



*INTERNATIONAL SYMPOSIUM*

**MICROBE-ASSISTED CROP PRODUCTION –  
OPPORTUNITIES, CHALLENGES & NEEDS**

**DEC. 4 – 7, 2017**

*SCHLOSS SCHÖNBRUNN - ORANGERIE  
VIENNA, AUSTRIA*

# ABSTRACT BOOK 2017



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# MICROBE-ASSISTED CROP PRODUCTION 2017

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OPPORTUNITIES, CHALLENGES & NEEDS

*DEC. 4 – 7, 2017*

*SCHLOSS SCHÖNBRUNN | ORANGERIE*

*VIENNA, AUSTRIA*

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## Table of Contents

<b>GENERAL INFORMATION</b>	<b>3</b>
<b>INTRODUCTION</b>	<b>5</b>
<b>INVITED SPEAKERS</b>	<b>6</b>
<b>SCIENTIFIC PROGRAM</b>	<b>12</b>
<b>DAY 1   MON, 4. DEC.   1<sup>P.M.</sup> - 8:30<sup>P.M.</sup></b>	<b>12</b>
<b>DAY 2   TUE, 5. DEC.   8:30<sup>A.M.</sup> - 3<sup>P.M.</sup></b>	<b>14</b>
<b>DAY 3   WED, 6. DEC.   8:30<sup>A.M.</sup> - 18:10<sup>P.M.</sup></b>	<b>16</b>
<b>DAY 4   THU, 7. DEC.   9:00<sup>A.M.</sup> - 15:00<sup>P.M.</sup></b>	<b>18</b>
<b>POSTERS TABLE – POSTER SESSION 1</b>	<b>20</b>
<b>POSTERS TABLE – POSTER SESSION 2</b>	<b>21</b>
<b>POSTERS TABLE – POSTER SESSION 3</b>	<b>22</b>
<b>LECTURES</b>	<b>23</b>
<b>OPENING LECTURE</b>	<b>24</b>
<b>SUCCESSFUL MICROBIAL PRODUCTS</b>	<b>26</b>
<b>MICROORGANISMS FOR RURAL DEVELOPMENT</b>	<b>29</b>
<b>POSTER TALKS I</b>	<b>34</b>
<b>NEW MECHANISMS INVOLVED IN BENEFICIAL PLANT-MICROBE INTERACTIONS</b>	<b>40</b>
<b>PLANT UNDERSTANDING AND IMPROVEMENT OF BENEFICIAL INTERACTIONS WITH MICROBES</b>	<b>46</b>
<b>POSTER TALKS II</b>	<b>50</b>
<b>THE HOLOBIONT PLANT: MULTITROPHIC INTERACTIONS</b>	<b>56</b>
<b>THE HOLOBIONT PLANT: MICROBIOME UNDERSTANDING</b>	<b>62</b>
<b>POSTER TALKS III</b>	<b>68</b>
<b>APPLICATION TECHNOLOGIES &amp; FORMULATIONS</b>	<b>74</b>
<b>PHYTOBIOMES ALLIANCE</b>	<b>78</b>
<b>FUTURE OF MICROBIAL PRODUCTS &amp; REGULATORY ISSUES</b>	<b>82</b>
<b>CLOSING LECTURE</b>	<b>84</b>
<b>POSTER PRESENTATIONS</b>	<b>86</b>
<b>POSTER SESSION 1: SUCCESSFUL MICROBIAL PRODUCTS</b>	<b>87</b>
<b>POSTER SESSION 1: NEW MECHANISMS INVOLVED IN BENEFICIAL PLANT-MICROBE INTERACTIONS</b>	<b>96</b>
<b>POSTER SESSION 2: PLANT UNDERSTANDING AND IMPROVEMENT OF BENEFICIAL INTERACTIONS WITH MICROBES</b>	<b>110</b>
<b>POSTER SESSION 2: THE HOLOBIONT PLANT: MULTITROPHIC INTERACTIONS</b>	<b>126</b>
<b>POSTER SESSION 3: THE HOLOBIONT PLANT: MICROBIOME UNDERSTANDING</b>	<b>138</b>
<b>POSTER SESSION 3: APPLICATION TECHNOLOGIES &amp; FORMULATIONS</b>	<b>153</b>
<b>POSTER SESSION 3: PHYTOBIOMES ALLIANCE</b>	<b>158</b>
<b>POSTER SESSION 3: FUTURE OF MICROBIAL PRODUCTS &amp; REGULATORY ISSUES</b>	<b>163</b>
<b>AUTHOR INDEX</b>	<b>165</b>
<b>PARTICIPANT INDEX</b>	<b>172</b>
<b>ACKNOWLEDGEMENTS</b>	<b>183</b>
<b>IMPRINT</b>	<b>183</b>

## General Information

### TAXI:

+431 40100 or  
+431 31300

### VENUE ADDRESS:

Schloss Schönbrunn Tagungszentrum | Orangerie | Schönbrunner Schloßstrasse – Entrance | 1130 Vienna, Austria



### miCROPe 2017 office at the venue:

The registration desk will be occupied throughout the symposium. Please contact us with any congress related queries in person or by e-mail.

E-mail: [office@micrope.org](mailto:office@micrope.org)

**WiFi** is available at the venue with this login data:

**WLAN: Meetings**  
**PW: Habsburg**

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The **language** of the meeting is English.

We reserve the right to use any photograph/video taken at the event without the expressed written permission of those included within the photograph/video.

## SHORT TALKS:

Please give your presentation in power point or pdf format to the technician in the break before the session via usb-stick. The program is very tight, so please take care of the prescribed talktime.

## POSTER TALKS:

Poster talk presentations must be submitted on December 4<sup>th</sup> 10-12 a.m. in power point format to the technician in lecture hall.

## POSTER SESSIONS:

Posters are only accepted in A0 upright format and in English. The poster sessions are organized in the poster session I, II and III and each poster has been assigned to a session. You can see the assignment within the poster table on page 21-23)

Postersession I Monday 4 Dec., 17:30 - 20:30 (Display posters from Dec. 4, 13:00 - Dec. 5, 12:45)

Postersession II Wednesday 6 Dec., 11:25 - 13:45 (Display posters from Dec. 5, 12:45 - Dec. 6, 15:25)

Postersession III Wednesday 6 Dec., 16:40 - 18:10 (Display posters from Dec. 6, 15:25 - Dec. 7, 11:40)

Posters from poster session I have to be removed on December 5th. Poster switch from poster session II and III is on December 6th in the coffee break from 15:25-15:50

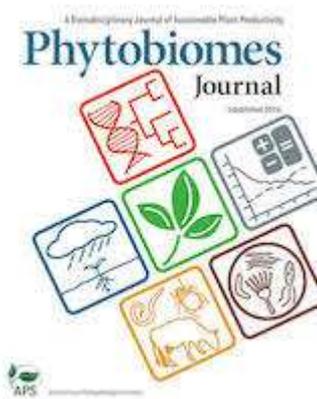
**5 posters will be awarded with Best Poster Awards.**

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### miCROPe 2017 Meeting Attendees:

#### Get Reduced Article Processing Charges (APCs) for *Our Conference Journal: Phytobiomes*

*Potential to produce virtual issue based on our meeting and its research*



*Phytobiomes*, the official conference journal of our upcoming miCROPe meeting, has received a lot of exposure among scientists for presenting new and novel research on microbes and their role in sustainable production in agriculture.

As such, *Phytobiomes* makes a fitting venue for researchers attending the upcoming miCROPe meeting.

To help stimulate submissions for attendees, *Phytobiomes'* Editor-in-Chief Carolyn Young has approved a significant drop in article processing charges to \$1,200 per paper.

This discount is only for those who have registered for the 2017 miCROPe meeting, and the article processing charge (APC) discount applies from today through June 1, 2018. To qualify, you will need to be a first or corresponding author who attended

the miCROPe meeting. This offer is not transferrable to non-attendees. If enough submissions are accepted, a special virtual issue will be produced representing miCROPe 2017.

If you are not attending but still wish to submit, the still-discounted article processing charge of \$1,350 currently applies.

To learn more about *Phytobiomes*, visit [www.phytobiomesjournal.org](http://www.phytobiomesjournal.org).

# Introduction

Dear colleagues,

Global demographic development and climate change create challenges for crop production in many areas. Difficulties to be overcome include world-wide population increases, extreme weather events and highly variable weather conditions, emerging pathogens and pests, and diminishing land resources. Furthermore, the use of chemical pesticides poses a threat to human health, animal welfare, and biodiversity.



Various strategies are currently being developed to improve sustainable agricultural production. It is now widely recognised that plant microbiota provide important functions for their host's performance, and mediate functions like nutrient delivery, fitness, stress tolerance, and pathogen or pest control. Current understanding of plant-microbe interactions is helping to develop microbial products, new applications to improve crop production, and create alternatives to chemicals. Microbial ecology is an important asset for understanding the fate of applied microorganisms in a natural environment, and for affecting product development. Greater understanding of microbiome functioning will also lead to new routes of exploration.

The symposium "Microbe-assisted crop production – opportunities, challenges and needs" (miCROPe 2017) addresses basic and applied aspects of applying beneficial microorganisms in crop production. Scientific sessions address mechanistic understanding of beneficial plant-microbe interactions, microbiome interactions, transfer of microbial applications from lab to field, formulations and registration issues. Our aim is to promote innovation as well as implementation of new technologies and to enable discussions between academia and industry.

Around 180 abstracts were submitted by scientists working in academia and industry around the globe. We would like to thank all authors for their valuable contribution to provide new results for further scientific discussion and to make this symposium highly interesting for different stakeholders. This exchange will lead in the future to a better implementation of microbe-based crop production. We would like to particularly thank the scientific committee for their excellent support in organizing a high-quality program. The contributions of all invited speakers are highly appreciated. All authors are invited to submit their contribution to a special thematic issue of the *Phytobiomes* journal (<https://apsjournals.apsnet.org/page/aboutphytobiomes>). We furthermore would like to thank all sponsors, partners and supporters of this symposium.

We wish you all an exciting symposium, time to interact with colleagues and friends as well as time to enjoy the Christmas atmosphere in Vienna!

**Angela Sessitsch** – AIT Austrian Institute of Technology, Austria  
on behalf of the Organizing Committee

## Invited Speakers

### **Gabriele Berg, TU Graz, AT**



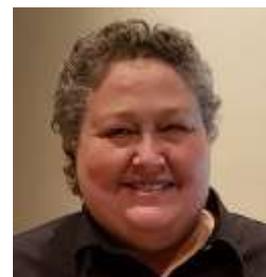
Gabriele Berg studied biology and biotechnology at the universities in Rostock and Greifswald obtained her Ph.D. in 1995 in microbiology from Rostock University (Germany). In 2003, she got a Heisenberg grant from the DFG (Deutsche Forschungsgemeinschaft), and in 2005 she became a full professor in environmental biotechnology at Graz University of Technology (Austria). Her interests are focused on microbiome research and translation of the results into new biotechnological concepts for our environment as well as for plant and human health. Results have published in more than 200 peer-reviewed papers and in several patents. For her results and developments she received numerous awards, e.g. Science2Business Award Austria and “ÖGUT Umweltpreis” (2011) and Fast Forward Award Styria (2015).

### **Paola Bonfante, University of Torino, IT**



Her research is focused on the biology of symbiotic associations, mainly mycorrhizas. She has studied the intimate interactions that occur between mycorrhizal fungi and plants, mostly focusing on cell plant re-organization upon AM fungal entry by using cellular and molecular approaches. Applying DNA technologies, early in the nineties, she provided contribution to the knowledge of mycorrhizal diversity in natural and agricultural environments. She has discovered a group of endobacteria which live inside mycorrhizal fungi and may modulate some of the functional traits of their fungal hosts. Her major current projects are focused on: Understanding the Bacterial and fungal microbiomes in rice, tomato and wheat; Dissecting the role of the fungal intracellular microbiota; Revealing Rice and tomato responses to AM fungi; Developing Genomics and Functional genomics approaches for *Gigaspora margarita*; Systemic effects of mycorrhizal fungi on plant traits. She has published more than 200 papers in refereed journals and her H index is 79.

### **Kellye Eversole, International Phytobiomes Alliance, US**



Kellye Eversole is Executive Director of the International Alliance for Phytobiomes Research (the Alliance) and President of Eversole Associates, an agricultural science and technology consulting firm ([www.eversoleassociates.com](http://www.eversoleassociates.com)).

She co-led the development of the Phytobiomes Roadmap (<http://www.phytobiomes.org/Roadmap/Pages/default.aspx>) and, in 2016, established the Alliance ([www.phytobiomesalliance.org](http://www.phytobiomesalliance.org)), a public-private collaborative consortium focused on building a phytobiome-based foundation for accelerating the sustainable production of food, feed, and fiber.

An expert in sequencing technologies and strategies, Eversole has more than twenty years of experience in leading efforts to obtain genome sequences for agriculturally important crop and livestock species and has been the Executive Director of the International Wheat Genome Sequencing Consortium (IWGSC, [www.wheatgenome.org](http://www.wheatgenome.org)) since its inception in 2005.

In recognition of her work in plant and microbial genomics, Eversole was elected as a Fellow of the American Association for the Advancement of Science in 2013. Passionate about innovation for societal benefits, Kellye mentors entrepreneurs from emerging economies who are launching science and technology based businesses. Eversole has been involved actively in various aspects of the agricultural industry since childhood, including working 10 years in the US Senate as an agricultural policy specialist and 2 years as the head of a US study commission before launching her firm. She co-owns a farm in southwestern Oklahoma.

## Janet Jansson, Pacific Northwest National Laboratory, US

Janet Jansson obtained her Ph.D. in Microbial Ecology in 1988 from Michigan State University under the supervision of James Tiedje. From there she moved to Sweden for 20 years, at the end of which she held a position as Professor (Chair) of Environmental Microbiology at the Swedish University of Agricultural Sciences (SLU) and Vice Dean of the Natural Science Faculty. In 2007 she moved to Lawrence Berkeley National Laboratory (LBNL) where she held a position as Senior Staff Scientist and head of the Ecosystems Biology Program. In 2014 she moved to the Pacific Northwest National Laboratory (PNNL) where she currently holds a position as the Chief Scientist for Biology in the Earth and Biological Sciences Directorate. She recently completed a term as the President of the International Society for Microbiology (ISME). She is a fellow of the American Academy of Microbiology and of the Washington State Academy of Science. In 2016 she was among the top 1% cited researchers in the World and she has more than 150 publications, including several in Nature. Dr. Jansson's current research interests are in the application of molecular "omics" tools to gain an understanding of the function of microbial communities in complex environments, ranging from soil to the human gut. She is currently coordinating a large research initiative at PNNL focused on microbiomes in transition "MinT".



## Jürgen Köhl, Wageningen University & Research, NL

I focus my research on sustainable solutions ensuring plant health. My main topics are the biological control of fungal plant pathogens using antagonistic fungi and methods for disease prevention. Understanding disease epidemiology often is a first step in such studies. During the last years, I worked on the disease management in pome fruits, cereals and vegetables with emphasis on leaf and fruit diseases: Screening methods for the selection of biological control agents; Biological control of apple scab and powdery mildew; Risk assessment of microbial antagonists; Characterization of endophyte microbiomes; Detection, population dynamics and biological control of toxigenic *Fusarium* spp. in cereals; Life cycle of pear brown spot and storage diseases in pome fruit; Epidemiology of back spot disease (*Rhexocercosporidium carotae*) in carrots.

Currently, I am co-ordinator of EU FR7 project BIOCOTES ([www.biocotes.eu](http://www.biocotes.eu)) and convenor of the IOBC-WPRS working group 'Biological and integrated control of plant pathogens'. My institute at Wageningen University & Research is a private not for profit research institute specialized in strategic and applied research for industry and public institutions. I am also member of the Faculty of Agriculture, Rheinische Friedrich-Wilhelms-Universität Bonn, Germany since I finished my habilitation in 2003.



## Tuesday Simmons, UC Berkeley, US

Tuesday Simmons is a PhD candidate in Microbiology in the lab of Assistant Professor Devin Coleman-Derr in the Department of Plant and Microbial Biology at the University of California Berkeley. Our lab functions also as component of the Plant Gene Expression Center, funded through the Agricultural Research Service of the United States Department of Agriculture. The Coleman-Derr laboratory investigates the effects of drought and other abiotic stresses on the microbiomes associated with grass crops, with a particular emphasis on sorghum. Recent studies have demonstrated that individual microbial symbionts of crop plants are capable of enhancing the abiotic stress tolerance of their host, however, the complex dynamics of plant microbiome development and maintenance have yet to be fully uncovered and evaluated. Using a combination of metagenomics, metatranscriptomics, plant molecular biology and microbiology of culturable symbionts, we are working to uncover the genetic mechanisms in host and microbe that are responsible for recently observed shifts in the composition of the root microbiome during drought stress.



**Esperanza Martinez-Romero, Center of Genomic Science, UNAM, MX**



Dr. Esperanza Martínez-Romero discovered *Rhizobium tropici*, a species with outstanding characteristics (stress resistance, genomic stability and high nitrogen fixing capabilities) that changed the history of *P. vulgaris* bean inoculants. *P. vulgaris* bean is the most widely used legume for human nutrition worldwide. Inoculants allow the savings of millions of dollars in agriculture and are ecologically friendly. *R. tropici* is widely used for bean in Brazil and in Africa. She described the Nod factors (key molecules in symbiosis) from bean nodulating bacteria. Interestingly, she found that bean nodulating bacteria are also maize endophytes that contribute some nitrogen to maize and compete with fungal pathogens. Nitrogen fixation in cereals is a long-sought goal. In addition, she has described novel bacteria from tropical plants and a sister species of *Klebsiella pneumoniae*, *K. variicola*, which is found in human patients and also associates with different plant species. She has warned about the use of this human pathogen in agriculture.

She has also discovered novel insect endosymbionts, explored their functions by genomics and transcriptomics, and proposed parallelisms between plant and insect symbiosis. She has found novel (endo) symbionts from Monarch butterflies, from the carmine producing cochineal and a wax producing cochineal.

She has been a provider of *Rhizobium* strains (of the species she has described) to many different laboratories and bacterial collections for many years. She frequently gives conferences to farmers on the benefits of biofertilizers. Her research has included basic and applied work, highly required in developing countries, and she has received external funding from Belgium VLIR-Abos, Global Environmental Facility, and the European Union.

Dr. Martínez-Romero has been editor of different journals, like GBE, SAM ISME J, J Bacteriol, MPMI and AEM. She has over 13,000 citations to her work with an H index of 63 (Google citations).

**Birgit Mitter, AIT Austrian Institute of Technology GmbH, AT**



Birgit Mitter is microbiologist with strong experience in the field of plant microbiome research, in particular in studying bacterial endophytes. She studied endophytes in a variety of plants including plants of agricultural importance, such as potato, rice and maize but also in wild flowers and tropical trees. B. Mitter applies community sequencing as well as (meta)genome sequence and transcriptome analysis to study the mechanisms of beneficial plant-microbe interactions. Recently, B. Mitter focused in her work on the development of improved application strategies of endophytes for use in sustainable agriculture.

**Yvan Moënne-Loccoz, Université de Lyon, FR**



Yvan Moënne-Loccoz is professor at the Université de Lyon (France) and director of the UMR CNRS 5557 Microbial Ecology in Villeurbanne, France. He obtained a master degree in soil science at the Université de Montpellier and a Ph.D. in soil microbiology at Texas A&M university. He worked at CIRAD (Paris and Côte d'Ivoire), the UCC in Cork (Ireland) and the ETH Zurich (Switzerland) before moving to Lyon. His research interests include (i) the molecular ecology of PGPR in the rhizosphere, (ii) the functioning of disease-suppressive soils, (iii) rhizosphere metagenomics and crop breeding, and (iv) the development of bacterial seed inoculants for phytostimulation or biocontrol purposes.

### **Anant Patel, Bielefeld University of Applied Sciences, DE**

Anant Patel holds a research professorship of process engineering at his university and focusses on fermentation and formulation materials, methods and technology. His groups aims at understanding the relationship between material structure and properties, spatio-temporal phenomena that occur in the complex interaction of the biological ingredients with the formulation materials and the communication of the formulation with the environment. The novel award-winning biocontrol product ATTRACAP that attracts wireworms in soil by CO<sub>2</sub> is based on the encapsulation technology of the Patel group.

On-going research deals with fermentation and formulation of endophytic entomopathogenic fungi, attract-and-kill, push-and-pull and repellent formulations, drying of Gram-negative bacteria, endophytes from medicinal plants, seed treatment with bird repellants, nanocoating of biological surfaces, chemoenzymatic one-pot synthesis, entrapment of microalgae, azadirachtin production from plant cell cultures, and bioprinting of enzymes and living cells. He is holding 17 patents and consultant for the sustainable development of cultivation regimes for *Moringa oleifera* in Sierra Leone to combat malnutrition in children. He is also co-owner of a farm in India that was voted “Best farm of Asia” by the FAO.



### **Corné Pieterse, Utrecht University, NL**

Corné Pieterse (1964) is professor Plant-Microbe Interactions and scientific director of the Institute of Environmental Biology of the Faculty of Science. His research group investigates how the plant immune system protects plants against microbial pathogens and insect herbivores, and how beneficial microbes in the plant root microbiome stimulate plant growth and health.

Current research is focused on plant-beneficial functions that are encoded by the root microbiome, the role of plant genes that aid in maximizing profitable functions from the root microbiome, and crosstalk between plant defense hormones. With his research he aims to contribute to grand societal challenges, such as food security and sustainable agriculture.

In 2004 he was appointed as full professor Plant-Microbe Interactions. In 2010, he was awarded an ERC Advanced Investigator grant by the European Research Council. His research group pioneered research on unravelling the rhizobacteria-induced systemic resistance signalling pathway and the role of phytohormone crosstalk in the regulation of plant immunity. In 2013 he was elected as a member of the Royal Netherlands Academy of Arts and Sciences (KNAW). Since 2014 he is a Thomson Reuters Highly Cited Researcher (World's top 1% in the field). Besides heading the Plant-Microbe Interactions group at the Department of Biology, Corné Pieterse is scientific director of the Institute of Environmental Biology of the Faculty of Science.



### **Claudia Preininger, AIT Austrian Institute of Technology, AT**

Claudia Preininger is a senior researcher at the AIT Austrian Institute of Technology, Tulln, Austria. She currently has responsibility for the development of bioformulations suitable for microbial seed coatings, foliar spray and soil amendment in the field of plant growth promotion and biocontrol. She has over 20 years experience in immobilization of enzymes, proteins and microbes, especially with biosensors and microarrays.

Current research includes the development and characterization of microbial micro- and nanocomposite seed coatings, granules and powders and the fabrication of microbial capsules with distinct function and properties. She is a chemist and actually coordinates two m-era.net projects on microbial seed coatings with main focus on material – microbe interaction.



## **Willem Ravensberg, Koppert Biological Systems, NL**



Willem is Regulatory and Governmental Affairs Manager and Member of the Management Team of the BU Microbials of Koppert Biological Systems with more than 30 years of experience in research and development of beneficial insects and microorganisms for crop protection products. He has ample experience in registration of microorganism in the European Union as well as in other countries. Willem has a Masters degree in Biology (Entomology) from Leiden University, and he did his PhD in 2010 on the development of microbial pest control products for control of arthropods at the University of Wageningen, the Netherlands. His thesis resulted in the following book: Ravensberg, W.J., 2011. A Roadmap to the Successful Development and Commercialization of Microbial Pest Control Products for Control of Arthropods. Springer, Dordrecht, the Netherlands.

The first part of his career he was mainly active in the field of beneficial insects and mites, the last 15 years this shifted to microbial biopesticides where he was R & D Manager. Willem has a long experience in the biocontrol industry and has been active in many areas of biocontrol in research, product development and practical applications in greenhouse crops and field crops.

Willem has been active for many years in industry associations in the Netherlands, EU and USA. Since 2013 he is President of IBMA (the International Biocontrol Manufacturers Association). He has many contacts within the industry, with research institutions and with regulatory authorities. Among these are the EU (DG SANTE), EFSA, Member State authorities, US EPA, and OECD. In 2016 Willem is the first President of the newly formed Federation of Biocontrol and Biopesticides Associations called Bioprotection Global. he is now acting as the Immediate Past President.

## **Alia Rodriguez Villate, Universidad Nacional de Colombia, CO**



Alia Rodriguez Villate obtained her PhD from the University of Kent at Canterbury in 2001, where she worked on the diversity of mycorrhizal fungi. After postdoctoral positions at University of Höhenheim (Germany) and the Consejo Superior de Investigaciones Científicas (CSCIC) in Granada (Spain) she became associate professor at the National University of Colombia. In Colombia she leads a research group on the biotechnology of the mycorrhizal symbiosis. Her main research interest is on the development of biotechnological applications of mycorrhizal fungi to increase crop productivity in low fertility acidic soils, which account for almost 40% of arable soils globally. Thus, her focus has been soils with high aluminium concentrations, low pH and low phosphate availability, and crop plants of both local and global importance, i.e. cassava. She is working on the application genetically different mycorrhizal fungi in the field, for improving productivity in agricultural systems in Colombia, Kenya and Tanzania in collaboration with Prof. Ian. Sanders (University of Lausanne, Switzerland).

## **Ian Sanders, University of Lausanne, CH**



Ian Sanders obtained his PhD from the University of York in 1991, where he worked on the ecology of the mycorrhizal symbiosis. After postdoctoral positions at INRA Dijon and the Pennsylvania State University, he obtained a junior group leader position at the University of Basel, Switzerland. In 2000, he went to the University of Lausanne as a Swiss National Science Foundation Fellow and since then has become a full professor. His main research group focuses on molecular genetics, genomics and transcriptomics of the mycorrhizal symbiosis. A part of his research is dedicated to applying knowledge about the genomics of mycorrhizal fungi to improve productivity in globally important crops in the tropics, particularly cassava and how variation in plant genomes affect the outcome of the symbiosis with fungi. His work in the tropics is conducted in close collaboration with the group of Prof. Alia Rodriguez Villate (National University of Colombia) in Colombia, Kenya and Tanzania.

### **Leo van Overbeek, Wageningen University and research, NL**



Leo van Overbeek is senior scientist at Wageningen UR. His main scientific interest is on the roles that micro-organisms play in plant production systems. These interactions can be positive from a human perception such as plant growth stimulation, but also negative such as contamination with human pathogens. He received his Master degree in molecular microbiology at Utrecht University in 1992 and his PhD degree on the fate of microbial inoculants in soil at Leiden University in 1997. He performed post-doctoral research at Wageningen University on the microbiology of the human gut system and at the Institute for Plant Protection, later Plant Research International (PRI), on the fate of the potato brownrot-causative agent, *Ralsonia solanacearum* biovar 2, in Dutch potato fields for three years. After his post-doctoral positions he became principle investigator in endophyte research at PRI and was involved, and later acting as leader, in many projects about endophytes and bacterial plant pathogens, horizontal transmission of mobile genetic elements between plant-associated bacteria, isolation and characterization of hitherto uncultured species from plant microbiomes, presence of human pathogens in *Ixodes ricinus* ticks from Dutch forests, and ecology of human pathogens in plants. Currently, he is involved in public-private collaborative projects on human pathogens in plant and food production systems, seed coating with *Rhizobium* strains, plant root growth stimulation by microbials, and plant microbiome composition and functioning. Further, he is involved in the EU Biofactor project and in two Global One Health projects on the role of midge (*Culicoides* species) midgut microbiome composition in Arbovirus transmission, and on the impact of antibiotic residues in manure on plant microbiome composition. He recently was elected as chair of Cost Action 16110 on 'control of human pathogenic micro-organisms in plant production systems (HUPLANTcontrol)'.

### **Laure Weisskopf, University of Fribourg, CH**



Laure Weisskopf obtained her PhD in plant-microbe interactions in 2005 at the University of Neuchâtel (Switzerland). After a one-year post-doctoral stay in Kenya, she became a junior group leader at the University of Zurich. She stayed there until 2012 investigating the effect of bacterial volatiles on plant growth and writing her habilitation (venia legend), which she later obtained in the field of plant-microbe interactions. After 4 years of applied research at Agroscope and at the University of Applied Sciences in Western Switzerland, she moved to the University of Fribourg (Switzerland), where she was appointed as professor for plant-microbe interactions in 2017. Her main research interest lies in understanding how organisms communicate with each other to establish mutualistic associations. She focusses on the emission of volatiles by plant-associated bacteria and on their protective effect against plant diseases, such as potato late blight. Her research combines basic projects aiming at understanding the regulation of bacterial volatile emission and their physiological impact on target organisms, and more applied projects aiming at mobilising the potential of protective bacterial volatiles for sustainable crop protection.

# Scientific Program

**DAY 1 | Mon, 4. Dec. | 1<sup>P.M.</sup> - 8:30<sup>P.M.</sup>**

10:00 - 12:00 Arrival, registration

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13:00 - 13:15 WELCOME

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13:15 - 14:00 **OPENING LECTURE** Supported by   
**Esperanza Martinez-Romero (Center of Genomic Science, UNAM, MX)**  
Beneficial effects of microbes on plants

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## **SESSION 1 SUCCESSFUL MICROBIAL PRODUCTS**

14:00 - 15:10 **Session chairs: Jürgen Köhl & Angela Sessitsch**

14:00 - 14:25 **Jürgen Köhl (Wageningen University & Research, NL)**  
The challenge of developing microbial biocontrol products for disease control

14:25 - 14:40 **Christoph Lehnen**  
BVT fungus *Clonostachys rosea* CR-7 vectored by bumble bees protects plants from Grey Mold infection and enhances yields

14:40 - 14:55 **Brenda Loznik**  
Improving the efficacy of organic fertilizers with protozoa

14:55 - 15:10 **Ross Mann**  
Isoprene identified in the volatolome of endophytic fungi as a promising phosphine alternative

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15:10 - 15:30 **Coffee break**

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## **SESSION 2 Microorganisms for rural development**

15:30 - 16:40 **Session chairs: Ian Sanders & Alia Rodriguez**

15:30 - 15:55 **Ian Sanders (University of Lausanne, CH)**  
A microbial green revolution: Breeding microbes to feed the world

15:55 - 16:10 **Didier Lesueur**  
Importance of rhizobia in agriculture: potential of the commercial inoculants and native strains for improving legume yields in different land-use systems

16:10 - 16:25 **Maged Saad**  
Desert rhizosphere microbes for future sustainable SMART agriculture

16:25 - 16:40 **Valentina Riva**  
Plant growth promoting bacteria: a sustainable tool to minimize water footprint in agriculture in arid and semi-arid zones

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16:40 - 17:30 POSTER TALKS I:

Andrea Kunova: *Exploring plant endophytic Streptomyces for their use in sustainable agriculture*

Daniel Bravo: *Assessing cadmium-tolerant endophytic bacteria as a strategy to bio-remediate cadmium presence in Cacao crops from Colombia*

Gabriele Schiro: *Spatial distribution of phyllosphere fungi in a heterogeneous wheat field*

Zhibo Li: *Improving pasture quality by beneficial Bacillus sp. for optimized milk quality for use in dairy industry*

Sandra Bredenbruch: *Microbial rhamnolipids mediate control of plant parasitic nematodes through several mechanisms*

Sebastian Schneider: *Nitrogenase biosynthesis of Rhizobium (Mesorhizobium loti) is limited by the symbiotic sulfate transport in root nodules of the legume Lotus japonicus*

Anna Clocchiatti: *Stimulating saprotrophic fungi to control soil-borne fungal crop diseases*

Caroline Baudson: *Impact of plant growth promoting rhizobacteria on phosphorus use efficiency in the model grass Brachypodium distachyon (L.) Beauv.*

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17:30 - 20:30 **Poster Session 1 & Networking**

**DAY 2 | Tue, 5. Dec. | 8:30<sup>A.M.</sup> - 3<sup>P.M.</sup>**

**SESSION 3 New mechanisms involved in beneficial plant-microbe interactions**

08:30 - 10:20

**Session chairs: Paola Bonfante, Laure Weisskopf & Barbara Reinhold-Hurek**

08:30 - 08:55 **Paola Bonfante (University of Torino, IT)**

Looking at the plant side: plant crop responses to arbuscular mycorrhizal fungi

08:55 - 09:20 **Laure Weisskopf (University of Fribourg, CH)**

News from the volatile warfare between potato-associated *Pseudomonas* and the late blight causing agent *Phytophthora infestans*

09:20 - 09:35 **Lara Martin-Sanchez**

The role of bacterial terpenes in microbial interactions in the soil

09:35 - 09:50 **Antoine Desrut**

Unraveling the importance of sugar transport in plant-beneficial rhizobacteria interactions

09:50 - 10:05 **Monika Schmoll**

*Trichoderma reesei* prioritizes plant sensing and the presence of a mating partner over nutrient sensing to achieve optimal adaptation to the plant by sexual development

10:05 - 10:20 **Alberico Bedini**

Biostimulants for biostimulants: oligosaccharides as a new opportunity to enhance mycorrhizal responses, theoretical basis and practical cases

10:20 - 10:40 **Coffee break**

**SESSION 3 New mechanisms involved in beneficial plant-microbe interactions**

10:40 - 11:20

**Session chairs: Paola Bonfante, Laure Weisskopf & Barbara Reinhold-Hurek**

10:40 - 11:05 **Barbara Reinhold-Hurek (University of Bremen, DE)**

To be or not to be inside: A complex role of bacterial factors for endo- or epiphytic colonization

11:05 - 11:20 **Lilach Iasur Kruh**

Substance secreted by the endophytic *Dyella*-like bacterium as a potential solution against yellows disease of carrots

**SESSION 4 Plant understanding and improvement of beneficial interactions with microbes**

11:20 - 12:45

**Session chairs: Corné Pieterse & Angela Sessitsch**

11:20 - 11:45 **Corné Pieterse (Utrecht University, NL)**

The root microbiome and plant health

11:45 - 12:00 **Abhishek Shrestha**  
On a search to unravel the differences in molecular events between primable and non-primable barley varieties

12:00 – 12:15 **Pierre Hohmann**  
Breeding for microbiome-mediated disease resistance

12:15 - 12:30 **Philipp Franken**  
Petunia as model to breed for new crop cultivars with improved responses to root-endophytic fungi

12:30 - 12:45 **Davide Gerna**  
Bacteria on the surface of wheat seeds affect hydrogen peroxide production during early seedling growth

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12:45 - 13:30 **Lunch break**

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**SESSION 5 The holobiont plant: multitrophic interactions**

13:30 - 14:10 **Session chairs: Leo van Overbeek & Gabriele Berg**

13:30 - 13:55 **Leo van Overbeek (Wageningen University and research, NL)**  
Occurrence of human pathogenic micro-organisms in plant production systems

13:55 - 14:10 **Paloma Duran**  
Dissecting the multispecies interaction network at the Arabidopsis root-soil interface

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14:10 - 15:00 **POSTER TALKS II:**

Souvik Kusari: *Ecological role of maytansine is revealed by in situ MALDI-HRMS-imaging of Maytenus senegalensis during the germination process*

Andrea Manzotti: *How do phytohormones influence the composition of fungal endophyte communities in tomato roots?*

Carmen Bianco: *Effects of structural and functional analogues of IAA in triggering biological nitrogen fixation in non-legume plants*

Olga Calvo: *Effects of plant growth-promoting rhizobacteria on barley under global warming-associated environmental factors*

Nurmi Pangesti: *Back-to-the-roots: unraveling the endophytic microbiome in banana germplasm to control Fusarium wilt*

Guofen Li: *Cooperation of Trichoderma spp. and Bacillus spp. in biocontrol applications*

Klara Bradacova: *Bio-effectors in the rhizosphere: Microbial consortia as inoculants for improved plant growth and mineral nutrition*

Mark Winter: *Biocontrol of Fusarium crown and root rot in wheat by inhibitory Streptomyces isolates – it's complicated*

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15:00 **SOCIAL EVENT: Vienna tours (optional)**

**DAY 3 | Wed, 6. Dec. | 8:30<sup>A.M.</sup> - 18:10<sup>P.M.</sup>**

**SESSION 5 The holobiont plant: multitrophic interactions**

08:30 - 09:55 **Session chairs: Leo van Overbeek & Gabriele Berg**

08:30 - 08:55 **Gabriele Berg (TU Graz, AT)**

From seeds to postharvest: the impact of microbial diversity on plant health

08:55 - 09:10 **Adam Schikora**

Diversified microbiome bears the potential to protect crop plants against human pathogens

09:10 - 09:25 **Robert Czajkowski**

Ecological interaction of lytic bacteriophages and plant pathogenic bacteria – a case study involving lytic phages and pectinolytic plant pathogens in plant-associated environment

09:25 - 09:40 **David Ezra**

Synthetic volatile mixture, from a fungal origin, controls *Sclerotium rolfsii* both *in vitro* and in soil

09:40 - 09:55 **Roeland Berendsen**

Disease-induced assemblage of a plant-beneficial bacterial consortium

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09:55 - 10:20 **Coffee break**

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**SESSION 6 The holobiont plant: microbiome understanding**

10:20 - 11:25 **Session chairs: Yvan Moënne-Loccoz & Birgit Mitter**

10:20 - 10:45 **Tuesday Simmons (UC Berkeley, US)**

Drought alters the development of the grass root microbiome

10:45 - 11:10 **Yvan Moënne-Loccoz (Université de Lyon, FR)**

Plant-beneficial bacteria in the rhizosphere microbiome of cereals

11:10 - 11:25 **Viviane Radl**

From seeds to roots: the role of seed-borne endophytes for the composition of the active microbiome of barley roots

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11:25 - 13:45 **Poster session 2 & Lunch break**

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**SESSION 6 The holobiont plant: microbiome understanding**

13:45 - 15:25 **Session chairs: Yvan Moënne-Loccoz & Birgit Mitter**

13:45 - 14:10 **Birgit Mitter (AIT Austrian Institute of Technology GmbH, AT)**

Understanding the plant microbiome

14:10 - 14:25 **Saskia Bindschedler**

The oxalate-carbonate pathway: an ecosystem service for sustainable agriculture

14:25 - 14:40 **Klaus Schlaeppli**

Smart farming: manipulating root and soil microbiomes through specific cropping practices

- 14:40 - 14:55 **Linda Thomashow**  
Phenazine-1-carboxylic acid influences biofilm development and turnover of rhizobacterial biomass in a soil moisture-dependent manner
- 14:55 - 15:10 **Enoch Narh Kudjorjie**  
Effects of plant secondary metabolite benzoxazinoids on the plant microbiome
- 15:10 - 15:25 **David Hallahan**  
Contrasting soil microbial community profiles associated with outliers in early growth of corn (*Zea mays*)
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- 15:25 - 15:50 **Coffee break**
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- 15:50 - 16:40 **POSTER TALKS III:**  
Saranya Kanukollu: *Unravelling the key players of grassland plant microbiomes behind methanol consumption*  
Valeria Verrone: *Engineering the plant microbiome to improve crop quality and yield*  
Natacha Bodenhausen: *Root microbiome dynamics in response to phosphate*  
Soumitra Paul Chowdhury: *Legacy effects of long-term organic or conventional farming practices on the rhizosphere microbiome and health of the next plant generation measured under controlled conditions.*  
Robert R. Junker: *Asymmetric dependencies – a novel statistic to reveal non-reciprocal relationships between plant associated microbes*  
Thijs Van Gerrewey: *Biostimul'eau: crop quality improvement in (vertical) hydroponics through transplantation of a soil rhizosphere microbial community*  
Julia Hengl: *Microbial nanoclay and nanocellulose composite formulations using Gram negative endophytic bacteria for maize*  
Kristin Dietel: *The project "SaatMaisPlus": Development of non-chemical seed treatments for maize*  
Natalia Hatrakova: *Two-way protection of Gram-negative beneficial soil bacteria *Paraburkholderia phytofirmans* in dry alginate formulations: inducing the cell adaptation in tolerance to desiccation*
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- 16:40 - 18:10 **Poster Session 3**
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- 19:00 - 24:00 **miCROPe Party (optional)**

**DAY 4 | Thu, 7. Dec. | 9:00<sup>A.M.</sup> - 15:00<sup>P.M.</sup>**

**SESSION 7 Application technologies & formulations**

09:00 - 10:35 **Session chairs: Anant Patel & Claudia Preininger**

09:00 - 09:25 **Anant Patel (Bielefeld University of Applied Sciences, DE)**

Formulations for novel biological pest control strategies

09:25 - 09:50 **Claudia Preininger (AIT Austrian Institute of Technology, AT)**

Design of microbial formulations – connecting material properties with bacterial viability and overall treatment performance

09:50 - 10:05 **David Schisler**

Non-viable antagonist cells are associated with reduced biocontrol performance by viable cells of the yeast *Papiliotrema flavescens* against Fusarium head blight of wheat

10:05 - 10:20 **Ahmed Idris Hassen**

Co-inoculation of symbiotic rhizobia and free-living rhizobacteria (PGPR) have a role in enhancing growth in the legumes lucerne (*Medicago sativa* L.) and cowpea (*Vigna unguiculata* L.)

