Although studies are beginning to unravel the intricacies and complexities of plant defenses against pathogens, we know very little about the relationship between plant innate immunity and the plant microbiome. Here, we demonstrate that a soil-derived microbial community promotes seedling growth of the model angiosperm Arabidopsis thaliana. We take a 16S rRNA gene culture-independent approach to determine alpha- and beta-diversity of Arabidopsis phyllosphere bacterial communities. By correcting for 16S rRNA gene copy number, we achieve a more accurate picture of bacterial diversity and true community evenness as compared to traditional analysis approaches. Using a highly controlled growth system, we examine the phyllosphere communities of various natural Arabidopsis accessions (Swedish, Italian, and German) and defense mutants, and find a significant role for salicylic acid signaling in associated phyllosphere bacterial communities. Most notably, the ratio of certain Gammaproteobacteria (Pseudomonas, Xanthomonas) to Bacteroidetes (Sphingobacteria, Flavobacteria) is reproducibly shifted upon manipulation of salicylic acid signaling, with increased salicylic acid levels favoring Bacteroidetes. Furthermore, we demonstrate that Pseudomonas isolates from apparently healthy Arabidopsis plants can have significant differences in pathogenic potential, despite effectively identical 16S rRNA gene sequences.

547A  Stimulation of microbial metabolism by inefficient herbivory
Jennifer Krumins1, Valdis Krumins2, Wim van der Putten3
1Montclair State University, USA, 2Rutgers University, USA, 3Netherlands Institute of Ecology, Netherlands

A growing body of theory studies the paradoxical idea that herbivory can have positive feedbacks on nutrient flow to plants. Specifically, the relationships between root grazing herbivores and microbial decomposers may heavily influence plant primary production. By developing a theoretical model we tested the idea that plants, microorganisms and plant herbivores are in a delicate balance between maximizing nutrient mineralization, consuming resources and maintaining plant biomass. Plants tend to leak carbon through their roots. This is ultimately beneficial to the plant by supporting a robust microbial flora. Further carbon is released into the soil when herbivores graze on roots, and the efficiency with which they graze affects the amount of carbon available to the microbial flora. Inefficient grazing in the rhizosphere may stimulate microbial growth through an increased availability of root organic matter. The increase in microbial metabolism likely results in more mineralized nutrients available to plants. However, we hypothesize that the degree of stimulation interacts with the stoichiometric quality of the organic matter coming off of the root. We have designed a steady state model consisting of differential equations relating biomass of plants, microbes, herbivores, and masses of organic carbon, nitrogen and inorganic nitrogen. In our model we manipulate grazing inefficiency and the stoichiometric quality of the host plant. We parameterize the model and the equations with values from the literature and our own glasshouse experiment. The results of our model show that herbivore inefficiency can have direct impacts on the microbial metabolism and feedbacks to plant growth. Specifically, when herbivores are less efficient with their consumption and more carbon is leaked to the soil, microbial activity increases, nutrients are mineralized and plant growth increases. However, this varies with plant quality (C:N ratio). As host plant quality decreases, more organic carbon and nitrogen is released to the surrounding environment with increasing herbivore inefficiency. Likewise, microbes excrete more nitrogen when plant quality is highest with increasing availability of organic matter from inefficient grazing. Microbial biomass declines as microbes assimilate less nitrogen with increasing plant quality. These results affirm the notion that microbes are typically carbon limited. This model was parameterized with data from soil communities. However, by accounting for
Endophytic bacteria can be defined as bacteria that live inside plant tissues without causing symptoms of infection on their hosts. Within this close relationship, plants provide nutrients and a residency to the bacteria while these latter can improve plant health and growth either directly or indirectly. Moreover, the endophyte-plant interactions can play an important role in promoting phytoremediation of soils polluted by either toxic hydrocarbons or heavy metals.

Polycyclic aromatic hydrocarbons (PAHs) represent a wide class of organic compounds that are ubiquitous in the natural environments. Since PAHs behave as recalcitrant compounds, contamination by these molecules is of a growing concern. In the last few years, many studies have been focusing on endophyte-assisted phytoremediation, evidencing that microorganisms can enhance the remediation efficiency either in annual and perennial plants, such as poplar. Burkholderia fungorum DBT1 is a bacterial strain previously isolated from an oil refinery wastewater which grows on dibenzothiophene, phenanthrene and naphthalene as sole source of carbon and energy. Its catabolic genes (dbt genes) have been identified, resulting to be harbored in two separate operons and showing low similarity to both nah-like and phn-like genes.

The present study reports a comparative analysis of B. fungorum DBT1 with an endophyte Burkholderia strain isolated from the roots of hybrid aspen (Populus tremula x Populus tremuloides), harboring genes for PAHs degradation. Surface-sterilized leaves, stems and roots were macerated and plated. Selective growth media were used and several endophytic bacterial strains were isolated. Among these, strains 95 resulted to be capable of growing on naphthalene, fluorene, dibenzothiophene, and phenanthrene as sole source of carbon and energy. Phylogenetic investigations carried out by means of 16S rDNA and recA sequencing showed an average of sequence identity of 99.8% and 98.6% respectively to Burkholderia fungorum LMG 16225T. DNA-DNA hybridization analysis showed a complementation of 89.8 ± 5.80%, confirming the affiliation of strain 95 to B. fungorum species. PCR analysis carried out in order to detect the presence of specific PAHs catabolic genes, revealed the presence of dbt genes previously identified in Burkholderia fungorum DBT1. Restriction and sequence analysis of the internal fragment of dbt genes of the endophytic strain and B. fungorum DBT1 proved the existence of two distinct isoforms of dbt genes: the first harbored in the DBT1 strain, while the second occurring in the newly isolated aspen strain. Moreover, growth tests performed in presence of phenanthrene, dibenzothiophene, fluorene and naphthalene as sole source of carbon and energy demonstrated that B. fungorum DBT1 is more efficient in degradation of PAHs tested than B. fungorum 95. Thus, the difference between B. fungorum 95 isolated in aspen and DBT1 is not only at the genetic level.

This investigation demonstrates that a bacterial strain isolated from hybrid aspen roots 1) is capable of transforming PAHs, 2) harbors a new isoform of dbt catabolic genes, and 3) belongs to Burkholderia fungorum. These results open interesting perspectives in the use of natural endophytic bacteria equipped with appropriate degradation pathways to improve in planta PAHs degradation.
traditional groves and new intensive orchards. During the last two decades, several major technological changes have occurred in olive production in Spain to increase and maintain stable yields. Furthermore, new soil management systems aimed to minimize soil erosion, and new environmental-friendly practices such as organic and integrated production have been adopted. The objective of this study was to analyze the effects of olive management systems on microbial diversity and functionality of the olive rhizosphere by using classical and metagenomic approaches.

For this purpose, a collection of rhizosphere soils from 90 olive orchards under different management systems and six rhizosphere soils from wild olive havens in Andalusia were characterized using several approaches including: physicochemical characteristics, functional diversity (API ZYM and Biolog Ecoplates), and population density of culturable heterotrophic bacteria and of Pseudomonas. Additionally, we used a bar-coded pyrosequencing technique to characterize bacterial, fungal, and arbuscular mycorrhizal (AM) communities from those soils. Sequences generated from pyrosequencing of rRNA gene amplicons were processed using the Quantitative Insights Into Microbial Ecology (QIIME 1.4.0) pipeline. Flowgrams were clustered into OTUs at 97% pairwise identity using the seed-based uclust algorithm, and representative sequences from each OTU were aligned to the Greengenes (bacteria), Silva (mycorrhiza) or Unite-Taxonomy (fungi) databases using PyNAST or UCLUST. Chimeras were removed from the reference set via Chimera Slayer. In addition, α diversity (PD_Whole_Tree, observed species count, Shannon and Chao1 richness estimators) and β diversity (unifrac weighted and unweighted) metrics together with rarefaction plots were also calculated using QIIME.

Canonical discriminant analyses have allowed identifying some functional indicators, including enzyme activities and physiological profiles that are differentially associated to olive management systems (organic, conventional or wild olives). Data from pyrosequencing detected, after removing low-quality sequences, a total of 514274 sequences from which 3054, 527, and 206 were bacterial, fungal and AM on average per orchard. Results from α diversity and β diversity indices indicated that olive rhizosphere harbor a high microbial diversity that in general terms is highly stable. Thus, we did not find clear differences in microbial structure (particularly bacteria) among the several orchard management systems tested, but to some extent, rhizosphere communities were related to olive variety, soil texture (clay content), soil management and irrigation system. Multivariate analyses are being conducted to identify biotic or abiotic factors that could be used as an indicator of the different olive management systems and which specific microbial groups, if any, could be correlated with those orchard management systems as well as with the level of suppressiveness of those soils to Verticillium wilt, the most important disease of this crop in the Mediterranean basin.

550A Proteome analysis of Arabidopsis seedlings exposed to bacterial volatiles
Soohyun Lee*, Young Sang Kwon*, Hyo Bee Park*, Ki Soo Han*, Jung Han Lee*, Kyunghee Lee†, Woo Sik Chung†, Mi-Jeong Jeong*, Hee Kyu Kim†, Dong-Won Bae†, Choong-Min Ryu†
†KRIBB, South Korea, ‡Gyeongsang National University, South Korea, §Yeungnam University, South Korea, †Plant Molecular Biology and Biotechnology Research Center, South Korea, ‡Rural Development Administration, South Korea

Plant root-associated bacteria (rhizobacteria) elicit plant basal immunity referred to as induced systemic resistance (ISR) against multiple pathogens. Among multi-bacterial determinants involving such ISR, the induction of ISR and promotion of growth by bacterial volatile compounds was previously reported. To exploit global de novo expression of plant proteins by bacterial volatiles, proteomic analysis was performed after exposure of Arabidopsis plants to the rhizobacterium Bacillus subtilis GB03. Ethylene biosynthesis enzymes were significantly up-regulated. Analysis by quantitative reverse transcriptase Polymerase Chain Reaction confirmed that ethylene biosynthesis related genes SAM-2, ACS4, ACS12, and ACO2 as well as ethylene response genes, ERF1, GST2, and CHIB were up-regulated by the exposure to bacterial volatiles. More interestingly, the emission of bacterial volatiles significantly up-regulated both key defense mechanisms mediated by jasmonic acid and salicylic acid signaling pathways. In addition, high accumulation of antioxidant proteins also provided evidence of decreased sensitivity to reactive oxygen species during the elicitation of ISR by bacterial volatiles. The present results suggest that the proteomic analysis of plant defense responses in bacterial volatile-mediated ISR can reveal the mechanisms of plant basal defenses orchestrated by endogenous ethylene production pathways and the generation of reactive oxygen species.
551A Foliar aphid feeding recruits rhizosphere bacteria and primes plant immunity against pathogenic and non-pathogenic bacteria in pepper

Soo hyun Lee*, Boyoung Lee, Choong-Min Ryu
University of Science and Technology / KRIBB, South Korea

Plants modulate defence signalling networks in response to different biotic stresses. The present study evaluated the effect of a phloem-sucking aphid on plant defence mechanisms in pepper (Capsicum annuum) during subsequent pathogen attacks on leaves and rhizosphere bacteria on roots. Plants were pretreated with aphids and/or the chemical trigger benzothiadiazol (BTH) 7 d before being challenged with two pathogenic bacteria, Xanthomonas axonopodis pv. vesicatoria (Xav) as a compatible pathogen and X. axonopodis pv. glycines (Xag) as an incompatible (non-host) pathogen. Disease severity was noticeably lower in aphid- and BTH + aphid-treated plants than in controls. Although treatment with BTH or aphids alone did not affect the hypersensitive response (HR) against Xag strain 8ra, the combination treatment had a synergistic effect on the HR. The aphid population was reduced by BTH pretreatment and by combination treatment with BTH and bacterial pathogens in a synergistic manner. Analysis of the expression of the defence-related genes Capsicum annuum pathogenesis-related gene 9 (CaPR9), chitinase 2 (CaCHI2), SAR8.2 and Lipoxygenase1 (CaLOX1) revealed that aphid infestation resulted in the priming of the systemic defence responses against compatible and incompatible pathogens. Conversely, pre-challenge with the compatible pathogen Xav on pepper leaves significantly reduced aphid numbers. Aphid infestation increased the population of the beneficial Bacillus subtilis GB03 but reduced that of the pathogenic Ralstonia solanacearum SL1931. The expression of defence-related genes in the root and leaf after aphid feeding indicated that the above-ground aphid infestation elicited salicylic acid and jasmonic acid signalling throughout the whole plant. The findings of this study show that aphid feeding elicits plant resistance responses and attracts beneficial bacterial populations to help the plant cope with subsequent pathogen attacks.

552A Spatiotemporal variation in bacterial community composition on leaves of field-grown lettuce

Johan Leveau*, Gurdeep Rastogi, Adrian Sbodio, Jan Tech, Trevor Suslow, Gitta Coaker
University of California - Davis, United States

The presence, size, and importance of bacterial communities on plant leaf surfaces are widely appreciated. However, information is scarce regarding their composition and how this composition changes along geographical and seasonal scales.

We collected 106 samples of field-grown Romaine lettuce from commercial production regions in California and Arizona during the 2009/2010 crop cycle. We used both culture-dependent and -independent methods to determine the size and composition of bacterial communities associated with lettuce foliage at time of harvest.