10:20 - 10:35 **Virginia Estévez**

Shelf-life and storage of *Trichoderma asperellum* strain T34 applied as a seed treatment on experimental corn seeds

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10:35 - 11:00 **Coffee break**

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11:00 - 12:25 **Phytobiomes Alliance Session**

**Session chairs: Kellye Eversole & Angela Sessitsch**

11:00 - 11:25 **Kellye Eversole (International Phytobiomes Alliance, US)**

Building a foundation to combine site-specific biological and physical data for next-generation precision agriculture

11:25 - 11:40 **Slavica Djonovic**

Streptomyces as endophytes: understanding the mode of action of plant growth promotion under abiotic stress

11:40 - 11:55 **Jim Germida**

Bacterial and fungal endophytes increase growth and yield of agricultural crops at heat and drought stressed field sites

11:55 - 12:10 **Michael Ionescu**

Harnessing plant understanding and microbiome functional diversity to stimulate plant yield production

12:10 - 12:25 **Kateryna Zhalnina**

Root exudate chemistry and microbial substrate preferences drive rhizosphere microbial community assembly during plant development and under nutrient and water stress

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12:25 - 13:15 **Lunch break**

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13:15 - 13:55 **Future of microbial products & regulatory issues**

13:15 - 13:55 **Willem Ravensberg (Koppert Biological Systems, NL)**

The future of microbial products and regulatory issues

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13:55 - 14:40 CLOSING LECTURE

**Janet Jansson (Pacific Northwest National Laboratory, US)**

Phenotypic response of soil microbiomes to environmental change

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14:40 - 15:00 **Closure**

## Posters Table – Poster Session 1

Poster Session 1 Monday 4 Dec., 17:30 - 20:30 (Display posters from Dec. 4, 13:00 - Dec. 5, 12:45)

Poster #	Successful microbial products	Poster #	New mechanisms involved in beneficial plant-microbe interactions		
PP-SMP-01	Dorota M. Krzyzanowska	PP-NMI-01	Manuela Rändler	PT-NMI-19	Anna Clocchiatti
PP-SMP-02	Or Sharon	PP-NMI-02	Gilles Vismans	PT-NMI-20	Sebastian Schneider
PP-SMP-03	Rajnish Khanna	PP-NMI-03	Yunuen Tapia Torres	PT-NMI-21	Zhibo Li
PP-SMP-04	Michael Przyklenk	PP-NMI-04	Sebastian Baier	PT-NMI-22	Sandra Bredenbruch
PP-SMP-05	Brenda Loznik	PP-NMI-05	Edward Camilo Rojas	PT-NMI-23	Gabriele Schiro
		PP-NMI-06	Michael Opitz	PT-NMI-24	Caroline Baudson
Poster #	Microorganisms for rural development	PP-NMI-07	Annemieke van der Wal		
PP-MRD-01	Sandra Matthijs	PP-NMI-08	Krzysztof Wieczorek	PP-V-01	Hyun Kim
PP-MRD-02	Tinatin Doolotkeldieva	PP-NMI-09	Sylwia Jafra		
PP-MRD-03	Jolien Venneman	PP-NMI-10	Vincent Greffe		
PP-MRD-04	Nongcebo Sandra Memela	PP-NMI-11	Peter Kusstatscher		
PP-MRD-05	Ernestina Galdeano	PP-NMI-12	Bernard Dumas		
PP-MRD-06	Vishal Prasad	PP-NMI-13	Abdul Aziz Eida		
PT-MRD-08	Andrea Kunova	PP-NMI-14	Meike A. C. Latz		
PT-MRD-09	Daniel Bravo	PP-NMI-15	Orna Liarzi		
		PP-NMI-16	Lu Zhou		
		PP-NMI-17	Danny Vereecke		
		PP-NMI-18	Marine Valette		

## Posters Table – Poster Session 2

Poster Session 2 Wednesday 6 Dec., 11:25 - 13:45 (Display posters from Dec. 5, 12:45 - Sept. 6, 15:25)

Poster #	Plant understanding and improvement of beneficial interactions with microbes				
PP-PU-01	Bliss Furtado	PT-PU-25	Souvik Kusari	PT-MI-19	Klara Bradacova
PP-PU-02	Robert Koller	PT-PU-26	Carmen Bianco	PT-MI-20	Guofen Li
PP-PU-03	Anton Prof. Dr. Hartmann	PT-PU-27	Andrea Manzotti	PT-MI-21	Mark Winter
PP-PU-04	Evelyn Hackl	PT-PU-28	Olga Calvo	PT-MI-22	Nurmi Pangesti
PP-PU-05	Walter Chitarra				
PP-PU-06	Anna Andreozzi	<b>Poster #</b>	<b>The holobiont plant: multitrophic interactions</b>		
PP-PU-07	Karolin Pohl	PP-MI-01	Pascal Mülner		
PP-PU-08	Natalia Valdespino	PP-MI-02	Wietse de Boer		
PP-PU-09	Julian Preiner	PP-MI-03	Christina Schönhuber		
PP-PU-10	Lempie Ekandjo	PP-MI-04	Andreea Cosoveanu		
PP-PU-11	Helena Moreira	PP-MI-05	Victor Gonzalez Menendez		
PP-PU-12	Everardo V.S.B. Sampaio	PP-MI-06	Constanze Hauser		
PP-PU-13	Negar Ghezel Sefloo	PP-MI-07	Myrto Tsiknia		
PP-PU-14	Sofia Pereira	PP-MI-08	Shubhangi Sharma		
PP-PU-15	Andrés Sauvêtre	PP-MI-09	Luca Nerva		
PP-PU-16	Ana D.S. Freitas	PP-MI-10	Anna Kaja Hoeyer		
PP-PU-17	Giampaolo Buriani	PP-MI-11	Ana Fernández Scavino		
PP-PU-18	Fani Ntana	PP-MI-12	Ezgi Özkurt		
PP-PU-19	Cristina Rodriguez-Gil	PP-MI-13	Gisell A. Garcia		
PP-PU-20	Su-May Yu	PP-MI-14	Abdoul Razack Sare		
PP-PU-21	Youry Pii	PP-MI-15	Vincenzo De Rocchis		
PP-PU-22	Shu-Hua Hsu	PP-MI-16	Alejandro del Barrio Duque		
PP-PU-23	Honghong Li	PP-MI-17	Tomasz Maciąg		
PP-PU-24	Fabio Valentinuzzi	PP-MI-18	Karin Hage-Ahmed		

## Posters Table – Poster Session 3

Poster Session 3 Wednesday 6 Dec., 16:40 - 18:10 (Display posters from Dec. 6, 15:25 - Sept. 7, 11:40)

Poster #	The holobiont plant: microbiome understanding			Poster #	Phytobiomes Alliance
PP-MU-01	Günter Brader	PT-MU-22	Soumitra Paul Chowdhury	PP-PA-01	Markus Weinmann
PP-MU-02	Vasvi Chaudhry	PT-MU-23	Valeria Verrone	PP-PA-02	Gregory Sword
PP-MU-03	Denise Bachmann	PT-MU-24	Saranya Kanukollu	PP-PA-03	Samuel Jacquiod
PP-MU-04	Matthieu Barret	PT-MU-25	Robert R. Junker	PP-PA-04	Sofie Goormachtig
PP-MU-05	Alessandro Bergna	PT-MU-26	Thijs Van Gerrewey	PP-PA-05	Nikolaus Pfaffenbichler
PP-MU-06	Chrysi Sergaki	PT-MU-27	Natacha Bodenhausen	PP-PA-06	Malay C. Saha
PP-MU-07	Lucia Ferrando			PP-PA-07	Emina Mulaosmanovic
PP-MU-08	Tim Dumonceaux	<b>Poster #</b>	<b>Application technologies &amp; formulations</b>		
PP-MU-09	Franziska Buchholz	PP-ATF-01	Isha Jamil	<b>Poster #</b>	<b>Future of microbial products &amp; regulatory issues</b>
PP-MU-10	Xu Cheng	PP-ATF-02	Marie-Emmanuelle Saint-macary	PP-FR-01	Ignacio Gonzalez
PP-MU-11	Sofie Thijs	PP-ATF-03	Sirisha Kanugala	PP-FR-02	Miranda M. Hart
PP-MU-12	Nina Bziuk	PP-ATF-04	David Weller		
PP-MU-13	Morgan McPherson	PT-ATF-05	Natalia Hatrakova		
PP-MU-14	Laura M Kaminsky	PT-ATF-06	Julia Hengl		
PP-MU-15	Antonio Cellini	PT-ATF-07	Kristin Dietel		
PP-MU-16	Xiaoxia Zhang				
PP-MU-17	Suliana Teasdale				
PP-MU-18	Wouter Sillen				
PP-MU-19	Felix G. Moronta-Barrios				
PP-MU-20	M. Haïssam Jijakli				
PP-MU-21	Carolina Escobar				

# LECTURES

Opening Lecture

**Microbe-assisted crop production, opportunities, challenges & needs**

**Vienna, Austria, 4- 7 December 2017**

*Opening Lecture*

Supported by



## PL-01 Beneficial effects of microbes on plants

### Esperanza Martínez-Romero

Ecological Genomics, Center of Genomic Science, UNAM, Mexico

Crop yields may be severely reduced by pathogenic fungi or bacteria and insects, as well as by nutrient limitations. Microbes in plants may promote health as probiotics do in animals. Thus, bacteria and fungi are our allies in the quest for food production when antagonizing pathogens, fixing nitrogen or solubilizing soil nutrients. The diversity of bacteria associated with plants has been extensively studied and bacteria beneficial to plants have been identified. Novel metagenomic approaches are contributing a growing list of potentially beneficial symbionts. The prevailing questions are which microbes are effective crop inoculants and what is their real performance in the presence of locally adapted bacteria under field conditions. Although we do not have a recipe for choosing ideal inoculants, there are some microbes that have been chosen based on previous field trials or phenotypic characteristics. For example, nitrogen fixing bacteria are good candidates in low nitrogen soils. Many beneficial isolates excrete phytohormones such as auxins that promote plant growth, and there seems to be functional redundancy in plant associated bacteria having this stimulatory capacity. To improve plant growth promotion, novel methods are needed to supply probiotics; for example irrigation water may be a good source of bacteria. Additionally, microbial consortia are being tested for their potentially synergistic beneficial effects. Furthermore, the possibility to evolve or construct more adapted beneficial plant symbionts is being followed. Natural seed transmission of bacteria would give seedlings an adaptive advantage if the inherited bacteria antagonize pathogens or promote plant growth. Our studies on seed bacteria allowed the isolation of many *Bacillus* species, one of which turned out to be identical to a commercial product that is used for fungal biocontrol in different crops in Mexico.

On the other hand, there is not a super microbe to fight all pathogens and thus we face a very complex and difficult problem, especially considering the large densities of genetically uniform plants in crops with homogeneous disease susceptibilities. It is uncertain whether microbes selected for their beneficial effects will fulfill our expectations, especially in view of the ongoing arm race with pathogens and considering that our enemies, the pest insects, also associate with bacteria or fungi that confer adaptive advantages to them.

Financed by CONACyT 253116 and PAPIIT IN207615.

**Microbe-assisted crop production, opportunities, challenges & needs**

**Vienna, Austria, 4- 7 December 2017**

***Successful microbial products***

**Chairs: Jürgen Köhl & Angela Sessitsch**

## SMP-01 The challenge of developing microbial biocontrol products for disease control

Jürgen Köhl

Wageningen University & Research, Netherlands

Commercial biological control products for the use against plant diseases must be highly efficient against the targeted diseases. However, the used antagonists have to fulfil a broad range of requirements besides their ability to control pathogens. Major requirements concern the market size for the envisaged product, ecological characteristics and production costs of the antagonists, safety, toxicological and eco-toxicological risks and protection of intellectual property rights. Each of these requirements can be even important for the impact of a biological control product on the market. Consequently, a broad range of criteria has to be considered during the selection of new antagonists. Screening programs can use a stepwise approach to assess candidate antagonists for this broad range of criteria in order to exclude unwanted candidates in an early stage. The antagonists selected with such a screening strategy fulfilling major criteria will combine suitable characteristics for registration and marketing as commercial biocontrol products. Essential decisions at the beginning of new screening programs are to collaborate with biocontrol industries from the beginning, to include relevant commercial questions early during the screening program and to combine the expertise in plant pathology with expertise in several other disciplines, e.g. biotechnology, agronomy, microbiology, toxicology, registration, marketing and product development.

Hundreds or thousands of candidates are usually tested in screening programs to find new antagonists against a targeted pathogen. Examples of screening programs for the development of new biocontrol products will be given. Screening strategies applied for the selection of antagonists against *Rhizoctonia solani* in lettuce, *Botrytis cinerea* in various crops, *Venturia inaequalis* in apple and *Blumeria graminis* in wheat ([www.biocomes.eu](http://www.biocomes.eu)) will be discussed and their results will be compared.

Considering the wide range of commercially important criteria already during screening programs can further increase the impact of biocontrol science on plant protection.

The project BIOCAMES has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no 612713.

## SMP-02 BVT fungus *Clonostachys rosea* CR-7 vectored by bumble bees protects plants from Grey Mold infection and enhances yields

Christoph Lehnen<sup>1</sup>, John C. Sutton<sup>2</sup>, Sherri Tedford<sup>2</sup>, Dr. Albert Bassi<sup>2</sup>

<sup>1</sup> Bee Vectoring Technologies / Amanzi AG, Switzerland

<sup>2</sup> 4160 Sladeview Crescent, #7, Mississauga, Ontario, L5L 0A1, Canada

**The cycle of Grey Mold in berry crops** Grey Mold is an extremely common disease that affects many crops around the world, and is often seen on strawberries. Caused by the fungus *Botrytis*, the infection begins on dead leaves and flowers, eventually affecting the berries themselves. Like many fungi, *Botrytis* grows by producing hyphae and is spread when spores from the hyphae become airborne, landing on nearby flowers. The spores germinate inside the new flower, stimulated by the nutrient rich tissue. As *Botrytis* grows, it produces toxins that destroy the cell membranes of the flower, and later on, the berry.

**How does the BVT fungus control *Botrytis*?** *Clonostachys* is a naturally occurring fungus that forms a symbiotic relationship with plants it contacts. Once established inside a flower, *Clonostachys* helps prevent the growth and development of *Botrytis* without harming the plant in any way. *Clonostachys* protects plants from *Botrytis* infection in several ways: **Healthy colonizer** When plant tissues first show signs of stress or injury from *Botrytis*, *Clonostachys* is naturally triggered to rapidly grow in the affected tissues, blocking further development of the pathogen. Similarly, *Clonostachys* is triggered to occupy senescing plant tissues before a harmful pathogen can colonize the dying area. **Rapid growth** As *Clonostachys* grows, its tiny colonies send out signals in the plant both locally and systemically, which produce enhanced levels of natural resistance to disease organisms. As induced resistance increases, the plant is protected from potential infections. **Plant vigour** *Clonostachys* colonies actually assist in maintaining plant vigour, almost like an anti-aging treatment for plants! This is commonly observed as a delay in the yellowing of green plant tissues. Not only does this delay the onset of *Botrytis* infection, but it may also increase nutrient uptake due to prolonged functional lifespan of the plant tissues. *Clonostachys* spores are delivered directly to a crop's flowers by the bees when they forage for nectar and pollen. Within a few hours, the *Clonostachys* spores that land on plant tissue germinate and form colonies between the superficial plant cells. *Clonostachys* instantly begins working in the plant's favour, increasing crop yield and mitigating spread of *Botrytis*. Trials in Europe and North America show good performance in reducing Grey Mold infections and enhancing marketable yields, and give indications of extended shelf life of strawberries.

### **SMP-03 Improving the efficacy of organic fertilizers with protozoa**

**Brenda Loznik**

Technical specialist Plant-Micorbes, ECOstyle, Netherlands

Organic fertilizers are considered an environmentally friendly way of providing essential nutrients to plants. Nutrients are present in organic forms, which reduces leaching and problems associated with overfertilization. This however can become a problem when microbial activity is low. Reduced mineralization can result in a potential shortage of plant nutrients, with negative impacts on plant growth. Protozoa are unicellular eukaryotes that have previously been described to stimulate the mineralization of organic materials by grazing on soil bacteria, thereby stimulating the activity of the bacteria as well as releasing nutrients from bacterial biomass. The aim of the present study was to determine whether protozoa could be used to improve the efficacy of a commercially available organic lawn fertilizer. To test the hypothesis, a trial was designed using two types of sandy soil sown with *Lolium perenne*, 4 species of protozoa (*Cercomonas lenta*, *Rosculus terrestris*, *Bodomorpha* sp. and *Rosculus elongata*) and three concentrations of each protozoa ( $10^2$ ,  $10^3$  and  $10^4$  cysts per gram of fertilizer). Three weeks after applying the treatments, *Lolium perenne* was clipped and the fresh and dry weight of the clippings were measured. Six weeks after the application of the treatments, *Lolium perenne* was harvested and fresh and dry weight of the above and belowground plant parts were determined. The results showed that SP1 (*Cercomonas lenta*) and SP2 (*Rosculus terrestris*) significantly increased aboveground plant biomass. A follow-up study in 2015 confirmed synergistic effects when both species were combined. Additionally, the different species of protozoa were observed to show clear differences in feeding patterns when added to a monoculture of 6 different *Bacillus* species commonly added to organic fertilizers to stimulate mineralization. Furthermore it was shown that protozoa had a significant positive or negative effect on the *Bacillus* density in the soil according to the protozoa species used. It can be concluded that protozoa can be used to improve the efficacy of organic fertilizers. A patent for this invention was published in June 2017 (WO2017/105238). The results of the present and follow-up studies also showed potential for the use of protozoa in improving the establishment and possibly the efficacy of biostimulants and fertilizers containing *Bacillus* in the field.

### **SMP-04 Isoprene Identified in the Volatolome of Endophytic Fungi as a Promising Phosphine Alternative**

**Ross Mann**, Christian Krill, Desmond Auer, Mofakhar Hossain, Simone Rochfort, Jacky Edwards, Ian Porter, German Spangenberg

Agriculture Victoria Research Division, AgriBio, Centre for AgriBioscience, Australia

Fungal endophytes are known to confer enhanced tolerance towards both biotic and abiotic stresses to their host plants. Recently, bioactive secondary metabolites produced by these fungi have come into focus for potential application in agriculture. In particular, fungal volatile organic compounds (VOC) have shown application as fumigants to control fungal pathogens and insect pests of stored grain. These fungal VOCs open up new avenues to overcome resistance of stored grain pests to the widely used fumigant phosphine.

Using Solid-Phase Microextraction Gas Chromatography – Mass Spectrometry, a number of potential biocidal VOCs were identified in the volatolome of native endophytic fungi – *Muscodor* sp. and *Nodulisporium* sp. We evaluated these compounds in isolation and combination for their biocidal activity against major insect pests of stored grains such as *Tribolium castaneum*, *Cryptolestes ferrugineus* and *Rhyzopertha dominica*, and fungal pathogens such as *Fusarium verticillioides*. From the compounds tested, isoprene, produced by *Nodulisporium* sp, emerged as the most promising candidate as a stored grain fumigant. Small scale, mock grain silo experiments demonstrated good efficacy and low residue levels. Combining isoprene with other fungal VOCs further increased insecticidal activity, including at reduced rates.

Isoprene is highly volatile and produced commercially in large quantities as a building block for organic synthesis of rubber. It is naturally produced by many plant species, and is commonly found in low levels in many foodstuffs. It is the most abundant hydrocarbon detectable in human breath and has no acute toxicity, demonstrating its relative safety compared to other fumigants, including phosphine.

**Microbe-assisted crop production, opportunities, challenges & needs**

**Vienna, Austria, 4- 7 December 2017**

***Microorganisms for rural development***

**Chairs: Ian Sanders & Alia Rodriguez**

## MRD-01 A microbial green revolution: Breeding microbes to feed the world

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Today we face an unprecedented challenge to feed the growing global human population that can only be achieved with major changes in how we combine science with agronomy. The green revolution is thought to have saved approximately 1 billion lives and was largely fuelled by effective plant genetic improvement (breeding) programs. All plants live with microbes and it is well known that many rhizosphere microbes can be beneficial for plant growth. However, microbiologists have largely ignored the principals used by plant breeders of taking naturally occurring genetic variation as a base to genetically improve microorganisms to increase crop production.

Mycorrhizal fungi form symbioses with all our major crops. They help plants obtain phosphate from the soil; an essential nutrient that limits crop production in the tropics. In recent years, our group, has shown that naturally occurring processes in these fungi can be used to develop genetically novel varieties. These novel fungal genotypes have been shown to induce enormous changes in rice growth (up to five fold) in greenhouse conditions.

Cassava is an obvious target for the application of mycorrhizal fungi. Cassava is globally important, annually feeding almost a billion people in 105 countries. It is an important crop for subsistence farming throughout tropical and subtropical regions for smallholder farmers, but especially in sub-Saharan Africa. In this presentation, I will demonstrate that genetic improvement of mycorrhizal fungi in a lab-based *in vitro* system can be realistically and practically used to achieve large increases in cassava production in real farming situations in tropical regions.

Our research demonstrates the power of using microbial genetics to improve food production and that this could potentially lead to more rapid improvements than conventional genetic plant improvement programs. Furthermore, variation in growth response of crop varieties to inoculation with mycorrhizal fungi likely has a genetic basis and responsiveness to these microbes is a trait that could also be incorporated into crop breeding programs leading to further increases.

## **MRD-02 Importance of rhizobia in Agriculture: potential of the commercial inoculants and native strains for improving legume yields in different land-use systems**

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Legumes play an important role in the traditional diets of many regions throughout the world. The ability of legumes to fix atmospheric nitrogen in association with rhizobia gives them the capacity to grow in very degraded soils. But do we have to systematically inoculate legumes? Our results suggested that the systematic inoculation of both cowpea and green gram with commercial inoculants to improve yields is not really justified, native strains performing better than inoculated strains. But when native rhizobia nodulating legumes are not naturally present, application of rhizobial inoculants is commonly used. We showed that the utilization of effective good-quality rhizobial inoculants by farmers have a real potential to improve soybean yields in unfertile soils requesting high applications of mineral fertilizers. For example an effective commercial inoculants was tested in Kenya. Application of the rhizobial inoculant significantly increased the soybean yields in all mandate areas (about 75% of the farms). But nodule occupancy analysis showed that a high number of nodules occupied by the inoculated strain did not obviously lead to an increase of soybean production. Soil factors seemed to affect the inoculant efficiency whether the strain is occupying the nodules or not. Soil pH significantly affected nodulation and yield, though the effect was variable depending on the region. We concluded that the competitiveness of rhizobial strains might not be the main factor explaining the effect (or lack of) of legumes inoculation in the field.

Meanwhile, we assessed if several factors such as cropping systems, N fertilization and application of crop residues affect the genetic diversity of native strains of rhizobia nodulating soybean in Kenya without any inoculation. Results showed that nodulation was not significantly affected by the different factors except N fertilization, regardless the season and also the occurrence of low diversity of native rhizobial strains capable to nodulate soybean. The factors didn't increase the diversity of the rhizobia but results indicated an effect on the distribution of the 3 profiles within the nodules of the plants. It suggests that cropping systems and both N and crop residues applications affect more specifically plant growth and grain yields than the diversity of the native rhizobia nodulating soybean.

Our work shows how diverse are the factors influencing the success of the field rhizobial inoculation of legumes.

### MRD-03 Desert rhizosphere microbes for future sustainable SMART agriculture

**Maged M. Saad**<sup>1</sup>, Abdul Aziz Eida<sup>1</sup>, Ameerah Bokhari<sup>1</sup>, Hanin Alzubaidy<sup>1</sup>, Rewaa Jalal<sup>1</sup>, Yakun Xie<sup>1</sup>, Cristina Andres-Barrao<sup>1</sup>, Lukas Synek<sup>1</sup>, Axel De Zelicourt<sup>2</sup>, Ihsanullah Daur<sup>3</sup>, Heribert Hirt<sup>1</sup>

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A major global challenge of the 21<sup>st</sup> century is achieving global food security. The mission of feeding a rapidly growing human population, which is projected to reach 9.7 billion by the year 2050, is largely hindered by adverse environmental stresses. Drought, heat, nutrient deficiency and salinity are primary causes of poor crop yields, accounting for more than 60% yield losses of major crops. These stresses are predominantly present in regions where agriculture is currently not possible, such as deserts which cover more than 20% of Earth's land surface. Although major crops lack the ability to survive under these extreme, harsh environmental conditions, a variety of plants are able to adapt and cope with these stresses by establishing associations with beneficial microbes in the rhizosphere. In the DARWIN21 project (<http://www.darwin21.net>), the biodiversity of the pioneer plant root microbes and the mechanisms of enhancing the tolerance of plants to different abiotic stresses are investigated. By exploring the roots of pioneer plants from various deserts, we collected and characterized a large variety of bacterial endophytes. High throughput screening assays were developed and successfully used to identify bacterial strains with the ability to promote growth and/or enhance salt stress tolerance on the model plant *Arabidopsis thaliana*. Different biochemical and molecular approaches were used to understand the mechanisms of the growth promoting activity of selected strains including the comparative genome sequencing of bacterial strains, cell-imaging techniques, dual RNAseq transcriptional profiling and genome-wide association studies on different *Arabidopsis* accessions. Furthermore, the performance of successful bacterial candidates was evaluated under field conditions with three economically important crops: alfalfa, barley and wheat. The results demonstrate the great potential of using bacteria as a growth promoting bio-agent under extreme environmental conditions offering a promising solution for long-term sustainability of food production and SMART Agriculture practices as a key solution to eradicate hunger in poor developing countries.

## MRD-04 Plant growth promoting bacteria: a sustainable tool to minimize water footprint in agriculture in arid and semi-arid zones

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Impact of climate change in arid and semi-arid zones is leading us to deliver innovative strategies in agriculture to increase safe food production and at the same time minimizing water footprint. Desert farming, combined with an improved wastewater recycling, might represent a smart solution to guarantee food access in water stressed countries to an increasing population. Plant Growth Promoting (PGP) microorganisms, recruited as components of the plant microbiome, exert several mechanisms to improve plant growth and health and could therefore be exploited to improve plant productivity and services.

We aimed at the study of PGP bacteria associated to plants growing in Mediterranean African Countries (MACs): plants of interest for desert farming and/or adapted to arid/saline soils (*Sorghum* sp., *Argania spinosa*, *Salicornia* sp.) and plants cultivated in constructed wetland facilities having phytodepuration activity.

A large collection of rhizosphere and endosphere bacterial isolates was established, dereplicated and identified. The phylogenetic and phenotypic characterization led to select strains for the *in vivo* assessment of PGP activity: i) classifiable as GRAS (Generally Regarded As Safe), basing on the literature screening about their species, ii) resistant to osmotic and saline stresses and iii) sensitive to different classes of antibiotics. The *in vivo* PGP assays were performed on potted tomato plants under greenhouse conditions, artificially inducing water stress. Several bacterial strains, including isolates belonging to the *Bacillus* and *Pseudomonas* genera, demonstrated to significantly increase plant growth compared with non-inoculated controls.

Besides corroborating previous findings on the potential of extremophilic plants as source of PGP bacterial strains exploitable under adverse condition, we identified promising candidates for the future development of biofertilizers tailored on the need of MACs, exploitable to sustainably improve desert farming and phytodepuration.

Acknowledgements

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Poster Talks I

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***Poster Talks I***

## **PT-MRD-08 Exploring plant endophytic *Streptomyces* for their use in sustainable agriculture**

**Andrea Kunova**, Jana Stecova, Cristina Pizzatti, Elena Maria Colombo, Matias Pasquali, Marco Saracchi, Paolo Cortesi

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Streptomycetes, together with other bacterial species such as *Bacillus* spp. and *Pseudomonas* spp., have been widely studied as valuable sources of strains able to promote plant growth and control plant pathogens in agriculture. *Streptomyces* spp. are fundamental in pharmaceutical industry, however, their practical use in agriculture as plant growth promoting bacteria (PGPB) and biocontrol agents (BCAs) is still in its infancy.

Based on the *in vitro* results, the most promising *Streptomyces* strains were tested in controlled conditions to evaluate their effect on germination and plant growth of various horticultural crops, including monocots, i.e. maize, wheat, and rice; and dicots, i.e. lettuce, lamb lettuce, rocket, savoy cabbage, soybean, onion, and tomato. The strains showed species-specific promotion of seed germination and plant growth. These effects were dose-dependent, however, higher inoculum dose not always resulted in better performance of the strain. The biocontrol potential of *Streptomyces* strains was tested on the pathosystem *Lactuca sativa* – *S. sclerotiorum*. The most active strains were able to reduce the disease incidence by ca. 50%. The disease risk was reduced when the strains were applied to the *S. sclerotiorum*-infected soil one week prior to lettuce sowing.

Our results confirm that *Streptomyces* have a great potential to act as BCA and PGPB, although, to be able to obtain their optimal performance, the inoculum dose, timing and the application method of individual strains need to be fine-tuned for specific crops.

## PT-MRD-09 Assessing cadmium-tolerant endophytic bacteria as an strategy to bio-remediate cadmium presence in Cacao crops from Colombia

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Cadmium is a heavy metal that, in high concentrations, might be toxic to all living organisms. It has been found that in some South American soils cultured with Cacao, cadmium could reach elevated concentrations. Although one of the strategies to deal with high cadmium concentration that migrate from soil to the Cacao grains is to explore the endophytic populations of the so-called cadmium-tolerant bacteria (E-CdtB), very few is known regarding this functional group of bacteria. Therefore, our goal in this study was to isolate and identify E-CdtB and characterize their metabolic capacity to immobilize cadmium using molecular and calorimetric techniques. We have set a pool of 45 E-CdtB isolated from 23 varieties of Cacao represented in 7 genera. The strains *Bacillus* sp. ECdtB1, 2, 3, *Herbaspirillum* sp. ECdtB14 and *Ralstonia* sp. ECdtB5 were selected due the amplification of *cad* and *smt* genes, related with cadmium efflux and its chelation in the bacterial cytoplasm. The strains were also selected due their higher immobilization ratios of Cd<sup>2+</sup> (1.04, 1.08, 1.07, 1.2 and 1.9 mg l<sup>-1</sup> of Cd in 12 days, respectively) in Batch experiments. On the other hand, the strain ECdtB2 has shown a major activity during the immobilization process and minor biomass production (1050 J s<sup>-1</sup> and 0.8 OD<sub>600nm</sub>, respectively), whereas the strains ECdtB3 and 5 have lower activity and higher biomass production (both 580, 405 J s<sup>-1</sup> and 1.4, 1.1 OD<sub>600nm</sub>, respectively). Therefore, the strain *Bacillus* sp. ECdtB2 has been selected for a further greenhouse experiment in order to determine endophytic growth in rootstocks and its immobilization ratio and maximum metabolic capability under controlled conditions.

## PT-NMI-23 Spatial distribution of Phyllosphere fungi in a heterogeneous wheat field.

**Gabriele Schiro**, Thomas Müller, Marina Müller

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The phyllosphere of common wheat (*Triticum aestivum*) is colonized by various microorganisms, such as bacteria, yeasts and filamentous fungi. The wheat ear (the grain bearing top part of the plant) in particular, hosts a microbial community composed, among others, by various species of fungi well known due to their phytopathogenicity for the host plant. Besides their parasitic activity, they also produce toxic metabolites (mycotoxins) dangerous to end consumers once the harvest has been processed into food. A deeper understanding of the mechanisms leading influencing the microbial community composition is therefore needed to shed light on the infection dynamics influencing the presence of such pathogens.

In my work I analysed the spatial distribution of *Alternaria spp.* and *Fusarium spp.* in different fields in the region of Brandenburg (Germany). The fields were chosen for their topographic heterogeneity, which caused differences in the microclimatic conditions among various points within them. The central aim of my study is to observe how the microbial community composition varies spatially, using different microclimatic conditions as proxies. Samples were collected from 1 square meters sampling areas distributed in the tested fields. The fungal presence was analysed using qPCR techniques, mycotoxins measured with GC-MS. Other parameters such as microclimatic data, field productivity and bacterial presence were also taken into account as response variables. The fungal abundance has shown to differ greatly between different points of the field, with microclimatic conditions influencing the abundance of the genetic markers measured and the presence of mycotoxins. The samples showing interesting results will be further analysed with high throughput methods.

This study aims at serving as a base for a more comprehensive understanding of infection dynamics at field level, showing that microclimatic variables need to be taken into account when considering this study system. A deeper knowledge of the dynamics affecting this microbial community will in future help to develop more sustainable managing practices or even to manipulate the microbial community into a hostile environment for those pathogenic fungi.

## **PT-NMI-21 Improving pasture quality by beneficial *Bacillus* sp. for optimized milk quality for use in dairy industry**

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With the vast daily consumption of dairy products worldwide, it is important to ensure the quality of the products. Pasture quality control is the first step to the success of this food web. Perennial ryegrass (*Lolium perenne*) is one of the most important pasture plants and widely distributed throughout the world due to its high levels of quality, palatability, digestible energy (fiber), protein, minerals and total usable carbohydrates. It is the main food for cattle, also determining milk quality. However, both susceptibility of plant diseases and potential causes of ruminant diseases (some plant origin pathogens can cause disease of the ruminant) are main limitations to expand its use. Therefore, in this study, we aim at i) seeking sustainable means for pathogen control and unraveling the mechanisms underneath. ii) increasing the biomass and quality (such as nutritional values) of the perennial ryegrass.

To this end, we isolated 119 *Bacillus* strains from healthy perennial ryegrass rhizosphere in the Netherlands. In vitro antagonistic assay showed that one of the strains, *B. amyloliquefaciens* HS9 can inhibit a wide range of plant pathogens, including both Gram-positive and Gram-negative bacteria, as well as fungi and oomycetes. To further investigate the compounds that responsible for the antagonistic activities, the genome of *B. amyloliquefaciens* HS9 was sequenced and genome mining was conducted. It revealed that *B. amyloliquefaciens* HS9 has potential to produce a wide variety of secondary metabolites, including the known surfactin, iturin A, fengycin B, bacilysin, bacillibactin, bacillaene, difficidin, macrolactin, 2,3-butanediol and acetoin. More interestingly, it harbors several potential novel NRPs. Furthermore, direct root tip inoculation of *B. amyloliquefaciens* HS9 on ryegrass seedlings and its volatile effect were studied. In both conditions, *B. amyloliquefaciens* HS9 can increase shoot and root biomass of *L. perenne* on half strength MS medium, which indicates that *B. amyloliquefaciens* HS9 plays a crucial role in the promotion of ryegrass growth. What exactly caused the plant growth promoting effect and how those effects are related to the quality of the ryegrass are currently under investigation.

## **PT-NMI-22 Microbial rhamnolipids mediate control of plant parasitic nematodes through several mechanisms**

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Plant parasitic nematodes (PPNs) are a global threat to crop production due to severe damages they cause to roots. Treatment with common nematicides, which are banned in most countries, is rather a risk to the environment than yielding a profit. Crop rotation is partly effective, but demands to be integrated in agricultural practice. The urge for alternatives raises the awareness for ecologically friendly approaches clearing the way from synthetic pesticides for agents of biological origin. Biosurfactants (BSFs) are a promising example for such an alternative in crop protection as a natural weapon to antagonize plant pathogens. These secondary metabolites, produced by various ubiquitous microorganisms, show an imposing efficiency against numerous economically relevant pathogens in agriculture. We demonstrate that rhamnolipids (RLs), one prominent representative of BSFs, have the capability to counteract PPNs by hampering the infestation via complementary activities. Plant infection by the cyst nematode *Heterodera schachtii* and the root-knot nematode *Meloidogyne incognita* is inhibited at RL concentrations far below the ecotoxicological level. We proof that RLs reinforce plant defense already in advance to a pathogen attack thus enabling the plant to react more appropriate. Even in the case that the parasite is able to overcome this first hurdle, there is strong evidence that RLs modify the quality of the plant as a host. In this respect, galls of *M. incognita* are reduced in size and most importantly they cause a drop in the reproduction of both PPNs. Attractivity assays with *H. schachtii* indicate that a RL-treatment induces disorientation of the parasite further extending the mode of operation by including actions prior to infection.

## **PT-NMI-20 Nitrogenase biosynthesis of *Rhizobium (Mesorhizobium loti)* is limited by the symbiotic sulfate transport in root nodules of the legume *Lotus japonicus***

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Symbiotic interaction between *Lotus japonicus* and *Mesorhizobium loti* results in the development of nitrogen-fixing root nodules expressing the nitrogenase enzyme complex. Enclosed in symbiosomes and surrounded by the plants-derived peribacteroid membrane (PBM), the rhizobacteria are able to reduce molecular nitrogen. The metabolic exchange of carbon and nitrogen between plant and bacteria takes place via this structural and functional border of both symbionts. Besides the transport of nitrogen and carbon, sulfur (S) transport and metabolism is crucial for the symbiotic interaction: the symbiotic sulfate transporter (SST1) is one of the most abundant PBM proteins and nitrogen-fixing nodules are enriched in reduced S compounds. However, the exact function of sulfate during nodule protein-synthesis and -functional maintenance remains unclear.

<sup>34</sup>S-metabolic labelling and mass spectrometry was applied to analyze the incorporation of reduced sulfur into plant and bacteroid proteins of the wild-type and the SST1 mutant. Samples were harvested 24, 48, 72 and 96 hours after pulse labelling. Sulfur turnover dynamics and the incorporation into key proteins for symbiotic nitrogen fixation in different plant organs were determined. Additionally NanoSIMS analysis was used to analyze the <sup>34</sup>S/<sup>32</sup>S ratio of the different symbiosome components.

## **PT-NMI-19 Stimulating saprotrophic fungi to control soil-borne fungal crop diseases**

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Natural protection of plants against soil-borne fungal diseases can result from the inhibitory activities of the soil microbiome. Indeed, bacteria with antagonistic activity against pathogens are often isolated from the soil. These strains are also marketed as bio-pesticides. Unfortunately, under field conditions microbial inoculants often fail to establish. Our study aims to stimulate members of the resident soil microbiome, that are already adapted to the local conditions, in order to improve the suppression of soil-borne fungal pathogens. Specifically, we propose to activate saprotrophic fungi, whose abundance is very low in intensively managed arable soils as compared to (semi-)natural ecosystems. We expect that an increase of saprotrophic fungi will raise the competition by fungi for root-derived energy sources. As a response, we expect that antagonistic functions against fungi will be triggered in the rhizosphere bacterial community, thereby forming a shield around roots that suppresses soil-borne plant-pathogenic fungi. Practical measures are explored to enhance growth of saprotrophic fungi in arable soils. Firstly, a range of organic residues of different composition was mixed with an agricultural soil. Woody materials and cellulose pulp were found to be the best amendments for stimulating fungal growth, as compared to waste materials, composts and plant residues. Analysis of the fungal community composition indicated that these fungus-stimulating amendments did not increase potential pathogens. Currently, wood- and cellulose-based amendments are tested in bioassays using pathogen-infested agricultural soils. Another approach to stimulate saprotrophic fungi in the rhizosphere is via modification of composition of root exudates e.g. by breeding. Here we focus on the role of phenolic acids. When combined with a simple artificial mixture of primary metabolites, some phenolic compounds stimulated the growth of fungi.

Reference: De Boer, W., Hundscheid, MPJ, Klein Gunnewiek PJA, de Ridder-Duine AS, Thion C, van Veen JA, van der Wal A (2015) Antifungal rhizosphere bacteria can increase as response to the presence of saprotrophic fungi. PlosOne 10: e0137988

**PT-NMI-24 Impact of plant growth promoting rhizobacteria on phosphorus use efficiency in the model grass *Brachypodium distachyon* (L.) Beauv.**

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In the present context of global change, reaching crop production sustainability is of main concern. This encompasses a better use of plant fertilizers, in order to reduce nutrient leaching and accumulation in the environment (e.g. nitrogen) and save limited natural resources (e.g. rock phosphate). Improvement of plant nutrient use efficiency (NUE), by acting on the plant ability to take up nutrients and produce biomass, is a promising way to achieve this goal. Plant growth promoting rhizobacteria (PGPR) are able to impact NUE. Regarding plant phosphorus (P) nutrition, PGPR can modulate the plant ability to take up P through several mechanisms including the increase of P bioavailability in soils, the modulation of the root system development for foraging and the alteration of the expression of P responsive genes. Our project aims at studying the impact of PGPR strains on the P use efficiency of *Brachypodium distachyon* (L.) Beauv. The ability of PGPR strains to increase P bioavailability is studied by performing P solubilization tests in a liquid medium. As several P forms are present in soils, three poorly available P forms are used. The selected strains exhibit contrasted solubilization and acidification activities. In parallel, the impact of PGPR on *Brachypodium* growth is studied in Magenta boxes, in a total interaction system under gnotobiotic conditions. Plants and PGPRs are exposed to contrasted P conditions including the same P sources as for the solubilization tests. Biomass production and allocation are measured after 30 days. The major effect is an increase in root biomass production under P deficiency conditions when PGPRs are applied. This can negatively impact shoot biomass production. The PGPR effect on root system development will be further studied by measuring root system architecture parameters. Finally, the plant response to contrasted P conditions in the presence/absence of PGPR will be considered at the molecular level by quantifying the expression of P responsive genes.

**Microbe-assisted crop production, opportunities, challenges & needs**

**Vienna, Austria, 4- 7 December 2017**

***New mechanisms involved in beneficial plant-microbe interactions***

**Chairs: Paola Bonfante, Laure Weisskopf & Barbara Reinhold-Hurek**

## **NMI-01 Looking at the plant side: plant crop responses to arbuscular mycorrhizal fungi**

**Paola Bonfante**

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In natural and agricultural ecosystems, rhizospheric microbiota influences plant growth and health. Bacteria and fungi with beneficial functions, such as root symbionts and growth-promoting rhizobacteria, coexist with endophytes, saprotrophic microbes, and pathogens. While several studies have investigated soil microbial biodiversity, mostly focusing on prokaryotes, our comprehension of the mechanisms underlying plant responses remains in its infancy. The aim of the presentation will be to summarize the benefits provided by arbuscular mycorrhizal fungi (AMF) to crops like tomato and wheat, thanks to their role of biofertilizers and bioprotectors against pathogens. Using transcriptomics supported by proteomics, metatranscriptomics, and biochemistry, we describe some of the molecular mechanisms elicited by AMF, by investigating plants inoculated with a single AMF isolate (*Funneliformis mosseae*) or growing in native substrates which contain not only AMF but also a complex microbiota. The activation and regulation of phosphate transporters emerge among the more important tools to improve plant mineral nutrition, with systemic impacts which move from roots to fruits and seeds in both tomato and wheat. *Funneliformis mosseae* also acts as a bioprotector against a wheat pathogen thanks to the activation of defense reactions which are elicited at local and systemic level (roots and leaves). When AMF are present together with all the other members of a native soil microbiota, they also elicit defense responses in tomato. We detected an activation of defence pathways, including phenol biosynthesis, lignin deposition, and innate immunity in two tomato genotypes. Taken in the whole, the results suggest that AMF together with the other soil microbes elicit a "state of alert" in crops which may provide a protection against bacterial and fungal pathogens.