Total bacterial populations averaged between $10^5$ and $10^6$ per gram of tissue, whereas counts of culturable bacteria were on average one (summer season) or two (winter season) orders of magnitude lower. Pyrosequencing of 16S rRNA gene amplicons from 88 samples revealed Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria as the most abundantly represented phyla. At the genus level, Pseudomonas, Bacillus, Massilia, Arthrobacter, and Pantoea were the most consistently found across samples, suggesting that they form the bacterial 'core' phyllosphere microbiota on lettuce. The foliar presence of Xanthomonas campestris pv. vitians, the causal agent of bacterial leaf spot of lettuce, correlated positively with the relative representation of bacteria from the genus Alkanindiges, but negatively with Bacillus, Erwinia and Pantoea. Summer samples showed an overrepresentation of Enterobacteriaceae sequences and culturable coliforms compared to winter. The distance between fields or the timing of a dust storm, but not Romaine cultivar, explained differences in bacterial community composition between several of the fields sampled.

As one of the largest surveys of leaf surface microbiology, this study offers new insights into the extent of variability in bacterial community composition on plant leaves as a function of time, space, and environment. Such insights are important for a better understanding of the establishment of unwanted plant and human pathogens on leafy greens.
**552B**  
**Phylogenetic distribution of traits associated with plant colonization in Escherichia coli**  
Sacha Lucchini¹, Guillaume Meric¹, Katherine Kemsley¹, Laura Searle¹, Ida Porcelli¹, Daniel Falush², Elizabeth Saggers¹  
¹Institute of Food Research, United Kingdom, ²Max-Planck Institute for Evolutionary Anthropology, Germany  

*Escherichia coli* is routinely isolated from crops and there is increasing evidence that plants are a secondary reservoir for commensal and pathogenic strains. However, the ecological factors involved in the association of *E. coli* with plants are not clear. To investigate the factors influencing *E. coli* persistence, we undertook a comparative approach combining phenotypic and phylogenetic analyses of *E. coli* isolates from crops and mammalian hosts. We speculated that each environment influences the properties of the *E. coli* population and thus a comparative approach would reveal possible specificities. Phenotypic profiling revealed significant differences according to the source of isolation. Isolates from plants displayed greater motility, higher biofilm production, and better growth on the aromatic compound p-hydroxyphenylacetic acid, sucrose and raffinose. However, when compared with mammalian-associated strains, they reached lower growth yields on many C-sources commonly used by *E. coli* and showed reduced siderophore production. Strikingly, we observed a strong association between phenotypes and *E. coli* phylogenetic groups. Strains belonging to phylogroup B1 were more likely to harbour traits indicative of a higher ability to colonize plants, whereas phylogroup A and B2 isolates displayed phenotypes linked to a host-associated lifestyle. This work provides clear indications that *E. coli* phylogroups are differentially affected by niche-specific selective pressures, and provides an explanation on why *E. coli* population structures vary in different environments, implying that different *E. coli* lineages differ substantially in their transmission ecology.

**553A**  
**Selective mobilisation of rhizosphere bacterial populations upon groundwater recharge**  
Tillmann Lueders¹, Doerte Dibbern¹, Andreas Schmalwasser², Kai-Uwe Totsche²  
¹Institute of Groundwater Ecology, Germany, ²Institute for Geosciences, Germany  

Plants introduce abundant carbon into soil, where it is sequestered into microbial biomass and recalcitrant organic matter. However, considerable fractions of this carbon pool (up to ~20% of root exudates) can be relocated, by event-driven transport to deeper soil layers and also the groundwater during groundwater recharge, such as after heavy rainfalls or snowmelt. It is postulated that large amounts of this efflux consist of mobile organic particulate substances (MOPS), including biocolloids or microbial biomass itself. This may represent a direct and dramatically under-investigated link in organismic and carbon transfer between soil horizons and distinct meta-communities. Relevant questions are, whether selected microbial populations are flushed from top soils during such events, and what the fate of this biomass is in deeper zones and groundwater.

Here, at an experimental agricultural maize field, we analyzed the composition of mobilised bacterial communities collected in seepage water directly after recharge events at different soil depths (35 and 65 cm), and compared them to communities from corresponding rhizospheric, bulk soil and vadose zone depths. Using qPCR, fingerprinting and pyrotag sequencing, we reveal that specific top soil bacteria were purged selectively. Surprisingly, mobilised populations were dominated by members of the *Oxalobacteraceae, Comamonadaceae, Bradyrhizobiaceae, Sphingomonadaceae, Pseudomonadaceae* and *Bacteroidetes*. They were thus highly related to the microbial assemblages typical found on plant roots at the site, rather than bulk soil or vadose zone communities. Likely, this effect was caused by the predominant flow of seepage water along macropores, which include earthworm burrows and especially root channels. In summary, we show that specific bacteria are selectively mobilised from top soil rhizosphere compartments upon groundwater recharge, and that the nature and composition of mobilised communities is mainly controlled by water flow paths. These findings greatly extend our understanding of the mechanisms of event-driven efflux of organisms and carbon from plant-influenced soil into the subsurface, and open a door to estimating their contribution to net carbon flux between soil horizons.
Evaluation of mutants from Rhizobium tropici with biotechnological potential
Tereza Cristina Luque Castellane, Eliana Gertrudes de Macedo Lemos, Manoel Victor Franco Lemos*
UNESP / FCAV, Brazil

Rhizobium tropici and other bacteria belonging to the order Rhizobiales are extracellular polysaccharides (EPS) producers, which possess the function of the receptor molecule to function as micro-symbiont, making an interaction cell/cell and triggering the nodulation process. The diversity of structures and chemical composition of EPS presented by molecules is reflected by the diversity of enzymes responsible for its synthesis. In this study, we sought to inactivation by insertional mutagenesis by "in vitro" transposition of genes from wild type Rhizobium tropici SEMIA 4080, responsible for synthesis of polyhydroxybutyrate, in order to obtain microorganisms with high mucosal aspect producer of exopolysaccharide compared to wild type. Plasmid isolation, DNA ligation, restriction mapping, transformation and genomic DNA extraction, and plasmid profile were performed by standard methods. The construct was transferred to R. tropici by electrottransformation. EPS-producing strains had a mucoid phenotype that could easily be distinguished from mutants deficient in exopolysaccharide synthesis. The mutants showed mucous colonies when grown in culture media PsyA, demonstrating that mutant strains are able to form biofilm "in vitro", and to evaluate the relative efficiency in the production of exopolysaccharide, these mutants showed the best results. For the two samples of exopolysaccharide was possible to observe a flow behavior of pseudoplastic fluids, and also the influence of exopolysaccharide concentration on the apparent viscosity of aqueous solutions. For the study the interactions of mutant strains with in IAC Carioca bean through nodulation tests and efficiency of biological nitrogen fixation, conducted under controlled conditions in a greenhouse at UNESP, Jaboticabal, São Paulo, Brazil. The parameters evaluated were: plant height at 35 days after sowing, the dry matter shoot and root dry matter, number and dry matter weight of nodules per plant and nitrogen content in the leaves of Phaseolus vulgaris. The wild type and mutants induced the formation of nodules in common bean, suggesting that inactivation by insertional mutagenesis are not affect in the processes of infection and nodule formation, but affects the nitrogen fixation.

Investigation of key species of dark septate endophytic fungi from different habitats
Rola Mahmoud¹, Kazuhiko Narisawa²
¹Tokyo university of agriculture and technology, Japan, ²Ibaraki university, Japan

Dark septate root endophytes (DSE) are dematiaceous fungi that occur with some regularity in the roots of apparently healthy plants, were they usually form distinctive inter- or intra-cellular structures or colonization patterns. Endophytic fungi have mostly been reported for their behavior to enhance plant growth, and suppress plant diseases. In this study we tried to assess the key species of (DSE) from different apparently healthy fields using T-RFLP (terminal restriction fragment length polymorphism), and evaluate their ability to promote plant growth, and suppress the plant diseases. Soil samples were collected from woody field dominated with mature cedar tree (F), asparagus field (H), and organic field (O), and used for genetic analysis, and isolating DSE using baiting method. Scolecobasidium humicola was found to be the common isolate in all three fields (S. humicola F-1-3, H-2-2, and O-MH respectively). The T-RFLP results showed the abundance of S. humicola H-2-2 as dominant species in asparagus field. The role of S. humicola on host plant performance and Fusarium disease control on Tomato were investigated in vitro. The Results showed that S. humicola H-2-2, and F-1-3 significantly improved tomato biomass in comparison with control. when the inorganic nitrogen replaced with amino acids in oat meal agar media. Production of plant growth promotion IAA were detected in all isolates in the presence of tryptophan, however S. humicola H-2-2 showed 2 times higher concentration of IAA. The antagonistic effect against Fusarium oxysporum f.sp. lycopersici results showed that only S. humicola H-2-2 significantly decrease disease severity. The ability of S. humicola to deliver nitrogen from different sources for plant and their production of IAA, which explain their ability to promote plant growth. However more research need to be done to make clear understanding about the mechanism of suppressing the disease, and environmental or biological conditions from where dark septate root endophytes (DSE) isolated could influenced their behavior with plant.
Anthropogenic influence on the environment as well as climatic changes lead to water deficiency, which restricts essentially the realization of the productivity potential of important agricultural plants. This problem requires active implementation of the high effective technology for agriculture to save the world resources.

Unique way to enrich the soil with biological nitrogen as well as to increase yield capacity and quality of agricultural products is the creation of effective symbiotic systems of legumes with nodule bacteria. The peculiarities of metabolic state of the symbiotic system during different periods of symbiosis establishment reflect the character of plant-microbe interactions especially under the acting of abiotic factors including insufficient water supply.

Studying the composition of soybean symbiotic system metabolites under the formation of defense reactions on negative actions of biotic and abiotic factors environment allows understanding the mechanisms of plant physiological adaptation.

The aim of our investigations was the study of metabolic profile of soybean (Glycine max L.) roots inoculated with strains of Bradyrhizobium japonicum possessing different symbiotic properties under both optimal and insufficient water supply of substrate for plant cultivation. The metabolites were extracted from the soybean roots. The derivatives of metabolites were estimated by GC/MS.

The results showed that there were approximately fifty compounds in the samples of metabolites including organic acids, amino acid, sugars, alcohols etc. The amounts of malonic and amber acids as well as some sugars and polyatomic alcohols which play role in the plant defense were increased in control plant roots under water stress and inoculated plant roots under the different levels of water supply (40 and 60 % of soil moisture). The control and inoculated plant roots under the different levels of water supply had similar contents saturated and unsaturated fatty acids. Among them were prevailed stearic and palmitic fatty acids. The content of these acids was increased essentially during intensive nodule formation. The increase of proline amount occurred in all samples under water stress whereas this amino acid was absent in the roots of uninoculated plants cultivated under optimal water supply.

The formation of free fatty acids may be due increasing activity of cell biosynthesis of membrane lipids. It is known that the level of membrane fatty acids influences essentially on plant resistance to drought. The increase of contents of osmoprotector proline under water stress promotes the water retention in plant cells and prevents protein dehydration as well as increases the irrigation of membranes and stabilizes the structure of latter.

The studies suggest that the effective inoculation of soybean seeds induces the synthesis of physiological active products in plants affected by stress and thereby creates conditions for increasing plant resistance to moisture deficiency.

Endophytic microorganisms colonise plant tissues, and some endophytic bacteria have plant growth promoting properties. Current research focuses on the exploitation of their activities (biocontrol, biofertiliser and/or phytohormone production) as an environmentally-friendly strategy to improve plant health and growth. These strategies could be part of a solution to the food shortage problems faced in
developing countries such as South Africa. However, in order to achieve this, the underlying biology of these microorganisms and their complex interactions with the plant hosts need to be thoroughly understood.

Metagenomic analyses have broadened the understanding of endophytic bacterial communities, but various DNA extraction procedures have been described and used for the study of these communities. Therefore, the aim of this study is to compare the efficiency of CTAB and SDS-based DNA extraction protocols and different DNA extraction kits (MoBio PowerSoil™ DNA Purification Kit, MoBio UltraClean® Soil DNA Isolation Kit, MoBio PowerPlant Pro® DNA Isolation Kit, QIAGEN DNeasy® Plant Mini Kit, Fermentas GeneJET Plant Genomic DNA Purification Kit) to study endophytic communities from the root and stem tissues of three different staple food plants: sorghum (Sorghum bicolor), pearl millet (Pennisetum glaucum) and groundnut (Arachis villosulicarpa). Extracted DNA yields and purity as well as the endophytic community diversities (using t-RFLP) are compared to determine the best DNA extraction protocol for the recovery of endophytic microbial communities metagenomic DNA.

558A How can plant-associated pseudomonads with antifungal activity become insect pathogens
Monika Maurhofer1, Beat Ruffner1, Maria Péchy-Tarr2, Peter Kupferschmied2, Pascale Flury*1, Christoph Keel2
1Swiss Federal Institute of Technology Zürich / Plant Pathology, Institute of Integrative Biology, Switzerland, 2University of Lausanne/Department of Fundamental Microbiology, Switzerland

Root colonizing fluorescent pseudomonads are well known for their ability to improve plant growth by the suppression of soilborne diseases. These bacteria produce a wide array of antifungal compounds they use as weapons to protect roots against the attack of plant-pathogenic fungi. To our surprise we detected some years ago in Pseudomonas fluorescens CHA0, a strain with well-described antifungal activity, a genomic locus encoding a protein with high similarity to the Mcf insect toxin produced by Photorhabdus luminescens. This protein, termed Fit, represents a novel insect toxin in root colonizing pseudomonads. Indeed, CHA0 exhibits potent oral activity against larvae of major lepidopteran insect pests, when sprayed on plant leaves and has the capacity to multiply and persist within insects. This finding prompted us to investigate the occurrence, abundance and origin of insect pathogenicity in plant-associated pseudomonads. To this end we performed a PCR based screening on a large collection of pseudomonads isolated not only from plants but from different environments followed by sequencing and phylogenetic analysis of Fit producing Pseudomonas strains in order to reconstruct the evolutionary history of the Fit toxin gene and to analyse its mode of evolution. Our results revealed that Fit is present in Pseudomonas chlororaphis and in a small subgroup of fluorescent pseudomonads producing the antifungal compounds 2,4-diacetylphloroglucinol and pyoluteorin. We found this group to be closely associated with P. chlororaphis when analyzing both, four housekeeping and the nucleotide sequences of the Fit toxin gene. In addition, a more detailed sequence analyses suggest that Fit was acquired by horizontal gene transfer from entomopathogenic Photorhabdus with subsequent rearrangements of the toxin cluster. Testing our Pseudomonas collection for insecticidal activity revealed that only strains harboring Fit are toxic to insects.

In summary we identified a genetically distinct subgroup of biocontrol pseudomonads as exclusive carriers of the Fit toxin gene, a marker for insecticidal activity of plant growth-promoting pseudomonads. Our findings suggest that a specific group of root-associated pseudomonads acquired a potent insect toxin enabling them to kill insects and colonize a new ecological niche.

237B Microbial endophytes from Warburgia ugandensis - diversity and adaption to host drimane sesquiterpenes
Birgit Mitter*1, Sigrid Drage2, Alice Muchugi3, Rhamni H. Jamnadass3, Franz Hadacek2, Angela Sessitsch4
1AIT Austrian Institute of Technology, Austria, 2Department of Chemical Ecology and Ecosystem Research, University of Vienna, Austria, 3World Agroforestry Centre (ICRAF), Kenya, 4AIT Austrian Institute of Technology, Austria

Endophytes encounter in plants a range of complex organic compounds that are not found in soils or other environments, some of them showing antibacterial and/or antifungal activities. It is still poorly
understood how and to which extent microbes make use of plant metabolites or how endophytes can withstand the chemical defense mechanism of plants towards microbes.

In this project, we aimed at answering the following questions: (1) Does the composition of secondary metabolites in a plant affect the endophytic community? (2) Which strategies have been developed by endophytes to deal with putatively antimicrobial substances produced by the host plant?

In order to address these questions, we studied the composition and physiology of culturable and non-culturable endophytes colonizing Warburgia ugandensis, a tropical tree which is highly regarded as remedy of various human diseases caused by bacteria, fungi or viruses in traditional folk medicine. Phytochemical studies on W. ugandensis led to the identification of drimane sesquiterpenes with broad antibiotic activities.

Metabolite profiles (GC-MS), drimane sesquiterpenes, sugars and sugar alcohols, were compared with bacterial and fungal endophyte communities (T-RFLP, DNA clones, qPCR) in leaves and roots of the pepper bark tree, Warburgia ugandensis (Canellaceae), ten individuals each were assessed from two location east and west of the Great Rift Valley, Kenya, Africa. We found qualitative as well as quantitative differences in the metabolite composition of individual trees and tissues. The fungi as well as bacteria communities in W. ugandensis were characterized by a high species variation and distinct compositions in roots, leaves and fruits of individual trees. Pseudomonadaceae and Enterobacteriaceae are the far most dominant bacterial endopyhytes in W. ugandensis trees. The main difference between the bacterial community profiles was the presence or absence of 16S rDNA genes originating from Firmicutes and Actinobacteria.

We found correlation between the occurrence of certain microbial groups and certain plant metabolites in W. ugandensis. Correlations were found with all analyzed types of plant metabolites (sesquiterpenes, sugars and fatty acids) and fungal endophytes responded more often than the bacteria.

Screening of isolated fungal and bacterial endophytes for their sensitivity towards plant drimane sesquiterpenes revealed Warburgia endophytes to be significantly more resistant than the control strains. As the bacteria resisted also other strong pro-oxidative compounds we assume that their ability to avert the toxic effect of the plant drimane sesquiterpenes is due to a high tolerance of oxidative stress.

558B  Phenazine antibiotic production regulated by two quorum sensing systems in Pseudomonas chlororaphis subsp. aurantiaca StFRB508 and its antifungal activity against Phytophthora infestans

Tomohiro Morohoshi*1, Wen-zhao Wang1, Tomonori Sutou1, Nobutaka Someya2, Tsukasa Ikeda1

1Utsunomiya University, Japan, 2Hokkaido Agricultural Research Center, Japan

A number of gram-negative bacteria have a quorum-sensing system and produce N-acylhomoserine lactones (AHLs) that they use them as a quorum-sensing signal molecule. AHLs are required for diverse behaviors, including bioluminescence, the formation of biofilms, and the production of pathogenic factors, antibiotics, and other secondary metabolites. Phenazine antibiotics are synthesized by a number of bacteria and known for their antimicrobial and antifungal activities. Pseudomonas chlororaphis subsp. aurantiaca StFRB508 is isolated from the root of potato and has an ability to produce phenazine antibiotics. In previous study, it has been reported that quorum-sensing system regulates phenazine antibiotic production in P. chlororaphis subsp. aureofaciens 30-84, but not in P. chlororaphis subsp. aurantiaca. In this study, we report the identification of the AHL synthase genes in StFRB508 and investigated the relationship among AHL-mediated quorum sensing, phenazine antibiotic production, and antifungal activity.

First, we screened for the AHL production of StFRB508 by cross streaking against AHL reporter strain based on Chromobacterium violaceum. Because StFRB508 stimulated violacein production of AHL reporter strain, it was revealed that StFRB508 had the ability to produce AHLs. To identify the AHL synthetase gene, whole genome sequencing of StFRB508 was performed by using pyrosequencing technology. We searched for the homologues of the reported AHL synthase genes in the draft genome
of StFRB508. As the results, two predicted ORFs, designed phzI and aurI, showed high similarity to phzI from P. chlororaphis subsp. aureofaciens 30-84 and afmI from P. fluorescens BL915, respectively. To investigate the relationship between these genes and phenazine antibiotic production, genomic phzI and aurI genes were disrupted in StFRB508. The aurI mutant showed obvious phenazine antibiotic production as well as the StFRB508 parent strain, but the phzI mutant showed slightly lower phenazine antibiotic production. Phenazine antibiotic production was drastically decreased in phzI and aurI double mutant, but restored by addition of exogenous AHL. These results demonstrated that phenazine antibiotic production was regulated by AHLs produced by both PhzI and Aurl.

To evaluate the antifungal activity of StFRB508, Phytophthora infestans, which causes late blight of potato, was used. When cross-streaked against StFRB508, P. infestans showed a severe growth defect. However, phenazine-deficient mutant of StFRB508 did not show any antifungal activity. Interestingly, phzI and aurI double mutant of StFRB508 produced a small amount of phenazine antibiotics and showed antifungal activity against P. infestans. When phzI and aurI double mutant was cross-streaked against AHL reporter strain, double mutant slightly stimulated violacein production. These results assumed that the double mutant could produce small amounts of AHL by a third AHL synthase gene and induces phenazine antibiotic production by AHL-mediated quorum sensing.

559A Symbiotic associations with beta-rhizobia are deep-rooted within the tribe Mimoseae
Lionel Moulin1, Caroline Bournaud2, Miguel Santos3, Pierre Tisseyre2, Michele Silva4, Sergio M. de Faria1, Eduardo Gross3, Euan K. James5, Yves Prin2
1IRD-LSTM, France, 2LSTM-UMR113 CIRAD/IRD/UM2/SupaGro Usc INRA, France, 3Universidade Estadual de Santa Cruz, Brazil, 4Laboratório de Leguminosas Florestais-EMBRAPA, Brazil, 5The James Hutton Institute, United Kingdom

Rhizobial symbioses with legumes are not restricted to Alphaproteobacteria, but also occur with two genera (Burkholderia and Cupriavidus) of the Betaproteobacteria, so-called β-rhizobia. β-rhizobia were found mainly to associate with Mimosa (sub-family Mimosoideae). The β-rhizobia/Mimosa symbiosis being ancient and stable (Bontemps et al., 2010), suggesting co-evolution, we wondered if genera phylogenetically close to Mimosa (i.e. the "Piptadenia group" within the tribe Mimoseae) could also associate with β-rhizobia. To test this hypothesis, a systematic characterization of the taxonomic and symbiotic diversity of rhizobia isolated from nodules of members in this group was undertaken, and patterns of specificity were assessed. On neutral genes analyses (16S rRNA and recA), 202 rhizobial strains isolated from 12 Piptadenia species, were clustered into 7 genotypes, mostly within the genus Burkholderia and a few in the genera Rhizobium and Bradyrhizobium. Phylogenetic analyses were performed on the symbiotic genes (nodC and nifH) to evaluate their origin and evolution. Finally, the proportions of genotypes in the nodules of 6 Piptadenia species were evaluated to assess host-rhizobial specificity following coinoculation with 13 rhizobial genotypes. The results showed that: (i) Association with β-rhizobia is deep-rooted within the tribe Mimoseae, but not exclusive, since α-rhizobia were also found in nodules of some species; (ii) Vertical transfer was the main mechanism maintaining symbiotic genes within Burkholderia species; (iii) Diverse symbiotic behaviours were found in Burkholderia, with some genotypes being specific to Piptadenia species, but with others having a large host range and being highly competitive, thus underlining that the local selection of bacterial genotypes can explain the diversity data. We conclude that co-evolution between the Mimoseae and Burkholderia is possible, with the appearance of nod genes in a Burkholderia ancestor followed by diversification at c. 30 mya, which is compatible with speciation in the Mimoseae (c. 10-40 mya).

560A Bacterial succession in Populus rhizosphere in rhizoremediation
Shinjini Mukherjee1, Hyunseok Choi1, Timo Sipilä2, Jaak Truu1, Pertti Pulkkinen3, Kim Yrjälä1
1University of Helsinki, Finland, 2University of Tartu, Estonia, 3Finnish Forest Research Institute, Finland

Anthropogenic activities such as oil extraction, refinement and transportation have resulted in surface and near-subsurface soil contamination with petroleum hydrocarbons. Rhizoremediation is emerging as one of the most effective means by which plants can affect the remediation of organic contaminants. Hybrid poplars are promising candidates for rhizoremediation of petroleum hydrocarbon-contaminated soils in the boreal climate zone. Due to a continuous supply of organic compounds from the root tissues and other favorable physical and biological factors, rhizosphere...
Thursday 23 August

displays an increased microbial abundance and activity. Oil pollution can be considered as a fundamental driver of secondary bacterial succession and the introduction of plant in the polluted soil is a major factor regulating succession. There is little experimental evidence of predictable patterns in microbial community structure or composition during rhizoremediation.

A rhizoremediation experiment is conducted with Aspen (Populus tremula) and hybrid aspen (Populus tremula x tremuloides) clones planted in soil from an accidental oil spill site. For monitoring of the degradation process chemical analysis of hydrocarbon fractions, most probable number (MPN) of oil degraders and qPCR of catabolic genes in the rhizosphere was conducted. The dynamics of structural and functional bacterial communities over two growing seasons were analyzed with RFLP and T-RFLP fingerprinting. Microbial diversity associated with rhizosphere was studied using a 16S rRNA 454 pyrosequencing approach.

Analyses of petroleum hydrocarbon content after each growing season showed higher degradation rates in the rhizosphere as compared to the un-vegetated soil. The extradiol dioxygenase gene copy numbers were also higher in the rhizosphere throughout the growing seasons indicating a higher abundance of PAH degraders. A rhizosphere effect in the Populus rhizosphere was observed as an altered diversity and structure of extradiol dioxygenase genes as compared to the bulk soil. Treatment specific and seasonal variations in the structural and functional (extradiol dioxygenase) gene communities were shown.

This study recognized seasonal variation in microbial community structures and identified potential degraders in polluted soils. Analyses of the co-occurrence patterns of phylotypes and correlation of these patterns with specific factors gave an insight to community structure-function relationships in the polluted rhizospheres.

561A  Evidence for presence and dehalogenation activity of chloromethane-degrading bacteria in the Arabidopsis thaliana phyllosphere: strain isolation and molecular analysis
Thierry Nadalig*1, Farhan Ul Haque1, Sandro Roselli1, Hubert Schaller2, Françoise Bringel1, Stéphane Vuilleumier1
1University of Strasbourg, UMR 7156 CNRS, France, 2University of Strasbourg, UPR 2357 CNRS, France

Chloromethane, the most abundant volatile halocarbon in the atmosphere (600 ppt), is responsible for about 15% of chlorine-catalysed ozone destruction in the stratosphere. Chloromethane gas is produced by the biosphere, especially in the phyllosphere, the environmental compartment defined as the above-ground parts of vegetation, and in particular the surface of leaves, which hosts a rich bacterial flora. The bacterial pathway of chloromethane aerobic degradation was elucidated in Methylobacterium extorquens strain CM4, and chloromethane utilisation genes (cmu) were identified.

The aim of our works is to prove the presence of chloromethane-degrading bacteria on leaf surfaces of Arabidopsis thaliana by strain isolation and by detection of dehalogenation genes and quantification of their expression.