## **NMI-02 News from the volatile warfare between potato-associated *Pseudomonas* and the late blight causing agent *Phytophthora infestans***

Mout De Vrieze<sup>1</sup>, Gfeller Aurelie<sup>2</sup>, Chinchilla Delphine<sup>3</sup>, Bailly Aurelien<sup>1</sup>, L'Haridon Floriane<sup>3</sup>, **Weisskopf Laure<sup>3</sup>**

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During the last decade, the importance of bacterial volatiles in cross-kingdom interactions has become evident. In addition to promoting plant growth and root development, bacterial volatiles have been repeatedly shown to inhibit the growth of phytopathogenic fungi, although the molecules responsible for this effect are still largely unknown, with the notable exception of hydrogen cyanide. Our recent work has shown that oomycete pathogens such as *Phytophthora infestans*, causing late blight in potato, are particularly sensitive to the volatiles of potato-associated *Pseudomonas* strains, when compared to other potato disease-causing agents such as *Rhizoctonia solani* or *Helminthosporium solani*. In a screen aimed at identifying the chemical composition of the volatile blends from those efficient anti-oomycete *Pseudomonas*, Sulphur-based compounds were identified as potent inhibitors of all life stages of the pathogen, including mycelial growth, sporangia production and germination, as well as zoospore motility. Some of these Sulphur-containing volatiles were able to prevent disease establishment on infected plant material. One important and so-far unresolved question concerns the ability of bacteria to emit those bioactive volatiles when living in natural conditions, e.g. on leaf surfaces. We are currently investigating this question using sterile potato plantlets inoculated with bacterial strains of known volatile blend emission. First results indicate that typical bacterial smells such as the long-chained alkene 1-Undecene previously shown to inhibit growth and sporulation of *P. infestans* can be detected on inoculated plants. This highlights the so-far underexplored potential of bacterial volatile compounds for sustainable crop protection.

### **NMI-03 The role of bacterial terpenes in microbial interactions in the soil**

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Soil bacteria produce a wide array of secondary metabolites that they use to interact with other organisms in their surroundings. Many of those metabolites are volatile compounds and facilitate long-distance microbe-microbe and plant-microbe interactions. These volatile organic compounds belong to different chemical classes including alkenes, alcohols, ketones, benzenoids, pyrazines, sulfides and terpenes. Terpenes are an interesting class of volatile compounds that are best known to humans as plant metabolites. In plants, they play important roles as phytohormones, and in interactions with insects, pathogens and other plants. In recent years, due to advances in bacterial genome sequences, it became clear that terpene synthases are widely distributed in bacteria and therefore bacteria can be a rich source of terpenoid metabolites. While there is evidence that terpenes may be involved in different kinds of microbial interactions in the rhizosphere, to date, the precise ecological role of bacterial terpenes in microbe-microbe and plant-microbe communication remains largely unknown.

The main aim of our study is to understand the function of bacterial terpenoid metabolites and their role in soil microbial interactions. Using targeted mutagenesis of terpene synthases and phytoene synthases in the mycophagous soil bacteria *Collimonas pratensis* Ter91 we compared the behaviour of wild type and mutants under different conditions. Our results revealed that the mutant strains were affected in motility, quorum sensing, production of exopolysaccharides, biofilm formation and antifungal activity. Here we present our recent findings about the role of bacterial terpenes in the belowground microbe-microbe (fungi, bacteria, protists) and microbe-plant interactions.

### **NMI-04 Unraveling the importance of sugar transport in plant-beneficial rhizobacteria interactions**

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Plant Growth Promoting Rhizobacteria (PGPR) are able to confer to plants an improved growth and/or tolerance to various biotic and abiotic stresses. Understanding the molecular mechanisms involved in these biological processes should help develop novel and environmentally friendly strategies for crop protection and productivity improvement and contribute to the rise of a more sustainable agriculture system. A growing body of evidence demonstrates the importance of sugar transport in plant pathogen resistance and in plant-microorganism mutualistic symbioses (Chen, 2014; Doidy *et al.*, 2012). In contrast, the role and regulation of sugar transporter activities in plant-PGPR interactions remain to be investigated. To address this issue, we have set up an *in vitro* experimental system that allow a detailed study of the different PGPR modes of action and mechanisms involved in their beneficial effects on plant growth and development (ie. in physical contact with the plant roots or via the emission of volatile compounds). Using this system, the model plant *Arabidopsis thaliana*, and a collection of PGPR strains, we have carried out a comprehensive targeted gene expression analysis (by quantitative RT-PCR). From this work, we have identified several plant sugar transporter genes whose expression is induced or repressed by the PGPR strain(s). We are currently studying the function of some of these candidate genes by a reverse genetic approach and extending the study to other genes by carrying out a genome-wide gene expression analysis (by RNA-sequencing). In order to achieve a sound understanding of changes in carbon allocation and sugar transport taking place during plant-PGPR interactions, we will also measure sugar content and fluxes within the plant and at the plant-PGPR interface. Results from this study should identify novel molecular mechanisms (either conserved between the PGPR strains or strain-specific) and essential genes involved in plant-PGPR interactions and their beneficial effects on plant growth.

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## **NMI-05 *Trichoderma reesei* prioritizes plant sensing and the presence of a mating partner over nutrient sensing to achieve optimal adaptation to the plant by sexual development**

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The natural environment of the potent cellulase producer *Trichoderma reesei* (syn. *Hypocrea jecorina*) is a tropical forest, where it degrades cellulosic plant biomass. However, related *Trichoderma* spp. are also known as efficient plant protection agents, antagonizing fungal pathogens.

In the plant pathogen *Fusarium oxysporum*, plant sensing is accomplished by pheromone receptors responding to secreted plant peroxidases. Here we investigated the relationship between plant sensing, sexual development and nutrient detection in *T. reesei*.

We found that *T. reesei* is able to sense plant root exudates and peroxidases via pheromone receptors, suggesting that this mechanism is conserved between *Trichoderma* and *Fusarium*. Moreover, *T. reesei* also showed a concentration dependent chemotropic response to glucose. Interestingly, a nutrient-rich medium such as malt extract (ME) resulted in bipolar and multipolar germination without discernible chemotropism, whereas exudates from soybean roots or from a compatible mating partner in the presence of ME caused unipolar germination with a robust chemotropic response. Hence, signals related to sexual development and plant sensing override nutrient regulated germination.

Accordingly, we found that the presence of soybean germlings promotes sexual development of *T. reesei* as do compounds releasing nitric oxide, a signaling molecule, which is produced by plants to counteract oxidative stress and involved in plant pathogen interaction. Also reactive oxygen species were found to play a role in sexual development, which may explain the relevance of sensing of a peroxidase by pheromone receptors.

We propose that *T. reesei* is attracted to plants by a mechanism that integrates directed hyphal growth and sexual development for efficient adaptation to the host and potentially also for optimized antagonism of competitors and plant pathogens. Thereby, association with a plant has higher priority than availability of nutrients.

## **NMI-06 Biostimulants for biostimulants: oligosaccharides as a new opportunity to enhance mycorrhizal responses, theoretical basis and practical cases**

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Harder regulatory constraints in Europe, implemented in the last decade, had reduced the use of fertilizer and chemical inputs. In this frame, the need to develop new agro-ecological strategies to optimize the nutrient use efficiency, promoted the interest in biostimulants as a promising elements of sustainable plant production systems for the near future.

Arbuscular mycorrhizal (AM) fungi are a widespread class of microorganisms that establish a symbiosis with the roots of majority of land plants. These fungi are an important ecosystem services tool able to promote plant growth and increase the quality of plant production *via* optimization of nutrient uptake. For these characteristic, AMF are usually included in the definition of biostimulants. However, mycorrhizal inoculum application faces many challenges, namely: the inoculum quality (in terms of richness and effectiveness), the application systems and the costs. Moreover, high soil phosphorus concentration, often encountered in soils used for conventional crop production, inhibits AM fungal colonization and AM responsiveness of plants.

Here, we discuss about possibilities to induce a specific favourable plant metabolic context to improve AM fungal root colonization and responses, by low dose application of signalling molecules. We focus in particular to the potentials of oligosaccharides, which are known as elicitors for plant defence systems, as emerging theory and evidences indicate that they could act as inductors for AM formation and function. We present the theoretical basis about the functioning of these molecules and a series of practical case in which the use of oligosaccharides have been successfully used to stimulate AM development and plant growth responses.

## **NMI-07 To be or not to be inside: A complex role of bacterial factors for endo- or epiphytic colonization**

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The diazotrophic model endophyte of grasses, *Azoarcus* sp. strain BH72, colonizes roots of its original host plant Kallar grass and rice in similar patterns. The molecular mechanisms by which endophytes interact with their host are not yet well understood. How do bacteria adapt to their endophytic lifestyle in comparison to free-living growth, and which proteins do they require for endophytic competence?

Among the bacterial factors required for establishment in the endophytic compartment, attachment factors and protein secretion systems of Type 6 (T6SS) will be covered here. In a transcriptome microarray study (1), gene expression of *azo1653* and *azo1684* was found to be responsive to O<sub>2</sub> concentration. Mutational analysis revealed that both encoded proteins, putative attachment factors, have a complex role in different steps of colonization. They have a negative impact on rhizosphere competence (surface colonization), however contribute to endophytic establishment inside roots. Another important bacterial tool appears to be the type VI secretion system, which is known to be involved in other bacteria in the interaction between different bacteria or between bacteria and their eukaryotic hosts. The genome of *Azoarcus* contains two gene clusters encoding for putative T6SSs, termed *sci* and *imp*. Secretion of the T6SS hallmark protein HCP was shown previously. Here we unraveled the structural components and expression patterns of the T6SSs in more detail. The *Sci*-system appeared to be constitutively active. For the *Sci*-system, TagF, a homolog to a *Pseudomonas aeruginosa* inhibitor of T6SS protein secretion, led to hypersecretion of Hcp also in *Azoarcus* strain BH72. However, even then we found no evidence of the *Imp*-system secreting its cognate Hcp protein, although the respective genes were strongly induced under conditions of nitrogen fixation. Surprisingly, strain BH72 carrying a gene knockout in this apparently inactive *Imp*-system showed a strong reduction in endophytic rice root colonization, indicating it is important for the interaction with the host. To visualize the secretion process, we constructed VipA-sfGFP fusions for both T6SSs, and can thus follow the process of active secretion in vivo. Regulatory cues and the role of T6SS in endophyte-plant interaction will be discussed.

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## **NMI-08 Substance secreted by the endophytic *Dyella*-like bacterium as a potential solution against yellows disease of carrots**

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Yellows disease of carrots is a widespread and destructive disease causing a wide range of symptoms such as deformation of edible root and atypical growth of leaves in the form of "witch's broom". This disease may cause total yield loss. *Ca. Liberibacter solanacearum* (Lso) and *Ca. Phytoplasma*, the causative agents of yellows diseases, inhabit the phloem of plants or the inner tissues of insects that transmit the disease and cannot be grown in culture. Therefore, in the current study, a culturable model of these fastidious phytopathogens (the bacterium *Spiroplasma melliferum*) was examined. In a previous study, we isolated a beneficial endophyte - DLB (*Dyella*-like bacterium) that have shown to reduce symptoms of grapevine yellows caused by *Ca. Phytoplasma*.

The aim of this study was to examine whether substances, secreted by DLB, can inhibit the development of *S. melliferum in vitro* and Lso infection *in planta*.

The effect of DLB's supernatant on *S. melliferum* growth was examined, and the substances secreted by DLB were identified by HPLC and GC-MS. Synthetic compounds, analogues to those identified by the biochemical analysis, were used to determine influence on *S. melliferum* growth *in vitro*. In addition, the effect of these compounds on the titer of Lso was examined in infected carrots by qPCR.

DLB was found to secrete different substances that inhibit the growth of *S. melliferum*. A synergistic effect against *S. melliferum* was detected when two synthetic compounds were combined. Application of these compounds caused a significant reduction in the titer of Lso in infected carrots in comparison to untreated infected plants. Further research is required to examine the potential of these compounds as a treatment against yellows disease of carrot, as well as other phloem-restricted phytopathogens.

**Microbe-assisted crop production, opportunities, challenges & needs**

**Vienna, Austria, 4- 7 December 2017**

***Plant understanding and improvement of beneficial interactions with  
microbes***

**Chairs: Corné Pieterse & Angela Sessitsch**

## PU-01 The root microbiome and plant health

**Corné Pieterse**

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Plants excrete significant proportions of their photosynthetically fixed carbon sources into the root environment, thereby nurturing a large community of root-associated microbiota. In return, root-colonizing microbes provide plants with essential services, such as enhanced mineral uptake, improvement of root architecture, growth promotion, and protection against pathogens [1]. Plants can select specific microbes in their rhizosphere to enhance beneficial plant-microbe interactions. We showed that *Arabidopsis* promotes a plant-beneficial consortium of bacteria in its rhizosphere upon foliar infection by the downy mildew pathogen. In turn, this rhizobacterial consortium triggers induced systemic resistance (ISR) against downy mildew and promotes growth of the plant. The recruited consortium members interact synergistically in biofilm formation and together are better able to stimulate plant health and growth than each of the strains separately, thereby maximizing the chance of plant survival. Using the plant-beneficial microbe interactions *Arabidopsis-Pseudomonas simiae* WCS417, we identified the root-specific transcription factor MYB72 as a central regulator in the onset of ISR [2]. MYB72 is specifically activated in epidermal root cells in response to ISR-inducing root microbiota members [3]. MYB72 controls the biosynthesis of iron-mobilizing phenolic compounds (coumarins) that are excreted in the rhizosphere, where they aid in iron uptake and support plant growth. Metabolome analysis of wild-type and *myb72* root exudates revealed scopoletin as the dominant MYB72-dependent metabolite. Scopoletin has an antimicrobial activity to which WCS417 is insensitive, providing this ISR-inducing rhizobacterium with a selective advantage over scopoletin-sensitive soil microbiota. Microbiome analysis of wild-type and *myb72* mutant plants revealed that MYB72-dependent metabolites function in rhizosphere community assembly, possibly to promote recruitment of ISR-inducing rhizobacteria into their rhizosphere. Understanding the mechanistic basis of early root-microbiome interactions will provide a firm knowledge basis for the sustainable development of improved crop systems that maximize profitable functions from the root microbiome.

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## PU-02 On a search to unravel the differences in molecular events between primable and non-primable barley varieties

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Priming of crop plants for enhanced resistance is an efficient strategy for disease management. Plants can be primed through different biological and chemical inducers. One such biological inducer is oxo-C14-HSL, *N*-acyl homoserine lactones (AHL), a quorum sensing molecule produced by the bacterium *Ensifer meliloti*. Prior, our studies demonstrated that barley, *Hordeum vulgare* cultivar Golden Promise, can be primed for augmented resistance with AHL against *Blumeria graminis* (Hernández-Reyes *et al.* 2014). In the current study, we investigated the impact of this AHL, oxo-C14-HSL, on different cultivars of winter barley and their capacity to induce priming.

The selection of susceptible and resistant cultivars that are primable or non-primable was carried out phenotypically using a detached leaf assay and studies of plant cell-conidia interactions. Furthermore, we assessed the correlation of priming capacity with molecular and metabolic events. To this end, in selected barley cultivars, we analyzed the differences in activation of mitogen activated protein kinases (MAPKs) through western blot and in expression of several defense related genes. In addition, we analyzed the differences in the generation of reactive oxygen species and in production of defense-related secondary metabolites.

The aim of this study is to understand the priming mechanism in barley, and also in other crop plants. Since different barley cultivars of varying genetic background differ in the priming capacity, we can utilize this information for a possible target for breeding approaches. The use of biologicals or beneficial bacteria represents a good alternative strategy for sustainable plant protection measures.

Publication bibliography

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### PU-03 Breeding for microbiome-mediated disease resistance

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Plant-associated microbial communities play a crucial role for the expression of various plant traits including disease resistance. Increasing evidence suggests that host genotype influences the composition and function of certain microbial key groups, which, in turn, effects how the plant reacts to environmental stresses. Several studies indicate that modern plant breeding may have selected against plant traits essential for hosting and supporting beneficial microbes. However, they also highlight the presence of an exploitable genetic base for the regulation of the rhizosphere microbiota.

We illustrate the concept of breeding for microbial symbioses with pea (*Pisum sativum* L.). Firstly, genotypic variation for the efficiency of a mycorrhizal symbiosis is shown, as measured by an estimation of the plant benefit per symbiotic unit. Secondly, we extend the view towards the wider fungal community using ITS amplicon sequencing. Two pea genotypes with contrasting resistance levels against pathogen complexes are investigated to provide information on the functional diversity of the rhizosphere microbiome in a naturally infested agricultural soil. In the near future, microbial hubs and diversity indices will be linked with root exudation in order to elucidate the plant's capacity to influence the microbial composition leading to disease susceptibility or resistance. Current and future research activities of our group aim to make use of plant-microbiome interactions to develop advanced screening tools for breeders for an improved expression and stability of important plant traits.

### PU-04 Petunia as model to breed for new crop cultivars with improved responses to root-endophytic fungi

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Root-endophytic fungi are able to improve nutrient and water use efficiencies of plants at the same time increasing their tolerance and resistance to abiotic stress. They could be therefore integral elements of sustainable plant production systems. The outcome of plant-endophyte interactions is, however, not always predictable and this drawback often prevents their application. How a particular interaction is positioned along the mutualism-parasitism continuum depends on environmental conditions and on the genotypes of the fungus and the plant. Among others, it is therefore necessary to define positive growth responses as a trait to aspire for breeding of new crop cultivars.

*Petunia hybrida* is used since ten years as model in research of interactions between plant roots and mycorrhizal fungi. This research is currently extended to the interactions with other root endophytes. The genomes of the progenitor species of *P. hybrida* have been recently sequenced. Together with available gene maps and markers, this data set now opens up new ways in breeding research. In a first step, the response of wild species to inoculation with mycorrhizal fungi, with Sebaciniales and with Dark Septate Endophytes was compared. Differences were revealed concerning root colonization and growth responses. In ongoing experiments, the phenotypes of crossing populations are analyzed in order to map QTLs for the different responses. Using the genome sequences and available expression data sets, candidate genes can be identified in these QTLs which are involved in the responses of petunia to root colonization. Due to the availability of a natural population with transposon insertions covering the whole genome, confirming the role of these genes is a relative fast procedure. These genes can be used in future breeding programs as functional markers for achieving new cultivars with a stable positive response to root colonization by endophytic fungi.

## PU-05 Bacteria on the surface of wheat seeds affect hydrogen peroxide production during early seedling growth

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Seed performance underpins agricultural productivity, and hence food security. Although the increasing number of reports on seed-microbe associations, including bacterial endophytes, their contribution to seed germination and vigour remains less clear. Here, the seed-microbe interaction was investigated in bread wheat (*Triticum aestivum* L.). Seeds were surface sterilised with a hot steam treatment, which significantly reduced the number of microbes, increased the speed of germination, and decreased rates of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) production, in comparison to non-surface-sterilised seeds. Considering that an oxidative burst leading to extracellular H<sub>2</sub>O<sub>2</sub> production is a typical biotic stress response of plants, it was hypothesised that bacterial infections were associated to elevated levels of H<sub>2</sub>O<sub>2</sub> production by wheat seedlings. 16S rRNA sequencing revealed that the microbiome of dry wheat seeds was dominated by *Gammaproteobacteria*, in the families of *Pseudomonadaceae* or *Enterobacteriaceae*. Typical for wheat seeds, the community was abundant in the genus *Pantoea*, which is known to infect different crops and induce systemic acquired resistance. Restriction fragment length polymorphism and sequencing of the intergenic spacer region, and sequencing of the *gyrase B* gene, identified two unique species of *Pantoea* (spp. *agglomerans* and *eucalypti*). Surface-sterilised seeds were inoculated with individual isolates, and the level of extracellular H<sub>2</sub>O<sub>2</sub> was measured after 48 h from the onset of imbibition. Interestingly, *Pantoea* spp. *agglomerans* and *eucalypti* induced wheat seedlings to increase and decrease extracellular H<sub>2</sub>O<sub>2</sub> production, respectively. If these differential responses of wheat seedlings to distinct *Pantoea* species are related to successful endophytic colonisation and changes in seedling growth rates is under investigation. Whether alterations in the microbiome structure may affect wheat seed physiology during imbibition and seedling growth is being assessed. Furthermore, the effects of other endophytic bacterial strains with proven growth-promoting abilities are evaluated in other crops. Our overall aim is to improve our understanding of plant-bacterial interactions in seeds and seedlings, and decipher how the seed microbiome can be modulated to enhance seed germination and vigour.

Poster Talks II

**Microbe-assisted crop production, opportunities, challenges & needs**

**Vienna, Austria, 4- 7 December 2017**

***Poster Talks II***

## PT-PU-25 Ecological role of maytansine is revealed by *in situ* MALDI-HRMS-imaging of *Maytenus senegalensis* during the germination process

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MALDI-HRMS-imaging (matrix assisted laser desorption ionization high-resolution mass spectrometry imaging) is an important tool for visualizing, both spatially and temporally, metabolites at the interface of plants and microbes (e.g., endophytes and/or pathogens). Furthermore, the chemical communication between plant-associated microbes can be elucidated at the cellular level. We employed MALDI-HRMS imaging to study the ecological role of maytansine, an important anticancer drug used against breast cancer [1,2]. Since the discovery of maytansine in the 1970s in Celastraceae plants such as *Maytenus* and *Putterlickia* species, its role as a chemical defense compound was hypothesized [3]. After more than four decades of discovery of this important antineoplastic drug, we provide a proof-of-concept of ecospecific and tissue-specific production and *in situ* spatial/temporal distribution of maytansine in *Maytenus senegalensis* plants. We have used MALDI-HRMS-imaging to visualize the occurrence and spatial/temporal distribution of maytansine in the leaves, stems, and roots of *M. senegalensis* plants, seeds obtained from the mother plants, through the germination process, and finally to the establishment of new seedlings or daughter plants. The mother plant was devoid of maytansine in all tissues. However, maytansine was produced and distributed in the cotyledons and the endosperm of the seeds with an augmented accretion towards the seed coat. Furthermore, maytansine was always detected in the emerging seedlings, particularly the cortex encompassing the radicle, hypocotyl, and epicotyl. The typical pattern of production and accumulation of maytansine not only in the seeds but also during germination provides evidence that *M. senegalensis* is ecologically primed to trigger the production of maytansine in vulnerable tissues such as seeds during plant reproduction. By utilizing maytansine as chemical defense compound against predators and/or pathogens, the plant can ensure viability of the seeds and successful germination, thus leading to the next generation of daughter plants with an evolutionary advantage of survival [4].

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## PT-PU-26 Effects of structural and functional analogues of IAA in triggering biological nitrogen fixation in non-legume plants

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We have verified that the endogenous overproduction of the main auxin indole-3-acetic acid (IAA) in diazotrophic endophytes isolated from rice plants led to a significant up-regulation of nitrogen fixation: the *nifH* gene expression and the nitrogenase enzyme activity increased in both bacterial cultures and inoculated host plants as compared to the wild-type ones. When rice plants inoculated with wild type and IAA-overproducing nitrogen-fixing endophytes were compared significant changes in root morphology were observed: rice plants inoculate with the IAA-overproducing strains showed a more branched root system with abundant lateral root and significant changes in the primary root length. The aim of this work was to test the specificity of IAA effects on nitrogen-fixing apparatus in diazotrophic endophytes. Chemically or functionally similar molecules, such as indole (IND), indole-3-carboxylic acid (ICA), and 2,4-dichlorophenoxyacetic acid (2,4-D) were selected and exogenously added as purified substances into both liquid cultures and hydroponic systems of inoculated rice plants. Acetylene reduction assay (ARA) was carried out after treatment with the selected molecules. A significant increase of nitrogenase activity was measured only after the exogenous IAA-treatment in both conditions above described. This result leads us to say that the observed effect was specifically due to the hormonal activity of IAA. Studies to see if a similar effect can be obtained through co-infection of rice plants with nitrogen-fixing and IAA-producer endophytes are still underway. Selecting the best endophytic bacterial consortium could offer new perspectives to enhance nitrogen-fixation in non-legume crops.

## **PT-PU-27 How do phytohormones influence the composition of fungal endophyte communities in tomato roots?**

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Endophytes are microbes capable of colonizing the inner part of different plant tissues without causing disease symptoms. In some cases, they have beneficial effects for the host plant such as biotic and abiotic stress resistance and plant growth promotion. For this reason, the use of these microbes could have a major impact on agriculture worldwide. However, the plant-endophyte interaction involves very complex mechanisms starting from the recruitment of the microorganisms to the colonization of the surface of the plant tissue and then the inner part, with the need to escape the plant immune system. All these processes are regulated by different plant and endophyte signalling molecules necessary for the establishment of the plant-endophyte interaction. Phytohormones are among the signalling compounds known to play a significant role in this interaction, but little is known about the specific ways by which they influence recruitment and colonization of the host tissues. The aim of the current project is to go deeper into the role of these signalling compounds in plant-endophyte interactions.

A community analysis (endophyte isolation and amplicon sequencing) of endophytic fungi was conducted on roots of tomato (*Solanum lycopersicum*) mutants impaired in synthesis of specific phytohormones (specifically ethylene and jasmonic acid) in order to understand how these compounds influence the composition of the endophytic communities. After the characterization of the endophytic communities, fungal isolates whose root-colonization frequency appears to be influenced by the presence/absence of specific phytohormones were selected. In order to obtain a deeper understanding of the role of these compounds in the plant-endophyte interaction, the selected isolates are currently being screened by confocal microscopy and qPCR in order to identify candidates whose colonization rate is critically affected by the phytohormones of interest.

A transcriptomic analysis of tomato plants inoculated with the isolates selected from the screening will provide further clues as to which physiological mechanisms, associated with endophyte recruitment, are influenced by phytohormones.

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## **PT-PU-28 Effects of plant-growth-promoting rhizobacteria on barley under global warming-associated environmental factors**

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Atmospheric concentration of CO<sub>2</sub> is continuously increasing since the industrial revolution and may reach 550 ppm by the year 2050. Furthermore, given that environmental factors like CO<sub>2</sub>, temperature, and water availability will likely change simultaneously, it is difficult to make accurate predictions about crop production under elevated CO<sub>2</sub>, reaching implications for food security. Plant-growth-promoting rhizobacteria (PGPR) colonize the rhizosphere of many plant species and confer beneficial effects under environmental stresses. Furthermore, root exudates play a role in interactions between plant roots and other organisms present in the rhizosphere.

Only few reports have been published on PGPR as elicitors of tolerance to abiotic stresses, such as drought. Furthermore, little is known about the influence of environmental factors on root exudation patterns. Therefore, this study was conducted in order to investigate the effect of two commercially available PGPRs on the growth and root exudation of barley (*Hordeum vulgare* L.) under different CO<sub>2</sub> and water treatments.

In a growth chamber experiment climatic conditions of a field site close to Stuttgart were simulated. Barley plants were grown in pots filled with sand and exposed to ambient (380 ppm) or elevated (550 ppm) CO<sub>2</sub>. Plants received the normal daily amount of rainfall in the region of Stuttgart or 33% less. Plants were harvested at the stem elongation growth stage and when the inflorescences emerged. At both dates, data were collected on above and belowground variables. In addition, analyses of water use efficiency and composition of root exudates were performed. Preliminary results showed significant effects of the factors and their interactions on some of the measured variables.

In the context of climate change and agricultural sustainability, further studies with other crop plants are needed to demonstrate whether PGPR cause a range of crops to be tolerant to environmental stresses improving crop production.

## PT-MI-19 Back-to-the-roots: unraveling the endophytic microbiome in banana germplasm to control *Fusarium* wilt

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Banana is one of the world's most important staple crop in the tropics and sub-tropics. Banana originates from South/South-East Asia and is now also cultivated and commercially produced in Central and South America, Africa and Australia. The majority of commercial banana varieties are genetically identical, making them vulnerable to a myriad of pests and diseases. *Fusarium* wilt or Panama disease caused by soil-borne fungus *Fusarium oxysporum* f.sp. *cubense* (Foc) has been a major threat for banana production worldwide. In the 1950's, devastating Foc race 1 strains emerged and wiped out variety "Gros Michel" from banana plantations in Central and South America. Following this pandemic, the new resistant "Cavendish" varieties were introduced. Decades later, another strain of Foc, commonly referred to as Tropical Race 4 (TR4), emerged in South-East Asia and destroyed Cavendish plantations in the region. The epidemic also spread to the Middle-East, the Indian subcontinental and Africa. Currently, there are no effective measures to control Panama disease and new sustainable methods are urgently needed. This project aims to elucidate the diversity of endophytic bacterial communities in banana germplasm, including wild relatives, and their metabolic potential to control Panama disease. We focus our study in Indonesia, one of the banana gene centers, and sampled roots and pseudostems of susceptible and resistant banana varieties Cavendish and Rejang, respectively, from Foc-endemic fields in Sumatra. Exploring endophytic microbial diversity of the resistant Rejang banana may unravel competitive bacterial genera to protect the plant from Panama disease. The taxonomic diversity of the banana endophytic bacterial communities was assessed by 16S rRNA amplicon sequencing followed by targeted isolation of different endophytic bacterial genera. Results on the diversity and antifungal activity of the endophytic bacteria sampled from diverse banana germplasm will be presented.

## PT-MI-20 Cooperation of *Trichoderma* spp. and *Bacillus* spp. in biocontrol applications

Guofen Li, Lisa Marie Pleyer, Günter Brader, Monika Schmoll

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*Trichoderma* spp. and *Bacillus* spp. are known as potent plant beneficial organisms, which can alleviate abiotic stress and exert a protective effect against pathogens. Therefore we aimed at selection of appropriate strains from both genera, which are suitable for combined application in agriculture for plant protection. For biocontrol assays, we isolated *Diaporthe sojae* from contaminated soybean seeds with deleterious effect on seed germination.

Efficient and poor biocontrol strains of *Trichoderma* as selected from plate assays and greenhouse tests were included in the assays in order to determine characteristic effects for efficient plant protection. Strains were additionally tested for growth at low temperatures, when seeds are particularly vulnerable to pathogens. Moreover we tested, whether the strains were able to recognize organisms in their environment by analyzing their chemotropic response to exudates of *D. sojae*, *Bacillus* spp. and soybean.

Then we selected efficient *Trichoderma* and *Bacillus* strains and tested whether they antagonize each other or are suitable for co-cultivation. Suitable combinations were then tested individually and in combination in greenhouse assays, which revealed a positive effect on germination individually as well as a protective function against *D. sojae* in several cases. Thereby we could identify a favorable combination of *Trichoderma* and *Bacillus*, which will be subject to further testing towards applications in agriculture.

## **PT-MI-21 Bio-effectors in the rhizosphere: Microbial consortia as inoculants for improved plant growth and mineral nutrition**

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In contrast to selection of single microbial strains with high efficiency for plant growth promotion, the use of large consortia of different (Plant Growth-Promoting Microorganisms, PGPMs) may be an alternative strategy to induce beneficial effects on plant performance, with the advantage of higher flexibility towards variability of environmental conditions due to selective promotion of the best-adapted strains, depending on the respective rhizosphere conditions. In this study, the effects of a commercial microbial consortium product (MCP) with different fungal and bacterial PGPMs on early growth of maize were tested in pot experiments with different levels of N and P supply, including a functional characterization of inoculant effects on marker enzymes for N, P and C cycling in the rhizosphere.

Maize plants (cv. Jessy) were cultivated on two soils with different soil properties. Fertilization with N140 in nitrate and stabilized ammonium form, K150, Mg50 and P50; P30 respectively as reduced P fertilization was performed. The MCP was applied by fortnightly fertigation. Measurements of shoot and root growth and functional characterization of C,P,N turnover in the rhizosphere using marker enzyme assays with fluorogenic substrates and auxin production potential were conducted in 3 and 6 weeks after sowing (WAS). Culture-dependent re-isolation of rhizosphere bacterial populations was performed with standard media. The auxin production potential of re-isolated populations was evaluated spectrophotometrically.

The application of the MCP induced significant stimulation of shoot and root biomass production and could compensate for reduced input of N and/or P. This was associated with increased fine root production and improved P-nutritional status, particularly in the inoculated variants with reduced nutrient input. Re-isolation of cultivable bacteria revealed a significant increase in total colony forming units in the inoculated variant even 6 WAS.

The results suggest that the MCP-induced beneficial effects on plant growth and nutrient acquisition in maize could be mainly attributed to root growth stimulation, while indications for direct interactions of the inoculants with nutrient turnover in the rhizosphere were partially confirmed only in the early growth stages. These findings demonstrate a clear rhizosphere effect of the inoculants with impact on the native microflora which deserves a more detailed investigation e.g by a metagenomics approach.

## **PT-MI-22 Biocontrol of *Fusarium* crown and root rot in wheat by inhibitory *Streptomyces* isolates – it's complicated**

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The predominant causal agents of *Fusarium* crown and root rot (FCR) in wheat, along with *Fusarium pseudograminearum*, are *F. graminearum* and *F. culmorum*. Members of the genus *Streptomyces* have been shown to inhibit various *Fusarium* species, and *Fusarium* is also able to inhibit *Streptomyces* in vitro. However, little is known about these complex interactions and the potential for inhibitory *Streptomyces* to reduce FCR in wheat. The aim of this study was to analyze whether inhibitory *Streptomyces* isolates affect the disease development of FCR in wheat, and reduce root and stem base colonization by *Fusarium* spp. We enriched sterilized potting soil with spore suspensions of *Streptomyces* isolates, inoculated the soil with *F. culmorum*-colonized wheat straw, and planted pre-germinated wheat seedlings. At four weeks, inoculation with *F. culmorum* led to significant disease symptoms on roots and reduced the fresh weight of roots and above-ground plant biomass compared to the non-inoculated controls. Enrichment of soil with inhibitory *Streptomyces* isolate reduced levels of *F. culmorum* DNA in roots and stem bases by 75% compared inoculation with *F. culmorum* alone. Interestingly, co-inoculation of *F. culmorum* with the non-inhibitory *Streptomyces* isolate led to the highest levels *F. culmorum* DNA in stem base tissue and *Streptomyces* densities (CFU/g soil) in the rhizosphere five times greater than inoculation with *Streptomyces* only. In vitro assays testing the inhibition of *Fusarium* against *Streptomyces* revealed that *F. culmorum* showed a strong inhibitory activity against the inhibitory *Streptomyces* isolate but not against the non-inhibitory isolate. Sensitivity to inhibition by *Fusarium* varied widely among *Streptomyces* isolates (inhibition zones ranged from 0 to 10.7 mm). In contrast, there was little variation among *Fusarium* isolates in capacities to inhibit the collection of *Streptomyces* (inhibition zones ranged from 2.4 to 3.5 mm). The results of this study illustrate the inhibitory potential of *Streptomyces* for biocontrol of FCR in wheat, while suggesting that *Streptomyces-Fusarium* interactions appear to be highly isolate-specific. In conclusion, a broader understanding of the variation in susceptibility within *Fusarium* populations to *Streptomyces* inhibition, as well as the capacity of *Fusarium* spp. to inhibit antagonistic *Streptomyces* is needed, which are both keys to biocontrol advances.

**Microbe-assisted crop production, opportunities, challenges & needs**

**Vienna, Austria, 4- 7 December 2017**

***The holobiont plant: multitrophic interactions***

**Chairs: Leo van Overbeek & Gabriele Berg**

## MI-01 Occurrence of human pathogenic micro-organisms in plant production systems.

### Leo van Overbeek

Wageningen University and research, Netherlands

Contamination of food and feed of plant origin with human pathogens incidentally occur. The sources of bacterial and fungal contaminants in these products are not always clear, but nowadays it is accepted that micro-organisms threatening human health do occur in plant microbiomes. Likely, the presence of human pathogenic strains in plants is an intrinsic property of plant microbiomes and thus a negative aspect in plant microbiome studies. It is of our interest to establish whether accumulation of human pathogenic strains in plants can be stimulated under specific agricultural practices. For instance, it is still uncertain what the consequences will be of the use of recyclable materials and of innovative plant growth facilities on plant contamination with human pathogenic strains. European research on plant contamination with human pathogens is recently aggregated under COST Action 16110, 'Control of human pathogenic micro-organisms in plant production systems (HUPLANTcontrol)'

In studies conducted at Wageningen UR the fate of human pathogenic strains in plants that can be used as food (pea, lettuce, endive) and as cattle feed (grass, maize) sources is investigated. *Escherichia coli* is the most commonly used model species because of its potential threatening nature when associated with Shiga-toxin production genes and other virulence traits (STEC), but also because of the high prevalence of *Enterobacteriaceae* (the taxonomic class to which *E. coli* belong) in plants. One of the key observations in our studies is that *E. coli* can survive over remarkably long periods in time in plants, especially in rhizosphere soil, albeit at low cell numbers. Contaminating sources in plant production systems are manure and water and we found that attachment of *E. coli*, and also of *Salmonella* Typhimurium cells on leaf surfaces occur within seconds, after which both cell types are protected against disinfecting reagents such as chlorine dioxide and peracetic acid. Further, we isolated new *E. coli* types from plant tissue and rhizosphere soil that are putative natural residents of plant microbiomes.

In my presentation I will give an overview on the ecological behaviour of *E. coli* in plants, grown under constrained greenhouse and open field circumstances.

## MI-02 Dissecting the multispecies interaction network at the *Arabidopsis* root-soil interface

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In nature, healthy plants cohabit with a plethora of microbes, forming complex microbial consortia that impact host growth and health. Here we aimed at investigating the complex microbial communities that are established on plant roots, where bacteria, fungi and oomycetes are not considered as separated entities but rather as internal drivers of the overall microbial community structure. In a first step, we selected 17 natural *A. thaliana* populations across a transect line in Europe and profiled corresponding root-associated bacterial (16s rRNA), fungal and oomycetal (ITS) communities in 3 consecutive years. Despite large geographical distances, root-associated bacterial communities show a highly reproducible taxonomic distribution and converge in the root endosphere. Consistent with that, the sample type (i.e. compartment) has a higher impact on bacterial community structure (25%) than the biogeography of the host (17%). On the other hand, fungal and oomycetal communities are strongly impacted by the local environment (19-25%) and show little compartmental footprint (1.2-3.7%). Furthermore, reciprocal transplant experiments conducted in growth chambers mimicking the environment of two contrasting sites, revealed that climate has a strong impact on soil microbial communities (6-24%), but that host genotype drives the strongest microbial community shift, only when the host is growing in its native climatic conditions (12-34%). In a second step, using comprehensive *A. thaliana* root-associated bacterial, fungal and oomycetal culture collections and gnotobiotic plant systems, we investigated how interkingdom microbe-microbe interactions contribute to microbiota establishment and what consequences on plant health are. We showed that re-colonization of *A. thaliana* by oomycetal and fungal communities have a strong deleterious impact on plant growth (0-24% survival rate, SR) compared to germ-free plants (80-84% SR). Remarkably, this phenotype is rescued in the presence of a bacterial community (87-100% SR), indicating that a major physiological function of the bacterial root microbiota is to protect plant roots from extensive colonization by filamentous eukaryotes. This bacteria-driven plant growth rescue is associated with fungal and oomycetal community shifts marked by decreased relative abundance of specific taxa. Our results demonstrate that inter-kingdom microbe-microbe interactions are key for maintaining microbiota balance in roots and promoting plant health

### MI-03 From seeds to postharvest: the impact of microbial diversity on plant health

Gabriele Berg

Environmental Biotechnology, TU Graz, Austria

The microbiome of plants plays a crucial role in plant health. Rapid advances in multi-omics and computational tools are dramatically increasing access to the plant microbiome and consequently leading to the identification of its links with diseases and to the control of those diseases. Plants have to be considered as co-evolved species assemblages and form functional holo-bionts. The plant microbiome harbor species-specific microbial diversity consisting of bacterial, archaeal, and diverse eukaryotic species. Diverse abiotic and biotic factors influence microbial diversity but the plant development stage is a key factor. From seeds to fruits - plants harbor specific microbes. The beneficial interplay of the host and its microbiome is responsible for maintaining the health and functioning of the holo-biont. At each plant stage, diseases are correlated with microbial dysbioses. Microbial diversity was identified as a key factor in preventing diseases and can be implemented as a biomarker in plant protection strategies. Targeted and predictive biocontrol approaches are possible by developing microbiome-based solutions. Moreover, combined breeding and biocontrol strategies maintaining diversity and ecosystem health are required. The analysis of plant microbiome data brought about a paradigm shift in our understanding of its role in health and disease and has substantial consequences for biocontrol and health issues.

### MI-04 Diversified microbiome bears the potential to protect crop plants against human pathogens

Helena J. Papathanasiou<sup>1</sup>, Jasper Schierstaedt<sup>2</sup>, Abhishek Shrestha<sup>1</sup>, Rita Grosch<sup>2</sup>, **Adam Schikora**<sup>1</sup>

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Outbreaks of food-borne diseases involving pathogens such as *Salmonella enterica* have been increasingly associated with raw produce. Even though plants perceive *Salmonella* and induce immune response, *S. enterica* is able to colonize plants. In order to establish in a plant environment *Salmonella*, as other human pathogenic bacteria, needs to cope with and outcompete the innate microbiome. The later however, can act as direct antagonist of human pathogens or induce plant immune system. Here, we analyzed the tripartite interactions between crop plants (tomatoes, lettuce and corn salad), the human pathogen *Salmonella enterica* and the microbial community. In first experiments we evaluated the survival of *Salmonella* in rhizosphere. We observed that *Salmonella* persisted in the rhizosphere of lettuce and tomato. In contrast, its numbers declined in the rhizosphere of corn salad. Very important was the observation that a drastic reduction of microbial diversity in soil, for example through autoclaving, dramatically increased the ability of *Salmonella* to persist in this environment. These results clearly show a wide range of niches, which can be colonized by *Salmonella* as well as its high physiological plasticity. In the following, we focused on the impact of induced resistance on the colonization. One of the means to enhance immune responses in plants is priming through *N*-acyl-homoserine-lactones (AHL), an efficient strategy to control plant pathogens. The main objective was to assess whether crop plants can be primed for enhanced resistance against *Salmonella*.