Enrichment cultures with A. thaliana leaves and chloromethane as sole carbon source allowed to obtain three chloromethane-degrading strains affiliated to the genus Hyphomicrobium basing on their 16S rRNA gene sequence and the presence of characteristic hyphae. The cmu genes of these isolates were amplified and sequenced using newly designed PCR primers, and the three isolates featured a consecutive and colinear cmuBCA gene arrangement similar to that of all previously characterised strains including Hyphomicrobium sp. MC1 whose genome sequence was recently determined, with the exception of Methylobacterium extorquens CM4 of known genome sequence.

Detection and quantification of chloromethane-degrading bacteria in the phyllosphere of A. thaliana was achieved by specific quantitative PCR and RT-PCR of the cmuA gene encoding the two-domain methyltransferase corrinoid protein of chloromethane dehalogenase. Three variants of A. thaliana (wild type, hol1 mutant without the HOL gene responsible for a major part of chloromethane production in A. thaliana and HOL overexpressor) were investigated. In addition, bacterial diversity of the phyllosphere
of these different variants was analysed by tag-based pyrosequencing of 16S ribosomal RNA and cmuA genes.

The results obtained provide evidence for the presence and dehalogenation activity of chloromethane-degrading bacteria on *A. thaliana* leaves, and suggest a link between expression of HOL by *A. thaliana*, bacterial expression of cmuA, and bacterial diversity in the phyllosphere.

**562A  Phenazine-1-carboxylic acid production by *Pseudomonas* spp. LBUM223 contributes to repressing thaxtomin A biosynthesis in the pathogen *Streptomyces scabies* under soil conditions**

Amy Novinscak¹, Tanya Arseneault¹, Claudia Goyer², Martin Filion¹
¹Université de Moncton, Canada, ²Agriculture and Agri-Food Canada, Canada

Previous experiments have revealed that *Pseudomonas* spp. LBUM223, which produces the antibiotic phenazine-1-carboxylic acid (PCA), is able to significantly reduce the development of common scab symptoms on potato tubers. This disease is caused by the bacterial pathogen *Streptomyces scabies*. *In vitro* studies have also shown that LBUM223 is able to reduce the growth and the production of the phytotoxin thaxtomin A by the pathogen, which is required for pathogenesis. A mutant of LBUM223, incapable of producing PCA, was not able to trigger these responses or to reduce disease symptoms. In order to better understand the impact of PCA production by LBUM223 on the biocontrol of *S. scabies* under soil conditions, we characterized the population dynamics of *S. scabies* in the rhizosphere and geocaulosphere of potato in soil, as well as the impact of PCA production on the expression of txtA, involved in thaxtomin biosynthesis in *S. scabies*. Potato plants were grown in controlled conditions and inoculated with different treatment combinations of *S. scabies* and LBUM223 (wild type and mutant) and harvested after 5, 10 and 15 weeks. Geocaulosphere and rhizosphere soils were collected and submitted to DNA and RNA isolation. Nucleic acids were then analyzed by qPCR and qRT-PCR using txtA specific primers and a TaqMan probe to quantify the population dynamics and gene expression leading to thaxtomin A production in *S. scabies*. Results showed that similar amounts of *S. scabies* (approx. $10^7-10^8$ gram of soil) were detected in the rhizosphere and geocaulosphere at all harvesting dates. Although no change in *S. scabies* population was observed in the rhizosphere in the presence of LBUM223 (wild type or mutant), in the geocaulosphere, the population of *S. scabies* was significantly increased when LBUM223 (wild type or mutant) was present. At all harvesting dates, txtA was expressed in both the rhizosphere and geocaulosphere soils. While no significant changes were detected in the rhizosphere, inoculation of potato plants with PCA-producing LBUM223 significantly repressed the expression of txtA in *S. scabies* in the geocaulosphere, which was not the case for the non-PCA producing mutant. These results suggest that antibiosis by LBUM223 does not play a central role in the biocontrol of *S. scabies* under soil condition, but instead, the alteration of txtA gene expression leading to a reduction in thaxtomin A production in soil is more likely involved in the biocontrol of *S. scabies* by PCA-producing LBUM223.

**563A  Development of the root microbiome: from seed to root systems, and emphasis on characteristics of plant-beneficial bacterial communities**

Maya Ofek¹, Dror Minz², Yitzhak Hadar³
¹Agricultural Research Organization of Israel, Israel, ²Institute of Soil, Water and Environmental Sciences, Agricultural Research Organization, Israel, ³The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Israel

The outcome of plant-microbe and microbe-microbe interactions at the soil-root interface can be detrimental or beneficial towards plant health and development, and ultimately affect yield. In agrosystems, compost amendments are used for improvement of plant nutrition and in order to promote a plant-beneficial root microbiome. Our aim was to follow the development of root microbiome from early stages of seed germination to a fully developed root system. We have examined the development and characteristics of a plant-beneficial (disease suppressive and growth promoting) root bacterial community. Those were compared to a root community developing in a poor-quality disease conducive plant growth medium. A simple clear-cut model was used: cucumber (*Cucumis sativus*) seeds were germinated and grown in perlite based potting medium, under controlled conditions. This basic medium was modified by addition of compost. Several growth stages were examined: seed germination, extension of primary root, first leaf and vine tip-over. Fluorescence in situ
hybridization, quantitative real-time PCR and high-throughput sequencing were used to determine spatial organization, size, structure and composition of bacterial communities.

A defined succession process was outlined: seed colonization by bacteria seems to result mainly from passive encounter between bacteria and the germinating seed, conveyed by imbibed soil solution. In accordance, the seed-associated bacterial community composition directly reflected that of the germination medium and was characterized by low dominance. Furthermore, the size and richness of the seed associated bacterial community were clearly determined by the community in the germination medium. Evident selection commenced with the extension of the primary root. At this stage, the bacterial community was dominated by few copiotrophic species, belonging to b- and g-proteobacteria (predominantly Massilia, Pseudomonas and Cellvibrio), which produced high biomass and formed dense biofilms on the root. Development and maturation of the root system was followed by reduction in bacterial density, reduction of species richness, but also increased evenness. The relative abundance of oligotrophic, slow growing groups (members of Rhizobiaceae, Chloroflexi, Verrucomicrobia and Actinobacteria) gradually increased. Plant-beneficial communities were distinguished by pronounced acceleration of the succession process delineated: the switch from copiotrophs to oligotrophs dominated community occurred much more rapidly.

In conclusion, a successive process of root microbiome development was featured. Beyond shifts in composition, plant-beneficial soil treatment was reflected in the rate and magnitude of bacterial community processes.

564A  Baseline survey of the anatomical microbial ecology of an important food plant: Solanum lycopersicum
Andrea Ottesen*, Antonio Pena, James Robert White, Cong Li, James Pettengill, Sarah Allard, Steven Rideout, Thomas Hill, Marc Allard, Errol Strain, Rob Knight, Eric Brown
1FDA, USA, 2University of Colorado, USA, 3University of Maryland School of Medicine, USA, 4Virginia Polytechnic Institute and State University, USA

Research to improve the understanding of microbiological risks associated with fresh fruits and vegetables has examined many points in the farm to consumer continuum. A data gap exists however at an important baseline - the anatomical microbial ecology associated with food plants themselves. Using culture independent methods, we examined microbial species associated with tomato organs; leaves, stems, roots, flowers and tomatoes from Virginia grown tomato variety; Solanum lycopersicum (BHN602), to provide baseline data to describe "native" bacterial microflora in for diverse anatomical parts of Virginia grown tomatoes. Tomatoes have been implicated in Salmonella illness outbreaks numerous times over the past 15 years. Whether or not there is something distinctive about tomatoes or their growing environments that facilitates persistence of Salmonella is still unknown. Plants were taken from a site in close proximity to commercial farms, which have been implicated in FDA trace-back investigations following tomato-Salmonella illness outbreaks. DNA was extracted from a wash of plant parts and described using 16S and 18S rRNA gene amplicons as well as shotgun sequenced metagenomes of epiphytic microflora.

Unique bacterial phylotypes of Microvirga, Pseudomonas, Sphingomonas, Brachybacterium, Rhizobiales, Paracoccus, Chryseomonas and Microbacterium were found associated with fruits and flowers of tomato plants. Many unique phylotypes were also observed in root samples. Most frequently observed bacterial phyla across aerial plant regions were members of Proteobacteria - such as Pseudomonas and Xanthomonas. Sooty molds such as Taphrina (Capnodiales) were prevalent across aerial samples - while Phoma and Hypocrean were well represented in roots. This work demonstrates the utility of metagenomic methods to provide valuable baseline ecological data pertaining to pre- and post harvest risks associated with important food plants. Significantly different communities were observed in different tomato plant organs and a gradient of compositional similarity could be correlated to the distance of the plant part from the soil. Future comparisons to additional bio-geographical datasets of Solanum lycopersicum microflora will identify whether or not a "core" microbiome can be ascribed to tomato plants and how if at all - that flora may play a role in the persistence of human pathogens such as Salmonella in tomato crops.
Many phyllosphere fungi were proven to have biocontrol activity which can lead to industrial applications. Because the nutrient is limited on the leave surface, colonization of nonpathogenic fungi can effectively inhibit the growth of pathogenic ones. In this study, we investigated the fungal diversities and abundance in the phyllosphere at the different growth stages of Bt cotton strains SGK321 and XP188, using strains SHIYUAN321 and JM20 as controls. Enzyme-linked immunosorbent assay (ELISA) and 454 sequencing were employed for the shift of fungal diversity and abundance and changes in plant Cry1Ac protein content. Shannon diversity index showed that the phyllosphere fungal diversities of the Bt cotton SGK321 were less complex than controls at the budding stage and blossoming and boll-forming stage, except for the seeding stage and boll opening stage. However, there are no obvious decrease of phyllosphere fungal diversities of the Bt cotton XP188 at seeding stage, obvious increase was observed at budding stage, blossoming and boll-forming stage and boll opening stage. Abundance index showed that the fungal aboundance of Bt cotton SGK321 phyllosphere were lower than controls at the blossom and boll-forming stage and boll opening stage, but higher than his control at the seeding stage and budding stage. For Bt cotton XP188, the phyllosphere fungal abundance were higher than controls at most of stages, except for a slight decrease in boll opening stage. Both the abundance index and diversity index showed the same trend of the fungal community shifts. ELISA showed that the concentrations of Cry1Ac protein in the leaves of Bt cotton strains reached the highest point at the seeding stage, decreased to the lower point at the budding stage, then increased to the higher point at the blossoming and boll-forming stage, decreased to the lowest point at the boll opening stage. There are no obvious direct relationship between the expression of Bt protein and the variation on phyllosphere fungal community in Bt cotton. In a summary, the shift of development stage of cotton was the key factor for fungal community diversity and abundance rather than the expression of Cry1Ac protein in transgenic cotton.

Temperature and climate affect the endophytes community in grapevine

Science has just started understanding how the environment drives the composition of microbial communities. Endophytes, as host-associated microbes, respond to environmental stimuli in a host-mediated fashion. To study how temperature and climate may affect endophytic microbial communities we studied grapevine-associated microbial populations using a cultivation independent approach.

Grafted cuttings were used to analyse how temperature affects microbial endophytic communities in a controlled environment. The composition of microbial endophytes in the field was assessed by surveying potted plants at different altitudes representing diverse climatic conditions. Seasonal fluctuations in the microbial endophytes were also considered by sampling test plants at different times throughout the year.

We adopted a DNA-based, cultivation-independent approach to the analysis of microbial populations variability in the conditions considered in this study. The analysis of DNA amplified by PCR involved the use of both Automated Ribosomal Intergenic Spacer Analysis (ARISA) and Roche 454 GS FLX+ technology.

Influence of Bacillus amyloliquefaciens FZB42 on the rhizosphere bacterial community of field grown lettuce (Lactuca sativa)

Bacillus amyloliquefaciens FZB42 is a widely used commercial phytostimulant producing several secondary metabolites having strong antifungal and antibacterial activities. The biological control
activity of FZB42 is apparently based on these secondary metabolites, which enable the bacterium to successfully colonize the rhizosphere habitat. A field study was conducted with lettuce plants grown in a field showing natural infestation with the pathogenic fungus *Rhizoctonia solani*. Two different treatments were examined in the study, namely plants inoculated with *B. amyloliquefaciens* FZB42 versus uninoculated controls in natural field conditions and in the presence of enhanced fungal pathogen *R. solani*. Plants were harvested after 6 weeks and a significant disease suppressive effect was found by the application of FZB42. An increased intensity of infestation was recorded in variants with additional inoculation of *R. solani*. To study the influence of application of *Bacillus amyloliquefaciens* FZB42 on the rhizosphere microbial community, samples were collected from the field 2 weeks and 5 weeks after planting. The 16S rRNA gene based DNA fingerprinting method terminal restriction fragment length polymorphism (T-RFLP) was used to generate community patterns using the restriction endonuclease MspI. Raw data were processed and analysed using the online software T-REX. The data were subjected to several quality control procedures and the analysis of data matrices used the additive main effect and multiplicative interaction (AMMI) model. The T-RFLP pattern as revealed by the ordination results of the AMMI showed that the T-RFs respond differentially to the treatments and change with time. There was as such no major effect of the inoculation of *B. amyloliquefaciens* FZB42 on the response of microbial community profiles as it does not show a major source of variation in the pattern. The presence of the enhanced fungal pathogen however has an influence on the response of microbial community profiles. This was also revealed by other ordination methods like NMDS and was statistically verified. The temporal change in the community profile as well as its response to presence of enhanced *R. solani* was reflected by the T-RFs and the Interaction effects. Thus, *B. amyloliquefaciens* FZB42 is able to control *R. solani* infestation in field grown salad without showing measurable effects on the rhizosphere bacterial community.