## **MI-05 Ecological interaction of lytic bacteriophages and plant pathogenic bacteria – a case study involving lytic phages and pectinolytic plant pathogens in plant-associated environment**

**Robert Czajkowski**

Department of Biotechnology, University of Gdansk, Poland

Bacteriophages (phages) are viruses able to exclusively infect and kill bacteria. Although phage-bacterial interactions have been studied since the beginning of their discovery, still little is known about their global ecological impact. It is generally accepted that bacteriophages are associated with most of known bacterial species, even as far evolutionally as cyanobacteria, archaea or mycoplasmas. Bacterial viruses are able to shape the structure of host population and therefore may influence bacterial virulence and plant infection. In this study we aimed to acquire knowledge on the specific ecological interaction between the pectinolytic bacterium soft rot *Dickeya solani* and broad host lytic bacteriophage  $\phi$ D5 isolated in our former studies. We also aimed to assess the  $\phi$ D5 viability in natural ecosystem (potato plants) as a preliminary step in the possibility to better understand how bacterial viruses affect populations of plant pathogens *in situ*. For this,  $\phi$ D5 was characterized in details for stability in solutions containing copper ions, in potato tuber extracts, in rain water and in soil. The phage was also tested for the ability to survive on the surface of (detached) potato leaves and potato tubers in storage at 8 °C. In tissue culture grown potato plants as well as in experiments in which tubers were grown in potting soil, we tested if application of  $\phi$ D5 presiding application of *D. solani* can protect growing plants from development of disease symptoms.  $\phi$ D5 remained infectious in potato tuber extract, soil and rain water in time but was inactivated in solutions containing copper ions. Both, in tissue culture and soil-grown potato plants,  $\phi$ D5 protected plants from *D. solani*, reducing infection caused by the pathogen by more than 50%. This is a case study describing viability and interaction of lytic bacteriophage with plant pathogen in their natural environment and may serve as a starting point for similar studies involving different viruses, bacterial hosts and crop systems with the overall aim to use the generated knowledge for development of new environmentally friendly control applications.

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## **MI-06 Synthetic volatile mixture, from a fungal origin, controls *Sclerotium rolfsii* both *in vitro* and in soil**

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*Sclerotium rolfsii* (also called *Athelia rolfsii*) is a phytopathogenic fungus and is the causal agent of 'southern blight' disease in economically important crops. The facts that this fungus has an extremely wide range of hosts, and its capacity to form sclerotia, hamper its eradication. Recently, we have shown that the endophytic fungus *Daldinia cf. concentrica* emits biologically active volatile organic compounds (VOCs). We also demonstrated that a synthetic volatile mixture (SVM), comprise from 4-heptanone and trans-2-octenal in volumetric ratio of 1:1, was the most effective against various phytopathogenic fungi. The objective of this work was to evaluate the potential of this SVM to control *S. rolfsii*, in the form of hyphae and sclerotia, both *in vitro* and in soil. We found that the SVM fully controlled hyphae and sclerotia in Petri plates. We used a controlled laboratory system to simulate soil conditions, and found that the SVM affected the viability of both hyphae and sclerotia of *S. rolfsii* whereas the former being more susceptible to the SVM than the latter. We also examined the ability of the SVM to affect the viability of the fungus in different soils. The soil that enabled the most efficient control against the fungus was loam soil, followed by sand. In contrast, planting mixture was less suitable for this purpose with nearly no effect of the SVM on hyphae and sclerotia of the fungus. Based on our result, we suggest the use of our SMV, from a fungal origin, as an alternative approach to controlling the phytopathogen *S. rolfsii*.

## MI-07 Disease-induced assemblage of a plant-beneficial bacterial consortium

Roeland Berendsen<sup>1</sup>, Gilles Vismans<sup>1</sup>, Ke Yu<sup>1</sup>, Yang Song<sup>2</sup>, Corne Pieterse<sup>1</sup>, Peter Bakker<sup>1</sup>

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Disease suppressive soils typically develop after a disease outbreak due to the subsequent assembly of protective microbiota in the rhizosphere. The role of the plant immune system in the assemblage of a protective rhizosphere microbiome is largely unknown. In this study, we demonstrate that *Arabidopsis thaliana* promotes a plant-beneficial consortium of bacteria in the rhizosphere upon foliar defense activation by the downy mildew pathogen. In turn, this rhizobacterial consortium induces systemic resistance against downy mildew and promotes growth of the plant. The recruited consortium members interact synergistically in biofilm formation and together are better able to stimulate plant health than each of the strains separately. Our results indicate that plants can cry out for help upon pathogen infection and recruit a synergistic consortium of disease resistance-inducing beneficial microbes to maximize their chance of survival.

**Microbe-assisted crop production, opportunities, challenges & needs**

**Vienna, Austria, 4- 7 December 2017**

***The holobiont plant: microbiome understanding***

**Chairs: Yvan Moënne-Loccoz & Birgit Mitter**

## MU-01 Drought alters the development of the grass root microbiome

### Tuesday Simmons

Plant and Microbial Biology, UC Berkeley, United States of America

Root associated microbes have been shown to play key roles in determining host fitness under periods of drought stress, and yet the effect of drought on the broader root endosphere bacterial community remains largely uncharacterized. We present an analysis of bacterial communities associated with drought-treated root and rhizosphere tissues of eighteen species of plants with varying degrees of drought tolerance belonging to the Poaceae family, including important crop plants. Through 16S rRNA gene profiling of samples taken across two distinct watering regimes and multiple developmental time points, we demonstrate that there is a strong correlation between host phylogenetic distance and the microbiome dissimilarity within root tissues, and that drought weakens this correlation by inducing conserved shifts in bacterial community composition. We identify a significant enrichment in a wide variety of Actinobacteria during drought within the roots of all hosts, and demonstrate that this enrichment is higher within the root than it is in the surrounding environments. Furthermore, we show that this observed enrichment is the result of an absolute increase in Actinobacterial abundance and that previously hypothesized mechanisms for observed enrichments in Actinobacteria in drought-treated soils are unlikely to fully account for the phenomena observed here within the plant root.

## MU-02 Plant-beneficial bacteria in the rhizosphere microbiome of cereals

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Understanding root microbiome assembly and dynamics is of fundamental interest and could help design microbiome-based strategies for crop management. We have addressed this issue in two ways. First, the extent to which microbial inoculants might be useful to modify microbiome composition of cereal crops was investigated, based on the rationale that inoculants could change microbiome composition and functioning. When focusing on particular functional microbial groups of relevance for plant fitness, such as those involved in nitrogen fixation or deamination of the ethylene precursor 1-aminocyclopropane-1-carboxylate, it appeared that the effect of the maize seed inoculant *Azospirillum lipoferum* CRT1 was significant but largely field dependent. Second, the significance of cereal genotype for root-microbiome interactions was studied. A relationship was found when comparing rhizobacterial communities with the phylogenetic distance between the corresponding plant genotypes, but this relationship held true only for a fraction of the community. It prompted us to assess how plant hosts selected for bacteria with plant-beneficial potential. This type of screening showed that the ability to interact with plant-beneficial bacteria had been largely counter-selected in modern cultivars, yet it had been retained in some of them. The implications for plant-microbe interactions will be discussed.

### **MU-03 From seeds to roots: the role of seed-borne endophytes for the composition of the active microbiome of barley roots**

Luhua Yang, Jasmin Danzberger, Yvonne Biggot, Michael Schloter, Peter Schröder, **Viviane Radl**

Comparative Microbiome Analysis, Helmholtz Zentrum München, Germany

Endophytes are microorganisms colonizing plant internal tissues. They are ubiquitously associated with plants and play a major role in plant growth and health. Among all endophytes, those found inside seeds are particularly interesting, as they are the first to colonize the tissue of the seedlings. Although significant efforts have been made to determine the composition of the endophytic bacterial communities, less is known about their activity. Therefore, we investigated the active bacteria communities residing in seeds and their relevance for composition of root tissues of barley in different growth stages, using an RNA-based approach. Moreover, metagenomic analyses of seed-borne root endophytes were carried out to obtain information about their genomes. We found out that members of the genera *Phyllobacterium*, *Paenibacillus* and *Trabusiella* were the most abundant active bacteria inside germinating seeds. The presence of the latest families was also confirmed by cultivation (e.g. *Panaenibacillus hordei* and *Pantoea agglomerans*). Though some of these populations were also found in the roots, we found a greater influence of soil microbes on the composition of the community, particularly at later growth stages. Metagenomic analysis corroborated the great dominance of Enterobacteraceae (*Pantoea*) and Pseudomonaceae (*Pseudomonas*) in roots of plants growing in axenic systems. In general, we detected genes related to functions assumed to be essential for bacteria colonizing plant tissue, such as antioxidative stress enzymes, osmoprotectants, biofilm formation, etc.. However, bacterial endophytes might possess different strategies to “communicate” with the host plant and evade their immune system, as we detected different pathways for the synthesis or regulation of plant signaling molecules, e.g. indole acetic acid and ACC deaminase. We hypothesize that the seed endophytes play a role during germination, but becomes less relevant as the plant develops, where endophytic bacterial populations most probably originates from the soil.

### **MU-04 Understanding the plant microbiome**

**Birgit Mitter**<sup>1</sup>, Carolina Escobar-Rodriguez<sup>1</sup>, Stefan Pfeiffer<sup>1</sup>, Raheleh Sheibani-Tezerji<sup>1</sup>, Alexandra Petric<sup>1</sup>, Livio Antonielli<sup>1</sup>, Thomas Rattei<sup>2</sup>, Angela Sessitsch<sup>1</sup>

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Similar to the human gut are plants tightly colonized by complex microbial communities. Microbiomes of both gut and plants are known for their importance for healthy growth and development of the host. Similar to modern therapeutic concepts in human and veterinary medicine modifications of plant microbiomes will be an essential component of tomorrow's crop production and protection. It will also allow for making use of the full potential of naturally occurring microbes and microbial functions. Microbiome modulation is a new approach, which currently receives extremely high attention by science and industry.

However, targeting the plant microbiome for improved crop production requires profound knowledge on plant microbiome functioning and dynamics. We apply (meta)genomic approaches to studying the bacterial community compositions and dynamics in different crops and model plants and modern transcriptomics to elucidate the genetic basis of beneficial plant-microbe interaction and communication mechanisms. Our results showed that the cultivation soil, plant genotype, tissue, the vegetation stage as well as stress factors influence the structure and possibly functioning of plant-associated microbial communities. Metagenome analysis of a rice root community revealed characteristics like various plant growth promoting characteristics, cellulolytic enzymes, quorum sensing, degradation of aromatic compounds, methane oxidation, nitrogen fixation as well as nitrification and denitrification. Transcriptome profiling of *Paraburkholderia phytofirmans* PsJN colonizing potato tubers showed the bacterium is metabolically active inside plants and that it actively senses the plant environment and changes its metabolism in response to plant drought stress.

## MU-05 The oxalate-carbonate pathway: an ecosystem service for sustainable agriculture

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The sustainable exploitation of soils is essential to maintain crop production and to provide good quality food on the long term. However, soil degradation has become a major threat to soil sustainability, partly due to the continuous use of agrochemicals to fight pathogens and to increase soil fertility. In sustainable agriculture, the use of beneficial microbes to support plant health and growth is promoted. In this research, we investigate the feasibility of harnessing complex natural microbial communities that are known for their positive impact on soil properties and functioning. The oxalate-carbonate pathway (OCP) is typically an example of a complex array of varied and interconnected microbes. This biogeochemical process described in tropical environments combines the action of plants, fungi, and bacteria in soils. In the OCP, bacterial oxidation of biogenic calcium oxalate (Ca<sub>ox</sub>) leads to the modification of soil physicochemical factors, and particularly soil pH. This change in soil pH, in turn, modifies the availability of several nutrients and positively impacts soil functioning and fertility. A key element at the centre of a functional OCP is the presence of oxalotrophic microorganisms capable of oxidizing Ca<sub>ox</sub>. However, Ca<sub>ox</sub> is a poorly soluble compound. Its bioaccessibility, and thus turnover, is enhanced by the presence of fungal mycelial networks that act as dispersal networks for oxalotrophic bacteria (fungal highways). Therefore, bacterial-fungal interactions are crucial for the functioning of the OCP. Because of their central role in the modification of soil pH and on soil nutrient bioavailability, the biological interactions of the OCP constitute a prime example of an ecosystem service that could be harnessed for sustainable agriculture in tropical environments.

## MU-06 Smart farming: manipulating root and soil microbiomes through specific cropping practices

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Harnessing beneficial microbes presents a promising strategy to optimize plant growth and agricultural sustainability. Little is known to which extent and how specifically soil and plant microbiomes can be manipulated through different cropping practices. Here, we investigated soil and wheat root microbial communities in a cropping system experiment consisting of conventional and organic managements, both with different tillage intensities. While microbial richness was marginally affected, we found pronounced cropping effects on community composition, which were specific for the respective microbiomes. Soil bacterial communities were primarily structured by tillage, whereas soil fungal communities responded mainly to management type with additional effects by tillage. In roots, management type was also the driving factor for bacteria but not for fungi, which were generally determined by changes in tillage intensity. To quantify an 'effect size' for microbiome manipulation, we found that about 10% of variation in microbial communities was explained by the tested cropping practices. Cropping sensitive microbes were taxonomically diverse and they responded in guilds of taxa to the specific practices. These microbes also included frequent community members or members co-occurring with many other microbes in the community, suggesting that cropping practices may allow manipulation of influential community members. Understanding the abundance patterns of cropping sensitive microbes presents the basis towards developing microbiome management strategies for *smart* farming. For future targeted microbiome management – e.g., to foster certain microbes with specific agricultural practices – a next step will be to identify the functional traits of the cropping sensitive microbes.

## MU-07 Phenazine-1-carboxylic acid influences biofilm development and turnover of rhizobacterial biomass in a soil moisture-dependent manner

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Phenazine-1-carboxylic acid (PCA) is a redox-active metabolite produced in measurable quantities by rhizobacteria in dryland cereal cropping systems throughout the low-precipitation zone of the inland Pacific Northwest, USA. Long studied for its biocontrol activity against phytopathogens, PCA promotes the formation of biofilms in bacterial cultures and facilitates competitive colonization of wheat roots by bacteria that produce the compound. However, the impact of PCA upon biofilm development and the turnover of bacterial biomass have not been demonstrated in the rhizosphere of crops, nor have the mechanisms driving the accumulation of PCA and PCA-producing bacteria as a function of soil moisture been identified. To address these knowledge gaps, we compared the expression and degradation rates of PCA in the rhizosphere of wheat under dryland and irrigated conditions. We also compared biofilm development and the re-distribution of rhizobacterial nitrogen on the surfaces of dryland and irrigated wheat roots inoculated with the PCA-producing (PCA<sup>+</sup>) strain *Pseudomonas synxantha* 2-79RN10 or with its PCA-deficient (PCA<sup>-</sup>) mutant. The expression of PCA biosynthesis genes was increased over background expression levels in wheat rhizospheres maintained under dryland conditions relative to those under irrigated conditions, indicating that rhizobacteria increase or maintain PCA production at low soil moisture. The degradation of PCA was slightly accelerated in dryland relative to irrigated rhizospheres, likely due to decreased oxygen availability in wetter soils. Electron and ion microscopy of dryland and irrigated rhizospheres showed that PCA<sup>+</sup> colonies developed more robust biofilm matrices than PCA<sup>-</sup> colonies. Few colonies were observed on the surfaces of irrigated roots. NanoSIMS imaging of wheat rhizospheres demonstrated that <sup>15</sup>N was transferred directly from labelled bacterial biomass to root biomass, highlighting the importance of bacterial biomass as a source of organic nutrients in soil. NanoSIMS also showed that under dryland conditions, <sup>15</sup>N turnover was slower in some labelled PCA<sup>+</sup> bacterial biomass than in PCA<sup>-</sup> biomass. Collectively, these results indicate that PCA production is a rhizobacterial adaptation to water stress that promotes the formation and development of root-associated biofilms, potentially influencing the availability of bacterial N to crop roots.

## MU-08 Effects of plant secondary metabolite benzoxazinoids on the plant microbiome

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The plant root actively shapes its microbiome for its own benefit to prevent root disease, mainly by exuding specific compounds into the rhizosphere. Root exudates secreted into the rhizosphere act as substrates for growth and mediate the complex signaling mechanisms that occur between plant roots, environmental stressors and soil microbes. Benzoxazinoids are a class of secondary metabolites widely distributed in cereals found to possess defensive and antimicrobial properties that protect the plant against pathogens. The aim of this study was to identify potential influence of benzoxazinoids in shaping the root and rhizosphere microbiome. For this, we grew five maize genotypes with different benzoxazinoid exudation profiles in rhizo-boxes with natural soils as planting media. Samples were collected from plant leaves, roots, rhizosphere and surrounding bulk soils at day 0 (before sowing) and 4 different growth stages, 10, 20, 30 and 40 days after sowing. We are currently employing metabolomics techniques to quantify the amount of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and 6-methoxy-benzoxazolin-2-one (MBOA), the main benzoxazinoids in the maize roots. Metabarcoding will be employed to elucidate the effect of benzoxazinoids on bacterial and fungal community structure. Results from this experiment will provide an in-depth knowledge of the role of secondary metabolites present in root exudates implicated in plant defense.

Keywords: root exudates, secondary metabolites, rhizosphere microbiome, benzoxazinoids

## **MU-09 Contrasting soil microbial community profiles associated with outliers in early growth of corn (*Zea mays*)**

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That the soil microbiome impacts plant performance is now well known, and significant efforts are being made to identify beneficial soil microbes and microbial consortia for crop yield improvement. Key to such efforts are sequence-based profiling techniques, which provide insights into microbial communities associated with improved performance, often under stress conditions. Using inocula prepared from field soils, corn plants were grown under modest nutrient (nitrogen) stress and outliers (for growth and biomass accumulation) identified. 16S ribosomal RNA gene profiling of soils associated with these outliers was conducted, and clear differences observed between the profiles associated with plants growing well and poorly under the conditions of the experiment.

Poster Talks III

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## **PT-MU-24 Unravelling the key players of grassland plant microbiomes behind methanol consumption**

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Managed grasslands are global sources of atmospheric methanol. Methanol is one of three most abundant reactive volatile organic compounds (VOCs) and contributes to the overall oxidative capacity of the troposphere and also to ozone depletion. Grasslands exhibit higher methanol emissions than forests and may be net sinks, i.e. they are globally affecting the methanol budget. Often the emitted methanol is of plant origin (~80-90%). The phyllosphere can be regarded as a favoured habitat of methanol-utilizing microorganisms being as abundant as up to 17% of the total phyllosphere microbiome. But the information regarding rhizosphere methanol utilizers is still scarce. Few studies suggested that the estimated global emission rate of methanol is considerably higher than the observed emission rates of terrestrial ecosystems. Thus, methanol utilizers of the plant microbiome are crucial in mitigating the emission rates through methanol consumption. The current study aims to identify the key methanol utilizers of a managed grassland in Germany. Hence, a central motivation of our study was to test the hypothesis if specific plant species are associated with specific methanol utilizers. We address this objective by the identification of active methanol utilizers of four grassland plant species (i.e. *Poa trivialis*, *Festuca arundinacea*, *Trifolium repens*, *Taraxacum officinale*) by DNA and RNA stable isotope probing (SIP) and metatranscriptomics at different growth stages (germination and flowering). We used gas-tight plant growth chambers to investigate the phyllosphere, endosphere, and rhizosphere of complete and intact grassland plants to avoid physical harms, which may lead to change of methanol fluxes. We measured the phyllospheric and rhizospheric methanol production and will identify those methanol utilizers that will have incorporated  $^{13}\text{C}$  from supplemented [ $^{13}\text{C}_1$ ]-methanol. Additional to the taxonomic identities, the metatranscriptome data of the labelled microbiome will deliver insights (i) in preferred metabolic pathways for methanol assimilation and dissimilation and (ii) in further metabolic interactions with the host plants.

## **PT-MU-23 Engineering the plant microbiome to improve crop quality and yield**

**Valeria Verrone**, Robert Edwards, Sanjay Swarup, Matthew Chang, Anil Wipat

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New approaches are required to meet the demand for an increased global food supply that do not harm the environment and are produced in a sustainable fashion. This study proposes to examine how the microorganisms associated with plants, the plant microbiome can be engineered to improve crop quality and yield using synthetic biology approaches. The overall aim of the project is to contribute to an alternative and more sustainable future for agriculture.

The methodology used in this study is based on the CRISPR-Cas9 genome editing approach, which is increasingly being adopted in microbiology due to its ability to allow the genetic manipulation of previously genetically intractable organisms. We are applying the technique to modify the genomes of plant growth-promoting rhizobacteria and also the metagenome of the rhizosphere community in order to obtain a synthetic bacterial consortium with improved capabilities of promotion of disease resistance or resource utilization in the partner crop. As a result, we also hope to gain a broader understanding of the interactions occurring between bacteria and plants in this complex environment.

**PT-MU-27 Root microbiome dynamics in response to phosphate**Natacha Bodenhausen<sup>1</sup>, Marcel van der Heijden<sup>2</sup>, Klaus Schlaeppi<sup>2</sup><sup>1</sup> Department of Soil Sciences, Research Institute of Organic Agriculture (FiBL), Switzerland<sup>2</sup> Department of Agroecology and Environment, Agroscope, Switzerland

Plants require phosphorus (P) for growth. A small fraction of soil P is directly available for plant growth while the larger fraction is held to minerals and inaccessible for plants. Farmers compensate the lack of phosphate with fertilizers; however, the world reserves for phosphate fertilizers will be depleted within the next 50 to 100 years. On the other hand, microbes are able to solubilize the inorganic P fraction. Understanding how plants depend on microbes for P uptake is crucial for a more sustainable agriculture. In this study, we investigated the effect of P availability on the bacterial and fungal communities associated with *Petunia* roots using 16S rRNA and ITS gene sequencing. We found that the phosphate gradient affected both richness and community composition. We identified differentially abundant OTUs under high and low P conditions. Interestingly, the response to P availability differed between *Petunia*, which is colonized by arbuscular mycorrhizal fungi (AMF), and *Arabidopsis*, a plant that does not form symbiosis with AMF. Finally, through network analysis, we identified fungal-bacterial microbial hubs affected by the P gradient. Together these results suggest that the root microbiome responds dynamically to P availability in soil and that this response differs between mycorrhizal and non-mycorrhizal plants. P-dependent OTUs/hubs identified in this study could be used as target to develop new biofertilizers.

**PT-MU-22 Legacy effects of long-term organic or conventional farming practices on the rhizosphere microbiome and health of the next plant generation measured under controlled conditions.**Soumitra Paul Chowdhury<sup>1</sup>, Doreen Babin<sup>2</sup>, Martin Sandmann<sup>3</sup>, Jörg Geistlinger<sup>4</sup>, Kornelia Smalla<sup>2</sup>, Michael Rothballer<sup>1</sup>, Rita Grosch<sup>5</sup><sup>1</sup> Helmholtz Zentrum München (GmbH), Germany<sup>2</sup> Julius Kühn-Institut, Federal Research Centre for Cultivated Plants (JKI), Institute for Epidemiology and Pathogen Diagnostics, Braunschweig, Germany<sup>3</sup> Leibniz Institute of Vegetable and Ornamental Crop (IGZ), Großbeeren, Germany<sup>4</sup> Anhalt University of Applied Sciences, Bernburg, Germany<sup>5</sup> Leibniz-Institute of Vegetable and Ornamental Crops, Großbeeren, Germany

There is an increasing world-wide demand for sustainable food supply and the current challenge is to enhance the agricultural productivity, while avoiding long-term negative consequences for the quality of soils and the soil microbiome. To understand how long-term fertilization strategies affect the microbiome and plant health, soils were collected from two long-term field experiments (LTEs) established in 1978 (DOK, Therwil, Switzerland), and 2006 (HUB, Thyrow, Germany) having a documented history of conventional or organic farming. Lettuce (*Lactuca sativa*) plants were grown for 6 weeks in these soils under controlled conditions and leaves and rhizosphere sampled. The expression of 21 putative genes involved in the plant response to stress conditions was monitored using a RT-qPCR based system. Although, no clear effect of organic vs. mineral fertilization on plant growth was observed, comparing gene expression, similar patterns were observed in plants grown on organically managed soils despite different types and origins of soils. The expressions of several genes with documented roles in oxidative and biotic stress were up-regulated in plants grown in soils with long-term organic management. This might be triggered by the rhizosphere microbiome. Results from denaturing gradient gel electrophoresis and Illumina sequencing of 16S rRNA gene amplicons showed significant differences in bacterial and archaeal communities between soils that originated from different sites and different long-term fertilization. Interestingly, organic fertilization resulted in a higher rhizosphere bacterial diversity compared to mineral fertilization in both LTEs. A higher relative abundance of *Firmicutes* (mostly belonging to genera *Bacillus* and *Paenibacillus*) in rhizosphere of both organically fertilized soils was also observed. It is well known that a number of beneficial bacteria belong to this phylum and that may positively influence plant defense priming. Our results strongly support the hypothesis that soil properties are characterized by the legacy of agricultural management. Additionally, there seems to be a soil memory effect on the rhizosphere bacteria that might result in altered performance and health of the next plant generation.

## **PT-MU-25 Asymmetric dependencies – a novel statistic to reveal non-reciprocal relationships between plant associated microbes**

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Bacterial communities associated to plants are highly diverse assemblages that are formed by plant-based factors as well as by interactions between bacterial species. The latter have the effect that species' abundances often do not vary independently of each other. Abundances of mutualistic partners can be expected to exhibit positive correlation, whereas the abundances of competitors may be negatively correlated. Such linear or at least monotone associations are well quantified by Pearson's *R* or Spearman's *rho*. Additional known dependence measures allow for an identification of non-linear relationships in community data. Unfortunately, all of these dependence measures are symmetric (i.e. mutual), implying that the influence of species *X* on species *Y* coincides with the influence of *Y* on *X*. However, in many types of interactions one species may be more dependent on another species than *vice versa*, for instance if one bacterial species depends on a by-product of another species' metabolism. In order to facilitate detection of such non-mutual relationships in large datasets, we implemented an asymmetric copula-based dependence measure called *acdm*. With this measure it is possible to quantify dependence for any functional relationship in an asymmetric manner.

We will discuss the theoretical and ecological relevance of asymmetry in dependence measures and, using data on bacteria associated with plants, provide fundamentally new insights into the nature of associations between bacteria.

## **PT-MU-26 Biostimul'eau: crop quality improvement in (vertical) hydroponics through transplantation of a soil rhizosphere microbial community**

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Today, soilless cultivation systems are the most intensive production systems in the horticulture industry. Because there is an increasing demand for more tasteful and quality agricultural products with limited impact on the environment and on food quality further down the chain (e.g. presence of chemical residues), there is an opportunity to improve crop quality in hydroponics. The quality and taste of fruit and vegetables is to a large extent determined by the soil in which they are grown. This is due, in part, to the soil type, but also to soil life. Although there is a connection between a rich soil life and crop quality, it has not yet been introduced as a concept in hydroponics. If we transplant the microbial rhizosphere community from a soil grown crop to its hydroponics counterpart, this could have a significant impact on promoting the quality of the final product. This hypothesis was tested in a preliminary experiment on hydroponic lettuce (*Lactuca sativa* L.) in a vertical farm. The rhizosphere microbial communities of soil grown lettuce plants were extracted. Hydroponic lettuce seedlings were inoculated with the rhizosphere extract. A second inoculation took place two weeks later. To determine the effect of transplantation on the nutritional quality of the crop, several quality parameters were determined after harvest. Transplantation caused an increase in total phenolics (+15%), Fe (+14%), Ca (+24%), Mg (+17%) and Zn (+35%) content, and a decrease in nitrate content (-46%). This preliminary experiment shows the potential of transplantation of soil rhizosphere communities to hydroponics. With the Biostimul'eau project we aim to find the conditions that can optimize and stabilize the positive functioning of transplanted microbial communities in hydroponics. Further research will be focused on optimizing substrate composition next to identifying and isolating bacteria with quality-improving properties.

## PT-ATF-06 Microbial nanoclay and nanocellulose composite formulations using Gram negative endophytic bacteria for maize

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Formulation granting prolonged survival on storage and both survival and sufficient level of bio-activity in the field represents the bottleneck in the broad scale implementation of biological means in plant protection urged by the EU directive on Integrated Pest Management 2009/128/EG. A major reason is the fact that bacteria-loaded water-based biopolymer coatings are of low mechanical stability and are prone to increased permeability of small molecules like water vapor and oxygen including reactive oxygen species that dramatically reduce bacterial viability and functionality and furthermore shelf life of the formulated seed.

We therefore evaluate the integration of high performance composites based on nanoclay and nanocellulose to meet the requirements for a stable and high quality microbial formulation to be used as seed coating for maize. We present new material designs comprising biobased polymer composites on well-defined structure/property relationships to enable substantial improvement of microbial viability, mechanical performance and water vapor and O<sub>2</sub> barrier properties of microbial formulations. Biopolymers, such as alginate, methoxy pectin, starch, arabic gum and gelatin will be used as binders. Better mechanical performance is expected to drastically reduce abrasion of coated seeds, while enhanced water vapor and O<sub>2</sub> barrier properties shall significantly improve bacterial survival and storage stability of formulations and formulated seeds at ambient conditions.

We demonstrate using the Gram negative bacteria *Paraburkholderia phytofirmans* PsJN and *Pseudomonas* sp. that the integration of new biobased filler materials (hemicellulose, nanoclay and nanocellulose) to develop fully biobased composites for application in biofertilizer products is significantly improving microbial viability and shelf life of microbial formulations.

## PT-ATF-07 The project "SaatMaisPlus": Development of non-chemical seed treatments for maize

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Maize is one of the most important crops worldwide. In Germany it is grown on 2.5 Mill. Hectar, of which 84 % are silage maize and 16 % grain maize. Especially under conditions of cool and wet soils, seed germination and crop establishment of maize are often affected by pathogenic fungi like *Fusarium*, *Pythium* and *Rhizoctonia*. Maize seed is therefore routinely treated with chemicals, commonly TMTD (thiram). So far, non-chemical seed treatments for maize are not available. Based on previous positive results with electron seed treatment of small-grain cereals and vegetables the project "SaatMaisPlus" aims at developing the application of low-energy electrons in combination with micro-organisms as a routine seed treatment method for maize. The project consortium includes two research institutions (JKI, CAU), the company EVONTA specialized in electron seed treatment, a specialist for production of microorganisms for sustainable agriculture (ABiTEP) as well as a supplier of seeds of arable and forage crops (DSV).

Because seed treatment with electrons is expected to be primarily effective against seed-borne pathogens (e.g. fusaria), microorganisms will be employed to protect seedlings against soil-borne attack. For this purpose, bacteria and fungi from maize roots and other sources will be screened in bioassays for activity against soil-borne *Fusarium*, *Pythium* and *Rhizoctonia*. The method of electron seed treatment will be adapted and optimized for maize regarding safety of the treatment and efficacy against pathogens and combined with the most effective micro-organisms. The efficacy of the single and combined treatments will be evaluated in greenhouse and field experiments in relation to the standard seed treatment TMTD.

The project is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support programme.

**PT-ATF-05 Two-way protection of Gram-negative beneficial soil bacteria *Paraburkholderia phytofirmans* in dry alginate formulations: inducing the cell adaptation in tolerance to desiccation**

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The major drawback of Gram-negative bacteria to be applied as plant-growth promoters or biocontrol agents is their sensitivity to desiccation. Therefore, the aim of this study was to significantly improve desiccation tolerance of Gram-negative bacteria, such as *Paraburkholderia phytofirmans* PsJN and to develop functional alginate beads providing not only protection to the bacterial cells but also a gradual release of PsJN upon rehydration.

Two different approaches were considered for improvement of desiccation tolerance and storage stability at ambient conditions:

First, growing PsJN under mild stress conditions induced by osmotically active solutes such as glycerol, sucrose, NaCl and polyethylene glycol 600 with water activity values of 0.95, 0.96, 0.97 and 0.98 to allow the bacteria to adapt and secondly, to add protective compounds to the alginate prior to encapsulation. Using osmotic treatment with either glycerol or sucrose storage stability of encapsulated PsJN was enhanced by 10 times compared with PsJN cultured in basic LB medium. This was observed for beads of various size (150 µm to 450 µm). The second approach was based on addition of protective compounds to alginate prior to encapsulation. For this purpose, skimmed-milk, trehalose, betaine and molasses were tested. Only addition of skimmed milk and/or molasses led to a substantial improvement in cell viability: cell counts of PsJN in alginate beads decreased by only two orders of magnitude in comparison with six orders in pure alginate beads. The formulations remained stable for at least 70 days at ambient conditions with log 4.5 CFU PsJN/mL alginate. Finally, both approaches were combined in order to preserve cells, both from the “inside and outside”. Furthermore, different types of starch were added to alginate in order to modify the bead porosity and tune the time course of bacterial release upon rehydration. Release studies were performed in 100% humidity in presence of saline solution. Performance of beads was evaluated with respect to storage stability and release profile at ambient conditions in comparison with Gram-positive bacteria (*Paenibacillus* sp.).

In conclusion, applying a two-way approach for PsJN protection, we were able to maintain cell viability after air-drying: The storage time of alginate formulations was prolonged from 4 days in pure alginate beads to up to 70 days.

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***Application technologies & formulations***

**Chairs: Anant Patel & Claudia Preininger**

## ATF-01 Formulations for novel biological pest control strategies

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In recent years, the world-wide demand for biological pest control agents has risen dramatically. However, biological control remains challenging. New pest control strategies aim at exploiting synergies to increase efficacy of pest control agents. Promising ideas include attract-and-kill, push-pull, stress-and-kill strategies and combination of kill-components. Besides, endophytic microorganisms offer new options for biological pest control.

Formulation science is meeting these newly arisen challenges with equally new formulation approaches and thus helps in the implementation of new biological control measures. That is why our group set out to shed some light into complex formulations that combine the agents in form-giving materials such as beads that allow a multi-compartment system, protection, growth support in a "minifermenter", and slow and controlled release of agents as a function of the bead materials and additives [1].

Here we present research into CO<sub>2</sub>-releasing bead systems that attract pests like wireworms, western corn rootworm, psyllids and ticks with a CO<sub>2</sub> gradient and then intend to control the pest population with a biological agent such as entomopathogenic fungi or neem extract. Achievements and pitfalls in co-formulation of these agents with synergistic chemicals like arrestants and aggregation pheromones, volatile attractants, drying additives, reswellers, nutrients and spatio-temporal changes within and around the formulations will be introduced. Besides, we will introduce research into beads and sprays that enhance colonization of tomato, potato and oilseed rape plants with endophytic entomopathogenic fungi.

It can be concluded that novel agricultural bio-formulations will in the next decade contribute to significant advances in biological pest control strategies that work in the field if formulation succeeds in moving away from conventional empirical mixing of substances with an active ingredient to formulation science drawing upon progresses in chemistry and biotechnology.

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## ATF-02 Design of microbial formulations – connecting material properties with bacterial viability and overall treatment performance

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Microbial biofertilizers and biocontrol agents are promising alternatives to agrochemicals in sustainable agriculture; however the lack of effective formulations is a major limitation for their successful application in the field. Using seed treatment, bacterial viability is reduced by at least one log CFU after drying, reduction is even more dramatic (2-3 logs), if non-spore forming bacteria are employed. Besides selecting suitable materials with distinct properties the formulation process itself and environmental conditions during coating and storage are critically important. In fact, stability of microbial formulations is influenced by multiple factors. Some of them have been previously studied with respect to microbial viability and shelf life, however, no systematic studies have been performed that take into account complex and multiple interactions between seed or plant leaf surface and rhizobial cell biology, and the physical and chemical properties of employed materials. When using foliar spray, formulation development also has to consider successful delivery to the plant surface (droplet impaction, adhesion, biofilm formation).

The scientific challenge is therefore to elucidate microbial responses to multiple factors including the properties of the used materials, the formulation process itself and the environmental conditions during processing and storage, and to estimate the driving factors that impact stability (viability, shelf life, metabolic activity), performance (germination, plant penetration) and function (release efficiency) of microbial seed coatings and foliar sprays.

We will show for formulations made of different types of bacteria (e.g. *Paraburkholderia phytofirmans* PsJN, *Paenibacillus* sp., *Pseudomonas* sp.) that the material properties of adhesives and additives, the drying and storage conditions of liquid concentrates and coated seeds, and the use of micro- and nano-carriers strongly affect microbial viability and overall performance. Our results highlight the necessity of taking into account the interplay between materials and microbes, furthermore the interaction with the plant leaf surface in foliar spray and the seeds in seed treatment development. We will present data of microbial formulations to be applied on maize that we believe will provide guidance and decision-making criteria and have the potential to help other researchers to optimize their choices in this area.

### **ATF-03 Non-viable antagonist cells are associated with reduced biocontrol performance by viable cells of the yeast *Papiliotrema flavescens* against Fusarium head blight of wheat**

David Schisler, Miho Yoshioka, Martha Vaughan, Christopher Dunlap

Crop BioProtection Research, NCAUR, USDA-ARS, United States of America

Microbially-based plant disease control products have achieved commercial market success, but the efficacy of such biocontrol products is sometimes deemed inconsistent. Improper processing of harvested microbial biomass or long-term storage can increase the proportion of cells that are no longer viable and necessitate the use of more biomass to achieve the desired concentration of viable cells in the final formulated biocontrol product. We hypothesized that non-viable cells, when in sufficient quantity within a formulation, could provide a nutritive benefit to plant pathogen propagules and therefore reduce the efficacy of a biocontrol product. To test this hypothesis we used *Fusarium graminearum*, an important causal agent of Fusarium head blight (FHB) of wheat, and the yeast *Papiliotrema flavescens* (formerly *Cryptococcus flavescens*) strain OH 182.9 3C, a proven antagonist of the FHB pathogen. In glasshouse experiments, wheat florets were injected with fixed amounts of *F. graminearum* conidia ( $\sim 1 \times 10^4$  conidia/ml), viable cells of *P. flavescens* ( $\sim 1 \times 10^9$  cfu/ml) and varying concentrations of non-viable *P. flavescens* cells including 0 as a control,  $\sim 1 \times 10^9$ /ml, and  $\sim 2 \times 10^9$ /ml. In the absence of non-viable cells, *P. flavescens* consistently reduced FHB severity by more than 50%. However, when co-inoculated with low or high levels of non-viable cells, viable cells of *P. flavescens* did not suppress disease. Instead, disease increased by >150% ( $P < 0.05$ , FPLSD) compared to the treatment with only viable biocontrol cells. Interestingly, the wheat heads treated with viable and non-viable *P. flavescens* cells displayed greater disease severity than heads inoculated with only the *F. graminearum* pathogen. When *F. graminearum* was grown in a liquid basal salts medium without carbon or nitrogen but supplemented with increasing levels of non-viable cells of antagonist strain OH 182.9 3C, the amount of pathogen biomass increased ( $P < 0.05$ ) for each sequentially higher level of non-viable cell amendment tested. Scanning electron microscopy indicated that the presence of non-viable *P. flavescens* cells modified the ectotrophic growth of *F. graminearum* on wheat lemma tissue *in situ*. Results indicate that non-viable antagonist cells present in a viable biocontrol cell treatment can reduce disease control efficacy or even cause the biocontrol treatment to produce a net adverse effect on plant health.

### **ATF-04 Co-inoculation of symbiotic rhizobia and free-living rhizobacteria (PGPR) have a role in enhancing growth in the legumes Lucerne (*Medicago sativa* L.) and Cowpea (*Vigna unguiculata* L.)**

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Small-scale farmers in South Africa suffer from poor crop yields, as they cannot purchase expensive inorganic fertilizer to obtain high yields in their staple crops. At times when inorganic fertilizers are available to small scale farmers or when excessively used by commercial farmers coupled with poor agronomic practices, the establishment and performance of root nodules is negatively affected ultimately resulting in yield reductions. In addition to this, farmers face substantial crop losses because of several other abiotic stresses in the soil. In South Africa abiotic stress caused by acidic soils in Kwazulu Natal and Eastern Cape provinces, salinity in the Sunday River Valley regions, poor soil nutrition status in the Eastern Cape and Limpopo province have been reported, resulting in yield loss of more than 30% in susceptible legumes including beans, lucerne, clover, and ground nuts. A novel strategy to alleviate this problem especially in legume production under abiotically stressed soils could be the use of consortiums of rhizobia and free-living rhizobacteria. In this study, *Sinorhizobium* and *Bradyrhizobium* strains that specifically nodulate lucerne (*Medicago sativum* L.) and cowpea (*Vigna unguiculata* L.) respectively were co-inoculated with two strains of free-living rhizobacteria, namely *Pseudomonas chlororaphis* and *Burkholderia* sp in a glasshouse pot experiment. Statistically significant improvements were observed in the number, size, color and dry weight of the nodules in the legumes inoculated with the consortium of rhizobia and PGPR as compared to those treated with the rhizobium alone. The co-inoculation also resulted in significant increase in plant biomass as well as chlorophyll content of both lucerne and cowpea. The study also detected beneficial mechanisms of action by the PGPR strains that include production of siderophores, indoleacetic acids, phosphate solubilization and ACC-deaminase production, which helped the rhizobia to establish an improved symbiotic performance. The screening of plant growth promoting rhizobacteria (PGPR) and their utilization as inoculants in consortium with the symbiotic rhizobia in legume production is at its early stage in South Africa. Thus, the findings of this study have valuable contributions in microbe assisted legume production for sustainable agroecosystems.

## **ATF-05 Shelf-life and storage of *Trichoderma asperellum* strain T34 applied as a seed treatment on experimental corn seeds.**

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Corn is a very important crop mainly for human consumption. Application of beneficial microorganisms to seeds is an efficient mechanism to allow the microbial inoculant to colonise roots and protect them against soil-borne diseases. However, there are several issues to solve before making available seed treatment inoculants. Particularly maintaining viable the microbial inoculant on the seed throughout commercial seed processes and further shelf-life.

The aim of this work is to evaluate the viability to *Trichoderma asperellum* strain T34 (Biocontrol Technologies, S.L.) after the seed treatment and along 1 year storage. We developed a basic formulation protocol with an inert adhesive that served us to apply the T34 in corn seeds at the higher concentration possible without compromising seed germination and/or vigor. Once we established the optimal concentration of the fungus, we performed several assays to check the viable T34 concentration through time (0, 2, 4, 7, 12 months), four experimental corn varieties, three different formulations and two different storage conditions: room temperature (20-30°C, 50-70% HR) and optimal seed conservation conditions. Once the seeds were treated with the formulation compounds, those were dried at room temperature and then stored. The measurements of T34 concentration on the seed were performed through ten-fold serial dilution method using autoclaved saline solution (NaCl 9gr/L) and Malt extract solid media.

Results show a high concentration of T34 in the seed at initial time (*time 0*) up to  $10^6$  cfu/seed and then at the end (1 year after treating the seed), the analyses show a viable T34 concentration of  $5 \times 10^4$ - $10^5$  cfu/seed depending on storage condition and corn variety. It is known that T34 is able to perform its beneficial actions at an optimal dose of  $10^4$  cfu/mL of substrate which is lower than the results obtained in this work. To sum up, these results show a promising industrial use of *T. asperellum* strain T34 as a high value seed treatment in corn.