567A **Molecular characterization of the interaction between antimicrobial metabolite producing *Pseudomonas* spp. and *Clavibacter michiganensis* subsp. *michiganensis* in tomato**
Mélanie M Paulin*, Carine Lanteigne, Martin Filion
*Université de Moncton, Canada*

Bacterial canker of tomato caused by the Gram-positive bacterium *Clavibacter michiganensis* (Cmm) is one of the most important bacterial disease affecting tomato crops worldwide. The molecular mechanisms governing Cmm pathogenicity and virulence are not well characterized but a few genes key to disease development have been identified. *celA* encodes a secreted cellulase and is located on plasmid pCM1 while *pat-1*, found on plasmid pCM2, encodes a putative serine protease. Expression of both genes appears essential for pathogenesis. As no reliable classical control method exists for Cmm, biological control using antimicrobial metabolite-producing antagonistic organisms has been proposed as an alternative. In previous experiments, we have demonstrated that the antimicrobial metabolite-producing *Pseudomonas* sp. LBUM300 can control the development of bacterial canker of tomato and reduce Cmm populations under soil conditions. *Pseudomonas* sp. LBUM300 is known to produce the antimicrobial compounds 2,4-diacetylphloroglucinol (DAPG) and hydrogen cyanide (HCN) and experimental trials *in vitro* and *in planta* suggest that these compounds are involved in disease suppression. The objective in this study was therefore to investigate if *Pseudomonas* sp. LBUM300 and/or its capacity to produce DAPG and HCN can alter the transcriptional activity of the *celA* and *pat-1* genes in planta during interactions between the pathogen and wild-type *Pseudomonas* sp. LBUM300, as well as with recently developed isogenic mutant strains of *Pseudomonas* sp. LBUM300 incapable of producing DAPG (*phiD*) or HCN (*hcnC*). Tomato seeds (*Solanum lycopersicum* cv. Scotia) were germinated on watered compressed peat disks in growth chambers before being transplanted into pots filled with non-sterile agricultural soil. During transplantation, plantlets were inoculated with different combinations of *Pseudomonas* spp. or pathogen treatments as follows: Cmm, Cmm+LBUM300 wild-type, Cmm+LBUM300*phiD*, Cmm+LBUM300*hcnC*, no bacteria control. Plants were destructively harvested at 24h, 72h, 120h, 168h, 216h and 264h post-inoculation and rhizosphere soil was also sampled. Novel TaqMan quantitative reverse transcriptase PCR (RT-qPCR) assays were developed to follow in situ time-course expression of *celA* and *pat-1* genes in *Cmm*. Expression of *celA* and *pat-1* could not be detected in the rhizosphere soil of any treatment but results indicated changes in transcriptional patterns of *celA* and *pat-1* genes *in planta* during the interaction with *Pseudomonas* sp. LBUM300 producing DAPG and HCN. These results suggest that antibiosis not only can have a direct effect on Cmm’s survival but also indirectly can reduce disease development by altering the transcriptional activity of key genes in the pathogen.

PS27 – Plant-Microbe Interactions
568A  An α-tubulin of the ectomycorrhizal fungus Hebeloma cylindrosporum is essential for symbiosis establishment

Aurélie Perrin*1, Clément Pellegrin1, Jeanne Doré1, Cindy Dieryckx2, Vincent Girard2, Jean Phillippe Combier3, Roland Marmeisse1, Gilles Gay1

1Université Claude Bernard LYON1 UMR 5557, France, 2Université Claude Bernard LYON1 UMR 5240, France, 3Universtié Paul Sabatier UMR CNRS 5546, France

In all terrestrial ecosystems, plants live in close interaction with numerous fungi. At the individual level, the interaction has a negative or positive effect on the plant fitness depending on whether the fungus is pathogenic or mutualistic. The establishment of such interactions is based on a tightly regulated reorientation of both partner metabolisms. We developed an original approach to elucidate how mycorrhizal fungi interact with their host plants. An insertional mutant library of the ectomycorrhizal fungus Hebeloma cylindrosporum was generated using Agrobacterium tumefaciens. It was screened for symbiosis defect thus allowing the identification of myc- mutants unable to achieve symbiotic association with Pinus pinaster. One of these mutants harbours an insertion of mutagenic T-DNA in the promoter of Hctubα2 which encodes one of the two α-tubulins of H. cylindrosporum. As a result, the expression of this α-tubulin is reduced by six fold.

Ectomycorrhiza formation relies on a dramatic reorientation of hyphal growth polarity, leading to the differentiation of symbiotic structures. The studied mutant is impaired in its ability to differentiate the Hartig net which represents the interface where trophic exchanges between symbionts take place. As Hartig net formation is known to involve microtubule remodelling, we hypothesized that Hctubα2 plays a specific role in microtubule dynamics. The fact that hyphal growth in pure culture is not affected by the mutation suggests that the two α-tubulins could be redundant in free living stage, but not upon symbiosis establishment.

This was confirmed by RT-qPCR studies showing that, in the absence of host plant, the two α-tubulins are expressed at a similar level whereas they are differentially regulated during symbiosis establishment. Indeed, Hctubα1 is up-regulated at pre-infectious stage and upon hyphal adhesion on host roots whereas Hctubα2 is up-regulated during Hartig net differentiation. Thus, a high proportion of Hctub2 in microtubules could decrease their stability, thus making them more dynamic. We further investigated this hypothesis using a proteomics approach. Under pure culture conditions, Hctub1 was much more degraded than Hctub2. The down-expression of Hctubα2 in the mutant strain resulted in a reduction of both tubulins degradation. A semi-quantitative proteome sequencing showed that the whole tubulin homeostasis was indeed affected. Altogether, these results show that Hctubα2 is essential for ectomycorrhizal symbiosis establishment. It probably acts in close interaction with Hctubα1, the balance between both being crucial. Present results also show that this balance is to be considered in the general framework of tubulin homeostasis.

569A  Genotypic effects of Norway spruce host trees on the associated communities of ectomycorrhizal and needle endophytic fungi

Tiina Rajala*, Sannakajsa Velmala, Matti Haapanen, Taina Pennanen

Finnish Forest Research Institute, Finland

Trees are inhabited by diverse communities of ectomycorrhizal and endophytic fungi. The host tree is suggested to control the associations, but there is little information about the role of host genotype on fungal community structures, which may affect the host tree vitality and performance.

Effects of host genotype on ectomycorrhizal and needle endophytic fungal community structure were studied in a greenhouse experiment comprising rooted cuttings of Norway spruce (Picea abies). Cuttings from 55 spruce clones were inoculated with a mix of vegetative hyphae of five ectomycorrhizal fungal species (Laccaria sp., Amphinema byssoides, Piloderma sp. Cadophora finlandia, Paxillus involutus). Ectomycorrhizal community composition was analyzed using the internal transcribed spacer region of ribosomal DNA that was directly PCR-amplified from single root tips. Ectomycorrhizal species were identified by means of species-specific restriction profiles. Needle endophytes were analyzed using DNA directly isolated from the fresh surface-sterilized needles from 30 clones. The abundance of endophytes was estimated by means of quantitative PCR. Community structures were determined through denaturing gradient gel electrophoresis coupled with sequencing. Finally, multiple root and shoot characteristics were measured.
There was a significant host genotype effect on ectomycorrhizal formation. We found differences in the ectomycorrhizal fungal community structure between spruce clones in their early phase of development. The community composition and the abundance of endophytic fungi was also affected by the host genotype. Spatial distance between the cuttings and size of the needles also affected endophytic fungal communities. The strongest genetic host-tree effect was associated with short roots, i.e., the root-mycorrhizal interphase.

The results from this study suggest that host-genotypes influence the associated ectomycorrhizal and endophytic fungal communities. However, a large part of the variation in fungal communities is assigned to environmental factors thus hindering practical applications (early selection) in tree breeding. The heritability of short root density was moderate (H² = 0.41) and the highest of all the traits measured on the cuttings. We suggest that the genetic component determining root growth and short root formation is significant for the performance of young trees as these traits drive the formation of the below-ground symbiotic interactions. Thus, short root density could be a useful early selection criteria in Norway spruce breeding programmes.

**570A Enterobacter radicincitans sp. nov. DSM16656 a novel and efficient PGPB strain**

Silke Ruppel*, Katja Witzel, Anita Brock, Beatrice Berger  
Leibniz-Institute of Vegetable and Ornamental Crops, Germany

Endophytic plant growth promoting bacteria (PGPB) may have significant impact on both the plant physiology and the composition of the plant microbiome.

Many different bacterial species proved to induce these plant growth promoting effects on a variety of plant species. However, the regulatory pathways of the beneficial effects are still questionable. We described the strain Enterobacter radicincitans sp. nov. (DSM 16656) as a new species of the genus Enterobacter, which was isolated from the phyllosphere of winter wheat under temperate conditions. Growth promotion of root and shoot, along with increased yield, was conferred by inoculation of different crop and model plant species. Using a species specific molecular probe, quantitative real-time PCR and online emission fingerprinting at CLSM after in situ hybridization, we could demonstrate the plant colonization behaviour and its high competitiveness against the native plant microbiome. Up to 20% of the total bacterial population was earned by the inoculated strain within Brassica oleracea root and shoot tissues. Gene expression studies in tomato revealed different modes of plant growth promoting actions depending on the nitrogen availability to the plant. For example, transcripts of the LePR1, a pathogen-related marker gene, were more elevated in low nitrogen grown tomato plants than in high nitrogen grown plants when they were inoculated with the PGPB strain E. radicincitans.

Our comprehensive studies using this E. radicincitans strain in model, greenhouse and field experiments give first indications of altered modes of plant growth promoting actions in the plant bacterial association depending on the nutritional status of the plant. We suggest that plant hormone signalling networks are involved in the fine tuning of the endophytic colonization behaviour and the bacterial gene expression depends significantly on the plants nitrogen availability.

**570B The antifungal chitinases of soil isolated Bacillus sp. and its potential role in biological control**

Stefan Russel*1, Urszula Jankiewicz2  
1Swedspan Polska Sp.z o.o., Poland, 2Warsaw University for Life Science, Poland

Several diseases and damage caused by mold fungi and nematodes affect plant crops, resulting in losses and decreasing the quality and safety of agricultural products. The affected plantations of cultivated plants not only make economic losses but they are also a serious hazard for health of their consumers. The ability to improve the health of plants by the rhizosphere bacteria is a well documented fact in literature A type of direct interactions is associated with the production of lytic enzymes or substances that directly restrict the organisms that have a detrimental effect on the plants. Currently of great importance in biocontrol have bacterial chitinases. Chitinolytic enzymes are able to lyse the cell wall of many fungi.

The aim of the present study was to determine the potential anti-fungal activity of chitinases produced by soil isolated Bacillus sp.. The investigated strain was identified on the base of morphological and
biochemical characteristics. Identification of the strain was confirmed through the analysis of the 16S rRNA gene sequence.

Chitinolytic activity of Bacillus sp. strain was determined using colloidal chitin as a substrate. Increase in the amount of reducing sugars in the reaction mixture was measured using the MBTH method. Bacterial cultures were grown and maintained in medium composed of (g/liter): KH₂PO₄, 3; K₂HPO₄, 3; MgSO₄, 0.5; NaCl, 2; FeCl₃, 0.005; glucose, 5; colloidal chitin, 1; the pH of the medium was adjusted to 7.0. The bacterial culture was incubated for 4 days with shaking (120 rpm) at 28°C. Following incubation, the bacterial culture was centrifuged and the supernatant was filtered and used for determination of fungal lytic activity.

The chitinolytic crude supernatant was tested for lysis activity against the mycelium of the following fungal strains: Alternaria alternate, Fusarium oxysporum, F. solani, F. culmorum and Botrytis cinerea. Antifungal activity of Bacillus sp. chitinases was observed against F. culmorum, F. solani and A. alternate. The mycelium of Botrytis cinerea and F. oxysporum was not lysed.

Isolated from a soil strain of Bacillus sp synthesizes two forms of chitinases. These enzymes exhibited mycelium lysis activity against phytopathogenic mold. Chitinases produced by studied microorganism can be used for production natural biofungicides. Therefore, they may become tools for an alternative and environmentally friendly strategy to control the growth of organisms harmful for cultivated plants.