**Microbe-assisted crop production, opportunities, challenges & needs**

**Vienna, Austria, 4- 7 December 2017**

***Phytobiomes Alliance***

**Chairs: Kellye Eversole & Angela Sessitsch**

## **PA-01 Building A Foundation to Combine Site-Specific Biological and Physical Data for Next-Generation Precision Agriculture**

### **Kellye Eversole**

International Phytobiomes Alliance, United States of America

To meet the demands of a global human population expected to exceed 9.6 billion by 2055, crop productivity in sustainable agricultural systems must improve considerably in the face of a steadily changing climate and increased biotic and abiotic stressors. While significant advancements have been made in characterizing genetic and genomic resources for crops, we have limited knowledge of plants in their environment, i.e., “phytobiomes” for agricultural systems. Phytobiomes are plants in a biome (a specific geophysical environment - e.g., soil, weather - and all macro- and micro-organisms – e.g., microbes, insects, other plants, and animals associated with the specific site). Phytobiomes research is a holistic, systems level approach that will empower predictive and prescriptive analytics for the most sustainable management practices for crops. To achieve a systems level approach, however, we must increase significantly our understanding of the interactions between the biological components of agriculturally relevant phytobiomes, particularly the interactions between plants, associated microbes, and the environment. With this increased knowledge, we can combine site-specific biological data with physical data and empower next-generation precision agriculture. The development of standards, reference materials, practices, and a whole genome-based classification system for plant associated microbes are essential for understanding these interactions and developing a platform for application. In 2016, an industry-academic consortium, the International Alliance for Phytobiomes Research ([www.phytobiomesalliance.org](http://www.phytobiomesalliance.org)), was established to accelerate phytobiomes research and build a framework that will enable the development of optimized, phytobiomes-based, site appropriate solutions for farmers, ranchers, and foresters. An overview of the phytobiomes concept as well as the priorities and activities of the Phytobiomes Alliance will be presented.

## **PA-02 Streptomyces as endophytes: understanding the mode of action of plant growth promotion under abiotic stress**

### **Slavica Djonovic**

R&D, Indigo Ag, United States of America

Indigo is a company dedicated to harnessing nature to help farmers sustainably feed the planet. The company utilizes beneficial microbes residing within plant tissues to improve crop health and productivity. Evidence is accumulating that microbes can alter plant phenotypic responses in a broad range of environmental stressors. In a recent study, we set out to elucidate what makes a group of beneficial microbes advantageous to plants. One such way to recognize beneficial microbes is to look at plant responses via “Omics” profiling. We tested several endophytes of the genus *Streptomyces* with different ranges of beneficial activity in soybean (conferring drought tolerance and yield improvements). To elucidate mode of action of these endophytes, we conducted several different types of studies including plant transcriptomics analysis, microbial community sequencing, microbial whole genome sequencing, and microbial secretome analyses. This study deepens our understanding of how this group of beneficial endophytic microbes can alter plant phenotypic responses to alleviate the effects of environmental stress on plant performance.

### **PA-03 Bacterial and fungal endophytes increase growth and yield of agricultural crops at heat and drought stressed field sites**

**Jim Germida**, Vladimir Vujanovic

Soil Science, University of Saskatchewan, Canada

Climate change is affecting crop production worldwide. Increased temperature and drought are major factors affecting plant growth. Plants respond to stress in many ways, but manipulation of the root microbiome may be a significant mechanism to deal with stress. Our previous research demonstrates that bacterial and fungal endophytes can be used as inoculants to promote growth and yield of cereals, oilseeds and legumes under heat and drought stress in greenhouse and field conditions. In some cases this is linked to impacts of inoculants on the root microbiome. In order to fully understand and exploit this phenomenon it is critical to characterize the root microbiome of crops under various soil and environmental conditions and determine how inoculants alter this second genome. This presentation will review progress on specific bacterial and fungal inoculants that help plants overcome stress and current research directed on characterizing and linking the root microbiome to plant phenotype.

### **PA-04 Harnessing plant understanding and microbiome functional diversity to stimulate plant yield production**

**Michael Ionescu**, Avital Adato, Michal Shores, Adi Etzioni, Guy Migdal, Hagai Karchi

Evogene Ltd., Israel

Recent progress in microbiome research provides directions for how beneficial microbials can be harnessed to improve plant productivity in a sustainable agriculture. Evogene embarked with AgBiologicals research and development, utilizing plant and microbiome understanding through its predictive biology computational platform to discover and develop biostimulants for cereals with a focus on corn. Key-hypothesis is that microbiomes have co-evolved with plants to potentiate plant genetic traits under abiotic and biotic fluctuating environments. The interaction includes plant-microbial recognition, plant colonization and functional complementation of plant lacking functions by microbials. To discover microbial treatments that complement plant required functions, we employed a reverse MOA approach by identifying first strains that improve plant genetic traits under moderate drought stress and later studying the mechanism. Thousand strains representing the culturable microbiome diversity of wild and domesticated cereals in Israel were sourced, prioritized and screened in greenhouse trials in corn and in *Brachypodium* model plant and further validated in the field resulting with dozen 'Hits' consistently improving a define list of selected target sub-traits and traits. The impact of the inoculants on traits varied with relation to the severity of the water treatment. In addition, different corn varieties differently affected by the same inoculants with evidences for inoculant-responsive and unresponsive varieties, raising several hypotheses including that the functional requirements of plants of different genetic backgrounds, is different. Direct and indirect effects of the top inoculants on a responsive variety were recorded by detailed phenotyping, gene expression and microbiome analyses, revealing distinguished mechanisms to modulate plant development during vegetative growth and reproductive stage. In general, two distinguished groups of plant responses were observed, one relates to plant defense and the other to plant biomass buildup and assimilate partitioning. Another observed effect was control of rhizosphere community by inoculants and enrichment of microbial functions that might contribute to the observed effect on plant. These results provide evidence for what we believe is the 'tip of the iceberg' of functional diversity, versatility and limitation of plant beneficial microbials. New findings and contemporary hypotheses will be discussed in the talk.

## PA-05 Root exudate chemistry and microbial substrate preferences drive rhizosphere microbial community assembly during plant development and under nutrient and water stress

Kateryna Zhalnina<sup>1</sup>, Joelle Schläpfer Sasse<sup>1</sup>, Jian Gao<sup>1</sup>, Eden Tepper-Fobes<sup>1</sup>, Katherine B. Louie<sup>1</sup>, Ulas Karaoz<sup>1</sup>, Dominique Loque<sup>2</sup>, Benjamin P. Bowen<sup>1</sup>, Mary K. Firestone<sup>3</sup>, Eoin L. Brodie<sup>1</sup>, Trent R. Northen<sup>1</sup>

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<sup>3</sup> University of California, Berkeley, California

Rhizosphere bacteria impact important processes during plant development. In 'return' plants release substantial quantities of soluble C into the soil surrounding its roots, attracting bacteria and other soil organisms. We suggest that exudate composition acts to select specific organisms beneficial to the plant, and, consequently, we expect that substrate specialization exists within the rhizosphere microbiome. Here we provide evidence that in the rhizosphere of grasses, specific metabolites are exuded that are preferentially used by select bacteria and this substrate specialization, together with the changing composition of root exudates, drives the observed successional patterns during plant development, and under nutrient and water stress conditions.

To investigate the relationship between exudates and rhizosphere bacteria we first examined exudate composition of hydroponically grown annual grass, *Avena barbata*, and perennial grass, *Panicum virgatum* (switchgrass), using mass spectrometry-based metabolomics (LC-MS). We analyzed exudation patterns at different developmental stages as well as under nutrient and water limitations.

Next, we used exudate-based microbial growth media to examine the exudate consumption and substrate preferences of a diverse collection of bacterial isolates from the rhizosphere of *Avena* and switchgrass. We assessed the ability of these isolates to consume exudate components by LC-MS. Substrate consumption preferences were related to genomic features, the successional patterns of bacteria in the rhizosphere, and to plant exudate composition at different developmental stages, and under nutrient limitation.

The major fraction of exudates for both grasses composed of amino- and carboxylic acids, sugars, nucleosides, mostly exuded during early plant development, while quaternary amines were exuded most at later developmental stages. Amino acids, sugars and nucleosides were consumed by all bacteria analyzed. However, bacteria that were preferentially stimulated by plant growth, revealed substrate utilization preferences towards aromatic organic acids.

We suggest that this substrate specialization of rhizosphere bacteria together with changing composition of root exudates during plant development, and under different stress conditions, combine to recruit beneficial bacteria from the surrounding soil in order to mitigate stress, improve nutrient availability and suppress plant pathogens.

**Microbe-assisted crop production, opportunities, challenges & needs**

**Vienna, Austria, 4- 7 December 2017**

*Future of microbial products & regulatory issues*

## **FR-01 The future of microbial products and regulatory issues**

### **Willem Ravensberg**

BU Microbials, Koppert Biological Systems, Netherlands

The use of microorganisms in agriculture is growing steadily. Agricultural biologicals based on microorganisms are applied as bio-insecticide, bio-fungicides, plant biostimulants and biofertilizers, often called microbial inoculants. New innovative products are continuously being developed, new companies are founded and more companies enter this field of biologicals in agriculture. Alongside the traditional crops in which these products have been used in the past, we see an expansion to many other crops including large agricultural crops like cereals, corn, soya. Seed treatment with biologicals is a rapidly growing market. The use of microbes in non-crop applications is also expanding, examples are seen in vector control, in animal and human pests, in forestry and amenity uses. All these types of inputs are regulated in some way, but the procedures and data requirements differ greatly between functions, authorities, countries and regions. Developments in the regulatory environment will be discussed and the activities in the ag-bio industry to improve legislation. The role of industry associations will be illustrated with examples such as the new criteria for low-risk active substances, an EPPO guidance for efficacy assessment of biopesticides, and the development of a new biostimulant regulation, all in the EU, and attempts to define and regulate biostimulants and biofertilizers in the USA and Brazil. Participation in discussions with authorities is crucial on many levels to make regulations appropriate for the type of products our innovative industry is involved in. Examples will be provided of collaborations with the IGO's such as OECD, FAO, EPPO, with authorities such as the EU Commission, the US EPA, and with farmer organisations such as Copa & Cogeca. BioProtection Global (BPG) was formed as the Global Federation of Biopesticide Associations to discuss global matters with authorities, to advocate harmonization and to promote biocontrol. The view on future regulation from industry will be presented. This should allow innovative products to reach the market in a predictable, affordable and timely manner being based on risk and not function. Modern agriculture is in need of new and additional tools for the production of healthy, more productive and sustainable crops.

**Microbe-assisted crop production, opportunities, challenges & needs**

**Vienna, Austria, 4- 7 December 2017**

***Closing Lecture***

## PL-02 Phenotypic response of soil microbiomes to environmental change

### Janet Jansson

Chief Scientist for Biology, Pacific Northwest National Laboratory, United States of America

Soil microbial communities (microbiomes) play several key ecosystem services, including cycling of carbon and other nutrients and support of plant growth. Despite their essential role for life on our planet, the specific roles carried out by individual members of soil microbiomes has been challenging to dissect, due to the high complexity and high microbial diversity of soil ecosystems. To meet this challenge we have been applying a suite of omics tools to uncover both the phylogenetic representation and the functional properties of soil microbes in complex soil microbiomes. Most of our studies are based on the analysis of soil metagenomes that subsequently serve as a scaffold for understanding gene expression data (metatranscriptomes and metaproteomes). Deep soil metagenome sequencing has now developed to the point that it is possible to bin near complete and complete genomes from soil microbial communities; the majority of which represent novel species that have never been isolated or studied in the laboratory. Here we used a multi-omics to study the impact of different perturbations (changes in nutrients, soil moisture and temperature) on native prairie and permafrost soils. The results reveal the functional response of active soil microorganisms to changes in their environment.

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# Poster Presentations

Poster Session 1: Successful microbial products

**Microbe-assisted crop production, opportunities, challenges & needs**

**Vienna, Austria, 4- 7 December 2017**

***Poster Session 1: Successful microbial products***

## **PP-SMP-01 PATBIOCON: Development of a biocontrol product effectively suppressing bacterial soft-rot on potato tubers in storage**

**Dorota M. Krzyzanowska<sup>1</sup>, Tomasz Maciag<sup>1</sup>, Joanna Siwinska<sup>2</sup>, Sylwia Jafra<sup>1</sup>, Robert Czajkowski<sup>2</sup>**

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Bacterial soft rot is a plant disease causing significant losses in the storage of vegetables worldwide. The affected plants include potato – an important staple food crop. Depending on the region, the disease is caused by different species belonging to *Pectobacterium* or *Dickeya* genera (formerly pectinolytic *Erwinia* spp.). The means to fight these pathogens are scarce.

The aim of the PATBIOCON project is to develop a prototype of a microbe-based biocontrol product, effective against soft rot during storage of potato tubers. The study began with twenty-two bacterial strains of different species, all known to show antagonism towards *Pectobacterium* and/or *Dickeya* isolates. The pool of strains was used to create multiple random mixtures comprising 4-5 microorganisms. Each mixture was screened for biocontrol potential on potato tubers, infected, *via* vacuum infiltration, with a blend of six species of soft-rot pathogens. Upon inoculation, the samples were incubated under disease-conducive conditions (high temperature, high humidity). In general, the efficiency of random mixtures was low. However, preparations showing some degree of protection were split into individual strains, and their active components were identified in assays on wounded tuber tissue. Five strains were selected (O16, O17, O19, O20, O22), showing up to 97% reduction of soft rot symptoms in tuber slices assay and up to 87% reduction in an assay where potatoes were spiked with pipette tips containing suspensions of pathogens and the antagonists. Finally, the biocontrol potential of the five strains was assessed on intact, vacuum-infiltrated tubers – a test more faithfully reflecting the real-life situation. Most promising results were obtained for a two-component inoculant comprising O16 and O20, providing 89% reduction of soft-rot symptoms.

Currently, methods for formulation of the five strains are developed, in an effort to obtain a prototype of a commercial product. Other qualities of the strains, important for their commercialization, are also investigated. Two patent applications were already filed concerning solutions developed within the PATBIOCON. One of them concerns the protocol for testing potential biocontrol agents on intact tubers, the development of which is an important outcome of the project.

This study was funded by grant no. LIDER/450/L-6/14/NCBR/2015 "PATBIOCON" from the National Centre for Research and Development, Poland (Principal Investigator: R. Czajkowski)

## **PP-SMP-02 Fungal endophytes from goatgrass enhance wheat growth and shield plants from sensing stress.**

**Or Sharon, Eugenio Llorens, Amir Sharon**

Institute for Cereal Crops Improvement, Tel Aviv University, Israel

Fungal endophytes live in plant tissues as asymptomatic mutualists. The roles of endophytes in plant ecosystems are unclear, however it is known that some species contribute to plants' health and sustainability. For these reasons endophytes are attractive targets for crop improvement. Host genetics and environmental conditions affect the diversity and composition of endophytes communities. Both of these factors have been reduced in crop plants due to plant domestication and intensive agriculture, possibly reducing endophytes diversity within crop plants compared to their wild ancestors. Accordingly, endophyte communities in wild plants may be more diverse, and may include beneficial species that are not found in related crop plants.

In a previous study, we compared fungal endophytes communities in wheat and related wild species. Here we report on the effect of two endophytes from Sharon goatgrass (*Aegilops sharonensis*), on development of bread wheat (*Triticum aestivum* L). Both fungi could infect bread wheat and affected plant growth and development. Most significantly, the endophytes from the wild grass promoted wheat growth and helped plants to better cope with environmental stresses. The developmental changes were associated with changes in physiological parameters, hinting to a possible modification of the stress response of plants by the endophytes.

## PP-SMP-03 Critical insights from a commercially successful product: major shifts in root microbiome of field-grown strawberries

Rajnish Khanna<sup>1</sup>, Siwen Deng<sup>2</sup>, Heidi Wipf<sup>2</sup>, Grady Pierroz<sup>2</sup>, Ted Raab<sup>3</sup>, Devin Coleman-Derr<sup>2</sup>

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A fermented liquid product composed of a broad spectrum of microbes, fermentation by-products and organic acids, VESTA® (SOBEC Corporation, Fowler CA), is a highly commercially successful biological product. VESTA has been sold for over 20 years and applied successfully on more than 80 different commercial crops grown on large fields located in different geographical regions and exposed to a range of climatic conditions. In 2016, over 20 million gallons of VESTA was sold in the U.S. (in four states, CA, WA, CO and AZ). VESTA is now commercially available in Europe, Middle East, Africa, India and Australia. It has been observed to increase plant growth, and decrease disease in crops, including strawberries grown in the central valley in CA. Strawberry production in CA averages 44,000 pounds per acre each season and accounts for nearly 88% of nation's strawberries. We tested the hypothesis that VESTA's beneficial properties may be correlated with a modulation of the strawberry root and rhizosphere microbiomes. The microbial communities in VESTA treated and untreated soil, rhizosphere, and root endosphere were profiled at four different time points across the strawberry field season (11 months) using 16S rRNA gene sequencing on the Illumina MiSeq platform. The microbiome composition shifted in all sample types in response to VESTA treatment, and importantly the root endosphere displayed the greatest change in community structure. A comparison between community structure in the VESTA product and the VESTA treated samples revealed little overlap between the organisms within VESTA and taxa enriched in plant and soil following VESTA treatment, suggesting instead that VESTA rewires existing networks of organisms through an as yet uncharacterized mechanism. Taken together, these results demonstrate that a commercial microbial soil amendment can affect the community structure of the plant root and surrounding environment. Further, we investigated the effect of VESTA on nutrient exchange between soil and strawberry plants. There was increased root growth, increased % water in roots over time, and % Nitrogen and % Carbon (per dry weight) in leaves. Our results indicate that shifts in microbiota from the biological amendment can influence nutrient flux between the soil and the plant possibly through microbial activity.

## PP-SMP-04 ATTRACT and KILL: Innovative wireworm control

Michael Przyklenk<sup>0</sup>, Elisa Beitzen-Heineke<sup>0</sup>, Wilhelm Beitzen-Heineke<sup>0</sup>, Stefan Vidal<sup>1</sup>, Anant Patel<sup>2</sup>

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Wireworms, the polyphagous soil-dwelling larvae of click beetles (Coleoptera: Elateridae), are a major insect pest of worldwide relevance causing tremendous yield losses in several crop production systems, like potatoes. Biological control of wireworms with entomopathogenic fungi is challenging because of the lower efficacy, difficult handling and limited shelf life of these organisms compared to synthetic pesticides. Until now, promising entomopathogenic fungi such as *Metarhizium brunneum* have been applied in simple formulation types, mostly on barley or rice kernels. In the projects ATTRACT and INBIOSOIL we developed novel mechanically stable beads containing CO<sub>2</sub> emitting baker's yeast as an attract component, an isolate of *M. brunneum* as a kill component and a substrate as a nutrient source and drying aid. This beads, commercialized as ATTRACAP®, can be applied in innovative attract and kill strategies based on solely biological components.

ATTRACAP® beads are produced by bioencapsulation of the microorganisms and the substrate in alginate and dried with an innovative fluidized-bed drying process. As a result the beads are storable for 6 months at low temperatures.

The beads emit CO<sub>2</sub> after contact with soil humidity over a period of 5 to 6 weeks to attract the wireworms, where they will then be infected with *M. brunneum*, which grows out of the beads. The attract and kill formulation allows a lower application dose of 30 kg/ha with an active substance of 4,8 x10<sup>11</sup> conidia/ha, thus making the product cost-effective and eco-friendly. For 2016 an efficacy of 40-60% was reached in several field trials. In 2017, ATTRACAP® obtained for the second time the emergency registration for 7000 ha in Germany. In a recently granted BMEL project (Acronym: ATTRACAP) further work will focus on the improvement of the efficacy of ATTRACAP®.

To conclude, this novel CO<sub>2</sub>-releasing bead system is not only suitable for cost-effective delivery of low doses of fungal biological control agents to the soil, but this technology also can be transferred to other pest problems.

**Microbe-assisted crop production, opportunities, challenges & needs**

**Vienna, Austria, 4- 7 December 2017**

**Poster Session 1: Microorganisms for rural development**

**PP-MRD-01 *Pseudomonas arachidis* sp. nov. and *P. indiaensis* sp. nov. isolated from the rhizosphere and geocarposphere of *Arachis hypogaea* show biocontrol activity against the phytopathogen *Aspergillus flavus***

**Sandra Matthijs<sup>1</sup>**, Vanamala Anjaiah<sup>2</sup>, Nathalie Brandt<sup>1</sup>, Pierre Van Antwerpen<sup>3</sup>, Marc Ongena<sup>4</sup>, Christophe Flahaut<sup>5</sup>, Mickaël Chevalier<sup>5</sup>, Philippe Jacques<sup>6</sup>, Nico Koedam<sup>2</sup>

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*Arachis hypogaea* L., commonly known as groundnut or peanut, is a major oilseed crop in the world. In addition, groundnut seeds are eaten raw, boiled or roasted, and made into confectionery, snack foods or peanut butter, and are added to soups, cakes and other dishes. The crop is widely grown in tropical and subtropical regions and is important to the economies of many developing countries. After the crop harvest, haulm and the oil cake is used for animal feed. A major problem in peanut production worldwide is the contamination with *Aspergillus flavus* and aflatoxins. Aflatoxins produced by *A. flavus* have many toxicological effects, they are hepatotoxic, teratogenic, carcinogenic and immunosuppressive substances for many animal species. Aflatoxin can be transmitted through the food chain. Among several forms of aflatoxin, aflatoxin B<sub>1</sub> is more common in food and feed. Infection of *A. flavus* can occur before harvest, at harvest, and at post-harvest during drying and storage. Aflatoxin contamination in groundnut may not affect productivity of the crop, but it makes the product unfit for consumption.

The aim of this study was to identify and characterize bacterial strains with biocontrol properties against *A. flavus*. Therefore strains were isolated from the rhizosphere and geocarposphere (pod-zone) of groundnut from a field in India and tested for *in vitro* antagonism against *A. flavus* in a dual-culture plate assay. Strains showing a clear antagonism against *Aspergillus* were selected and finally four strains were chosen for further study, including two *Pseudomonas*, a *Bacillus* and a *Burkholderia* strain. In pot culture experiments (greenhouse) inoculation of these strains resulted in a significant reduction of *A. flavus* population in the geocarposphere of groundnut.

The genome of the two *Pseudomonas* strains has been sequenced and through *in silico* analysis and mass analysis compounds, which could potentially play a role in the interaction with *Aspergillus*, are currently being identified. Since the taxonomic position of both *Pseudomonas* strains was not clear this was studied through phenotypic and physiological characterization, ANI, GGDC, DNA-DNA hybridization, MLSA, determination of G + C content and chemotaxonomic analysis. This revealed that both strains represent novel *Pseudomonas* species for which the name *P. indiaensis* LMG 30116<sup>T</sup> and *P. arachidis* LMG 29990<sup>T</sup> is proposed, referring to their geographic and biological origin of isolation.

## PP-MRD-02 Beneficial microorganisms against apple and pear diseases in rural organic orchards

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The Kyrgyz Republic has significant potential for the cultivation of fruit crops.

Fire blight and scab are the most harmful diseases of cultivated varieties of apple and pear in Kyrgyzstan, and they affect leaves, stems, flowers and fruits of trees and resulting in the loss of 30-40% of the crop. Fire blight is difficult to control, as it is able to rapidly spread in the plant and effective control methods are still lacking. Suppression of the blossom-blight and shoot - blight phases of fire blight is a key point in the management. To control scab disease up to 20 treatments are applied per season by chemical fungicides.

There is a need for conservation of the environment, rural development and the standard life style of the rural population and the successful implementation of organic production. Alternative approaches to chemical use are highly appreciated due to the increasing importance of integrated management strategies relying on biocontrol.

The aim of this study was to develop environmentally friendly local microbial products and control measures against these destructive and increasingly significant diseases in apple and pear trees.

The results revealed the ability of *Streptomyces bio product* to decrease fire blight severity on pear and apple trees during the first stage of the fire blight disease. This product suppressed *E. amylovora* disease symptoms in the leaf tissues and excised apple and pear shoots. The incidence of fire blight on leaves and shoots was reduced by about 85- 92, 3 % with two applications. Also this antagonistic bio product caused a significant reduction of *V. inaequalis* (scab causative pathogen) on apple leaves, efficacies of this antagonist varied between 79-92% after two applications in organic orchards. The general conclusion of this study is that alone *Streptomyces bio product* used three times could replace of copper and could be developed as an alternative strategy in organic orchards.

**Key words:** apple and pear diseases, *Streptomyces bio product*, rural organic orchards

## PP-MRD-03 Extension of the elusive genus *Piriformospora* with plant growth-promoting endophytic fungi from Congolese rhizospheric soils

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In the last decade, there has been an increasing focus on the implementation of plant growth-promoting (PGP) organisms as a sustainable option to compensate for poor soil fertility conditions in developing countries. In an effort to obtain PGP fungi to apply in an integrated soil fertility management approach (ISFM) in the Democratic Republic of Congo, we discovered that rhizospheric soils collected in the region of Kisangani are a promising source of fungi of the elusive basidiomycetous genus *Piriformospora* (recently transferred to the genus *Serendipita*). Different environmental molecular analyses have shown the ubiquitous presence of fungi of the order Sebaciales, including *Piriformospora*, within the roots of terrestrial plants worldwide. Nevertheless, to date, the genus only comprises single isolates of both species *P. indica* and *P. williamsii*, possibly suggesting difficulties with direct isolation from field samples. Since its discovery in 1998, *P. indica* has attracted great attention due to its stimulating effect on plant growth and yield, and its capacity to confer systemic resistance against (a)biotic stress, which resulted in a strong interest for the implementation of the fungus as biofertilizer, bioprotector, and bioregulator.

With sudangrass-based trap systems, we managed to obtain a collection of 51 new axenic *Piriformospora* cultures which could be divided in seven closely related 'genetic groups'. Based on morphological data, inter simple sequence repeat (ISSR) fingerprinting profiles, and marker gene sequences, we propose that these isolates together with *P. williamsii* constitute a species complex designated *Piriformospora* (= *Serendipita*) '*williamsii*'. Furthermore, a selection of isolates strongly promoted plant growth of *in vitro*-inoculated *Arabidopsis* seedlings, which was evidenced by an increase in shoot fresh weight and number of lateral roots of up to 40% and 86%, respectively. With this collection of new *Piriformospora* isolates at hand, exciting challenges for future research will be to determine their exact taxonomic position, to unravel the mode of action behind their PGP capacity, and to assess their performance under adverse conditions. Moreover, the frequently observed coexistence with arbuscular mycorrhizal fungi (AMF) might offer interesting opportunities to develop novel inocula.

## PP-MRD-04 Inoculation of *Beauveria bassiana* into sugarcane plantlets (*Saccharum* spp.) for endophytic control of *Eldana saccharina*

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In South Africa, sugarcane (*Saccharum* Linnaeus spp. hybrids; Poaceae) agriculture suffers significant economic losses due to the stem borer *Eldana saccharina* Walker (Lepidoptera: Pyralidae). Entomopathogenic fungi such as *Beauveria bassiana* (Bals.-Criv.) Vuill (Ascomycotina, Clavicipitacea) are able to endophytically colonize plant tissues and enhance plant defense against insect herbivore feeding. The presence of this fungus in the plant suggests a promising approach to control, already shown in maize crops. Documented reports of this fungus as an endophyte in sugarcane plants for biological control are scarce. This study therefore assessed the endophytic interaction of two *B. bassiana* strains (*TL-leaf* and *N41SITI*) with sugarcane plants (var. N41) under greenhouse conditions. *Beauveria bassiana* conidial suspensions  $10^7$  conidia/ml were inoculated into sugarcane using two different inoculation techniques: sett dip and foliar spray. Hot water treated sugarcane setts were dipped in conidial suspension for 12 hours and planted in pots. For foliar sprays, setts were planted in pots and allowed to germinate and acclimatize; thereafter 5ml of  $10^7$  conidia/ml *B. bassiana* suspensions were sprayed on leaves and stems of sugarcane plants. Endophytic colonization was assessed three months post inoculation using molecular techniques; plant response in terms of plant growth (fresh weight and height) was also analysed. Plants inoculated with *B. bassiana* strains weighed more; *N41SITI* ( $12.20\text{g} \pm 0.63$ ) and *TL-leaf* ( $10.57\text{g} \pm 2.01$ ) compared to control plants ( $9.05\text{g} \pm 0.66$ ) treated with 0.1% Tween80. Furthermore plants inoculated with *B. bassiana* strains were taller; *N41SITI* ( $151.2\text{ m} \pm 6.33$ ) and *TL-leaf* ( $132.3\text{ m} \pm 2.67$ ) when compared to control plants ( $129.0\text{m} \pm 4.8$ ). In addition, plants inoculated with *B. bassiana* strain *N41SITI* were seen to weigh more and grew taller than those inoculated with strain *TL-leaf*. Molecular techniques (qPCR) will be used to quantify the level of colonization of *B. bassiana* in the plants. These experiments provide preliminary results on the inoculation of *B. bassiana* into sugarcane plants for endophyte colonization.

## PP-MRD-05 Dynamics and characterization of the endophytic community of *Melia azedarach* trees infected with phytoplasmas

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We studied *Melia azedarach* endophytic community considering the effect of phytoplasma infection, plant organ and sampling season on a two year-survey, and analyzed in vitro plant growth promoting activities.

403 isolates were characterized by Rep-PCR and 16S rDNA. Community diversity was affected by organ source, but not by season or infection status. Diversity was higher in root samples, being the most represented orders Bacillales (31%), Burkholderiales (30%), Rhizobiales (17%) and Pseudomonadales (13%). Enterobacteriales were present only in leaf samples, and Burkholderiales only in root ones. Within root isolates, the proportion of Burkholderiales and Rhizobiales was higher during the autumn season while Bacillales and Enterobacteriales increased during the spring. Leaf community was affected by phytoplasma infection since Bacillales and Enterobacteriales were found in higher proportion in infected leaves.

N fixation, P solubilization, indol acetic acid (IAA) production, ACC deaminase activity, siderophore and antibiotic production were assayed. *Bacillus* and *Burkholderia* isolates had the highest functional diversity. Isolates from *Bacillus*, *Pseudomonas*, *Enterobacter*, *Burkholderia* and *Paenibacillus* grew on Nfb medium and were positive for *nifH* gene PCR amplification. 97% of the isolates had P solubilizing activity, being *Pseudomonas* and *Kosakonia* highly efficient. 50 isolates showed IAA production, 3 from *Enterobacter*, *Pseudomonas* and *Bacillus* produced twice the reference strain *Azospirillum brasiliensis*. 38 isolates produced siderophores and 16 had acc deaminase activity. In both activities, most of the positive isolates corresponded to order Burkholderiales and showed considerably higher production than the control strain (*Pseudomonas putida*). 15 of the 23 *Pseudomonas* isolates were PCR positive for genes (*phl*, *prn*, *phz*, and *plt*) related to antibiosis activity while only one *Bacillus* isolate showed amplification of *zma* gene (zwitermycin).

In conclusion, *M. azedarach* root and leaf endophytic communities are different in structure, diversity and composition as well as in their response to phytoplasma infection and seasonal conditions. Besides, they have high PGPB potential as reflected by their diverse and efficient functional activities. Selected isolates of *Burkholderia*, *Bacillus* and *Pseudomonas* are being tested now for their growth promoting activities after plantlet inoculation.

## PP-MRD-06 Isolation and functional characterization of fluorescent pseudomonads from drought prone regions of hamirpur for plant growth promoting traits

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Water availability in adequate quantities is very crucial for proper growth and development of crop plants. Hamirpur is a district of Uttar Pradesh in India which has been reported to experience situations of drought due to low precipitation, causing severe crop loss and a socioeconomic impact on farmers and local residents. Rhizospheric microorganisms especially fluorescent pseudomonads have potential to sustain crop growth and development even under limited supply of water. The present work was carried out with an objective to isolate and screen rhizobacterial populations with special reference to fluorescent pseudomonades from different locations of Hamirpur and functionally evaluate their plant growth promotion capabilities. A total of 32 isolates were obtained from rhizospheric soil collected from 5 different locations of Hamirpur having various crop cultivated. Following the screening of these 32 isolates on King's B agar media 9 possible fluorescent pseudomonad isolates were obtained. Amongst these 9 isolates based on the biochemical tests 8 were identified to belong to genera *Pseudomonas*. When these 8 isolates were tested for indole-3-acetic acid (IAA) production using L-tryptophan all were found positive. The amount of IAA produced ranged from 21 mg/L to 148 mg/L and the isolate HS4-7 was observed to produce the maximum amount of IAA (148 mg/L). These isolates were also tested for their *in-vitro* P-solubilisation potential using NBRIP broth containing tri calcium phosphate as the sole source of phosphorus. From the results obtained it was observed that the amount of phosphate solubilised ranged from 3.54 mg/L to 8.23 mg/L and the isolate HS5-6 exhibited maximum amount of phosphate solubilisation (8.23 mg/L). Qualitative testing for salinity tolerance by these isolates was also carried out on Pikovskaya agar media supplemented with bromocresol purple and 4 levels of NaCl (250, 500, 750 and 1000 mM) respectively. The results obtained exhibited proper growth and organic acid production by all the isolates on all 4 levels of salinity tested. This observation put forward the simultaneous ability of phosphate solubilisation and salinity tolerance by the isolates. Overall these observations suggest that these fluorescent pseudomonad isolates may serve as promising inoculants for growth promotion of crop plants in the soils of Hamirpur.

## PT-MRD-08 Exploring plant endophytic *Streptomyces* for their use in sustainable agriculture

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Streptomycetes, together with other bacterial species such as *Bacillus* spp. and *Pseudomonas* spp., have been widely studied as valuable sources of strains able to promote plant growth and control plant pathogens in agriculture. *Streptomyces* spp. are fundamental in pharmaceutical industry, however, their practical use in agriculture as plant growth promoting bacteria (PGPB) and biocontrol agents (BCAs) is still in its infancy.

Based on the *in vitro* results, the most promising *Streptomyces* strains were tested in controlled conditions to evaluate their effect on germination and plant growth of various horticultural crops, including monocots, i.e. maize, wheat, and rice; and dicots, i.e. lettuce, lamb lettuce, rocket, savoy cabbage, soybean, onion, and tomato. The strains showed species-specific promotion of seed germination and plant growth. These effects were dose-dependent, however, higher inoculum dose not always resulted in better performance of the strain. The biocontrol potential of *Streptomyces* strains was tested on the pathosystem *Lactuca sativa* – *S. sclerotiorum*. The most active strains were able to reduce the disease incidence by ca. 50%. The disease risk was reduced when the strains were applied to the *S. sclerotiorum*-infected soil one week prior to lettuce sowing.

Our results confirm that *Streptomyces* have a great potential to act as BCA and PGPB, although, to be able to obtain their optimal performance, the inoculum dose, timing and the application method of individual strains need to be fine-tuned for specific crops.

## **PT-MRD-09 Assessing cadmium-tolerant endophytic bacteria as an strategy to bio-remediate cadmium presence in Cacao crops from Colombia**

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Cadmium is a heavy metal that, in high concentrations, might be toxic to all living organisms. It has been found that in some South American soils cultured with Cacao, cadmium could reach elevated concentrations. Although one of the strategies to deal with high cadmium concentration that migrate from soil to the Cacao grains is to explore the endophytic populations of the so-called cadmium-tolerant bacteria (E-CdtB), very few is known regarding this functional group of bacteria. Therefore, our goal in this study was to isolate and identify E-CdtB and characterize their metabolic capacity to immobilize cadmium using molecular and calorimetric techniques. We have set a pool of 45 E-CdtB isolated from 23 varieties of Cacao represented in 7 genera. The strains *Bacillus* sp. ECdtB1, 2, 3, *Herbaspirillum* sp. ECdtB14 and *Ralstonia* sp. ECdtB5 were selected due the amplification of *cad* and *smt* genes, related with cadmium efflux and its chelation in the bacterial cytoplasm. The strains were also selected due their higher immobilization ratios of Cd<sup>+2</sup> (1.04, 1.08, 1.07, 1.2 and 1.9 mg l<sup>-1</sup> of Cd in 12 days, respectively) in Batch experiments. On the other hand, the strain ECdtB2 has shown a major activity during the immobilization process and minor biomass production (1050 J s<sup>-1</sup> and 0.8 OD<sub>600nm</sub>, respectively), whereas the strains ECdtB3 and 5 have lower activity and higher biomass production (both 580, 405 J s<sup>-1</sup> and 1.4, 1.1 OD<sub>600nm</sub>, respectively). Therefore, the strain *Bacillus* sp. ECdtB2 has been selected for a further greenhouse experiment in order to determine endophytic growth in rootstocks and its immobilization ratio and maximum metabolic capability under controlled conditions.

**Microbe-assisted crop production, opportunities, challenges & needs**

**Vienna, Austria, 4- 7 December 2017**

***Poster Session 1: New mechanisms involved in beneficial plant-microbe interactions***

## PP-NMI-01 Fragrant endophytes: VOC-producing endophytes and their potential for sustainable agriculture

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Bacterial endophytes often show plant growth promoting activities. They can produce phytohormones, make nutrients available and induce systemic resistance for their plant hosts. In addition, they are producer of bioactive metabolites including volatile organic compounds (VOCs), which can show antifungal and antibacterial activities. Until now the structure of approximately 1000 VOCs of bacterial origin was described; they can be responsible for communication and numerous interactions between plants, antagonists and microbes. However, a better understanding about the mode of action of VOCs and their beneficial effects on plant growth and plant health is necessary.

*Fusarium oxysporum* (causal agent of tomato wilt) is one of the most problematic plant pathogen for cultivation of tomato in greenhouses and fields. The infection with this pathogen is a latent infection and it stays unperceived in the first stages of development. *Fusarium oxysporum* infects the plant starting from the root and migrate then to the stem. The pathogen grows in the xylem and through that the plant seals the xylem as reaction to the fungus infection.

Our study focused on the analysis of the role of VOCs produced by endophytic bacteria in tomato on plant performance under biotic and abiotic stress. Therefore, a plant pot experiment with *Fusarium oxysporum* infected tomato cultivar 'Hildares' accompanied with salinity and drought stress was performed. In total, eight different treatments were conducted. From each of the eight different treatments bacterial endophytes were isolated, and a total of 804 isolates were preserved. An analysis by means of two clamp VOCs assay has identified bacterial endophytes, which produce VOCs and an abundance of plant growth promotion activities like indol-3-acetic acid, siderophores, exoenzymes (chitinase, phosphatase). It was found that a proportion of 15% of the promising candidates produce bioactive volatiles against the plant pathogen *Fusarium oxysporum*; 53% produce the phytohormone indol-3-acetic acid, 86% produce siderophores, 33% are able to solubilise organic phosphate and 76% are salt and drought tolerant. No significant difference was observed in the amount of volatile-producing endophytes among the various treatments. Altogether, several promising biocontrol agents towards *Fusarium* wilt and abiotic stress were selected and will be further evaluated under field conditions.

## PP-NMI-02 Disease induced recruitment of beneficial rhizobacteria

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Up to  $10^{11}$  microbial cells, belonging to thousands of bacterial and archaeal species, per gram of root are present in the rhizosphere, constituting the root microbiome. These microbes all interact with each other and with the plant. Root associated microbes can benefit plant health by inhibiting growth and activity of pathogens through competition for nutrients, antibiosis, or by eliciting induced systemic resistance. Interactions between pathogens and beneficial rhizobacteria have been described in detail for disease suppressive soils. In general a severe disease outbreak is required for disease suppressiveness to develop. Thus we postulate the hypothesis that plants cry out for help when under pathogen attack. In my PhD research I will test this hypothesis that plant beneficial bacteria can be actively recruited by plants under pathogen attack. I will study the mechanisms by which the plant can recruit specific bacteria in the rhizosphere. Moreover the mode of action of the beneficial bacteria to suppress plant diseases will be studied.

**PP-NMI-03 The capacity to release phosphorus from different soil compounds by bacteria: an alternative to increase crop production and reduce phosphate fertilization**

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Phosphorus (P) plays a fundamental role in the physiology and biochemistry of all living cells. Recent evidence indicates that organisms in the oceans can break down and use P forms in different oxidation states (e.g., +5, +3, +1, and -3); however, information is lacking for organisms from soil and sediment. We selected two different ecosystems: arid grassland (north of Mexico) and oak temperate forest (central Mexico) with different soil P limitation, providing a great opportunity to assess the various strategies that bacteria from soil and sediment use to obtain P. We measured the activities in sediment and soil of different exoenzymes involved in P recycling and evaluated 1,500 bacterial isolates (mainly *Bacillus* spp.) for their ability to use six different P substrates. Additionally, we conducted the soil P fractionation and related the bacterial capacity to use different P substrates with the most dominant P fraction in soil. In arid grassland, DNA turned out to be a preferred substrate, and in oak forest Iron Phosphate was the preferred substrate comparable to a more bioavailable P source, potassium phosphate. Phosphodiesterase and phosphomonoesterases activity, required for ester degradation, was observed consistently in the sampled-soil and sediment communities. A capability to use phosphite ( $\text{PO}_3^{3-}$ ) was observed mainly in sediment isolates, and calcium phosphate was used mainly by oak forest and arid sediment isolates. Phosphonates were used at a lower frequency by both soil and sediment isolates in arid grassland and oak forest, and phosphonate activity was detected only in soil communities. Our results revealed that soil and sediment bacteria are able to break down and use P forms in different oxidation states and contribute to ecosystem P cycling. Different strategies for P utilization were distributed between and within the different taxonomic lineages analyzed, suggesting a dynamic movement of P utilization traits among bacteria in microbial communities. Ongoing, the capacity of different microbial consortia to increase soil P availability, and plant growth are analyzed as an alternative to increase ecosystem P recycle and to reduce the use of phosphate fertilizers.

**PP-NMI-04 The soil and plant microbiome associated with rose replant disease**

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A soil-borne bacterial and fungal complex has been implicated as causative agent in rose replant disease, also referred to as 'soil sickness'. However, the specific microbial, plant genetic and environmental factors that are conducive to or antagonizing rose replant disease are not yet fully understood.

The present study explores soil and plant-associated bacteria and fungi with potential causative or antagonistic roles in rose replant disease. We analyzed the rhizosphere and endophytic bacterial and fungal communities of roses strongly affected by rose replant disease and compared them with those in less affected soils and soils not previously used for growing roses. Microbial community analysis was done by Illumina-based next-generation sequencing of bacterial 16S rRNA and fungal ITS genes as phylogenetic markers. Concomitantly, we studied the plant transcriptome response to the various replant disease conditions.