571A Archaea associated with Populus and its surrounding soil and trees
Migun Shakya⁎1, Christopher W. Schadt1, Mircea Podar1, Zamin Yang2, Marilyn Kerley2, Mitchel J. Doktycz2, Gerald Tuskan2
1University of Tennessee / Oak Ridge National Lab, USA, 2Oak Ridge National Lab, USA

Archaea are abundant in different soil and rhizosphere environments and are known to play a central role in nutrient cycling. Since the discovery of ammonia-oxidizing archaea, there have been many studies that described its diversity in soils and rhizosphere across the world. Moreover, it has been found that ammonia-oxidizing archaeas are more abundant in rhizosphere of certain plants than its corresponding bulk soil. However, mechanisms of selection of archaea to specialized environments like rhizosphere are poorly understood and studies that compare archaeal communities in rhizosphere and soil remain rare. One of the reasons behind this is the lack of robust model systems. Populus, a genetically diverse, ecologically widespread riparian species, with cellulosic biofuel potential and complete genome sequence, is a robust system to study the interactions between plants and microorganisms as these trees are host to a wide variety of symbiotic microbial associations within (endophytes and mycorrhizae) and area surrounding their roots (rhizosphere). We collected bulk soil and rhizosphere from six Populus deltoides and additional three non-Populus trees around it from the banks of Caney Fork River in Tennessee. We used barcoded pyrosequencing of 16S rRNA gene and amoA gene to describe and compare archaeal communities in rhizosphere, endophyte, and its corresponding bulk soil along with chemical and physical properties of tree and its environment. We also compared the abundance of ammonia-oxidizing archaeas using quantitative real-time polymerase chain reaction. As observed in previous studies, we detected high archaeal and amoA diversity in both soil and rhizosphere samples across all trees. Our data also indicated that archaeal community structure were different from a tree type to another, and from their corresponding soil. Similar to previous study in rice paddy soils, ammonia-oxidizing archaeas were more abundant in the Populus rhizosphere than in bulk soil. Although rare, we detected archaea in the endosphere of Populus. These results highlight the effects of specific soil and plant niches within the overall soil environment on maintaining and assembling archaeal communities.

571B Microbial mediation of litter decomposition in soil: the role of plant roots
Shengjing Shi⁎1, Donald Herman1, Katherine Louie2, Ben Bowen2, Trent Northen2, Eoin L. Brodie2, Mary Firestone1
1University of California, Berkeley, USA, 2Lawrence Berkeley National Laboratory, USA

Soil organic carbon (C) is the largest pool of terrestrial C, with fluxes through the C pool mediated by soil microorganisms and modulated by their interactions with plant roots. Root exudates are important mediators of this plant-microbial interaction. The presence of live roots can stimulate or suppress rates of litter decomposition by mechanisms that are poorly understood. Our studies use Avena barbata, a
common California grass, as a model system to investigate the role of plant roots in microbial mediation of litter decomposition.

We examined the effects of living A. barbata roots on $^{13}$C-labeled root litter mineralization in a California annual grassland soil over two growing seasons. Mineralization rates of root litter (total CO$_2$ and $^{13}$CO$_2$ fluxes) in the presence of plants were determined and compared to incubations without. $^{13}$CO$_2$ flux from soils without plants was significantly higher than from those with plants during the early litter decomposition stage, suggesting an initial negative effect of live roots on litter mineralization. However, the response changed after 10 weeks. During a 3-month dry season and the beginning of 2nd growing season, the $^{13}$CO$_2$ flux rates from the planted treatment were slightly higher than those without plants.

Root exudation may be one of the mechanisms that plants impact on litter decomposition. To further investigate this, profiles of A. barbata root exudates were collected from hydroponically grown plants and characterized using gas chromatography-mass spectrometry (GC-MS) and a recently-developed, spatially-explicit mass spectrometry technique, nanostructure-initiator mass spectrometry (NIMS). In addition, A.barbata seedlings grown in soil microcosms were pulse labeled with 99 atom% $^{13}$CO$_2$ and the exudates were sorbed to an initiator-treated silicon wafer. Organic compounds on the wafer were then analyzed by NIMS. A range of compounds were detected in exudate samples by GC-MS, including carbohydrates (for example glucose, fructose, galactose), low molecular weight organic acids (for example oxalic, malic, maleic acids), amino acids and amides (for example lysine, serine, glycine), fatty acids (for example arachidic, lauric, oleic acids), sterols (for example cholesterol) and others (for example hydroxylamine, glycerol). Compounds with a range of masses (atomic mass units, AMUs) were detected by NIMS both on wafers and in exudates extracted from both hydroponic and soil systems. Thirty top major AMUs only present in exudate samples were selected for further identification.

Together, these data are being used to develop a model for the temporal sequence of live root impacts on litter decomposition in this plant-soil system that relates root exudation to the suppression or stimulation of macromolecular C decomposition.

**572A Microbial community analysis of potato-associated bacteria using both culture-independent and -dependent methods**

Nobutaka Someya*, Yuki Ohdaira Kobayashi1, Akira Kobayashi1, Tomohiro Morohoshi2, Tsukasa Ikeda2, Seishi Ikeda1

1Hokkaido Agricultural Research Center, National Agriculture and Food Research Organization, Japan, 2Utsunomiya University, Japan

Potato is one of the world’s most important crops. We examined community structures of potato-associated bacteria by using both culture-independent and -dependent methods. At the flowering stage, plant (Solanum tuberosum L. cv. Matilda) tissues (leaves, stems, roots and tubers) were sampled, and clone libraries for 16S rRNA genes were constructed. Library coverage was 88.0, 94.8, 55.6 and 83.9% for leaves, stems, roots and tubers, respectively. The isolate collections were also made using the same plant samples by isolating potato-associated bacteria on R2A medium agar plates. Library coverage of collections was 98.5, 97.1, 87.4 and 87.3% for leaves, stems, roots and tubers, respectively. As a result, the most dominant phylum was shown to be Proteobacteria throughout all libraries (62.0 to 89.7%) and isolate collections (57.7 to 72.9%). Among the Proteobacteria, Alphaproteobacteria was the most dominant and was stably present in all libraries (32.5 to 50.0%) and collections (38.2 to 62.4% for collections). Gammaproteobacteria was also found to be the second dominant taxon in three libraries (leaf-, shoot- and root-clone libraries) and their abundance was comparable to Alphaproteobacteria in the leaf- and shoot-clone libraries. The phylogenetic analyses at lower taxonomic levels revealed that two genera Rhizobium and Sphingomonas were shown to be dominantly and stably present in all libraries (8.9 to 29.9% and 1.8 to 8.1%, respectively). The Genera Methylobacterium, Pseudomonas, Pantoea and Arthrobacter were found to be dominant taxa in the libraries for aboveground tissues (leaves and shoots). Meanwhile, the genera Phyllobacterium, Streptomyces, Paenibacillus and Bacillus were found to be dominant taxa in the libraries for belowground tissues (roots and tubers). The differences of phylogenetic compositions between the libraries and the collections were the decreasing of abundances for Rhizobium and Gammaproteobacteria in the collections compared to those in the libraries.
Conversely, the high abundance of *Methylobacterium*, Betaproteobacteria (Polaromonas, Variorvax and Pelomonas), Microbacterium, and Bacteroidetes (Pedobacter, Chitinophaga and Lacibacter) in the collections were observed compared to those in the libraries. Among the isolate collections, clustering analyses identified the OTU (operational taxonomic unit) BA13 was detected from only the isolate collection of tuber sample (4.7%). The blast and phylogenetic analyses revealed that this cultivable OTU is distantly related from known Chitinophagaceae bacteria and could represent a novel genus or family in the Sphingobacterialae. These results provide comprehensive information for the ecology of symbiotic bacteria in potato plants and would facilitate to develop more sophisticated management methods for utilizing beneficial microbes in agricultural environments.

573A  Growth enhancement of Aster spp. by Rhizobacteria for revegetation of barren lakeside area
Hong-Gyu Song*, Hyeok-Do Kwon
Kangwon National University, South Korea

Wild plants can be utilized as the pioneer species for the revegetation of barren lands. This study was conducted to examine the growth promotion of two species of Aster, common wild plants in S. Korea, by *Arthrobacter woluwensis* strain ED which was isolated from the rhizosphere of a wild plant Isachne globosa. The plant growth promoting capability of *A. woluwensis* ED was examined in a microcosm composed of soil collected from barren lakeside of Lake Paro in S. Korea. *A. woluwensis* ED applied at $10^6$ cells/g soil could improve the root length, shoot length, and dry weight of *Aster koraiensis* by 18.6, 22.8 and 26.2%, respectively compared to those of the uninoculated control. *A. woluwensis* ED could also improve the root length, shoot length, and dry weight of *Aster yomena* by 14.1, 11.2 and 31.8%, respectively compared to those of the uninoculated control. The plant growth promoting capability of *A. woluwensis* ED was also examined in a field study at barren lakeside of Lake Paro. In a gentle slope area, *A. woluwensis* ED enhanced the root and shoot length and dry weight of *A. koraiensis* by 27.2, 12.0 and 54.8%, respectively compared to those of the uninoculated control. It also improved the root length and dry weight of *A. yomena* by 22.5 and 22.8% respectively compared to the uninoculated control. In the case of a steep slope area, *A. woluwensis* ED promoted the root and shoot length and dry weight of *A. koraiensis* by 31.1, 10.2 and 33.9%, respectively compared to the uninoculated control. It also increased the root length of *A. yomena* by 17.1% compared to the control. These results suggest that *A. woluwensis* ED may be utilized as biofertilizer for the revegetation of barren lands including environmentally sensitive lakeside areas.

574A Symbiosis with *Mesorhizobium* sp. affects Zn accumulation patterns among four *Anthyllis vulneraria* subspecies
Souhir Soussou*, Stéphanie Mahieu2, Brigitte Brunel1, José Escarré2, Michel Lebrun1, Mohamed Banni3, Hamadi Boussetta2, Jean-Claude Cleynet-Marel4
1Montpellier SupAgro, UMR113, France, 2Centre d’Ecologie Fonctionnelle et Evolutive, CNRS, France, 3Institut Supérieur Agronomique de Chott Meriam, Tunisia, 4INRA, UMR113, France

The metallurgical industry uses heavy metals (lead, zinc, etcetera) present in veins that generate highly toxic tailings. To limit their dispersal by wind and water, the rubble mine can be stabilized by perennial vegetation. Leguminous plant species growing in severely heavy metal-polluted areas are of great interest because of their contribution to the improvement of soil fertility.

The aim of our study was to investigate the Zn accumulation in *Anthyllis vulneraria* – *Mesorhizobium* symbioses. To achieve our experiment, four subspecies of *A. vulneraria* were used: two from a contaminated soil (A.v. subsp. carpatica and A.v. subsp. boscii) and two from an uncontaminated soil (A.v. subsp. praepropera and A.v. subsp. vulneraria). Culture was conducted in hydroponics in the presence of 0, 250 and 1000 µM ZnSO4. They were either grown in presence of 3 mM KNO3 or either associated with one of two N2-fixing *Mesorhizobium* strains: STM2682 (non-metallicolous) or STM2683 (metallicolous (Vidal et al., 2009)).

Our data show that for different *Anthyllis vulneraria* subspecies exposed to 1000 µM Zn, the Zn concentration is higher in roots than in shoots and only the carpatica subspecies was able to accumulate a significant amount of Zn in shoots without damage. It appeared that, when exposed to 1000 µM Zn, the association between carpatica subspecies and the metallicolous or non-metallicolous *Mesorhizobium* caused drastic Zn content in both roots and shoots (up to 2.5-3 folds in shoots and 2.6
times in roots). Moreover, when the carpatica subspecies was nodulated by the metallicolous strain STM2683, a better tolerance to Zn was observed and this was confirmed by a high level of chlorophyll and absence of apparent phytotoxicity. By contrast, the association of boscii, praepropera and vulneraria subspecies with strain STM2683 was not able to increase in a significant way the Zn tolerance.

Following our experiment, we showed that the symbiosis of A. vulneraria subspecies carpatica with its symbiotic bacteria presents a new biological model for phytostabilization program.

It will be important in future to include the symbiosis of A. vulneraria subspecies carpatica with its symbiotic bacteria in the design of both research plans and applications, with the ultimate goal of increasing the efficiency of phytostabilization.

575A  Contribution of a biocontrol Pseudomonas strain to the total microbial load in bioaerosols during experimental seed inoculation

Ingvar Sundh*, Anna-Ida Johnsson Holmberg¹, Annika I. Nilsson¹, Petter Melin¹, Margareta Hökeberg², Anne Mette Madsen³

¹Swedish University of Agricultural Sciences, Sweden, ²Mase Laboratories, Sweden, ³National Research Centre for the Working Environment, Denmark

Bioaerosol formation during large-scale culturing, preparation and practical implementation of microorganisms for use in beneficial applications may cause increased exposure to microbes of workers or bystanders, and lead to spread of the microbe to the surrounding environment. In this study we used SCAR marker methodology and real-time PCR to quantify the presence of the biocontrol bacterium Pseudomonas brassicacearum MA250 (with effect against snow mould in cereals) in bioaerosols during experimental seed treatment with the bacterium in a model and a pilot-scale system. Bioaerosols were collected by air filtration and the abundance of MA250 was compared to background microbial communities on the seeds determined with quantitative PCR (qPCR) using group specific primers, plate counting, and indirectly with endotoxin analysis.

According to qPCR data obtained from the model system, Gram-negative bacteria, pseudomonads and strain MA250 increased with seed treatment/sampling time. The bacterial treatment generally gave up to ten-fold higher levels than controls for all three primer sets. In these samples, MA250 made up a major part of the total Gram-negative bacteria, whereas it was only very occasionally detected in control treatments. Plate counting for Gram-negative bacteria consistently gave 100-1000-fold lower levels than did qPCR, however many samples fell close to the detection limit. Nevertheless, a general trend was that the colony counts were 10-100 times higher for the bacterial than the control treatments. Endotoxin levels increased with treatment/sampling time in both bacterial treatments and controls, but with no clear effect of the MA250 addition. In contrast to the model set-up, qPCR and plate counts were below the detection limit in trials with the more realistic pilot-scale seed treater instrument, and endotoxin levels were also very low (below 6 EU m⁻³).

In conclusion, the qPCR data of the model set-up experiments where we provoked high bioaerosol concentrations, demonstrate that treatment with strain MA250 did result in increased total levels of Gram-negative bacteria/pseudomonads and hence potentially increased exposure of workers to microorganisms. We also conclude that the SCAR marker was necessary for reliable quantification of MA250 against the background of resident communities on the seeds. The plate counts seriously underestimated the microbial aerosol loads and could only register very general trends. Analysis of endotoxin was the most sensitive method for indicating presence of Gram-negative bacteria. In spite of the clear demonstration by the results from the model set-up, that treatment with a bacterium can result in increased bioaerosol loads of microbes, the low levels of all microbial parameters in the pilot set-up show that with normal precautions taken, it is unlikely that this type of seed treatment represents a human health concern.
575B Population dynamics and diversity of fungal communities in the rhizosphere soil of wild medicinal plants in the upper flood plains of Assam, North Eastern India
Sorokhaibam Sureshkumar Singh*, Chumi Khanikar
North Eastern Regional Institute of Science & Technology, India

Rhizosphere soils in the upper flood plains of Assam, North Eastern India were collected and analyzed to study the influence of four wild medicinal plants on population status and diversity of fungal communities for a period of four seasons between winter 2009 to summer 2011. Fungal colony forming units (CFUs) were counted and species diversity of soil fungi were analysed on triplicate plates of potato dextrose agar, Czapek Dox agar and malt extract agar under standard laboratory conditions. Highest fungal population was recorded in the rhizosphere soil of Asparagus racemosus (199 CFU/g soil) followed by Clerodendrum colebrookianum (185 CFU/g soil) and Tinospora cordifolia (180 CFU/g soil) during summer 2010 while the lowest population was recorded under the soil of Vitex negundo (97 CFU/g soil) during winter 2010. A total of 45 fungal species were recorded from the soil of A. racemosus, 37 species in the T. cordifolia during winter 2011, 34 species in the rhizosphere soil of C. colebrookianum during winter 2009 and a minimum of 24 fungal species were recorded under V. negundo rhizosphere during the summers of 2010 and 2011. Shannon-Weiner index of general diversity revealed higher fungal diversity in the rhizosphere soils of A. racemosus (1.59) while the lowest was recorded in the rhizosphere soils of V. negundo (1.05). The Simpson’s index of species dominance revealed highest value in the rhizosphere soil of V. negundo (0.56) while the lowest was recorded under C. colebrookianum and A. racemosus (0.21) respectively. Altogether 134 species of soil fungi under 23 genera under Basidiomycota, Zygomycota and Ascomycota groups were recorded from the rhizosphere soils of all the four wild medicinal plants. Fusarium with 34 species, Penicillium with 32 species, Aspergillus with 17 species and Trichoderma with 16 species were dominant genera recorded from the rhizosphere soils. There was statistically significant positive correlation (p=0.05 & p=0.01) between the fungal population and number of species in the rhizosphere soils of A. racemosus, C. colebrookianum and T. cordifolia, but no significant correlation was found in the soils of V. negundo. The total phosphorous content displayed significant positive correlation with fungal population and number of species in the rhizosphere soils of A. racemosus, C. colebrookianum and T. cordifolia during the two winter seasons. However, no significant correlation was recorded for fungal population or number of species with soil total phosphorous in the rhizosphere soil of V. negundo in all the seasons suggesting a possible role of soil phosphorous in growth and survival of fungal species in the soils of wild medicinal plants. It was concluded that A. racemosus influenced highest fungal population, number of species and species diversity while the V. negundo recorded with lowest population and species diversity index in the present study. Further investigation and analysis on the biochemical aspects of root exudates in the rhizosphere soil may help in establishing the functional relationship between soil microfungi and wild medicinal plants.

576A Plant surfaces select for ammonia oxidizing bacteria in alkaline aquatic environments
Rosalia Trias*, Aranžtazu García-Lledó, Ariadna Vilar-Sanz, Olaya Ruiz-Rueda, Sara Hallín, Lluis Bañeras

Institute of Aquatic Ecology. University of Girona, Spain, 2Swedish University of Agricultural Sciences, Sweden

Nitrogen is essential for plants and seaweeds. It is usually assimilated in the form of nitrate and requires two reduction steps to form ammonia. This fact, together with the oxygen release, makes the surface of plant tissues interesting hotspots for nitrification. The ammonia oxidation to nitrite is usually the rate limiting step for nitrification, and can be performed by the ammonia oxidizing bacteria (AOB) and archaea (AOA). The interaction between ammonia oxidizers and aquatic plants is not well understood, but previous reports have suggested that AOA could play an important role due to their dominance in comparison to AOB in the epiphyton and rhizosphere of freshwater macrophytes. However, the results of these reports have been limited to acidic and neutral environments, and to our knowledge, no information is available about the interaction between plants and ammonia oxidizers in alkaline environments.

We report the relative proportion of AOB and AOA amoA in the rhizosphere of aquatic macrophytes and the epiphyton of seaweeds in moderately alkaline environments. Sampled species included the seaweeds Osmundaria volubilis, Phyllophora crispa and Laminaria rodriguezii collected between 50 and 81 m depth around the Balearic Islands (Western Mediterranean Sea, water pH 8.1-8.3), and the
macrophytes *Phragmites australis*, *Ruppia* spp. and *Paspalum distichum* collected from nine coastal lagoons in Girona and Huelva (Spain, sediment pH 7.2-8.7; water pH 6.8-9.4).

In contrast to other marine environments, quantification of bacterial and archaeal 16S rRNA and *amoA* genes by quantitative PCR demonstrated that the macroalgae’s surfaces were dominated by bacterial *amoA* genes, with bacterial/archaeal *amoA* gene ratios varying from 4 to 100. As an average, the epiphytic AOB constituted 1% of the total bacterial community. Among emergent macrophytes, the relative abundances of AOB to AOA at the root surface were significantly higher than those found in the unvegetated sediment. Bacterial/archaeal *amoA* gene ratios increased from 0.02-0.4 in the sediments to 0.1-1.4 in the rhizosphere. These results show consistently the enrichment of ammonia oxidizing bacteria by different plant types in alkaline waters in a wide range of physicochemical conditions, although the plant effect was less evident among macrophytes in coastal lagoons compared to the epiphyte of marine macroalgae. We speculate that organic exudates produced by plants may act as a selection factor for mixotrophic nitrification. However, the mechanism remains elusive and effects of environmental variables, that is pH and oxygen release by the plant host, require further investigation.

577A  **Bacterial volatiles suppress growth of fungal pathogens**
Maaike van Agtmaal*1, Maria Hunscheid1, Angela Straathof2, Wietse de Boer1
1Department of Microbial Ecology, Netherlands Institute of Ecology, Netherlands, 2Department of Soil Quality, Wageningen University, Netherlands

Volatiles produced by soil bacteria did suppress fungal pathogens either by inhibiting growth or preventing spore germination. Results correlated with crop infection in bioassays

Bacterial volatiles might be components of soil suppressiveness. Previous studies showed differences in volatile profiles of soils with different degrees of fungistatic activity. In our experiments strong reduction of mycelial density of fungal pathogens by soil or extracted soil bacteria was found with a 2-side plate approach. Results from this experiment correlated with bioassays; increase in the number of disease symptoms and reduced root biomass was directly linked to reduced volatile suppression.

Future research is focusing on indentifying active compounds and the responsible microbes and genes. Furthermore, experiments will be set up to link volatile production to availability of organic resources to microbes e.g. sub fractions of soil organic matter. Understanding the relationships between plant pathogens, disease suppressive microbial populations, soil characteristics and bio-available substrates is essential to develop procedures for effective and consistent control.

578A  **Associations between bacteria and ectomycorrhizal roots investigated with high-throughput sequencing**
Unni Vik*1, Ramiro Logares2, Tor Carlsen3, Rakel Blaalid3, Ole Andreas Økstad4, Anne-Brit Kolstø5, Håvard Kauserud3
1University of Oslo, Norway, 2Institut de Ciències del Mar, Spain, 3University of Oslo/Department of Biology, Norway, 5University of Oslo/School of Pharmacy, Norway

The mycorrhizal symbiosis between fungi and plant roots are essential to plant growth and nutrient acquisition. However, this intimate partnership is also influenced third component, bacteria. Increasing evidence shows that bacteria-fungi interactions are more widespread than expected and that their dynamics may be important in the ectomycorrhizal symbiosis.

In this study we investigate the diversity of both fungi and bacteria associated with roots and analyze the taxonomic relationships in this prokaryote-eukaryote system. Our study system was the ectomycorrhizal microbiota of the arctic-alpine plant Bistorta vivipara, A total of 120 plants, as well as 64 soil samples, were collected from a 2 x 2 meter plot in an alpine area in Norway. The bacterial and fungal diversity in root and soil samples was analyzed using high-throughput 454 pyrosequencing of 16S (bacteria) and ITS (fungi) rDNA amplicons.

Filtering and subsequent clustering into operational taxonomic units were performed using the Qiime pipeline applying strict parameters to avoid overestimating the fungal and bacterial species richness. A
clustering level of 97% was used for both fungal and bacterial amplicons. Preliminary results indicate 6948 bacterial OTUs, with a dominance of the phyla Acidobacteria, Actinobacteria, Chloroflexi, Planctomycetes and Proteobacteria. The ectomycorrhizal basidiomycete fungi from the orders Agaricales, Thelephorales, and Sebacinales dominated the fungal OTUs.

We found clear differences between the bacterial communities in the root systems and soil samples. OTUs present in root systems differ from OTUs in soil samples. The diversity in soil samples was higher for both fungi and bacteria. The lower diversity of microbes and the taxonomical composition of these in the root systems, compared to the soil samples, indicate that the symbiosis is specific between the plant and certain OTUs of fungi and bacteria.

578B  First dip into rhizosphere microbial community transcriptome

Milana Voronov-Goldman1, Maya Ofek1, Noa Sela1, Yitzhak Hadar2, Dror Minz1
1Volcani center, Israel, 2Faculty of Agriculture, Hebrew University, Israel

High-resolution determination of environmental microbial community structure and function are highly coveted aims. We have tackled these questions in one of the most dynamic and complex environments- the plant rhizosphere. Root activity changes the chemical and physical properties of its surrounding soil. In response, the microbial community at the root-soil interface is re-structured and modulates root physiology, development and plant health. Therefore, revealing the structure and function of microbial communities is crucial for understanding this natural environment as well as management applications in agriculture.

Wheat (Triticum spp.) and Cucumber (Cucumis sativus) were selected as model plants, representing monocots and dicots respectively. A sandy loam soil was used for plants growth. The crucial step of the extraction of high quality total RNA and mRNA from roots and soil was overcome by using Mobio kit with modifications. Total community RNA was random reversely transcribed into cDNA from three repeats of each plant roots and its adjacent soil. Achieving the rRNA-depleted RNA and representative cDNA was executed by using AmpTec kit, which has strong selection against rRNA. cDNA rRNA-tags and cDNA rRNA-depleted RNA-tags were subjected to high throughput sequencing.

The results, based on cDNA, obtained from 16S rRNA, suggest that the similarity in microbial composition between roots- and soil-derived communities stands at about 50%, at the class level. More precisely, levels of Actinobacteria and Bacilli had decreased while levels of Proteobacteria, Flavobacteria and Planctomycetacia had increased in the root community compared to the soil. The data obtained supports previous DNA-based studies published in the literature. We observed high correlation between the cDNA based 16S phylogenetic profile and the mRNA-based phylogenetic profile of the root community, at the phylum level.

The predominance of rRNA in transcriptomes is a major technical challenge in sequence-based analysis of cDNA from microbial isolates and communities. Using the modern method mentioned above, we succeeded to decrease rRNA contamination to 30%, the lowest level reported up to date. The bacterial and fungal mRNA-tags represent about 14% (with 59% of the mRNA sequences originating from the host plant in root samples). The vast majority of these microbial mRNA sequences corresponded to catalytic activity, particularly hydrolases. Interestingly, fungal-bacterial comparison indicates that the catalytic and binding activities within bacteria were represented by 80% and 15% of the bacterial sequences respectively, compare to 40% and 40% catalytic and binding activities within fungal sequences.

The developed protocol and data obtained have the potential to provide us insights into community structure and function of the microbiome of this complex environments - the rhizosphere.
579A  AidC, a novel N-acylhomoserine lactonase from the potato root-associated Chryseobacterium sp. StRB126, a member of Cytophaga-Flavobacteria-Bacteroides (CFB) group
Wenzhao Wang1, Tomohiro Morohoshi2, Nobutaka Someya3, Tsukasa Ikeda2
1Utsunomiya University, Japan, 2Department of Material and Environmental Chemistry, Utsunomiya University, Japan, 3Hokkaido Agricultural Research Center (HARC), National Agriculture and Food Research Organization (NARO), Japan

Quorum Sensing (QS) is a cell-cell communication mechanism that depends on cell population density in bacteria. In many gram-negative bacteria, several kinds of N-acyl-l-homoserine lactone (AHL) have been identified as signal compounds involved in QS system. AHL-mediated QS regulates the expression of many genes, including those responsible for bioluminescence, the production of pigments and antibiotics and virulence. In general, AHL-negative mutants show defects in pathogenicity, so it is expected that disrupting or manipulating QS signals could inhibit the expression of virulence and infection of host cells. Recently, two classes of AHL-degrading genes, which encode AHL-acylase or AHL-lactonase, have been identified from various resources. AHL lactonases, which catalyze AHL lactone ring opening, have been identified from proteobacteria, acidobacteria, firmicute, actinobacteria and several eukaryotes. In previous study, we reported the AHL-lactonase activity in genus Chryseobacterium isolated from the potato roots. It was the first report of putative AHL-lactonase activity in Cytophaga-Flavobacteria-Bacteroides (CFB) group. In this study, we identified and characterized the novel AHL-degrading gene from the potato root-associated Chryseobacterium sp. StRB126.