Analysis of variance of abundance data and estimation of variance-mean dependences revealed significant differences of microbiomes in "healthy" versus affected soils. In disease-affected soils, we found higher abundances of specific taxa, among others in the *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Chloroflexi* bacterial groups. Characterizing the etiology of the disease and establishing relationships with the associated microbial communities shall help elucidate the underlying causes of low performance of ornamental roses growing on 'diseased' soil.

## PP-NMI-05 Fight fungi with fungi: endophytes as biocontrol agents on wheat

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Wheat is the most important crop in European agriculture. However, its high-yield production is strongly dependent on the use of pesticides. Thus, diseases like Fusarium Head Blight (FHB) (*Fusarium* spp.), require new control alternatives since traditional chemical methods are not efficient. Currently, there is an increased focus on the use of biological control in disease management and the use of endophytic microorganisms is an interesting option. Endophytes are microorganisms that colonize plant tissues internally without causing any visible symptoms. They can provide benefits such as stress tolerance, enhanced growth and nutrition, and disease resistance.

We have isolated several fungal endophytes from healthy wheat spikes in Swedish and Danish fields where FHB is present. We hypothesize that these organisms can work as biocontrol agents with potential for use in large-scale agriculture. In order to test the biocontrol properties of these endophytes, we have examined their behaviour against different *Fusarium* species associated with FHB.

Dual cultures test showed that several endophytes significantly reduced the growth of *Fusarium graminearum* in *in vitro* tests. Additionally, *in planta* tests were performed on spring wheat seedlings in order to estimate the effect of endophytic isolates on Fusarium root rot, caused by *Fusarium culmorum*. Likewise, spring wheat spikes were inoculated at flowering stage in controlled growth facilities with different endophytes to evaluate their performance in reducing FHB symptoms. Furthermore, field trials have been established on winter wheat plots during the 2016/2017 season in Denmark, spraying endophytes directly to spikes at the early anthesis stage. Disease and agronomical parameters will be measured as data will be collected at the end of the season.

A complete screening of these endophytic fungi will be performed, allowing the determination of their aptitude in reducing FHB in wheat and their potential use as biocontrol agents in European agriculture. Additionally, substantial knowledge about endophyte interactions with hosts and pathogens will be generated as we explore the possibility of using endophytic organisms as a source of environmentally friendly disease control organisms.

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## PP-NMI-06 Sucrose metabolism in *S. indica*-nematode-plant interaction

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The beneficial root endophyte *Serendipita indica* (former *Piriformospora indica*) is an orchid mycorrhiza, which forms mutualistic relationships with many different plants including the model plant *Arabidopsis thaliana*. During this interaction, the endophyte promotes host plant growth, biomass and seed production. Further, the resistance to abiotic (e.g. drought, salt stress, toxins and heavy metals) and biotic stresses (pathogenic organisms) is increased. It can be speculated that, similar to arbuscular mycorrhizal fungi, *S. indica* receives carbohydrates from the host in exchange for this service. To test this, qRT-PCR of several sucrose synthase (SUS) and invertase (INV) genes will be performed to reveal changes in their expression during different phases of plant-fungus interaction. Further, *A. thaliana* SUS and INV single and multiple mutant lines will be tested to verify the importance of these sucrose-modifying enzymes for successful colonization. Invertase activity assays will additionally shed light on the changes in enzyme activity triggered by *S. indica* in plant tissue. In contrast to the fungus, which most likely prefers simple sugars, the plant-parasitic nematodes favour unprocessed sugar. Hence, the impact of *S. indica* on nematodes will be tested. Nematode attraction and development assays will be carried out to assess the influence of *S. indica* on the attractiveness and development of cyst nematode *Heterodera schachtii*. This work will significantly increase our knowledge on *S. indica*-nematode-plant interaction. Further, obtained results could greatly contribute to a development of new agricultural strategies against plant-parasitic nematodes, in which *S. indica* is utilized as a biological agent controlling these important root parasites.

## PP-NMI-07 Green challenges: improving resilience of horticultural systems using concepts from soil microbial ecology

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The project Green Challenges aims to substantially reduce the use of chemical plant protection products by focussing on a number of innovations. One of these innovations is to use and stimulate the natural resilience of the plant- and soil system. In this study, we investigate the effect of natural products that can positively affect plant- and soil ecological functioning via different mechanisms:

1. Direct disease suppression by introducing antagonistic bacteria. The micro-organisms can produce enzymes, toxic volatiles or antibiotics that can suppress the growth of the pathogen. It is, however, challenging to let these bacteria colonize a new substrate. Competition between inoculant and indigenous microbial populations for available substrate and space can adversely affect the colonization and activity of inoculants.
2. Increase the competition between bacteria and fungi in the rhizosphere by introducing organic materials such as chompost and compost. Growth of bacteria with anti-fungal properties can increase as a response to the increase in growth of saprotrophic fungal hyphae in the rhizosphere, thereby reducing growth of fungal pathogens. The advantage of this mechanism is that indigenous micro-organisms are stimulated and no microbial inoculants are needed.
3. Increasing the general microbial activity in soils by adding organic materials. Fungal pathogens are in general weak competitors for organic substrate compared to saprotrophic micro-organisms. This makes it more difficult for a pathogen to colonize and grow.
4. Increasing the natural resistance of plants. Systemic Acquired Resistance (SAR) and Induced Systemic Resistance (ISR) can be induced by non-pathogenic micro-organisms, such as mycorrhizal fungi and rhizobacteria.

In autumn 2017, three different greenhouse crops (Kalanchoe, pot tomato, lisianthus) will be treated with natural products (microbial inocula, different organic materials, combinations ) and infected with different economically important fungal pathogens (*Phytophthora infestans*, *P. nicotianae*, *Fusarium oxysporum*, *F. solani*) in greenhouse experiments. Development of diseases and crop growth and resistance will be monitored over time to determine which natural product seems the most promising.

## PP-NMI-08 The role of nitrate reductase in interaction between *Arabidopsis thaliana* and root endophyte *Serendipita indica*

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Nitrogen is frequently the most limiting nutrient in plant growth and development. It is recruited by plants either as nitrate or ammonium or via nitrogen fixation with the help of rhizobia. Fungal endophytes (e.g. mycorrhiza fungi) also play an important role in delivering either nitrate or ammonium to the root cells. In *Arabidopsis thaliana* *NIA1* and *NIA2* genes encode the nitrate reductase (NR), a key enzyme in nitrate assimilation pathway, which reduces nitrate into toxic nitrite. Subsequently nitrite is further reduced to ammonia. *Serendipita indica* is an endophytic fungus from the order Sebaciales, which colonizes many monocots and dicots triggering plant growth promotion by enhancing, among others, plant nitrogen uptake. Thus, the aim of this study was to find out whether NR plays a role in the interaction between *S. indica* and *A. thaliana*. To do that, we used single *nia1* and *nia2* and the double *nia1nia2* mutants as well as *mpk6* line as the phosphorylation of *NIA2* by *MPK6* leads to an increase in NR activity. First we analyzed the expression of *Nia1* and *Nia2* in colonized roots of all mutant lines. Further, we checked the colonization rate of *S. indica* in roots of these plants. In addition, we analyzed changes triggered by the fungus in regard to growth such as fresh and dry weight, root length und the number of root tips. Finally, we performed the nitrate reductase assays with the shoot material at different time points corresponding to different colonization phases of the fungus. Our results demonstrate an important role of nitrate reductase especially at early stages of *S. indica* root colonization in interaction with *A. thaliana*.

## **PP-NMI-09 The role of the *aiiO* gene in the metabolism of *Ochrobactrum* sp. A44 and the ability of this bacterium to colonize the potato rhizosphere**

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Bacteria are able to sense changes in the environment and respond by modifying gene expression. Most signals reaching the bacterial cells are the direct environmental cues, providing information on the biotic and abiotic characteristics of the surrounding environment. The gene expression may be also regulated *via* quorum sensing (QS), a cell-to-cell communication system based on dedicated signal molecules. It is well established that many bacterial species use QS mechanism to regulate a broad spectrum of activities. One of the best studied QS systems is based on *N*-acyl homoserine lactones (AHLs) as signal molecules. Interference with QS, known as the quorum quenching (QQ), can be achieved i.a. *via* enzymatic inactivation of signal molecules. Numerous QQ-capable microorganisms have been described. However, despite extensive research on QS and QQ, the *in vivo* role of AHL-inactivating enzymes is not clear. It is not even certain whether the inactivation of AHLs is a "primary" function of the known QQ enzymes or only their minor side activity.

*Ochrobactrum* sp. A44, a bacterial strain obtained from the rhizosphere of potato, is able to inactivate AHLs due to the activity of the AiiO hydrolase. To elucidate the role of the *aiiO* gene in the biology of A44, two main research hypotheses have been stated: i) the role of *aiiO* in the metabolism of A44 is not limited to the inactivation of AHL-type signal molecules, and ii) the *aiiO* gene is important for efficient colonization of potato rhizosphere by A44. Therefore, the main aim of this study was to elucidate the function of the *aiiO* gene in the metabolism of *Ochrobactrum* sp. A44, as well as to assess the general contribution of this gene to the environmental fitness of A44. Elucidation of the *aiiO* role in the ability of A44 to colonize its native environment, the potato rhizosphere, shed a light on the function of QQ in the environmental fitness of QQ-capable microorganisms.

## **PP-NMI-10 Improving the shelf life of rhizobia inoculants: a pangenomics approach investigating stress-resistance in *Sinorhizobium meliloti***

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Rhizobia are gram-negative soil bacteria that can establish a symbiosis with plants from the family of *Leguminosae*. In structures in the plant root, called nodules, they fix atmospheric nitrogen which serves as a nitrogen source for the plant. Due to this beneficial effect on the plant, the interest in applying rhizobia in agriculture is high. Because of a low amount of rhizobia in natural populations and an overall low nitrogen fixing capacity, enough nitrogen supply can't be guaranteed. Therefore inoculation on seeds or directly in the soil is needed to have enough effective rhizobia strains in the rhizosphere. However loss by abiotic stress and highly competitive natural strains in the soil limit the efficiency of the inoculants. A deep understanding about genes that are important for survival under stress conditions is needed to select or create strains that are suitable for inoculants.

Phenotypic assays that focus on survival and growth under stress conditions were performed to observe characteristic traits of 78 *Sinorhizobium meliloti* strains collected from different regions. The genotype of a subset of strains, containing interesting phenotypes, was further characterized through whole genome sequencing. Paired-end illumina sequencing resulted in an average sequence coverage of 150. A phylogenetic tree based on the core genome was constructed to characterize the genomic diversity at strain level. Furthermore a pangenomics approach was used to identify the accessory genome, which is frequently associated with adaptation to the local environment. Linking the gene content with the phenotype will further elucidate how rhizobia deal with abiotic stress. Ultimately these new insights will serve as a basis for the creation of a successful rhizobia inoculant.

## PP-NMI-11 New way to control post-harvest losses in sugar beet

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Postharvest diseases of fruits and vegetables contribute significantly to a deterioration of quality and nutrient composition, mycotoxin contamination and reduction of the market value of natural products. Sugar beets stocked in clamps before processing are strongly affected by microbial spoilage mainly caused by saprophytic fungi such as *Botrytis*, *Fusarium* and *Penicillium*. Bacterial isolates from healthy field-grown sugar beets as well as fungal isolates from infected beets stored in clamps were sampled in Bavaria (Germany) and Lower Austria (Austria). Cultivation-dependent methods were applied to identify the antagonistic potential of bacterial isolates on solid medium and via volatile organic compounds (VOCs). A total ratio of 13.1% (257 of 1955) antagonistic bacteria was found in the isolates. Out of 30 fungal morphologic groups from clamp derived fungi, 10 genotypic groups were identified using 18S sequencing. Fungal isolates used for screening were further characterized by sequencing the ITS fragment. Out of the 257 potential bioactive bacteria, consortia were composed and tested for fungal growth inhibiting effects. About 30% (six consortia) showed significant VOC antagonistic effects against several fungal genotypes. Bacterial strains were genotypically characterized using BOX fingerprints and 16S sequencing. Potential bacteria were identified as *Bacillus subtilis*, *B. cereus*, *B. thuringiensis*, *B. amyloliquefaciens*, *B. safensis* and *B. simplex*. Using molecular methods the VOCs spectra of identified bioactive bacterial strains and consortia were analyzed. The developed sugar beet derived bioactive consortia will be applied on stored beets to decrease the fungal sugar loss during storage.

## PP-NMI-12 Identification of a new *Streptomyces* strain inducing plant defense responses and resistance against fungal infection

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A *Streptomyces* strain inducing plant defense responses against foliar pathogens was characterized. Multi-loci Sequence Typing and morphological characteristics identified this strain as a novel *Streptomyces* strain belonging to the *Streptomyces violaceusniger* subclade of the *S. hygroscopicus* clade. *Arabidopsis thaliana* plants treated with cell suspensions or culture filtrates of this strain and inoculated with various pathogens showed a significant reduction of symptom development. Microscopic examination of treated plant tissues revealed that *Streptomyces* mycelia efficiently colonized leaf surface leading to the production of spores. The effect of culture filtrate on the plant transcriptome was examined by RNA-Seq. Strong induction of defense genes expression was observed including markers of salicylic acid and jasmonate/ethylene pathways. Partial protection of immune-deficient *Arabidopsis* mutants inoculated with *C. higginsianum* or *A. brassicicola* conidies suggests that induction of defense responses is required to obtain high protection levels. Together, this work shows that *Streptomyces* bacteria produce compounds displaying strong elicitor activity and could be used as foliar treatment to efficiently protect plants against pathogen attacks.

## PP-NMI-13 Beneficial Rhizosphere Bacteria Isolated from Pioneer Desert Plants Induce Salt Stress Tolerance in *Arabidopsis thaliana*

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Salinity severely hampers crop productivity worldwide and plant growth promoting rhizobacteria (PGPR) could serve as a simple, sustainable solution to improve plant growth under salt stress. However, the molecular mechanisms underlying salt stress tolerance promotion by bacteria remain unclear. In this work, 116 rhizosphere bacteria were isolated from four desert plant species from Jizan, Saudi Arabia and screened for their PGP traits using biochemical assays. Six isolates qualified for analysis of their salt stress tolerance promoting activity on *Arabidopsis thaliana*, with five of them exhibiting increased shoot and root weight (up to 3-fold) under salinity stress. Further investigation into the root system architecture, salt ion content, and salt stress-related gene expression revealed common mechanisms of achieving salt stress tolerance, with some exceptions for certain isolates. The biochemical PGP traits or colonization abilities were not directly correlated with the growth promotion or root phenotypic changes. However, our results indicated that the transcriptional regulation of sodium and potassium transporters affected the uptake and distribution of sodium and potassium ions, thereby resulting in the overall improved health of the plants. We suggest that changes in sodium and potassium levels in the shoot are a crucial mechanism by which PGPR induce salt stress tolerance in *A. thaliana* and that bacteria isolated from pioneer desert plants provide a perfect opportunity to study abiotic stress tolerance mechanisms in plants. However, more experimental work using forward genetic approaches is needed to decipher the precise mechanisms.

## PP-NMI-14 Harnessing endophytes for biological control of *Septoria tritici* blotch

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Losses in wheat production due to pathogen infection represent a major threat to modern agriculture. *Septoria tritici* blotch (STB), caused by the fungus *Zymoseptoria tritici* (syn. *Septoria tritici*), is considered the most devastating foliar disease of wheat in Denmark and the EU. Fungicides are currently the primary control method of STB, as all commercially available wheat cultivars are susceptible to some extent. The increasing resistance of STB to all common fungicides therefore necessitates the development of innovative and sustainable strategies for effective disease control.

Endophytes are microorganisms, mainly bacteria and fungi, which inhabit inner parts of the plant without causing apparent symptoms on their host. Some endophytes have even been shown to have beneficial effects in different pathosystems, including conferring abiotic stress tolerance, stimulation of growth and decreasing disease incidence and severity. Due to these beneficial effects, there is an increasing economic interest in developing endophyte as biocontrol agents.

The aim of the project is to identify fungal endophytes that efficiently control STB in a reliable manner and that are adapted to the local environmental conditions. In a screening approach, fungal endophytes isolated from wheat were tested for control of *Z. tritici* *in vitro* and *in planta*, under both controlled and field conditions. Several endophyte strains were identified that significantly reduced disease symptoms under controlled conditions. To optimise the application of endophytes as biocontrol agents, the mechanisms of selected endophytes in controlling STB are investigated.

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## PP-NMI-15 Influence of the endophytic fungus *Daldinia cf. concentrica* and its volatiles on the model nematode *Caenorhabditis elegans*

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One of the major challenges in agriculture today is to discover novel nematocidal molecules that would be both efficient and environmentally friendly. The fungus *Daldinia cf. concentrica*, was isolated in our lab as endophyte from an olive tree (*Olea europaea*), and found to emit volatile organic compounds (VOCs), which were biologically active against a wide range of phytopathogenic fungi, and the rot-knot nematode *Meloidogyne javanica*. An initial step for understanding the mechanism of action of the VOCs against nematodes was to determine whether *D. cf. concentrica* VOCs could control the model nematode *Caenorhabditis elegans*. We found that exposure of *C. elegans* larvae to fungal volatiles caused 36±17% (mean ± SD) reduction in viability. Application of a synthetic volatile mixture, comprising 3-methyl-1-butanol, (±)-2-methyl-1-butanol, 4-heptanone, and isoamyl acetate, in volumetric ratio of 1:1:2:1, further reduced larvae viability by 86±4%. Each of the four constituents of the synthetic mixture significantly reduced larval viability relative to the control, however, only the compound 4-heptanone elicited the same effect as the whole mixture, with 76±9% reduction in larval viability. Future study will aim at elucidating the mechanism of these compounds to control nematodes by examining the response of mutant lines of *C. elegans* defected in neuronal pathways to the different VOCs in comparison to known mechanism of action of compounds such as organophosphate and carbamate, which are currently in use.

## PP-NMI-16 Identification and characterization of beneficial Bacilli for tomato disease control

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Tomato (*Solanum lycopersicum*) is one of the most important vegetable crops in the world and it has the highest acreage among all edible crops. However, tomato growing is frequently limited due to the high susceptibility to pests and diseases. The traditional chemical control causes a serious impact on both environment and human health. Therefore, seeking for environment-friendly and cost-effective green methods in agricultural production becomes crucial nowadays. Plant Growth Promoting Rhizobacteria (PGPR) can promote plant growth through direct solubilization of phosphate, production of phytohormones and fixation of nitrogen and/or inhibition of phytopathogens, and by triggering induced system resistance (ISR) of plants. It is considered to be a promising sustainable approach for crop disease control. In our research, we aim at isolating and characterizing novel PGPRs and deciphering the molecular mechanisms during plant-microbe interactions. To this end, we isolated 343 bacteria strains (of which 191 are *Bacillus* sp., and 28 endophytes) from healthy tomato, pepper and green onion rhizosphere soil and plant tissues of the Netherlands and Spain. *In vitro* antagonistic assays revealed that 20 strains have antimicrobial activity against *Rhizoctonia solani*; 24 against *Botrytis cinerea*; 30 are against of *Verticillium dahliae* and 36 act against *Phytophthora infestans*. Meanwhile, 16S RNA analyses of the endophytes showed that they are all gram positive bacteria, except 2 sequences from uncultured bacterium clones. More interestingly, 21 out of 26 from the Gram-positive bacteria fall into the *Paenibacillus* clade and one of them showed high antagonistic activity against all the tested pathogens. Currently, *in vitro* and *in vivo* plant assays, together with genome sequencing and chemical characterization are ongoing to discover the molecular mechanisms of the plant growth promoting effects.

## PP-NMI-17 *Rhodococcus fascians*, a rogue plant growth-promoting bacterium.

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*Rhodococcus fascians* is a phytopathogenic Gram-positive Actinomycete with a very broad host range encompassing especially dicotyledonous herbaceous perennials, but also some monocots, such as the *Liliaceae* and, recently, the woody crop pistachio. The pathogenicity of *R. fascians* strain D188 is known to be encoded by the linear plasmid pFiD188 and to be dictated by its capacity to produce a mixture of cytokinins. Here, we show that D188-5, the nonpathogenic plasmid-free derivative of the wild-type strain D188 actually has a plant growth-promoting effect. With the availability of the genome sequence of *R. fascians*, the chromosome of strain D188 was mined for putative plant growth-promoting functions and the functionality of some of these activities was tested. This analysis together with previous results suggest that the plant growth-promoting activity of *R. fascians* is due to production of plant growth modulators, such as auxin and cytokinin, combined with degradation of ethylene through ACC deaminase. Moreover, *R. fascians* has several functions that could contribute to efficient colonization and competitiveness, but there is little evidence for a strong impact on plant nutrition.

Additionally, several *Arabidopsis* mutants were inoculated with D188-5 to get a first indication of which pathways in the plant could be implicated in the plant growth promotion. Interestingly, all tested mutants, including a triple auxin receptor mutant, were responsive to the growth-promoting signals of D188-5.

Altogether, our data suggest that the mechanism underlying the improved plant performance will be multifactorial. Awaiting the generation of bacterial mutants in the putative plant growth-promoting genes, we postulate that the plant growth promotion encoded by the D188 chromosome is imperative for the epiphytic phase of the life cycle of *R. fascians* and prepares the plant to host the bacteria, thus ensuring proper continuation into the pathogenic phase.

## PP-NMI-18 Towards the identification of plant genes and metabolites potentially involved in the rice-PGPR association

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Plant growth and health is strongly influenced by interactions established between plant and microorganisms. Plant Growth-Promoting Rhizobacteria (PGPR) whose growth is stimulated by root exudates, are able to colonize plant roots and to enhance plant growth, representing a promising alternative to reduce chemical inputs in the context of sustainable agriculture. Plant growth-promoting effects of PGPR, notably of *Azospirillum* the main phytostimulator of cereals, have been shown to depend both on host plant genotypes and bacterial strains (Droque *et al.*, 2012) but the underlying molecular mechanisms remain largely unknown.

Using two rice cultivars and two *Azospirillum* strains isolated from rice, previous studies showed that promotion of root development was dependent upon the inoculated strain; profiling of plant secondary metabolites demonstrated strain-dependent modifications (Chamam *et al.*, 2012). Plant root transcriptome also revealed a strain-dependent response during the *Azospirillum*-rice association, with only a few genes commonly regulated in all tested conditions (Droque *et al.*, 2014).

In this work, we hypothesize that plant genes whose expression is modified upon inoculation might play an important role during the plant-PGPR interaction. We thus undertook to analyze rice response to ten different PGPR strains belonging to various genera (*Azospirillum*, *Herbaspirillum*, *Burkholderia*) and using different colonization strategies (endophyte or rhizospheric). Rice response is being analyzed in terms of root colonization (using *gfp*-tagged strains), of plant growth-promoting effects, of expression of a set of rice genes (selected from previous transcriptomic studies) and root metabolic profiling. This study should get insight into the molecular mechanisms involved in these interactions.

Chamam A. *et al.* 2013 *Phytochemistry*. 87, 65-77.

Droque B. *et al.* 2012 *Res. Microbiol.* 163, 500-510.

Droque B. *et al.* 2014 *Front. Plant Sci.* 5, 607.

## **PT-NMI-19 Stimulating saprotrophic fungi to control soil-borne fungal crop diseases**

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Natural protection of plants against soil-borne fungal diseases can result from the inhibitory activities of the soil microbiome. Indeed, bacteria with antagonistic activity against pathogens are often isolated from the soil. These strains are also marketed as bio-pesticides. Unfortunately, under field conditions microbial inoculants often fail to establish. Our study aims to stimulate members of the resident soil microbiome, that are already adapted to the local conditions, in order to improve the suppression of soil-borne fungal pathogens. Specifically, we propose to activate saprotrophic fungi, whose abundance is very low in intensively managed arable soils as compared to (semi-)natural ecosystems. We expect that an increase of saprotrophic fungi will raise the competition by fungi for root-derived energy sources. As a response, we expect that antagonistic functions against fungi will be triggered in the rhizosphere bacterial community, thereby forming a shield around roots that suppresses soil-borne plant-pathogenic fungi. Practical measures are explored to enhance growth of saprotrophic fungi in arable soils. Firstly, a range of organic residues of different composition was mixed with an agricultural soil. Woody materials and cellulose pulp were found to be the best amendments for stimulating fungal growth, as compared to waste materials, composts and plant residues. Analysis of the fungal community composition indicated that these fungus-stimulating amendments did not increase potential pathogens. Currently, wood- and cellulose-based amendments are tested in bioassays using pathogen-infested agricultural soils. Another approach to stimulate saprotrophic fungi in the rhizosphere is via modification of composition of root exudates e.g. by breeding. Here we focus on the role of phenolic acids. When combined with a simple artificial mixture of primary metabolites, some phenolic compounds stimulated the growth of fungi.

Reference: De Boer, W., Hundscheid, MPJ, Klein Gunnewiek PJA, de Ridder-Duine AS, Thion C, van Veen JA, van der Wal A (2015) Antifungal rhizosphere bacteria can increase as response to the presence of saprotrophic fungi. PlosOne 10: e0137988

## **PT-NMI-20 Nitrogenase biosynthesis of *Rhizobium (Mesorhizobium loti)* is limited by the symbiotic sulfate transport in root nodules of the legume *Lotus japonicus***

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Symbiotic interaction between *Lotus japonicus* and *Mesorhizobium loti* results in the development of nitrogen-fixing root nodules expressing the nitrogenase enzyme complex. Enclosed in symbiosomes and surrounded by the plants-derived peribacteroid membrane (PBM), the rhizobacteria are able to reduce molecular nitrogen. The metabolic exchange of carbon and nitrogen between plant and bacteria takes place via this structural and functional border of both symbionts. Besides the transport of nitrogen and carbon, sulfur (S) transport and metabolism is crucial for the symbiotic interaction: the symbiotic sulfate transporter (SST1) is one of the most abundant PBM proteins and nitrogen-fixing nodules are enriched in reduced S compounds. However, the exact function of sulfate during nodule protein-synthesis and -functional maintenance remains unclear.

<sup>34</sup>S-metabolic labelling and mass spectrometry was applied to analyze the incorporation of reduced sulfur into plant and bacteroid proteins of the wild-type and the SST1 mutant. Samples were harvested 24, 48, 72 and 96 hours after pulse labelling. Sulfur turnover dynamics and the incorporation into key proteins for symbiotic nitrogen fixation in different plant organs were determined. Additionally NanoSIMS analysis was used to analyze the <sup>34</sup>S/<sup>32</sup>S ratio of the different symbiosome components.

## **PT-NMI-21 Improving pasture quality by beneficial *Bacillus sp.* for optimized milk quality for use in dairy industry**

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With the vast daily consumption of dairy products worldwide, it is important to ensure the quality of the products. Pasture quality control is the first step to the success of this food web. Perennial ryegrass (*Lolium perenne*) is one of the most important pasture plants and widely distributed throughout the world due to its high levels of quality, palatability, digestible energy (fiber), protein, minerals and total usable carbohydrates. It is the main food for cattle, also determining milk quality. However, both susceptibility of plant diseases and potential causes of ruminant diseases (some plant origin pathogens can cause disease of the ruminant) are main limitations to expand its use. Therefore, in this study, we aim at i) seeking sustainable means for pathogen control and unraveling the mechanisms underneath. ii) increasing the biomass and quality (such as nutritional values) of the perennial ryegrass.

To this end, we isolated 119 *Bacillus* strains from healthy perennial ryegrass rhizosphere in the Netherlands. In vitro antagonistic assay showed that one of the strains, *B. amyloliquefaciens* HS9 can inhibit a wide range of plant pathogens, including both Gram-positive and Gram-negative bacteria, as well as fungi and oomycetes. To further investigate the compounds that responsible for the antagonistic activities, the genome of *B. amyloliquefaciens* HS9 was sequenced and genome mining was conducted. It revealed that *B. amyloliquefaciens* HS9 has potential to produce a wide variety of secondary metabolites, including the known surfactin, iturin A, fengycin B, bacilysin, bacillibactin, bacillaene, difficidin, macrolactin, 2,3-butanediol and acetoin. More interestingly, it harbors several potential novel NRPs. Furthermore, direct root tip inoculation of *B. amyloliquefaciens* HS9 on ryegrass seedlings and its volatile effect were studied. In both conditions, *B. amyloliquefaciens* HS9 can increase shoot and root biomass of *L. perenne* on half strength MS medium, which indicates that *B. amyloliquefaciens* HS9 plays a crucial role in the promotion of ryegrass growth. What exactly caused the plant growth promoting effect and how those effects are related to the quality of the ryegrass are currently under investigation.

## **PT-NMI-22 Microbial rhamnolipids mediate control of plant parasitic nematodes through several mechanisms**

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Plant parasitic nematodes (PPNs) are a global threat to crop production due to severe damages they cause to roots. Treatment with common nematicides, which are banned in most countries, is rather a risk to the environment than yielding a profit. Crop rotation is partly effective, but demands to be integrated in agricultural practice. The urge for alternatives raises the awareness for ecologically friendly approaches clearing the way from synthetic pesticides for agents of biological origin. Biosurfactants (BSFs) are a promising example for such an alternative in crop protection as a natural weapon to antagonize plant pathogens. These secondary metabolites, produced by various ubiquitous microorganisms, show an imposing efficiency against numerous economically relevant pathogens in agriculture. We demonstrate that rhamnolipids (RLs), one prominent representative of BSFs, have the capability to counteract PPNs by hampering the infestation via complementary activities. Plant infection by the cyst nematode *Heterodera schachtii* and the root-knot nematode *Meloidogyne incognita* is inhibited at RL concentrations far below the ecotoxicological level. We proof that RLs reinforce plant defense already in advance to a pathogen attack thus enabling the plant to react more appropriate. Even in the case that the parasite is able to overcome this first hurdle, there is strong evidence that RLs modify the quality of the plant as a host. In this respect, galls of *M. incognita* are reduced in size and most importantly they cause a drop in the reproduction of both PPNs. Attractivity assays with *H. schachtii* indicate that a RL-treatment induces disorientation of the parasite further extending the mode of operation by including actions prior to infection.

## **PT-NMI-23 Spatial distribution of Phyllosphere fungi in a heterogeneous wheat field.**

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The phyllosphere of common wheat (*Triticum aestivum*) is colonized by various microorganisms, such as bacteria, yeasts and filamentous fungi. The wheat ear (the grain bearing top part of the plant) in particular, hosts a microbial community composed, among others, by various species of fungi well known due to their phytopathogenicity for the host plant. Besides their parasitic activity, they also produce toxic metabolites (mycotoxins) dangerous to end consumers once the harvest has been processed into food. A deeper understanding of the mechanisms leading influencing the microbial community composition is therefore needed to shed light on the infection dynamics influencing the presence of such pathogens.

In my work I analysed the spatial distribution of *Alternaria spp.* and *Fusarium spp.* in different fields in the region of Brandenburg (Germany). The fields were chosen for their topographic heterogeneity, which caused differences in the microclimatic conditions among various points within them. The central aim of my study is to observe how the microbial community composition varies spatially, using different microclimatic conditions as proxies. Samples were collected from 1 square meters sampling areas distributed in the tested fields. The fungal presence was analysed using qPCR techniques, mycotoxins measured with GC-MS. Other parameters such as microclimatic data, field productivity and bacterial presence were also taken into account as response variables. The fungal abundance has shown to differ greatly between different points of the field, with microclimatic conditions influencing the abundance of the genetic markers measured and the presence of mycotoxins. The samples showing interesting results will be further analysed with high throughput methods.

This study aims at serving as a base for a more comprehensive understanding of infection dynamics at field level, showing that microclimatic variables need to be taken into account when considering this study system. A deeper knowledge of the dynamics affecting this microbial community will in future help to develop more sustainable managing practices or even to manipulate the microbial community into a hostile environment for those pathogenic fungi.

## **PT-NMI-24 Impact of plant growth promoting rhizobacteria on phosphorus use efficiency in the model grass *Brachypodium distachyon* (L.) Beauv.**

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In the present context of global change, reaching crop production sustainability is of main concern. This encompasses a better use of plant fertilizers, in order to reduce nutrient leaching and accumulation in the environment (e.g. nitrogen) and save limited natural resources (e.g. rock phosphate). Improvement of plant nutrient use efficiency (NUE), by acting on the plant ability to take up nutrients and produce biomass, is a promising way to achieve this goal. Plant growth promoting rhizobacteria (PGPR) are able to impact NUE. Regarding plant phosphorus (P) nutrition, PGPR can modulate the plant ability to take up P through several mechanisms including the increase of P bioavailability in soils, the modulation of the root system development for foraging and the alteration of the expression of P responsive genes. Our project aims at studying the impact of PGPR strains on the P use efficiency of *Brachypodium distachyon* (L.) Beauv. The ability of PGPR strains to increase P bioavailability is studied by performing P solubilization tests in a liquid medium. As several P forms are present in soils, three poorly available P forms are used. The selected strains exhibit contrasted solubilization and acidification activities. In parallel, the impact of PGPR on *Brachypodium* growth is studied in Magenta boxes, in a total interaction system under gnotobiotic conditions. Plants and PGPRs are exposed to contrasted P conditions including the same P sources as for the solubilization tests. Biomass production and allocation are measured after 30 days. The major effect is an increase in root biomass production under P deficiency conditions when PGPRs are applied. This can negatively impact shoot biomass production. The PGPR effect on root system development will be further studied by measuring root system architecture parameters. Finally, the plant response to contrasted P conditions in the presence/absence of PGPR will be considered at the molecular level by quantifying the expression of P responsive genes.

## PP-V-01 Unveiling bacterial and fungal communities residing in rice grain and endosphere of seedlings

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Plants have a close relationship with the surrounding environment including soil, climate, nutrients, animals, and microbiota during the lifetime. Among microorganisms associated with plants, microbes residing and proliferating in plant tissues are called endophytes. As high-throughput sequencing approaches have been introduced to microbiome research, many studies have shed light on the composition, diversity, and functions of endophytic microbial community. However, little is known about dynamics of endophytic microbiota during plant developments. In this study, we planned to inspect the structure of bacterial community (bacteriome) and fungal community (mycobiome) in rice grain and the endosphere of seedlings during early developmental stages of rice. To decipher origin and composition of both biomes residing in rice seedlings, time-series experiments were performed. When rice seedlings were grown up to 12 days in bed and paddy soils, bacterial OTUs were more abundant in seedlings grown in paddy soil than those grown in bed soil. Although Proteobacteria is commonly dominant in both seed and seedlings of rice at the phylum level, rice seedlings grown in field soil showed distinct diversity. Particularly, some OTUs were solely found in rice grown in soil. These results suggest that the OTUs only observed in rice grown in soil might originate from soil microbiome. In case of endophytic mycobiome, 37,151 sequences were detected and 1,086 OTUs were assigned at genus level. Genera *Sporothrix* and *Clonostachys* accounted for 96% of total sequences and were followed by genera *Epicoccum*, *Magnaporthe*, *Trichoderma*, *Pyrenochaetopsis*, and *Penicillium*. Among these genera, *Sporothrix*, *Clonostachys*, and *Magnaporthe* commonly existed in leaves, stems, and roots of rice. This result suggests three genera could reside systemically in rice endosphere. To validate these observations and decipher the origin of microbiome in seedlings, we plan to further investigate OTUs commonly detected among rice grain, seedlings, and paddy soil and their dynamics during developmental stages of rice. Ultimately, we aim to unveil effects of endophytic microbiome on physiological changes in rice.

**Microbe-assisted crop production, opportunities, challenges & needs**

**Vienna, Austria, 4- 7 December 2017**

***Poster Session 2: Plant understanding and improvement of beneficial interactions with microbes***

## **PP-PU-01 De novo transcriptome profiling of *Salicornia europaea* from salt affected locations.**

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*Salicornia europaea* L. belongs to the family Chenopodiaceae and is widely distributed in coastal and inland salt marshes. This halophyte can withstand more than 1000mM NaCl and is one of the most salt-tolerant plant species. Because of their high tolerance to salinity, *Salicornia* spp. could become models to study salt tolerance. The elucidation of its salt tolerance mechanism is of significance for generating salt-tolerant crops. Thus, to gain a better understanding of these mechanisms in *S. europaea*, we conducted a whole transcriptome study of this species using RNA sequencing approach.

*S. europaea* samples were collected in two seasons (autumn and spring) from two salt affected (natural and anthropogenic) locations in Poland. The physicochemical characteristics of the soil and plants were also analyzed. Total RNA was extracted from the shoot and root tissue of the plant. cDNA libraries were constructed and paired-end Illumina MiSeq sequencing was carried out. De-novo assembly was performed using the Trinity software platform.

A total number of 180401 and 181809 assembled contigs with N50 values of 1360bp and 1228bp were obtained for “S-I” and “S-II” locations in both seasons, respectively. The transcript abundance of the assembly contigs was estimated by Kallisto. The BLAST results and functional annotation data indicates that the transcriptome obtained from the present experiment nearly completely covers the gene space of *S. europaea*. This transcriptome may serve as a reference sequence for the study of other succulent halophytes. This work also identified potential genes in *S. europaea*, and has provided an outline of tools to use for de novo transcriptome analysis. Further, a detailed study of these genes will help us to understand the interactions between halophyte plants and its association with endophytic microorganisms.

Keywords: *Salicornia europaea*, salt stress, transcriptome

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## **PP-PU-02 New insights to below: phenotyping spatial and temporal dynamics of roots by Magnetic Resonance Imaging and Positron Emission Tomography**

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Lack of adequate methods for quantitative analysis of plant root architecture and function as well as their interaction with the dynamic environment hampers progress in fundamental and breeding related research. In recent years significant interdisciplinary approaches have been started to overcome this “phenotyping bottleneck”. Magnetic resonance imaging (MRI) allows for non-invasive detection of roots growing in soil and its high-contrast images facilitates quantification of plant root architecture (root lengths, diameters or angles) and special structures such as nodules. Coupling MRI with Positron Emission Tomography (PET) enables parallel monitoring of root and nodule development together with carbon tracer allocation along the root paths into active sink structures (e.g. root tips or nodules). This co-registration has high potential for gaining new insights into root growth and interactions with microbes for identifying novel traits demanded in breeding programs for future crops.

## PP-PU-03 QUORUM sensing homoserine lactone signal molecules play an important role in the cooperative interaction of beneficial endophytic bacteria with crop plants

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Specific signaling based communication between microbes and their plant hosts are of key importance for a successful establishment of symbiotic interactions. There are substantial evidences from many interactions of Gram-negative bacteria with host plants, that N-acyl-homoserine lactone (AHL) quorum sensing signal molecules play an important role in beneficial bacteria-plant interactions (1). However, detailed implications of AHL-triggered beneficial interactions of endophytic bacteria with plant hosts are not understood yet.

The interaction of the endophytic PGPR *Acidovorax radialis* N35 with barley seedlings as well as of the endophytic diazotroph *Gluconacetobacter diazotrophicus* PAL5 with rice seedlings was investigated by comparing the effects of wild type strains with AHL-deficient mutants in axenic systems. Root colonization behaviour was examined using GFP- or YFP-labelled strains and confocal laser scanning microscopy. In both bacteria, the AHL-deficient strains showed much different colonization behaviors and were much reduced in endophytic colonization. In the *G. diazotrophicus*-rice system, the AHL-deficient mutant was much reduced in the expression of oxygen-defense, phytohormone and exopolysaccharide production genes during plant colonization. In the *A. radialis*-barley system, gene expression in barley seedlings showed major changes upon bacterial inoculation using RNA seq-analysis and quantitative RT-PCR of selected plant transcripts. Most interestingly, the AHL-producing wild type *A. radialis* strain caused priming responses in several plant genes, but no defense response. In contrast, the AHL-deficient mutant caused a clear defense response, especially the expression of flavonoid biosynthesis and increased flavonoid production, which was proven by specific metabolite analysis.

Thus, AHL-signal molecules have an important role in the efficiency of colonization and the modulating the plant response. This may be exerted via QS-triggered gene expression of bacterial compounds, which are crucial in plant colonization or influence the perception by the plant themselves. In addition, a direct action of the AHL-molecule on plant gene expression could also be possible.

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## PP-PU-04 “MICROMETABOLITE” – Research Training Network on the Microbial Enhancement of Bioactive Secondary Metabolite Production in Plants

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Plant secondary metabolites are backbones of today's pharmaceutical and cosmeceutical industry, and evidence has shown that plant-associated microorganisms may induce or influence synthesis pathways. The MICROMETABOLITE training network aims at exploring the microflora associated with *Lithospermum* and *Alkanna* (*Boraginaceae*) plants for improving the production of high value-bioactive secondary plant metabolites. It focuses on shikonin and alkannin derivatives (A/S) already in use in pharmaceutical and cosmeceutical preparations or in the final stages of development. We analyse interactions between the A/S content, the plant genotype, and the plant-associated microflora, with the final aim of establishing novel production systems.

MICROMETABOLITE fosters synergistic cooperation between researchers in multiple scientific disciplines that are currently not well inter-linked. It promotes the advancement of plant biotechnology to meet the needs of the European pharmaceutical and cosmetic industries. As the first network in this technology field, it accomplishes that talented European young scientists (ESRs) will become future leading scientists, technologists and entrepreneurs, and enables leadership in scientific discovery and future pharmaceutical and cosmeceutical developments.

## **PP-PU-05 The molecular and physiological cross-talk between *Vitis vinifera* L. and Grapevine Virus B (GVB) affects berry secondary metabolism**

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A successful infection by a plant virus results from the complex molecular interplay between the host plant and the microorganism. Here, we present some advances in the study of the grapevine–virus interaction, considering it not only as a classical host–pathogen binary relationship. We discuss it from a new point of view, considering the plant and its endophytic microorganisms like a single organism or microecosystem adapting to the environment. Since great improvements in ‘-omics’ techniques have been obtained during the past decade, this topic deserves further attention in view of ongoing global change and to develop more sustainable management techniques. In the light of this, we studied the impact of a phloem-limited latent virus, Grapevine virus B (GVB), comparing infected and healthy *Vitis vinifera* L. wine red cultivar Albarossa in field conditions. This study was carried out following two consecutive summer seasons using molecular, biochemical and eco-physiological approaches. Results showed a GVB commensal relationship with no symptoms development and affecting eco-physiological performances, in terms of assimilation rates, particularly at the end of the season without compromising yield and vigour traits. Interestingly, in GVB-infected plants, soluble carbohydrates accumulation in leaves and expression profile of sugar- and Rubisco activase-related genes seem to activate moderate defence responses. Those responses did not cause detrimental phenotypic effects and, on the other hand, positively affected anthocyanin profiles in berries that are potentially involved in wine quality and stability. This work aims to improve the understanding of the multifaceted grapevine-virus interactions in response to a “natural” environmental condition, also in the light of the increasingly necessary adoption of sustainable viticulture approaches.