For cloning the AHL-degrading gene, we used the pLux28 reporter system. The pLux28 plasmid carries an 8.8-kb region of the lux operon of Vibrio fischeri. E. coli harboring pLux28 produces AHL and expresses luminescence under AHL-mediated regulation. Furthermore, a pUC118-based genomic library of StRB126 was prepared. The prepared genomic library was transformed into E. coli harboring pLux28. The formed colonies were grown and their luminescence activities were measured. As the results, one clone expressed luminescence to a very low degree. The sequence of the genomic DNA fragment in this clone contained five open reading frames (ORFs). One of them, the third ORF (ORF3) showed AHL-degrading activity. Although ORF3 encodes a metallo-β-lactamase homolog as the known AHL lactonases, ORF3 showed less than 13% identities to any known AHL-lactonases. ORF3 is the first characterized AHL-lactonase from CFB group bacteria and named as aidC (auto-inducer degrading gene from Chryseobacterium). Substrate specificity of purified AidC was determined by HPLC. Although MBP-AidC worked slightly better against the AHLS with short acyl chain than those with long acyl chain, the substitution at carbon 3 did not significantly affect enzyme activity. Additionally, the AHL-lactonase activity of AidC was inhibited by addition of EDTA, but restored by addition of zinc ion. These features demonstrated that AidC works as metallo-AHL lactonase as well as the known AHL lactonases such as AiiA from Bacillus cereus group.

In summary, AidC is the first reported AHL-lactonase from the CFB group. In previous study, AiiA-type AHL lactonases, which belong to metallo-β-lactamase superfamily, are divided into four groups. However, AidC showed very low similarity to these known AHL lactonases. Our results extended the resource of AHL-degrading enzymes and reinforced their ecological significance.

579B  Co-operation between PGPR containing ACC-deaminase and Rhizobium spp. for sustainable production of legumes
Zahir Zahir*1, Saqib Akhtar2, Maqshoof Ahmad3, Hafiz Asghar4
1Institute of Soil & Environmental Sciences, Pakistan, 2Department of Agriculture and Ecology, Faculty of Life Sciences, University of Copenhagen, Højbakkegaard Allé, Denmark, 3College of Agriculture, Islamia University Bahawalpur, Pakistan, 4Institute of soil & Environmental Sciences, Pakistan

The aim of this study was to evaluate microbial co-operation in order to select the effective combinations of PGPR containing ACC-deaminase and efficient rhizobia for sustainable production of legumes (lentil, chickpea and mung bean) under pot and field conditions. These PGPR were screened for their capability to produce ACC-deaminase in vitro and for their plant growth-promoting activity under gnotobiotic conditions. The results demonstrated that bacteria containing ACC-deaminase had impact on the improvement in the growth and nodulation of legumes. However, the degree to which
these inoculants imparted benefits to plant growth varied with the conditions and the ability of PGPR to utilize ACC as sole source of nitrogen. This premise was supported from the results of pot and field trials that microbial co-operation of PGPR and rhizobial spp. had promising effects for improving nutrient acquisition, N2 fixation, root system quality and productivity of legumes. Hence, cooperation of rhizobia and rhizobacteria containing ACC-deaminase along with some other growth promoting characters could be the most effective and win-win strategy for promoting growth, nodulation and yield of legumes.

580A Influence of root exudates on the structural development of microbial communities in the rhizosphere
Maren Ziegler*, Gerhard Welzl, Michael Schloter
Helmholtz Zentrum München / Environmental Genomics, Germany

The rhizosphere has been defined as the soil compartment which is highly influenced by the plant root system. Up to 40% of the carbon fixed by photosynthesis can be released by the plant root to the surrounding soil, i.e. in form of plant root exudates. Therefore, the rhizosphere is rich in easy degradable carbon rich compounds in comparison to bulk soil. The composition of these root exudates is highly dynamic in space and time and can vary depending on plant species, growth stage and the nutrient status of the plant, the root zone as well as external biotic and abiotic factors. Heterotrophic soil microorganisms use these compounds as a nutrient source, which leads to an increase in microbial biomass and activity in the rhizosphere (rhizosphere effect). Contrary, plants can also release compounds serving as antimicrobials which defend pathogens. Therefore, root exudates play a crucial role for plant-microbial interactions.

In this study, we developed an artificial root model with a continuous exudate flow offering a surface for microbial colonization. The model consists of glass slides covered with an agarose/artificial root exudate mix incubated in soil. The development of bacterial communities over time was studied by analyzing diversity of the 16S rRNA gene using T-RFLP fingerprinting. To calibrate our new model system we compared the development of bacterial community pattern in our artificial system with the rhizosphere microflora of Arabidopsis thaliana to the ARM. To test which exudates are key drivers for microbial community development in our systems, we performed experiments where glucose (a representative of carbohydrates), malic acid (organic acid) and serine (amino acid) were omitted from the artificial exudate mix of the ARM. Among the tested compounds only malic acid led to a significant shift in microbial community structure. This effect was detectable after 2 days and disappeared with ongoing incubation. Also, we could show that our new model generates a microbial community similar to a natural rhizosphere community of A. thaliana and hence serves as a valuable system for studying rhizosphere interactions.

581A Losing the partner - now what? Effects of host loss on arbuscular mycorrhizal fungi
Frank Zielinski*, Tina Netzker, Thomas Fester
Helmholtz Centre for Environmental Research - UFZ, Germany

Arbuscular mycorrhizal fungi are an ancient fungal phylum (Glomeromycota) that coevolved with plants for the last 400 million years. Today, these fungi associate with roots from about 80% of all plant species. The interaction is mainly characterized by fungal arbuscules, i.e. tree-shaped subcellular structures within plant root cells that are the main site of nutrient exchange. The fungal partner provides water, phosphate and other nutrients and obtains carbohydrates from the plant. Up to 20% of photosynthesis products are estimated to be consumed by arbuscular mycorrhizal fungi. Therefore, this symbiosis contributes considerably to global phosphate and carbon cycling, mediates CO2 sequestration, and influences primary productivity in terrestrial ecosystems.

Arbuscular mycorrhizal fungi are unable to complete their life cycles without forming a symbiosis. Noticeably, the symbiosis is accompanied by a constant turnover of arbuscules and hyphae which have relatively short life times, on average 5-6 days. While a continuous fungal turnover appears to result from a fixed symbiotic program, little is known regarding the fate of fungi that have established a symbiosis but are metabolically separated from their host due to termination of nutrient trading by the plant partner or as a result of mechanical separation. The aim of our experiments was thus to characterize the fungal reaction to separation from the plant partner.
Using root cultures mycorrhized with *Rhizophagus irregularis* (DAOM197198) we physically separated the fungal partner from its host and observed the hyphae over time. Surprisingly, host loss did not result in the formation of septated hyphae, as is typical for natural hyphal turnover, but in a continuous reduction of fungal cytoplasmic streaming. We eventually followed this process by counting functional hyphae (active cytoplasmic streaming) and non-functional hyphae (no cytoplasmic streaming). The half life of active fungal hyphae after separation from the host was between 2.1 and 6.5 days.

To study molecular factors involved in the transition from active to non-functional hyphae, we extracted RNA from fungal hyphae separated from their host after half of the hyphal population had become non-functional and hybridized this RNA with RNA extracted from a hyphal population not separated from the plant host using *Suppression Subtractive Hybridization*. The resulting differentially expressed gene fragments were subsequently cloned. A preliminary analysis of 287 clones revealed 204 distinct sequences with a mean length of 273 base pairs (+/- 140 bp). A sample based rarefaction analysis indicated that the pool of differentially expressed genes likely contains between 500 and 900 distinct sequences.

Comparison with nucleotide data bases revealed that 88% percent of the clone library had no matches, 10% matched ribosomal RNA sequences of *R. irregularis* (DAOM197198) and only 7 sequences (2%) had matches to functional genes. Comparison with protein databases gave a similar picture: 51% of all sequences were unknown, 39% yielded hypothetical proteins, 8% were ribosomal proteins and only 5 sequences (2%) had a match.

Currently, we sequence additional clones to analyze an extended set of differentially expressed genes. Promising gene candidates will then be evaluated regarding a possible role in the process described.

**582A  Effect of pathogenic soil bacteria and growth promoting bacteria on glucosinolate production in kale (Brassica oleracea var. Sabellica L.)**
Matthias Zielke*1, John Beck Jensen2, Jørgen Mølmann1
1Bioforsk - Norwegian Institute for Agricultural and Environmental Research, Norway, 2University of Tromsø, Norway

Glucosinolates and their hydrolysis products represent an important group of secondary metabolites found in almost all plants of the order Brassicales. It has been shown that these compounds have potential insecticidal, anti-cancer and anti-microbial effects. In the present study we investigated whether different soil bacteria in the plant's rhizosphere have an effect on the quality and quantity of the produced glucosinolates.

A sterile hydroponic system has been used to grow kale seedlings. Three days after germination the young roots were inoculated with different axenic cultures of common soil bacteria. Hereby both pathogenic and growth promoting bacteria have been used. After three weeks the plants were harvested and the quality and quantity of glucosinolates in the green parts and the roots of the kale plants have been determined by HPLC. The bacteria’s capacity of colonizing the plant roots has been studied using 16S rDNA sequencing.

**583A  PGPR characterization of strains isolated from goldenberry (Physalis peruviana L.) rhizosphere in highland zones**
Doris Zúñiga*1, Katty Ogata2, Ana Cumpa2, Lindacelia Flores2
1Laboratorio de Ecología Microbiana y Biotecnología Marino Tabusso, Universidad Nacional Agraria La Molina, Peru, 2Universidad Nacional Agraria La Molina, Peru

Goldenberry is a native peruvian crop that grows in the Andean valleys until 3300 m.a.s.l. This fruit has become increasingly important for international export market due to its high production of provitamin A, ascorbic acid and its medical properties. Unfortunately, farmers face serious problems because of the diseases originated by pathogenic viruses, fungus and bacteria. In order to produce bioinoculants that might be beneficial for goldenberry cultivation, bacteria were isolated from *P. peruviana* rhizospheric soil of Junin region. These strains were screened through the quantification of indolacetic acid (IAA) production, phosphate solubilization, percent inhibition of fungus *Fusarium* sp. and germination assay on the model plant *Trifolium pratense* (red clover). From 96 microorganisms isolated, 97% produced IAA; 27.1% of these produced the highest concentrations of this
phytohormone, with values between 25 µg/ml and 85 µg/ml. About the phosphate solubilization capacity, 52% solubilizes bicalcic phosphate as observed by a translucent halo on the NBRIP medium. In dual antagonism, only 11 of 26 strains inhibited one strain of *Fusarium* sp. Finally, strains of *Bacillus* BA18, *Pseudomonas* PA22 and diazotroph Da29 remarkably increased the seed germination percentage of red clover (incubated to 22°C) to 25 hours, therefore, these strains are plant growth promoting rhizobacteria (PGPR). The importance of studying PGPR bacteria is to obtain a biotechnological alternative for farmers growing this crop that would allow them to improve its yields inside sustainable agriculture.

584A  The effect of sodium chloride and urea in PGPRs isolated from cotton and lima bean from the valley of Ica, Peru

Doris Zúñiga¹, Elena Ramos¹, Carlos Arango*¹, Ricardo Santos¹, Juan Sanjuan²

¹Laboratorio de Ecología Microbiana y Biotecnología Marino Tabusso, Universidad Nacional Agraria La Molina, Peru, ²Estación Experimental del Zaidín, CSIC, España

In the valley of Ica, agriculture is the main economic activity, especially cotton (*Gossypium barbadense*) and legumes as lima beans (*Phaseolus lunatus*). The soil in these areas is sandy loam with low organic matter content, low in phosphorus and potassium intermediate levels. The climate is semi-warm with a minimum temperature of 9.8°C and maximum 32°C. In previous works, plant-growth promoting rhizobacterias (PGPR) were isolated from these crops. Five strains of *Bacillus* and four diazotrophs were tested in Triptone Yeast extract medium (TY) supplemented with urea (50 and 100 mM) or sodium chloride (50 and 100 mM). Bioscreen System was used in this study, which is an 12h controlled stirring. In TY medium, *Bacillus* isolates were inhibited by both salt concentrations, however only one strain was stimulated by 50 mM of urea and was resistant to 50 mM sodium chloride. In the case of diazotrophs strains, only the treatment with 100 mM of sodium chloride, affected drastically the growth.

On the other hand, *Bacillus* and diazotrophs strains were also tested in Yeast Extract Mannitol Agar (YEM) modified medium for carbon and amino acids sources, whose are low costs. Both bacterial groups grew optimally in the original and modified medium. In order to apply microbial inoculants to the crops, the levels of chemical nitrogen and salts would be care because it could significantly affect their growth and PGPR ability. These studies could serve to optimize and develop bioinoculants based on lower-cost inputs and can be applied into the cotton and lima beans crops which would benefit farmers in sustainable agriculture.