## **PP-PU-06 Effects of structural and functional analogues of IAA in triggering biological nitrogen fixation in non-legume plants**

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We have verified that the endogenous overproduction of the main auxin indole-3-acetic acid (IAA) in diazotrophic endophytes isolated from rice plants led to a significant up-regulation of nitrogen fixation: the *nifH* gene expression and the nitrogenase enzyme activity increased in both bacterial cultures and inoculated host plants as compared to the wild-type ones. When rice plants inoculated with wild type and IAA-overproducing nitrogen-fixing endophytes were compared significant changes in root morphology were observed: rice plants inoculate with the IAA-overproducing strains showed a more branched root system with abundant lateral root and significant changes in the primary root length. The aim of this work was to test the specificity of IAA effects on nitrogen-fixing apparatus in diazotrophic endophytes. Chemically or functionally similar molecules, such as indole (IND), indole-3-carboxylic acid (ICA), and 2,4-dichlorophenoxyacetic acid (2,4-D) were selected and exogenously added as purified substances into both liquid cultures and hydroponic systems of inoculated rice plants. Acetylene reduction assay (ARA) was carried out after treatment with the selected molecules. A significant increase of nitrogenase activity was measured only after the exogenous IAA-treatment in both conditions above described. This result leads us to say that the observed effect was specifically due to the hormonal activity of IAA. Studies to see if a similar effect can be obtained through co-infection of rice plants with nitrogen-fixing and IAA-producer endophytes are still underway. Selecting the best endophytic bacterial consortium could offer new perspectives to enhance nitrogen-fixation in non-legume crops.

## PP-PU-07 *PrimedPlant*: Priming for enhanced resistance in barley

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The primed state is a unique physiological state of the plant [1], induced upon a triggering stimulus. The primed plant is able to respond faster and stronger if compared to a naïve plant [2]. Therefore, priming is an efficient strategy for the plant to protect itself against e.g. pathogens. The knowledge of priming and induced resistance is currently mainly based on the model plant *Arabidopsis*. Our *PrimedPlant* project aims to expand the expertise and focuses on priming in barley (*Hordeum vulgare*), as one economically important cereal. Natural inducers of the primed state are for example bacterial quorum sensing molecules like the N-acyl homoserine lactone (AHL) oxo-C14-HSL which is produced by *Ensifer meliloti*. Here, we investigate the ability of barley to face the challenge against the powdery mildew causing fungus *Blumeria graminis f. sp. hordei* upon priming induced by oxo-C14-HSL. In this setting the priming capacity of different spring barley cultivars is investigated. For this purpose, two reference cultivars (Golden Promise and Morex) and five cultivars out of the spring barley GENOBAR collection (BCC768, BCC1589, BCC1415, BCC436 and HOR7985) were selected, based on their genetic distance. The infection rate and priming capacity were assessed phenotypically employing a detached leaf assay. The selected cultivars showed varieties regarding their resistance to powdery mildew and responded differently to the priming stimulus. Additionally, in order to localize and analyze the plant defense reaction, the production of reactive oxygen species in the leaves was visualized via DAB staining. As a second approach a luminometer-based assay was used to quantify the generation of reactive oxygen in leaves. Furthermore it is planned to investigate the expression of selected defense genes upon priming and a triggering stimulus to assess the cultivar dependent differences of the defense responses on transcriptional level. The aim is to obtain a detailed understanding of the processes and the cultivar dependent impact of priming in spring barley. In the future this information might be attractive to improve resistance of barley and other economically relevant cereals and to identify promising breeding targets.

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## PP-PU-08 Comprehensive management of maize cultivation, a trend of agri-food production in Mexico.

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Agri-food production has as objective to satisfy food demand and improve production systems. However, conventional farming methods have led to environmental problems such as water erosion, eutrophication, aquifer contamination and depletion and soil degradation. Maize cultivation in Mexico comprises almost one-third of agricultural production, with an average maize yield of 3.17 tons per hectare, which is 38% below the world average. Therefore, more than a third of the maize consumed in the country is imported. Under these conditions, it is required to increase productivity through an alternative production model incorporating the use of microorganisms and organic inputs such as biofertilizers, biostimulants, plant extracts and inducers of plant resistance, sustainable agricultural practices and balance. In the municipality of La Piedad, Michoacán which is a highly productive area, two plots of temporary maize of the pioneer variety 55-58 were selected. An area of 10 ha was delimited for each of these plots, in the first one a conventional agriculture management (CAM) was implemented, and in the second plot an integrated crop management plan was deployed (ICMP). Our results showed a reestablishment of the physico-chemical balance of the soil, increased plant resistance to the pests, a lower incidence of pests, greater productive and vegetative development (elongation, differentiation, flowering, fructification and ripening). Furthermore, the average production was 14.2 tons per hectare. The application of microbial communities played a fundamental role in increasing the health and quality of the soil as well as the growth, yield and quality of the crops. In the case of CAM, chemical fertilization, insecticides, herbicides and pesticides were applied to control pests and diseases, showing an average yield of 10.4 tons per hectare in addition to an increase in production costs.

## PP-PU-09 Tungsten (W) induces heavy metal stress by depleting primary metabolic processes, altering nutrient levels and causing oxidative stress in *Glycine max* - indices for modified stress response through rhizobia symbiosis

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Tungsten (W), a transition metal residing in the chromium group (IV Group) of the periodic table, finds increasing application in military, aviation and household appliance industry, opening new paths for the heavy metal into the environment. Since tungsten shares certain chemical properties with the essential plant micro nutrient molybdenum, it is proposed to inhibit enzymatic activity of molybdoenzymes by replacing the Mo-ion bound to the co-factor. However, recent studies suggest the inhibition of enzymatic activity might not be the only effect W has on plants and that, much like other heavy metals, tungsten exerts toxicity on its own. Still, our understanding of the mechanisms behind the apparent phytotoxicity remains limited. This study investigates the effects of W on growth, physiology as well as root and nodule proteome of *Glycine max* cv. Primus.

Plants were inoculated with *Bradyrhizobium japonicum* and grown in a semi hydroponic set up using three different tungsten concentrations (zero, 0.1 mM and 0.5 mM). To identify possible benefits of a functional bacterial symbiosis on W induced stress response, two different environmental growth conditions, one with suppressed N-fixation, supplied with Nitrate (10mM KNO<sub>3</sub>) and one solely relying on symbiotic N-fixation (zero KNO<sub>3</sub>), were applied.

The present study shows that *Glycine max* was able to take up considerable amounts of tungsten (318.13-355.46 mg kg<sup>-1</sup> at 0.1 mM W) without exhibiting reduced biomass production or impaired health. At the highest W concentration enzyme activity, nutrient concentrations and biomass production were significantly affected by the presence of tungsten. The proteomic analysis revealed similar defense mechanisms commonly found in plants in response to heavy metal toxicity such as the accumulation of various PR proteins. Additionally, indices for oxidative stress and radical scavenging via the phenol/ascorbate/peroxidases system as well as a stress induced response of secondary metabolism and peptidase inhibitors was found. Combined with a depletion in macro nutrient levels and starch accumulation in stems and roots, plants exhibited a failure of major metabolic pathways at the highest tungsten concentration, independent of N-regime. However, our results indicate that form and intensity of tungsten induced stress response seem to be different between plants living in intact and N-suppressed rhizobia symbiosis.

## PP-PU-10 Investigating two nitrogenase isozymes of *Kosakonia radicincitans* DSM16656<sup>T</sup>

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Plant growth-promoting (PGP) bacterial application in agricultural fields is a global concept to improve sustainability in contemporary agriculture. Biological nitrogen fixation (BNF) is one of the supposed PGP key factors of diazotrophic plant growth promoting bacteria (PGPB). *Kosakonia radicincitans* is a plant growth promoting bacterium that exhibits multiple plant growth promoting characters (Witzel, Gwinn-Giglio et al. 2012) and boosts growth of non-leguminous vegetables such as tomatoes (*Solanum lycopersicum*), *Brassica oleracea* and radish (*Raphanus sativus*) (Berger, Brock et al. 2013, Berger, Wiesner et al. 2015) upon inoculation in fields and greenhouses. *K. radicincitans* has the MoFe-nitrogenase and the FeFe-nitrogenase. Its BNF ability might be vital for yield increases in inoculated non-leguminous vegetables. In this study we conducted site directed mutation, delete *nifH* and *anfH* and created single and double nitrogenase mutants of *K. radicincitans*. We used natural <sup>15</sup>N and labeled <sup>15</sup>N to track BNF activities in the mutant strains: *DanfH*, *DnifH* and *DanfHDnif*. The mutants created in this study will be used to investigate whether the *K. radicincitans*' nitrogenases contribute to growth enhancements that are observed in *K. radicincitans* inoculated plants.

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## PP-PU-11 Bioinoculants as plants allies in phytotechnological approaches to soil requalification

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Soil functions and quality have been adversely affected by anthropogenic activities (eg. mining), and the resulting contamination is a serious and crosscutting problem in several countries, including those in the SUDOE territory. The presence of trace elements (TE) at high concentrations, cause negative effects on plants and animals, soil biodiversity and in aquatic environments, subsequently compromising human health. The urgent need for soil protection and conservation and the development of sustainable technologies to ensure the restoration of their environmental functionalities and services are a priority in European and national research programs and legislation. Thus, phytotechnologies (use of plants and microorganisms in the recovery of contaminated ecosystems) appear as sustainable alternatives to conventional remediation methods. Phytomanaging polluted soils can increase microbial diversity, carbon sequestration, soil quality and functionality.

The Borralha mine integrates soils with high TE concentrations that can benefit from the application of phytotechnologies in its remediation and requalification. In this context, energy crops such as sunflower (*Helianthus annuus*) and *Populus* sp., which have a high potential for success in adapting to soils of this nature, as well as bringing economic value, can be grown in these areas. In addition, microorganisms such as mycorrhizal fungi and plant growth promoting bacteria (PGPR) that stimulate crop growth may benefit these plants by reducing the stress promoted by the contamination. These bioinoculants can also modify the mobility of the TE in soil allowing their absorption and/or stabilization by the plants, reducing their losses to aquatic environments. Associated with these plants, other agronomic techniques such as intercropping with alfalfa and clover may be integrated in order to increase organic matter, promote the biological activity and nutritive status of soils.

The main goal of the work is to evaluate the potential of these energy crops and the application of microbial consortia in the improvement of the functionalities and mitigation of contamination of soil collected in the Borralha mine; and in the growth and improvement of plant development itself. Specific objectives include a) bioinoculant influence for improving plant performance and soil functionality/diversity; and b) effect of biostimulants on plant performance, TE mobility and uptake and soil functionality.

## PP-PU-12 Evaluating the potential of biological nitrogen fixation in C4 grasses by <sup>15</sup>N natural abundance technique

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Tropical grasses of C4 photosynthetic system have high photosynthetic efficiency and high potential for biomass production. They provide human food and animal fodder products and are important energy crops. Several nitrogen-fixing bacteria species, especially of genera *Azospirillum*, *Herbaspirillum*, *Gluconacetobacter* and *Burkholderia*, have already been identified colonizing the surface or the inner roots of several tropical grasses but it remains unclear whether and to what extent, grasses benefit from N fixed by endophytic diazotrophs (BNF) or if benefits are due to the production of indole-3-acetic acid (IAA) and other growth-promoting substances. We determined BNF of five sugarcane (*Saccharum* spp.) and two sorghum (*Sorghum bicolor* L. Moench) varieties, maize (*Zea mays* L.) and Guinea (*Panicum maximum* Jacq), elephant (*Pennisetum purpureum* Schum) and buffel grasses (*Cenchrus ciliaris* L.) in a pot experiment, using the <sup>15</sup>N natural abundance technique. Buffel grass, elephant grass and sugarcane variety RB92579 had  $\delta^{15}\text{N}$  values with no statistical difference from values of reference species and thus the hypothesis that they had absorbed N exclusively from the soil cannot be ruled out. Sorghum, maize, Guinea grass and four sugarcane varieties had  $\delta^{15}\text{N}$  signals significantly lower than both reference species (castor bean, *Ricinus communis* L., and cotton, *Gossypium hirsutum* L.). The absorption of atmospheric N through fixation contributes to the nitrogen nutrition (BNF of 13 to 31% of their N). The greatest proportion of N in nitrogen-fixing plants (>30%) was found in sorghum (variety IPA 1011), a species with a single previous fixation report. Confirmed fixation by these species represents the potential of great fertilizer economy and high yields in low input agriculture.

## PP-PU-13 Effects of endophytic fungi on host-pathogen interaction in tomato plants

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*Serendipitales* as root endophytic fungi can colonize the roots of different plant species and thus can lead to enhanced plant growth and also systemic resistance against pathogenic fungi. In previous studies *Serendipita indica* and *Serendipita vermifera* as the most well-studied members of this order promoted plant growth and increased resistance and tolerance to biotic and abiotic stresses. In this study, in order to assess the Biocontrol potential of these endophytic fungi to suppress pathogen activity of *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) and their impact on tomato plants the following four members of *Serendipitales* (formerly *Sebacinales* group B) were investigated under in vitro and in vivo conditions: *Serendipita williamsii*, *Serendipita herbamans* as European isolates, beside two well-studied members of this order, *S. indica* and *S. vermifera* for comparison. Three-week-old tomato seedlings were inoculated with selected endophytic fungi and/or *Fol*. 8 weeks later, the plants were harvested and assessed for growth parameters and disease development. In addition, to investigate the impact of these selected endophytic fungi on the root morphology of tomato plants in in-vitro experiments, tomato seedlings were placed on Knop medium, inoculated with selected endophytic fungi and were grown under a 10-h light/14-h dark photoperiod at 24°C. After 14 days, the roots were scanned and images were analyzed by using the software Win RHIZO Pro. The obtained results from greenhouse experiments showed positive effects of all selected endophytic fungi on tomato plants. There was not only a significant increase in growth but also a reduction in disease incidence of *Fol* by more than 50% in tomato plants co-inoculated with selected endophytic fungi and *Fol*. In addition, root analysis of inoculated and non-inoculated tomato plants under in vitro conditions showed a significant increase in tomato root length, root surface area of tomato plants inoculated with *S. indica*, *S. herbamans* and *S. vermifera* compared to the control. Furthermore, root surface area was increased in all *Serendipita* sp. treatments compared to the non-inoculated plants. In contrast, root diameter of tomato plants was affected only by *S. williamsii* and *S. vermifera*. Here, it was found that *S. williamsii* and *S. herbamans* can act as biostimulants and biocontrol agents, respectively, by promoting tomato plant growth and reducing the probability of disease incidence caused by the soil-borne pathogen *Fol*.

## PP-PU-14 The effect of bioinoculants on grapevine development

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The impact of abiotic stresses on crops is of great concern worldwide. In recent years several strategies have been designed to mitigate the negative effects caused by high temperatures, excessive salinity and extreme drought. Among these strategies is the application of organic and inorganic chemicals, such as osmoprotectants and plant hormones. However, the application of chemicals raises environmental concerns due to their persistence, fate and harmful effects on the environment and public health. The use of beneficial microorganisms, in particular plant growth promoting bacteria (PGPB) and mycorrhizal arbuscular fungi (AMF) appears as an environmentally friendly biotechnological tool, contributing to sustainable agricultural practices and to reverse the trend of applying large amounts of fertilizers and pesticides.

In the last years, we have used bioinoculation to increase the resilience of different plants to abiotic stresses, especially species of agronomic interest, such as maize. In particular, the strain *Pseudomonas reactans* EDP28 and the AMF *Rhizophagus irregularis* acted as plant growth-promoting inoculants, increasing root and shoot biomass of maize plants grown in degraded soil. Similar outputs were obtained with sunflower exposed to different levels of salinity where the application of bioinoculants enhanced biomass production and accumulation of K<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, and reduced Na<sup>+</sup> levels in maize tissues. However, the effectiveness of bioinoculants in enhancing grapevine performance is still poorly studied. Under this context, the present work aims to evaluate the effect of the bioinoculants already used in previous experiences on growth and development of grapevines in greenhouse and field experiments. Testing the same bioinoculants as growth promoting agents in different plants and across several climatic conditions may be of utmost importance to increase the success of their application, as the specificity of bioinoculants is seen as a factor to hamper their further use in different agricultural scenarios.

## PP-PU-15 Removal and metabolism of carbamazepine in plants assisted by reed endophytic rhizobacteria

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Carbamazepine (CBZ) and its metabolites are usually detected in sewage and surface waters. Because of its persistency in the environment, this antiepileptic and mood-stabilizing drug has been suggested as a good indicator of human influence on water systems. Since endophytes have been proposed to play an important role in phytoremediation, this study focuses on the identification and characterization of endophytic candidates for biodegradation of carbamazepine in wetland systems.

Endophytic bacteria were isolated from roots and rhizomes of reed (*Phragmites australis*) plants exposed to 5 mg/L CBZ, a concentration 20–80 times higher than usually found in municipal sewage water. The cultivable endophytic community was characterized regarding its phylogeny, its plant growth promoting traits and its ability for CBZ removal. *Rhizobium radiobacter* and *Diaphorobacter nitroreducens* were selected among the isolates for a comprehensive study of CBZ uptake and metabolism in interaction with plant roots. An axenic horseradish (*Armoracia rusticana*) hairy root (HR) culture was used as model to unravel which metabolic pathways are used for CBZ transformation by plants in the absence and presence of their endophytic partners.

Inoculation with *D. nitroreducens* and *R. radiobacter* led to a 2-fold and 4-fold increase in the removal capacity of HRs alone, respectively. In total, thirteen transformation products were identified in the liquid media by LC-UHR-QTOF-MS/MS. These metabolites were classified in four distinct metabolic pathways. For the first time, a CBZ-glutathione conjugate was found in plants. Glutathione and 10,11-diOH pathways were preferred by horseradish HRs while inoculation with *R. radiobacter* and *D. nitroreducens* favour the 2,3-diOH and the acridine pathway respectively.

It can be concluded that recalcitrant compounds such CBZ can be removed from wastewater treatment plants with the help of macrophytes and their endophyte partnerships. CBZ removal and degradation can be improved by enhancing the presence and functionality of selected beneficial strains among the endophytic bacterial community. Furthermore, CBZ metabolism and degradation pathways in plants can be modulated by the interaction with their endophytic community.

## PP-PU-16 Nitrogen input via diazotrophic bacteria symbiosis in irrigated mango crop

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Green manure is a well known practice to add organic matter and nitrogen to the soil before planting a commercial crop. When legume species are used as green manure enough N may be incorporated from symbiotic fixation to render unnecessary nitrogen fertilization of the commercial crop. Mixtures of several species planted together have the potential to maximize biomass production but little is known of their capacity to fix N. Irrigated fruit crops of high value are cultivated in the sandy soils of Northeast Brazil. In spite of green manure mixtures being a growing practice in the region, determinations of their biomass production and especially N fixation are scarce. This study aimed at estimating the amounts of biomass and nitrogen incorporated by a mixture of green manure species in a mango (*Mangifera indica* (L.)) crop, in the S  o Francisco river valley. Two treatments were composed of mixtures of green manure species and a third treatment of spontaneous species. The mixtures differed in the proportions of seeds planted in the plots. The first mixture was planted with 150 % of the amount of seeds recommended for each legume species and 50% of other species. The second mixture was planted with 150% of the amount of seeds recommended for each non-legume species and 50% of the legume species. The mixtures included the legumes: sunn hemp, *Crotalaria juncea* L. and *C. spectabilis* Roth; jack beans, *Canavalia ensiformis* (L.) DC.; lablab, *Dolichos lablab* L.; mucunas, *Mucuna aterrima* (Piper & Tracy) Holland and *M. nivea* (Roxb.) DC. ex Wight & Arn.); and pigeon pea, *Cajanus cajan* (L.) Huth. The amounts of fixed N were estimated using the <sup>15</sup>N abundance technique. All legume species had high proportions (> 70%) of their N derived from the atmosphere. Mucuna was the species that fixed most N: 76 kg ha<sup>-1</sup>. The mixture with the largest proportion of legume species produced the largest biomass (10 Mg ha<sup>-1</sup>). The input of fixed N by the legume mixtures resulted in higher mango production (14.4 Mg ha<sup>-1</sup>) than that of the spontaneous plant treatment (8.7 Mg ha<sup>-1</sup>).

## PP-PU-17 Role of plant associated microbiota in fruit aroma and plant defense in strawberry and raspberry

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The study of plant-associated microbiomes is a rapidly growing field of extraordinary interest. In the last few years, an increasing number of functions has been attributed to the microbiome associated to crop plants, including the promotion of host growth, the induction of resistance and the protection against abiotic and biotic stress. Some research has also focused on the contribution of the microbial community to fruit quality. Pioneer studies dealt with the influence of the association between *Methylobacterium* strains and strawberries to the composition of the aroma of fruit.

In this study, the fruit-associated microbiome of different strawberry and raspberry cultivars was characterized by a metagenomic approach, based on the sequencing of the 16S gene on Illumina platform. The profile of volatile organic compounds (VOCs) of the same fruits was previously characterized by PTR-ToF-MS and GC-MS to describe the main chemical components of the berry aroma and the data were correlated to the presence and abundance of microbial species capable of producing VOCs of interest. For the bacterial strains belonging to such species, the growth rates on the fruit and the production of VOCs were measured in order to quantify their contribution to the aroma composition of the fruit.

In addition, the metagenomic exploration highlighted the presence of bacterial species potentially acting as biological control agents (BCAs) or as plant-growth promoting bacteria (PGPB). Their populations were quantified by qPCR in various organs of the plant beside the fruit. Putative BCAs were tested against pathogens, on both plants and fruits in postharvest condition.

The ultimate/last aim of the present work was to identify useful bacteria in the resident microbiome of some valuable crop plants, in order to design a sustainable orchard management strategy, integrating crop associated functional microbes as one of the key components.

## PP-PU-18 Transcriptome analysis sheds light on the *Serendipita indica*-tomato interaction

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Alternative practices are urgently needed to transform current agriculture to more sustainable and environmentally friendly systems. Endophytes, a diverse group of bacteria and fungi growing asymptotically inside plant tissues, are often associated with enhanced plant growth and tolerance to abiotic and biotic stresses, indicating their potential for improving sustainability in agricultural systems. However, the exact mechanisms and effects of the complicated plant-endophyte interactions remain poorly understood and characterized.

*Serendipita indica* (syn. *Piriformospora indica*) is an endophytic fungus with several promising agricultural and biotechnological applications. The fungus can colonize the root cortex of a wide range of plants, enhancing plant growth and modulating plant specialized metabolism. Tomato (*Solanum lycopersicum*) is an important crop, often challenged by fungal pathogens and insect pests. The wide variety of specialised metabolites produced by the plant, and especially terpenes, plays a crucial role in plant defence, helping in repelling possible enemies.

This project involves establishing a balanced interaction between the fungal *S. indica* and tomato plants, providing a model system for studying general plant-endophyte interactions. However, our main focus is on the mechanisms used by the fungus to induce host specialized metabolism. Preliminary data suggesting that colonization by *S. indica* results in increased production of specific volatile terpenes in fungus-colonised tomato plants encouraged us to study the induction of these metabolites at transcription level. RNAseq analysis on fungus-associated and fungus-free plant tissues is currently ongoing to provide a more-in-depth view on the actual mechanisms underlying the *S. indica*-tomato interaction.

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## PP-PU-19 Drought tolerance of modern barley (*Hordeum vulgare* L.) varieties and influence of *Pseudomonas putida* KT2440 inoculation

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Despite predictions of increased occurrence of drought events, tolerance to this abiotic stress has received relatively little attention by plant breeders. Better understanding of the drought tolerance of modern barley (*Hordeum vulgare* L.) varieties is needed. Further, inoculation of bacteria to young plants could have a positive effect on how they face drought stress periods. In this work the leaf surface area and relative water content (RWC) in leaves and roots of four barley varieties (Concerto, Oddysey, Olympus and Sienna) were studied to evaluate the morphological responses to short period drought for 14 days. The varieties showed different responses to drought treatments. Concerto had the lowest root RWC 10% and leaf RWC 42% for drought plants while Sienna showed the highest leaf RWC 60% and root RWC 14%. Therefore, Concerto and Sienna were chosen for further study with inoculation of the bacterium *Pseudomonas putida* KT2440 four days after sowing the seedlings. The results showed significant increase ( $P=0.02$ ) in the leaf surface area for Concerto plants inoculated with *P. putida* KT2440 compared to corresponding non inoculated control plants. Specifically, average plant leaf surface area under drought stress without bacteria was 10.9 cm<sup>2</sup> compared to 18.2 cm<sup>2</sup> with the inoculant under the same conditions. By comparison there was no significant difference between root or leaf RWC for inoculated and non-inoculated plants under drought conditions. For Sienna, the leaf surface area of plants inoculated with *P. putida* KT2440 was significant bigger in watered plants, 21.8 cm<sup>2</sup>; than in drought plants with (14.4 cm<sup>2</sup>) or without (11.6 cm<sup>2</sup>) the bacteria. In Sienna the root RWC in control plants without bacteria was 68% dropping to 15% in plants that suffered drought. On the contrary in the plants that were inoculated with bacteria there were no significant differences in root RWC between controls and drought plants. *Pseudomonas* isolation agar plate counts were used to confirm survival of the *P. putida* KT2440 inoculum at the end of the experiment. The results suggest adding *P. putida* KT2440 to Concerto seedlings may have a positive impact on drought tolerance based on the specific plant growth indicator of leaf surface area. Further work is needed to fully elucidate the benefit of bacterial inocula on drought tolerance of different barley varieties.

## PP-PU-20 Mechanisms of Rice and Rhizobia Interaction

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The N-fixing bacteria, rhizobia, are able to infect the root of leguminous plants, induce the formation of root nodules, and convert atmospheric N into ammonia, a biological form that can be directly used by plants. Our previous studies have shown that despite being unable to induce nodulation, rhizobia are able to infect and colonize the rice roots. Such an "endophytic" interaction can disseminate in both below-ground and above-ground tissues and promote growth and productivity in rice. Rice plants inoculated with certain strains of green fluorescence protein (GFP)-tagged rhizobia produced significantly higher root and shoot biomass, increased photosynthetic rate, stomatal conductance, water utilization efficiency, flag leaf area (which possess the highest photosynthetic activity in a rice plant), and accumulated higher levels of indoleacetic acid (IAA) and gibberellin. Our recent study has focused on two specific aims: (1) Identification and investigation of rice genes regulating rice-rhizobia interactions for enhancing drought and flooding tolerance and root development - We are screening the Taiwan Rice Insertional Mutant (TRIM) population for lines that respond more favorably to rhizobia, so that we can identify rice genes involved in the rice-rhizobia interactions. We will also study the potential synergistic effect of rhizobia with rice mutants/transgenic plants with specific genes mutated or over-expressed, which serves as a foundation for investigating mechanisms underlying rice-rhizobia interactions. (2) Investigation of mechanisms of rice-rhizobia interaction under normal and stress conditions - The mechanism as to how different strains differentially colonize in different rice tissues, and how the bacteria interact with rice tissues, remain largely unknown. We will first determine the infection and colonization processes that favor the endophytic rhizobia-rice association, and then study the gene expression profiles involved in these processes. The function of potential genes could be further studied with TRIM lines corresponding to the target genes.

## PP-PU-21 Effects of *Azospirillum brasilense* on nitrate uptake in maize seedlings

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The global increasing population requires an enhancement in the agricultural productivity that today, as in the past, is obtained by high fertilizers applications. The over-exploitation of these intensive practices has a high environmental impact, threatening seriously the agricultural ecosystem. Therefore, agriculture has to promote more sustainable approaches, taking advantage, for instance, of the biological potential of the plant-soil-microorganism relationships occurring in the rhizosphere.

*Azospirillum brasilense* is a plant growth-promoting rhizobacterium (PGPR) that is able to colonize the root system of many crops and to promote root growth. Recently, it was also demonstrated that it can affect the molecular mechanisms of iron acquisition in cucumber plants, favouring a quicker recovery from the nutritional stress.

Therefore, the aim of this study was to investigate the influence of *A. brasilense* on  $\text{NO}_3^-$  uptake in maize plants, with particular focus on the high affinity transport system (HATS).

In one-day-old plants, HATS was induced by adding 0.5 mM  $\text{NO}_3^-$  to the nutrient solution; in addition, seedlings were either non-inoculated or inoculated with *A. brasilense*. At 0, 4, 8, 16 and 24h after the treatments, the  $\text{NO}_3^-$  uptake rate was assessed. The analysis confirmed the occurrence of the induction phenomenon; nevertheless, the microorganism caused a reduction in the uptake rate in induced plants, suggesting that *A. brasilense* may negatively affect HATS in the short period. Conversely, at 24 h the  $\text{NO}_3^-$  uptake rate was higher in inoculated control plants than in the other treatments. Plus, the expression of key genes involved in HATS (*NRT2.1*, *NRT2.2* and *NRT3.1*) as well as in the transport and assimilation of  $\text{NH}_4^+$  (*AMT1.1a* and *AMT1.3*, *GS1.2* GOGAT) was studied; the increased expression of *AMT1.1a* and *GOGAT* in inoculated plants suggested an active uptake and assimilation of  $\text{NH}_4^+$ .

Our results highlighted that maize plants could partly take advantage from *A. brasilense* inoculation. Indeed, *Azospirillum* increased  $\text{NO}_3^-$  uptake in control plants, but, on the other hand, the molecular data suggest that it might however provide plants with N, yet as  $\text{NH}_4^+$ . Therefore, this evidence demonstrates the potential of *A. brasilense* as biofertilizer that might allow a better utilization of naturally available resources, thus leading to a more sustainable agriculture.

## PP-PU-22 *Rhodopseudomonas palustris* NTUIOB-PS3 (PS3) promotes the growth of Chinese cabbage (*Brassica rapa chinensis* L.) by raising plant endogenous indole-3-acetic acid level in developing leaves.

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A phototrophic bacterium *Rhodopseudomonas palustris* NTUIOB-PS3 (PS3) has been shown to exert positive effects on the growth of non-heading cabbage. Yet, the underlying mechanism of its plant growth-promoting (PGP) ability is still unclear. Synthesis of microbial indole-3-acetic acid (IAA) is one of the major PGP traits among the well-known beneficial rhizobacterium. Yet, the relationship between host plant IAA homeosis and PS3 PGP ability is still unclear. The aim of this study is to gain more insight into the PGP ability of PS3 strain regarding the phytostimulation capacity. To this end, we assessed the IAA producing ability of PS3 strain and the IAA level in the developing leaves of Chinese cabbage (*Brassica rapa chinensis* L.) after PS3 inoculation in a hydroponic system. To eliminate the side effects of plant-rhizobacteria interaction, we used the *R. palustris* strain NTUIOB-C3 as an inoculation control treatment. C3 is 95% genetically similar to PS3, but owns no plant-growth promoting ability. Our results showed that the IAA synthesis rates of PS3 and C3 strains under sufficient IAA precursor Tryptophan were equal. However, the yield assessments showed that the shoot and root fresh weight of Chinese cabbage were significantly increased by PS3, not by C3, after the onset of the fast growth stage. By harvest, the shoot-root ratio of the PS3-treated group was substantially higher than those without inoculation, and the total leaf area of the former was increased by 122%, mainly due to newly opened leaves. Leaves endogenous IAA analysis showed that, the IAA concentration decreased as the young leaves expanded to their full size, regardless of inoculation treatments. Intriguingly, PS3 inoculation significantly raised the IAA concentration at the early stage during leaves expanding. From cell number measurement, we further confirmed that PS3 inoculation promoted the cell division rate of leaves. Our findings suggest PS3 enhances the IAA level and the cell number in leaf of Chinese cabbage, corresponding to a higher leaf area and photosynthetic capacity, which in turn enhances the accumulation of biomass.

## PP-PU-23 Feed production and soil microbial communities under different fertilizer-cropping managements in Finland

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Timothy (*Phleum pratense*) and red clover (*Trifolium pratense*) are important forage crops for livestock in Finland. In sustainable agriculture, we need to know the mechanisms between forage production and soil microbes to maintain soil health.

To assess the feed production and the response of soil microbial community to different fertilizer-cropping managements, we established a three-year (2013-2015) field experiment in Finland. In our split-plot design, fertilizer treatment (none, industrial (saltpeter), manure (cow manure)) was the main plot factor and cropping system (fallow, red clover, timothy, and a mixture of red clover and timothy) was the subplot factor. To detect the effect of different nitrogen fertilizer levels, the plots received 40 kg N ha<sup>-1</sup> in 2013-2014 and 150 kg N ha<sup>-1</sup> in 2015.

We harvested the crops twice per year in 2014-2015. We measured crop dry matter yield, C and N contents, and calculated C:N ratio and C and N yields. Also, we analyzed the soil NO<sub>3</sub>-N and NH<sub>4</sub>-N contents, pH, EC and soil moisture. After extracting soil DNA, we investigated the soil bacterial community by amplicon sequencing targeting the V3-V4 region of 16S rRNA gene.

The differences in dry matter, N content, C and N yield between the cropping systems were all statistically significant ( $P < 0.05$ ) except in the second harvest in 2015. At 40 kg N ha<sup>-1</sup>, dry matter and N yield were highest in the mixture plots. After increasing N level to 150 kg N ha<sup>-1</sup>, dry matter and N yield were highest in the red clover plots. The crop C:N ratios were different between cropping systems in the four harvests ( $P < 0.05$ ). The C:N ratios in the mixture at the first harvest and timothy at the second harvest were approximately ideal (25:1) for being decomposed quickly by microbes. The soil NO<sub>3</sub>-N content followed the order fallow > red clover > mixture > timothy ( $P < 0.05$ ). The effect of fertilizer on soil NO<sub>3</sub>-N, EC, and pH were significantly different after the N level was increased to 150 kg N ha<sup>-1</sup> ( $P < 0.05$ ). And the soil NO<sub>3</sub>-N and EC of industrial was highest, then the soil pH of manure was the highest.

The soil microbial community responded to the different cropping systems. Compared to other three cropping systems, in fallow the abundances of *Bacteroidetes*, *Chloroflexi*, *Fibrobacteres* and *Verrucomicrobia* were lower, and the abundances of *Armatimonadetes*, *Cyanobacteria* and *Saccharibacteria* were higher.

## PP-PU-24 Influence of plant growth-promoting rhizobacteria (PGPR) on the growth and quality of strawberries

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Several studies have shown the benefits of plant growth-promoting rhizobacteria (PGPR) on plant mineral nutrition suggesting their application as biofertilizers. PGPR can stimulate plant growth, increase plant resistance to abiotic and biotic stresses and might thus have a positive effect also on fruit quality.

The aim of this work was therefore to evaluate and compare the effects of beneficial microorganisms, supplied either as pure culture (*Azospirillum brasilense*) or as a commercial mixture (Effective Microorganisms – EM™), on the growth and quality of strawberry (*Fragaria ananassa* cv. Elsanta) fruits. Strawberries are in fact among the most popular fruits, because of their unique taste and health benefits for humans, due to a high content of micronutrients, phytochemicals and antioxidants.

Strawberry frigo-plants were hydroponically grown either in a complete nutrient solution, or in a nutrient solution inoculated with *A. brasilense* or with EM for 10 weeks (Pii et al., 2016). At harvest, biometric parameters, as shoot fresh weight, root fresh weight, and yield parameters, like number of fruits per plant and fruit weight, were recorded.

Growth parameter (e.g. biomass, leaf area) were not affected by the rhizobacteria. Even though PGPR-inoculated plants showed slightly reduced fruit yields in terms of average number of fruits/plant they delivered larger fruits as compared to controls. Fruits obtained from PGPR-inoculated plants had also a higher sweetness index in comparison to control fruits. The content of total phenols showed no significant difference between the different samples, whereas the concentration of flavonoids and flavonols was higher in fruits harvested from *A. brasilense*-inoculated plants. In addition, PGPRs also influenced the uptake and allocation of nutrients in fruits, in particular increasing the concentration of micronutrients (e.g. Fe).

In conclusion, our results demonstrate that the application of PGPR as biofertilizers might represent a sustainable agricultural practice to improve the nutraceutical value of strawberries, particularly concerning the content of flavonoids and micronutrients.

This work has been financially supported by the Free University of Bolzano (TN2023).

## PT-PU-25 Ecological role of maytansine is revealed by *in situ* MALDI-HRMS-imaging of *Maytenus senegalensis* during the germination process

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MALDI-HRMS-imaging (matrix assisted laser desorption ionization high-resolution mass spectrometry imaging) is an important tool for visualizing, both spatially and temporally, metabolites at the interface of plants and microbes (e.g., endophytes and/or pathogens). Furthermore, the chemical communication between plant-associated microbes can be elucidated at the cellular level. We employed MALDI-HRMS imaging to study the ecological role of maytansine, an important anticancer drug used against breast cancer [1,2]. Since the discovery of maytansine in the 1970s in Celastraceae plants such as *Maytenus* and *Putterlickia* species, its role as a chemical defense compound was hypothesized [3]. After more than four decades of discovery of this important antineoplastic drug, we provide a proof-of-concept of ecospecific and tissue-specific production and *in situ* spatial/temporal distribution of maytansine in *Maytenus senegalensis* plants. We have used MALDI-HRMS-imaging to visualize the occurrence and spatial/temporal distribution of maytansine in the leaves, stems, and roots of *M. senegalensis* plants, seeds obtained from the mother plants, through the germination process, and finally to the establishment of new seedlings or daughter plants. The mother plant was devoid of maytansine in all tissues. However, maytansine was produced and distributed in the cotyledons and the endosperm of the seeds with an augmented accretion towards the seed coat. Furthermore, maytansine was always detected in the emerging seedlings, particularly the cortex encompassing the radicle, hypocotyl, and epicotyl. The typical pattern of production and accumulation of maytansine not only in the seeds but also during germination provides evidence that *M. senegalensis* is ecologically primed to trigger the production of maytansine in vulnerable tissues such as seeds during plant reproduction. By utilizing maytansine as chemical defense compound against predators and/or pathogens, the plant can ensure viability of the seeds and successful germination, thus leading to the next generation of daughter plants with an evolutionary advantage of survival [4].

References: [1] Kusari et al. J. Nat. Prod., 2014, 77, 2577-2584; [2] Kusari et al. RSC Adv., 2016, 6, 10011-10016; [3] Kupchan et al. J. Am. Chem. Soc., 1972, 94, 1354-1356; [4] Eckelmann et al. Fitoterapia, 2017, 119, 51-56.

## **PT-PU-26 Effects of structural and functional analogues of IAA in triggering biological nitrogen fixation in non-legume plants**

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We have verified that the endogenous overproduction of the main auxin indole-3-acetic acid (IAA) in diazotrophic endophytes isolated from rice plants led to a significant up-regulation of nitrogen fixation: the *nifH* gene expression and the nitrogenase enzyme activity increased in both bacterial cultures and inoculated host plants as compared to the wild-type ones. When rice plants inoculated with wild type and IAA-overproducing nitrogen-fixing endophytes were compared significant changes in root morphology were observed: rice plants inoculated with the IAA-overproducing strains showed a more branched root system with abundant lateral root and significant changes in the primary root length. The aim of this work was to test the specificity of IAA effects on nitrogen-fixing apparatus in diazotrophic endophytes. Chemically or functionally similar molecules, such as indole (IND), indole-3-carboxylic acid (ICA), and 2,4-dichlorophenoxyacetic acid (2,4-D) were selected and exogenously added as purified substances into both liquid cultures and hydroponic systems of inoculated rice plants. Acetylene reduction assay (ARA) was carried out after treatment with the selected molecules. A significant increase of nitrogenase activity was measured only after the exogenous IAA-treatment in both conditions above described. This result leads us to say that the observed effect was specifically due to the hormonal activity of IAA. Studies to see if a similar effect can be obtained through co-infection of rice plants with nitrogen-fixing and IAA-producer endophytes are still underway. Selecting the best endophytic bacterial consortium could offer new perspectives to enhance nitrogen-fixation in non-legume crops.

## **PT-PU-27 How do phytohormones influence the composition of fungal endophyte communities in tomato roots?**

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Endophytes are microbes capable of colonizing the inner part of different plant tissues without causing disease symptoms. In some cases, they have beneficial effects for the host plant such as biotic and abiotic stress resistance and plant growth promotion. For this reason, the use of these microbes could have a major impact on agriculture worldwide. However, the plant-endophyte interaction involves very complex mechanisms starting from the recruitment of the microorganisms to the colonization of the surface of the plant tissue and then the inner part, with the need to escape the plant immune system. All these processes are regulated by different plant and endophyte signalling molecules necessary for the establishment of the plant-endophyte interaction. Phytohormones are among the signalling compounds known to play a significant role in this interaction, but little is known about the specific ways by which they influence recruitment and colonization of the host tissues. The aim of the current project is to go deeper into the role of these signalling compounds in plant-endophyte interactions.

A community analysis (endophyte isolation and amplicon sequencing) of endophytic fungi was conducted on roots of tomato (*Solanum lycopersicum*) mutants impaired in synthesis of specific phytohormones (specifically ethylene and jasmonic acid) in order to understand how these compounds influence the composition of the endophytic communities. After the characterization of the endophytic communities, fungal isolates whose root-colonization frequency appears to be influenced by the presence/absence of specific phytohormones were selected. In order to obtain a deeper understanding of the role of these compounds in the plant-endophyte interaction, the selected isolates are currently being screened by confocal microscopy and qPCR in order to identify candidates whose colonization rate is critically affected by the phytohormones of interest.

A transcriptomic analysis of tomato plants inoculated with the isolates selected from the screening will provide further clues as to which physiological mechanisms, associated with endophyte recruitment, are influenced by phytohormones.

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## **PT-PU-28 Effects of plant-growth-promoting rhizobacteria on barley under global warming-associated environmental factors**

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Atmospheric concentration of CO<sub>2</sub> is continuously increasing since the industrial revolution and may reach 550 ppm by the year 2050. Furthermore, given that environmental factors like CO<sub>2</sub>, temperature, and water availability will likely change simultaneously, it is difficult to make accurate predictions about crop production under elevated CO<sub>2</sub>, reaching implications for food security. Plant-growth-promoting rhizobacteria (PGPR) colonize the rhizosphere of many plant species and confer beneficial effects under environmental stresses. Furthermore, root exudates play a role in interactions between plant roots and other organisms present in the rhizosphere.

Only few reports have been published on PGPR as elicitors of tolerance to abiotic stresses, such as drought. Furthermore, little is known about the influence of environmental factors on root exudation patterns. Therefore, this study was conducted in order to investigate the effect of two commercially available PGPRs on the growth and root exudation of barley (*Hordeum vulgare* L.) under different CO<sub>2</sub> and water treatments.

In a growth chamber experiment climatic conditions of a field site close to Stuttgart were simulated. Barley plants were grown in pots filled with sand and exposed to ambient (380 ppm) or elevated (550 ppm) CO<sub>2</sub>. Plants received the normal daily amount of rainfall in the region of Stuttgart or 33% less. Plants were harvested at the stem elongation growth stage and when the inflorescences emerged. At both dates, data were collected on above and belowground variables. In addition, analyses of water use efficiency and composition of root exudates were performed. Preliminary results showed significant effects of the factors and their interactions on some of the measured variables.

In the context of climate change and agricultural sustainability, further studies with other crop plants are needed to demonstrate whether PGPR cause a range of crops to be tolerant to environmental stresses improving crop production.

**Microbe-assisted crop production, opportunities, challenges & needs**

**Vienna, Austria, 4- 7 December 2017**

***Poster Session 2: The holobiont plant: multitrophic interactions***

## PP-MI-01 Fighting against imperishable survival structures of phytopathogenic fungi

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Fungal pathogens such as *Sclerotinia sclerotiorum* and *Rhizoctonia solani* are the cause of extensive crop damage across the globe. Sclerotia, consisting of an outer black layer containing cells with high concentration of melanin and medullary hyphae with extended vacuoles, are formed by both fungi to persist severe environmental conditions. These survival structures preserve their viability and ability to infect potential hosts up to eight years. Microbial communities associated with both fungal pathogens were explored to identify potential biocontrol agents. Cultivation-dependent and -independent methods were combined to obtain deepening insights into the microbiome of sclerotia. Bacterial cultures were harvested from both model organisms and tested for their antagonistic potential against fungal pathogens. In a subsequent approach the most promising bacteria were screened for bioactive volatiles. Data from the 16S rRNA amplicon study showed that the microbiome associated with sclerotia of *Rhizoctonia solani* is influenced and closely related to the microbiota of the surrounding soil, while microbial communities of the potato peel display significant differences. Distinctive bacterial communities were associated with healthy and sclerotia-affected potato peel. *Flavobacteriaceae* and *Caulobacteraceae* were primarily found in unaffected areas, while *Phyllobacteriaceae* and *Bradyrhizobiaceae* were associated with the presence of sclerotia of *Rhizoctonia solani*. In addition to a wide range of *Bacillus* species, isolates assigned to the *Enterobacter*, *Pseudomonas* and *Buttiauxella* genus exhibited auspicious antagonistic activities. Among other substances, a wide range of alkylated pyrazines were detected in the volatilome of antagonists belonging to various species of *Bacillus*. Previous studies displayed the powerful antimicrobial effect of these diazine derivatives. These microbial volatile organic compounds (mVOCs) are able to alter the morphology of sclerotia and increase the layer of non-viable hyphae. Moreover, distinct combinations of preselected antagonists led to a significant increase in the pathogen inhibition. The so far conducted experiments set the basis for further molecular studies and will facilitate the identification of novel biocontrol agents.

## PP-MI-02 Use the soil's biocontrol force!

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The most common approach to use soil microbes for biocontrol of soil-borne diseases is to add pathogen-suppressing microbial strains to crops e.g. via seed coating. Whereas this approach can be successful, the inconsistency of effective control under different environmental conditions is often hampering broad-scale application.

Most strains in biocontrol products originate from soil environments. Therefore, it should also be possible to stimulate biocontrol microbes directly in the soil without a need to isolate them. The advantage is that such microbes are already adapted to local environmental conditions.

Here I will give an overview of the approaches that we examine to manage biocontrol activities of soil microbiomes: (1) stimulation of saprotrophic fungi, (2) stimulation of production of pathogen-suppressing volatiles, (3) stimulation of biocontrol strains via selective organic amendments.

Reference: De Boer, W (2017) Upscaling of fungal-bacterial interactions: from the lab to the field. *Curr. Opin. Microbiol.* 37:35-41.

### PP-MI-03 Investigations on the effect of potential biocontrol agents on stolbur phytoplasma infections

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Phytoplasmas are tiny wall-less bacteria that colonize and spread through the phloem system of plants causing symptoms like yellowing, phyllody, decline or dieback. Infections occur in many plants and can create substantial economic losses in high-value crops. As obligate trans-kingdom parasites, they inhabit several organs in insect vectors, which incorporate as well as transmit the disease while feeding on the plant's sap. In Europe, two candidate-species of phytoplasma are predominant on grapevine, associated with „Bois Noir“ (BN) and “*Flavescence dorée*” (FD). Containment options for this pathogen are so far largely limited to agricultural practices like pruning as well as the use of insecticides to reduce vector transmission. Both the difficulty of controlling the disease and the increasing demand for sustainable solutions in agriculture make microbial biocontrol agents an interesting source for developing new management strategies.

We are employing the model organism *Catharanthus roseus* to test the potential of biocontrol strains collected on healthy and from phytoplasma recovered fruit trees and grapevine plants to control BN. *C. roseus* were infected with the vector *Hyalesthes obsoletus* with phytoplasma strains prevalent in Austria and in Slovenia. Before graft transmission of these BN strains, test plants were treated twice with biocontrol strains. Along a visible symptom survey qPCR is performed to track the colonization capacity of the biocontrol strains as well as the spread of the phytoplasma invasion. Results obtained in these experiments will be presented and discussed.

Key words: *Candidatus* Phytoplasma solani, *Catharanthus roseus*, grapevine, Bois Noir, biological control agents

### PP-MI-04 A tale from Artemisia's haberdashery: secondary metabolites of an endophytic fungus generate ROS in Alternaria brassicicola

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Endophytic fungi are considered a spring of natural compounds with large applications in plant protection. One fungal endophyte, *Fusarium* sp. strain HRO8, was isolated from *Artemisia austriaca*, an aromatic and medicinal plant, from south-east coast of Romania. Preliminary assays of antagonist features showed this fungus interesting. Fungal solvent (methanol) extract was fractionated by vacuum liquid chromatography in silica gel. Fractions were eluted with mixtures of n-hex/AcOEt and AcOEt/MeOH, increasing the polarity over fractions. Resulted fractions were investigated for potential growth inhibitors of *Alternaria brassicicola*. Intracellular generation of oxidative products (ROS) was monitored on the strain *Abra43* by using dichlorodihydrofluorescein diacetate (H2DCF-DA). ROS were detected on 16h-old germinated conidia after exposure to 50 µg ml<sup>-1</sup> for 30, 60 and 120 minutes. After 30 minutes of incubation with ethyl acetate-methanol (90:10) fraction at 0.05 mg ml<sup>-1</sup>, H2DCF-DA-dependent fluorescence was observed in hyphae and conidia whereas no signal was recorded in untreated controls. This observation indicates that this polar fraction contains metabolites which are potential inducers of intracellular ROS in *Alternaria brassicicola*.

Key words: *Artemisia austriaca*, fungal endophyte, *Alternaria brassicicola*, oxidative stress, *Fusarium*

## PP-MI-05 Co-culturing of fungal strains against the phytopathogen *Botrytis cinerea* for the induction of chemical diversity, agrochemical and therapeutic agents

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Microbial communities have been successfully described as one of the most important resources for the discovery of novel drugs by the capacity to produce a broad diversity of secondary metabolites (SMs). A wide variety of applications are attributed to microbial natural products in human health, agriculture and other bio-industrial sectors. Microorganisms represent one of the most important resource to discover new bioactive SMs, but frequently are not expressed under standard laboratory conditions. Microbial interactions have been extensively reported in their ability to modulate fungal secondary metabolism and induce the production of new molecules<sup>1</sup>. Co-culturing of fungi has proved to be an efficient tool to simulate the physiological conditions that occur during microbial interactions in their natural environment and can be useful to induce the activation of cryptic biosynthetic pathways involved in antifungal SMs.

*Botrytis cinerea* is one of the most relevant phytopathogenic fungi in terms of scientific and economic impact<sup>2</sup>, and was selected as a model in this co-culturing approach for the induction of new SMs. A large set of fungal strains (762) ecologically-related with the plant pathogen were confronted using co-culturing methods on agar media, in order to identify potential producers of new antifungal SMs. A collection of extracts was generated from the antagonistic fungal interactions zones with potential of containing new bioactive molecules.

The antifungal specificity of active molecules induced after the fungal interaction with *B. cinerea* was screened against a panel of three further important plant pathogens (*Colletotrichum acutatum*, *Fusarium proliferatum* and *Magnaporthe grisea*) and their activity was also evaluated against the insect cell line (SF9) as potential pesticides. In addition, we analyzed and compared the chemical profiles of the co-culture antifungal extracts (93) with their counterpart fungal axenic extracts by LC-MS and identified a wide variety of induced known and novel compounds.

In conclusion, fungal co-culturing has permitted to trigger different microbial interactions that generated dramatic changes in the number and levels of SMs of chemical profiles of fungal strains. Then, setting up a methodology to induce new chemical diversity with wide potential both in drug discovery and crop protection.

## PP-MI-06 In-vivo monitoring of endophytic *Serendipita* spp. and the soilborne pathogen *Fusarium oxysporum* f. sp. *lycopersici* in tomato roots

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Sebacinales are a large order of fungi within the division of Basidiomycota known for their beneficial effects on a high number of vascular plants. Such effects comprise: (i) enhanced plant growth and yield, (ii) resistance against abiotic and biotic stress and (iii) altered root morphology and nutrient uptake. This is assumed to be achieved by a positive endophytism and various types of mycorrhizal associations (orchid mycorrhizae, ectomycorrhizae, ericoid and cavendishoid mycorrhizae, etc.). Previous experiments revealed bioprotective effects of selected *Serendipita* spp. against *Fusarium* wilt in tomato. However, the interactions of these fungi at the root surface and in the root cortex have not been investigated yet.

In this work, two candidates of the family Serendipitaceae (*Serendipita indica* and *S. vermifera*) were tested against the soilborne pathogen *Fusarium oxysporum* f. sp. *lycopersici* strain 007 (*Fol*). Agrobacterium-mediated transformation was performed to obtain expression of green fluorescent protein (GFP) in *Serendipita* spp. and tdTomato fluorescent protein (tdTom) in *Fol*, respectively. Thereafter, greenhouse experiments on tomato variety „Kremser Perle“ were performed where seedlings were co-inoculated with *Serendipita* spp. and *Fol*. Roots were removed from the substrate at regular intervals. Thereafter, pathogen and endophyte interactions on the root surface and inside the root cortex were visualized using confocal laser scanning microscopy (CLSM).

### **PP-MI-07 Investigation on the combined effect of Arbuscular Mycorrhiza Fungal (AMF) inoculation with compost on yield and nutrient uptake of *Lactuca sativa* plants.**

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To achieve global food security, we will need to produce more food, and do so in an environmentally sustainable manner. As inorganic fertilizers are becoming increasingly scarce and expensive, we also need to consider other options for providing agricultural plants with nutrients. To this end, there has been increased interest in making better use of the nutrients tied up in organic amendments like compost or/and to understand and use the beneficial activity of rhizosphere microorganisms that enhance plant nutrient acquisition, like arbuscular mycorrhiza fungi (AMF). However, limited information is available on the combined application of AMF inoculants and composted organic wastes and their effects on crop productivity and soil fertility. A pot experiment under greenhouse conditions was conducted to evaluate the effects of inoculation with three different autochthonous AMF inoculants, previously isolated from our lab and their mixture, in the presence and absence of a commercial compost (derived from poultry wastes and *Pleurotus* growth-substrate residues) on lettuce (*Lactuca sativa*) growth and nutrient status. Pots were filled with a sandy alkaline soil and three different doses of compost were applied including control (0%, 3%, 8%). Moreover, we investigated the effect of compost application dose on AMF colonization & spore density and we evaluated treatment effects on soil metabolic profiles. Compost applications alone resulted in reduced soil pH, increased P availability in soil and increased P in plants. While aboveground biomass of lettuce was generally increased by single AMF inoculations in the absence of compost (an effect combined with higher colonization and P in plants), the opposite trend was observed in the high compost application dose. Mixing the AMF inocula, however, does not appear to affect plant performance. The results are discussed in relation to changes in soil nutrient status and metabolic capacity.

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### **PP-MI-08 Interaction of *Piriformospora indica* with the mycophagous *Collimonas fungivorans* and the nitrogen fixing *Kosakonia radicincitans***

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Soil microorganisms play an important role in improving plant nutrition by providing key nutrients such as phosphate and nitrogen. Beneficial fungi have been used as bio-inoculants under a controlled environment to improve plant performance but these effects are often not reproducible in the field. One possible reason for this could be the attack by mycophagous bacteria, which can feed on living fungal hyphae. *Piriformospora indica* is a plant growth-promoting fungus with high potential for application in plant production systems. In order to improve its performance it will be important to know, if the fungus could be host for mycophagous bacteria and if hyphae-colonizing beneficial bacteria can protect the fungus against mycophagy. *Collimonas fungivorans* is a well-studied mycophagous bacterium which feed on different types of fungi by feeding on the fungal hyphae. These bacteria are well distributed in different ecosystems therefore it is important to understand if they can feed on beneficial fungus thereby affecting the stability of the fungal bio-inoculants in the field, while *Kosakonia radicincitans* is able to fix atmospheric nitrogen and could therefore support the beneficial effects of *P. indica* on plants.

In the present study, we are trying to understand the interaction between these two groups of microorganisms. The work here aims to elucidate the mechanism of interaction and its impact on the fungus. The preliminary data suggest a differential nutritional interaction between the two bacteria and the fungus.

Keywords: Bacterial-fungal interactions, Plant nutrition, mycophagous bacteria, endophytic fungi

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## PP-MI-09 Viruses can positively affect plant physiology: the case study of *Chenopodium quinoa* mitovirus 1

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The family *Narnaviridae* comprise two genera of positive single-stranded (ss) RNA viruses, the *Narnavirus* and the *Mitovirus*: both genera comprise species that replicate in fungal hosts. The mitoviruses described so far are able to infect only mitochondria of filamentous fungi and in many cases they were associated to hypovirulence. An intriguing observation is that the *Mitovirus* genus find the closest relative species in the *Leviviridae* family, the only known group of (+)ssRNA bacterial phages, which are more close to mitoviruses than the other genus *Narnavirus* in the *Narnaviridae* family. This fact could be explained by the theory of mitochondria derived from an alphaproteobacterial endosymbiont, and so mitoviruses appear most likely to be derived from an ancestral mitochondrial phage by losing the capsid protein (CP).

The use of next generation sequencing (NGS) technique revealed the presence of non-retroviral endogenous RNA viral elements (NERVES): complete genomes or partial/complete genes sequences of RNA viruses are found in almost all the eukaryotic nuclear genomes. Moreover the widespread occurrence of mitoviral sequences into many plant nuclear and mitochondrial genomes was demonstrated. Two different theories were proposed to explain such occurrence: the first hypothesizes the possibilities of diverse integration events of a fungal mitovirus, or of a native plant virus into the mitochondrial genome, and from here to the nucleus as a result of mitochondrial DNA transfer; the second proposes the possibility of integration of such sequences in plant genomes via fungal mediated horizontal gene transfer (HGT) during the long-term coevolution of fungi and plants.

We are here reporting for the first time the complete genome sequence of a “bona fidae” replicating plant mitovirus detected in two different commercial isolates of *Chenopodium quinoa* and designated as *Chenopodium quinoa* mitovirus 1 (CqMV1). We then assessed the interaction between the virus and the host plant observing positive effects during infection with other pathogenic viruses or after long exposure to heat stress.

## PP-MI-10 Maximising fungal endophyte discovery for biological control

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Endophytes are microorganisms that live inside plants without causing symptoms of disease and some can improve disease resistance. Although, the interest in using microorganisms as biological control agents of plant diseases is increasing, no common discovery strategy has been agreed upon within the scientific community to maximize endophyte discovery and explore their efficacy. In this project the three most commonly used culture media described in the literature were tested in order to determine and compare their suitability for fungal endophyte isolation. Furthermore three fungal DNA barcoding regions were used and evaluated for their specificity. The root endophytes were isolated from a wild relative of barley and wheat, *Elymus repens*, and sampled in a field that had high disease pressure from soil borne diseases such as Fusarium head blight and Take-all. The assumption is that the diversity of endophytes will be higher in perennial crop wild relatives compared to the annual crops themselves. Furthermore because of taxonomic similarity, and the fact that the wild relatives were taken from the environment the crops were growing in, it is hypothesized that the endophytes can be transferred to barley and work against soil borne diseases. The roots were surface sterilised using standard techniques and emerging fungi were cultured on the three different media, 2 % MEA, MEA and PDA. The fungal strains were identified using *TEF1α*, *ITS* and *LSU* DNA barcoding regions. Furthermore, root samples were prepared for next generation sequencing in order to primarily determine the full diversity of fungal root endophytes and secondly investigate the bias of the culturing method. To date, more than 140 endophytes have been cultured from 10 plants. 22 cultures were isolated on 2 % MEA, 61 cultures from MEA and 58 cultures from PDA. The fungal species richness will be discussed in relation to the individual plant, the different media and compared to the diversity discovered using next generation sequencing.

## PP-MI-11 Dynamics and diversity of native and inoculated diazotrophic bacteria associated to rice roots

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Cultivated rice (*Oryza sativa*) is an important staple crop in the world and Nitrogen is frequently the limiting nutrient for its production. Therefore, the exploitation of biological nitrogen fixation would significantly contribute to long-term rice productivity and sustainability. For this reason, diazotrophy is one of the main relevant traits searched in Plant Growth Promoting Bacteria (PGPB) and several successful commercial inoculants, used extensively in South America, contain diazotrophs. Many of these bacteria are strains of the genera *Herbaspirillum* or *Azospirillum* that interact with plants promoting its growth for more than one mechanism. These strains have been isolated from plant tissues of several crops and their success depends on their ability to overgrowth or survive facing to autochthonous bacteria from seeds or soil which are able to colonize vegetal tissues. The inoculation with diazotrophic PGPB certainly alters the native plant associated microbiome since the high amounts of the PGPB inoculated can replace native bacteria. In a long-term perspective, this practice could threaten the diversity of native bacteria that can eventually be better plant growth promoters or better adapted to the changing environmental conditions. In this work, we studied the dynamics of diazotrophic bacteria associated to rice roots in non-inoculated plants as well as in plants inoculated with *H. seropedicae* z67 or *A. brasilense* Az39. The density of diazotrophs, *Azospirillum* and *Herbaspirillum* bacteria in rice roots was monitored by qPCR along 75 days of cultivation in soil in a controlled experiment. The diversity of diazotrophs was analyzed by High Throughput Sequencing of the *nifH* gene after 10 days of seedlings transplanting to the soil and compared in non-inoculated and *Herbaspirillum* or *Azospirillum* inoculated plants. The results revealed that the inoculation with *Azospirillum* or *Herbaspirillum* did not exclude the colonization of rice roots by other diazotrophs. Moreover, non-inoculated plants showed similar density of *nifH* gene copies than inoculated plants along the all period of time analyzed. The inoculation did not exclude completely other diazotrophic bacteria, though *Azospirillum* and *Herbaspirillum* exhibited different ability for colonization of rice roots.

## PP-MI-12 Seed-borne Metaorganism Evolution of Wheat

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There is evidence that bacterial populations over time can be re-established in the phyllosphere to form the same composition as observed in previous plant generations. These findings suggest conserved mechanism of microbial uptake from the environment and by the maintenance of an internal reservoir transmitted from generation to generation via seeds. Here, we examine the microbial community composition of wheat seeds as a conceivable way of early introduction of endophytes into seedlings. We hypothesise that competition among endophytes is weak during early seedling growth. We aim to identify a core community of seed-associated microbes migrating to seedlings and we propose to unravel the dynamics of community composition across generations. To do this, we use wild and domesticated wheat seed material derived from Southeast Turkey located in the Fertile Crescent, the center of origin of wheat collected over different growth seasons from different soil types. We germinate seeds under sterile conditions and characterize microbes migrating from seeds to seedlings. Ultimately, we aim to characterize microbial species tightly associated with wheat seed. Microbial species is characterized by 16S and ITS sequencing using a MiSeq platform. Already first sequencing run has allowed to identify different microbial species in sterilized wheat seeds and seedlings including the fungal species from *Alternaria* and *Antrrodiaella* genus, the bacterial species from *Pseudomonas*, *Luteibacter* and *Halomonas* genus. Some of these microbes are associated with plant growth promotion in previous studies. As a future work, the species identified to be part of the core community will be isolated to obtain pure cultures. The culture collections will serve to decrease the inherent complexity of the communities and used for inoculation experiments to study binary plant-microbe interactions. Also, they will allow us to establish whole-genome reference sequences of the core community.

### PP-MI-13 Evaluating the native microbiota of *in vitro* banana plants and the effect of *B. subtilis* EA-CB0575 on the banana plant as holobiont

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The strain *B. subtilis* EA-CB0575, isolated from the rhizosphere of a banana plant in Colombia, is a plant-growth-promoting rhizobacteria of banana plants and other crops. In this study, we evaluated the composition of the native microbiota of banana plant *in vitro* and effect of the application of *B. subtilis* EA-CB0575 on the culturable endophyte microbiota of banana plants grown in Murashig Skoog (MS) medium. We determined that the application of EA-CB0575 affected the microbial diversity of the inoculated plants when compared to the diversity of the control (non-inoculated plants), reducing the richness and abundance of other species other than *Bacillus*. Non-inoculated plants had a population size of culturable endophyte microbes of  $7,76 \times 10^4$  CFU/g (fw) of root with a high proportion (46,4 - 92,4%) been *Bacillus* species, from which 83% of them were identified as *B. subtilis* by 16s rDNA sequencing. It was determined that the non-inoculated microbiota was composed, in a lower quantity, by the genera *Paenibacillus*, *Pseudomonas*, *Methylobacter*, *Microbacterium*, *Staphylococcus*, *Micococcus*, *Rhodopseudomonas*, among others. On the other side, the treated plants had a population size of  $1,35 \times 10^8$  CFU/g of root and a highest proportion of *Bacillus* species (around 99% respect to total culturable population). Finally, the presence of strain EA-CB0575 and other native microorganisms on banana plants grown on MS cultures *in vitro* was determined using FISH and CARD-FISH techniques. The strain EA-CB0575 was detected until 30 days post inoculation using the specific probe Bsub-ss-0463-aA-22A and the universal probe EUBMIX 338, and the presence of other native microorganisms were also observed as they did not hybridize with the specific probe. These results suggest that EA-CB0575 colonize the roots and compete for space and nutrients displacing other native species.

### PP-MI-14 Impact of apple microbiota on *Pichia anomala* strain K, a biocontrol yeast against apple major postharvest diseases

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The yeast *Pichia anomala* strain K is a Biological Control Agent (BCA) against two postharvest apple pathogens (*Penicillium* spp. and *Botrytis cinerea*). Progress has been made during the past two decades to understand the modes of action of the strain K through various studies (microbiology, enzymatic, genomic, transcriptomic and proteomic). Nevertheless, BCAs commercial application has been hampered by low or non-reliable efficacies in comparison to fungicide treatments (Droby et al., 2016). Massart et al. (2015) identified new alternatives to improve BCA efficacy using microbiota. Once applied on the fruit surface, a BCA will face a complex microbiota where ecological interactions such as parasitism, mutualism and commensalism occur, thus affecting its efficacy. In this study, we evaluate the potential of apple microbiota to influence the efficacy of strain K. Apple fruit samples of seventeen varieties grown in four disease management practices have been collected and their epiphytic microbiota harvested to create a bank of apple microbiota to be screened. Biological assays on apple fruits have been carried out by co-inoculating each apple microbiota with strain K and *B. cinerea*. The results of the preliminary assays revealed that the apple microbiota can either raise, drop or have no effect on the efficacy of the strain K. Study is ongoing to identify ecological strains/species or groups of taxa which are beneficial to strain K efficacy.

Keywords: Microbiota; Strain K; Apple

#### References:

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## **PP-MI-15 Carbohydrate metabolism and production of phytohormones in interactions of *Piriformospora indica* and *Sebacina herbamans* with their plant partner**

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Carbohydrate allocation in plants plays a primary role in economy and human food consumption. The storage of sugars in specific organs is an important driver to yield plant products with high nutritional value. *Piriformospora indica* (recently renamed *Serendipita indica*) and *Serendipita herbamans* are root endophytic fungi of the order Sebaciales; they can live in symbiosis with different species of plants without negative interference with plant health. *P. indica* possess growth promoting effects by changing the host metabolism probably through the secretion of particular proteins and metabolites including phytohormones. The resulting holosymbiont shows metabolic behaviours different from the plant alone. The current project is aimed to understand the relation between carbohydrate metabolism and phytohormone balance in order to reveal the mechanisms underlying the plant growth-promoting effects.

*P. indica* and *S. herbamans* show particular carbon source preferences. They produce most efficiently biomass if supplied with sucrose, glucose, fructose or mannose. Moreover, secondary metabolites of both fungi can boost carbohydrate metabolism with a significant turnover of sucrose, glucose and fructose in tomato roots. Genes for invertase and phosphoglucose-isomerase are present in their genomes and the corresponding proteins could be detected in the secretome of *P. indica* only in co-cultivation with the plant. Measuring extracellular enzymatic activities under different conditions show amongst other things that a particular levansucrase could be also involved in carbohydrate metabolism. The presence of genes for auxin biosynthesis have been already shown; in addition, genes encoding enzymes involved in the biosynthesis of cytokinins, gibberellins, brassinosteroids and ABA have been detected in *P. indica* and *S. herbamans* genomes. Interaction with plant mutants show that the fungi can produce compounds which complement phytohormone deficiencies of their plant partner. Keywords: endophytic fungi, carbohydrates, phytohormones, plant growth promoting effects.

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## **PP-MI-16 Bacterial helpers to make intimate association with the mycorrhiza-like *Serendipita indica* and to enhance plant resistance against plant pathogens**

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Some bacterial endophytes are known to increase plant growth and enhance plant fitness (tolerance to biotic and abiotic stresses). However, some fungi can do this as well. *Serendipita indica* (syn. *Piriformospora indica*) is, for instance, a root-colonizing endophytic fungus that boosts plant vigor and confers resistance against plant pathogens. This fungus is further known as having a bacterial endosymbiont living inside its hyphae. However, we aimed to boost effects of the fungus and its bacterial symbiont on plants by combining the fungus and its symbiont with some new bacterial helpers. A collection of bacteria from roots of potato and tomato plants were isolated in this way and combined them with the beneficial fungus *Serendipita indica* in order to study the type of interaction. Some endophytes belonging to *Tardiphaga*, *Mycobacterium*, *Burkholderia* or *Methylobacterium* can stimulate *Serendipita* growth, and colonize its hyphae. Some of these bacteria can further help to reduce tomato disease caused by *Fusarium oxysporum*, while others do not. Genomes of selected isolates have been sequenced and annotated to understand more the genomic contents of the bacterial helpers. The future transcriptomics analyses of tomato plants inoculated with these microorganisms will unravel the mechanisms behind these interactions, identifying genes up- and down-regulated during the interplay. The mechanisms of the bacterial helpers on the fungus and its symbiont will be further elucidated to understand better the multi-partite interactions between a fungus, bacterial symbiont, the helpers, and the plant.

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## **PP-MI-17 Antagonistic interaction between biocontrol strains, implications for biological plant control against soft rot disease**

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Plant pathogens can cause up to 40% of yield losses and huge deterioration in quality. Traditional methods used against plant pathogens, mainly dependent on the usage of chemicals, although reliable, pose a major threat towards natural environment. Because of that, biological plant protection, as an ecologically friendly alternative, is becoming more appreciated. It relies, inter alia, on the usage of beneficial bacteria to fight plant pathogens. Soft Rot Enterobacteriaceae (SRE) are one of the most important bacterial plant pathogens causing soft rot disease of potato tubers in storage and black leg of potato plants in the field. Strains used for biocontrol purposes must be highly competitive and able to contradict a broad range of pathogens. This raises a question if the usage of high inoculum of such bacteria would not wreck the natural balance of microorganisms in the rhizosphere. Volatiles are important secondary metabolites, that can play an important role in signaling and antimicrobial activity. In this project, I am studying antagonistic interactions between five biocontrol strains and SRE and the role of volatiles. Those antagonistic have antimicrobial properties against SRE species and suppress soft rot on potato. Interactions between antagonistic bacteria can alter their ability to suppress growth of pathogens. Examination of relations between microorganisms in the process of biological plant control may help in the development of new products. Usage of bacteria as biological control agents faces many limitations, and still, cannot be broadly used. Better understanding of biocontrol agents ecology will help to overcome those limitations.

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## **PP-MI-18 Improving the benefits of mycorrhizal associations in tomato plant health**

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Soilborne pathogens such as *Fusarium oxysporum* f.sp. *lycopersici* are difficult to control and can cause serious yield losses in tomato production worldwide. The use of arbuscular mycorrhizal fungi (AMF) with their bioprotective aspects shows great promise for the management of plant diseases in sustainable agriculture. However, bioprotective effects of AMF alone are limited but still can be effective elements in disease management strategies. Here, we investigated if combining AMF with intercropping or with the application of biochar impact on tomato plant health under *Fusarium* wilt disease stress.

In the first approach AMF were tested against *Fusarium oxysporum* f. sp. *lycopersici* (Fol) in tomato intercropped with either leek, cucumber, basil, fennel or tomato itself. In the second approach AMF were combined with two different types of biochar (greenwaste biochar, woodchip biochar) and were investigated in the same Fol-tomato pathosystem in the greenhouse.

The intercropping partners leek, cucumber, basil and tomato had no effect on Fol disease incidence or disease severity. Nonetheless, bioprotective effects of AMF resulting in the decrease of Fol disease severity were evident in treatments with AMF and Fol co-inoculation in the tomato/leek and tomato/basil combination. Furthermore, the intercropping partner affected the arbuscular mycorrhizal (AM) root colonization of tomato. Tomato intercropped with leek showed a 20 % higher AM colonisation rate than tomato intercropped with tomato. The addition of biochar did not alter AM root colonisation in tomato. However, a bioprotective effect of mycorrhization was evident in both biochar treatments.

In conclusion, combining AMF with other measures such as intercropping or biochar application can increase the bioprotective effects of AMF. Further studies are needed to test other combination strategies.

## PT-MI-19 Bio-effectors in the rhizosphere: Microbial consortia as inoculants for improved plant growth and mineral nutrition

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In contrast to selection of single microbial strains with high efficiency for plant growth promotion, the use of large consortia of different (Plant Growth-Promoting Microorganisms, PGPMs) may be an alternative strategy to induce beneficial effects on plant performance, with the advantage of higher flexibility towards variability of environmental conditions due to selective promotion of the best-adapted strains, depending on the respective rhizosphere conditions. In this study, the effects of a commercial microbial consortium product (MCP) with different fungal and bacterial PGPMs on early growth of maize were tested in pot experiments with different levels of N and P supply, including a functional characterization of inoculant effects on marker enzymes for N, P and C cycling in the rhizosphere.

Maize plants (cv. Jessy) were cultivated on two soils with different soil properties. Fertilization with N140 in nitrate and stabilized ammonium form, K150, Mg50 and P50; P30 respectively as reduced P fertilization was performed. The MCP was applied by fortnightly fertigation. Measurements of shoot and root growth and functional characterization of C,P,N turnover in the rhizosphere using marker enzyme assays with fluorogenic substrates and auxin production potential were conducted in 3 and 6 weeks after sowing (WAS). Culture-dependent re-isolation of rhizosphere bacterial populations was performed with standard media. The auxin production potential of re-isolated populations was evaluated spectrophotometrically.

The application of the MCP induced significant stimulation of shoot and root biomass production and could compensate for reduced input of N and/or P. This was associated with increased fine root production and improved P-nutritional status, particularly in the inoculated variants with reduced nutrient input. Re-isolation of cultivable bacteria revealed a significant increase in total colony forming units in the inoculated variant even 6 WAS.

The results suggest that the MCP-induced beneficial effects on plant growth and nutrient acquisition in maize could be mainly attributed to root growth stimulation, while indications for direct interactions of the inoculants with nutrient turnover in the rhizosphere were partially confirmed only in the early growth stages. These findings demonstrate a clear rhizosphere effect of the inoculants with impact on the native microflora which deserves a more detailed investigation e.g by a metagenomics approach.

## PT-MI-20 Cooperation of *Trichoderma* spp. and *Bacillus* spp. in biocontrol applications

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*Trichoderma* spp. and *Bacillus* spp. are known as potent plant beneficial organisms, which can alleviate abiotic stress and exert a protective effect against pathogens. Therefore we aimed at selection of appropriate strains from both genera, which are suitable for combined application in agriculture for plant protection. For biocontrol assays, we isolated *Diaporthe sojae* from contaminated soybean seeds with deleterious effect on seed germination.

Efficient and poor biocontrol strains of *Trichoderma* as selected from plate assays and greenhouse tests were included in the assays in order to determine characteristic effects for efficient plant protection. Strains were additionally tested for growth at low temperatures, when seeds are particularly vulnerable to pathogens. Moreover we tested, whether the strains were able to recognize organisms in their environment by analyzing their chemotropic response to exudates of *D. sojae*, *Bacillus* spp. and soybean.

Then we selected efficient *Trichoderma* and *Bacillus* strains and tested whether they antagonize each other or are suitable for co-cultivation. Suitable combinations were then tested individually and in combination in greenhouse assays, which revealed a positive effect on germination individually as well as a protective function against *D. sojae* in several cases. Thereby we could identify a favorable combination of *Trichoderma* and *Bacillus*, which will be subject to further testing towards applications in agriculture.

## PT-MI-21 Biocontrol of *Fusarium* crown and root rot in wheat by inhibitory *Streptomyces* isolates – it's complicated

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The predominant causal agents of *Fusarium* crown and root rot (FCR) in wheat, along with *Fusarium pseudograminearum*, are *F. graminearum* and *F. culmorum*. Members of the genus *Streptomyces* have been shown to inhibit various *Fusarium* species, and *Fusarium* is also able to inhibit *Streptomyces* in vitro. However, little is known about these complex interactions and the potential for inhibitory *Streptomyces* to reduce FCR in wheat. The aim of this study was to analyze whether inhibitory *Streptomyces* isolates affect the disease development of FCR in wheat, and reduce root and stem base colonization by *Fusarium* spp. We enriched sterilized potting soil with spore suspensions of *Streptomyces* isolates, inoculated the soil with *F. culmorum*-colonized wheat straw, and planted pre-germinated wheat seedlings. At four weeks, inoculation with *F. culmorum* led to significant disease symptoms on roots and reduced the fresh weight of roots and above-ground plant biomass compared to the non-inoculated controls. Enrichment of soil with inhibitory *Streptomyces* isolate reduced levels of *F. culmorum* DNA in roots and stem bases by 75% compared inoculation with *F. culmorum* alone. Interestingly, co-inoculation of *F. culmorum* with the non-inhibitory *Streptomyces* isolate led to the highest levels *F. culmorum* DNA in stem base tissue and *Streptomyces* densities (CFU/g soil) in the rhizosphere five times greater than inoculation with *Streptomyces* only. In vitro assays testing the inhibition of *Fusarium* against *Streptomyces* revealed that *F. culmorum* showed a strong inhibitory activity against the inhibitory *Streptomyces* isolate but not against the non-inhibitory isolate. Sensitivity to inhibition by *Fusarium* varied widely among *Streptomyces* isolates (inhibition zones ranged from 0 to 10.7 mm). In contrast, there was little variation among *Fusarium* isolates in capacities to inhibit the collection of *Streptomyces* (inhibition zones ranged from 2.4 to 3.5 mm). The results of this study illustrate the inhibitory potential of *Streptomyces* for biocontrol of FCR in wheat, while suggesting that *Streptomyces-Fusarium* interactions appear to be highly isolate-specific. In conclusion, a broader understanding of the variation in susceptibility within *Fusarium* populations to *Streptomyces* inhibition, as well as the capacity of *Fusarium* spp. to inhibit antagonistic *Streptomyces* is needed, which are both keys to biocontrol advances.

## PT-MI-22 Back-to-the-roots: unraveling the endophytic microbiome in banana germplasm to control *Fusarium* wilt

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Banana is one of the world's most important staple crop in the tropics and sub-tropics. Banana originates from South/South-East Asia and is now also cultivated and commercially produced in Central and South America, Africa and Australia. The majority of commercial banana varieties are genetically identical, making them vulnerable to a myriad of pests and diseases. *Fusarium* wilt or Panama disease caused by soil-borne fungus *Fusarium oxysporum* f.sp. *cubense* (Foc) has been a major threat for banana production worldwide. In the 1950's, devastating Foc race 1 strains emerged and wiped out variety "Gros Michel" from banana plantations in Central and South America. Following this pandemic, the new resistant "Cavendish" varieties were introduced. Decades later, another strain of Foc, commonly referred to as Tropical Race 4 (TR4), emerged in South-East Asia and destroyed Cavendish plantations in the region. The epidemic also spread to the Middle-East, the Indian subcontinental and Africa. Currently, there are no effective measures to control Panama disease and new sustainable methods are urgently needed. This project aims to elucidate the diversity of endophytic bacterial communities in banana germplasm, including wild relatives, and their metabolic potential to control Panama disease. We focus our study in Indonesia, one of the banana gene centers, and sampled roots and pseudostems of susceptible and resistant banana varieties Cavendish and Rejang, respectively, from Foc-endemic fields in Sumatra. Exploring endophytic microbial diversity of the resistant Rejang banana may unravel competitive bacterial genera to protect the plant from Panama disease. The taxonomic diversity of the banana endophytic bacterial communities was assessed by 16S rRNA amplicon sequencing followed by targeted isolation of different endophytic bacterial genera. Results on the diversity and antifungal activity of the endophytic bacteria sampled from diverse banana germplasm will be presented.

**Microbe-assisted crop production, opportunities, challenges & needs**

**Vienna, Austria, 4- 7 December 2017**

***Poster Session 3: The holobiont plant: microbiome understanding***

## PP-MU-01 Assessing above and below ground bacterial communities and their secondary metabolite gene diversity in Asteraceae and Poaceae

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Plant hosts rich and diverse microbial communities and members of these communities play key roles in plant growth and health. Bacterial plant colonization from soil is influenced by root exudates and can take place in the rhizosphere or as endophytic colonization inside the tissues of root, shoot and leaves. Although soil may serve as a common bacterial reservoir for belowground and aboveground plant microbiota, microbiota composition, in particular, lower taxonomic ranks (genus and species level) are largely influenced by host compartment and host genotype. Specific nature of these interactions, the factors driving these host–microbial associations and the consequence on the genetic capacity of the associated microbiome remain largely unexplored.

We aim to study plant host as the main driver of endosphere microbial community and also investigate the factors that impact the bacterial community structure and composition in endosphere. We investigated the bacterial diversities from root and shoot endospheres of different plant species collected at three natural sites representing clay, lime stone and loam soils, from the families Asteraceae (*Scorzonera purpurea*, *Hieracium* sp. and *Leontodon incanus*) and Poaceae (*Stipa pennata*, *Poa nemorosa* and *Sesleria albicans*) to evaluate host factors in two families at three different locations. Using Illumina Miseq platform we sequenced three molecular markers: the V5 and V6 region of 16S rRNA, a species-specific bacterial marker based on a fragment of *gyrB* and a functional gene targeting the ACP domain of polyketide synthase from phylum Firmicutes. The genetic diversity within the polyketide synthase is an indicative of the secondary metabolite diversity and host specific factors for secondary metabolite potential of microorganisms is not well understood.

Preliminary results based on CAP plot on Bray-Curtis dissimilarity values (taxonomy assignments based on 13.8 greengenes database) revealed that shoot endosphere associated microbiomes are mostly clustered by host plant while root associated microbiomes are clustered by sampling site indicating the impact of host genotype and soil roles in shaping the microbial community structure. We further aim to evaluate which plant-specific associations remain consistent regardless of geographic locations.

## PP-MU-02 GENOME based insights of Rice seeds borne bacterial endophytes

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Plants are home to a diverse bacteria community that resides both on and inside plant. Our work focuses on genomics based approaches to get an integrative understanding of the distribution, diversity and evolution of bacterial endophytes associated with healthy rice seeds. We have isolated and characterized more than 200 pure bacterial endophytes based on diverse morphology and 16S rRNA typing. We further focus on whole genome profiling of representative bacterial species belonged to Firmicutes and Proteobacteria phyla generated using in-house Illumina-MiSeq platform. Further, bioinformatics tools were used for phylogeny, taxonomy and comparative genomics of these bacterial endophytes. *In silico* prediction of genes important for plant growth, development and protection as well as for endophytic lifestyle was performed. From the whole genome information of rice seed endophytic bacterial strains, a set of bacterial genes orthologs was identified that are predicted to be involved in determining the endophytic behavior in these strains. Majority of the genes identified by bioinformatics analysis encode for function suggested to be involved in stress tolerance and survival. Interestingly, detection of these genes provide strong support for their role in endophytic lifestyle and their functional contribution to rice.

### **PP-MU-03 Responses of the *Brassica napus* L. holobiont with focus on chloromethane emission under salt and temperature stress**

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*Brassica napus* L., or rapeseed, is a major oilseed crop in Europe and is increasingly cultivated for food, feed, and biodiesel production. Chloromethane (CH<sub>3</sub>Cl) is the most abundant halogenated organic compound in the atmosphere and is responsible for chlorine-mediated destruction of the ozone layer. Anthropogenic sources of CH<sub>3</sub>Cl became negligible since the Montreal Protocol (1987) came into force. Thus, natural sources, such as plants, became again substantial for the global budget which is imbalanced due to unrecognized sinks. The only known mechanism for plant-induced CH<sub>3</sub>Cl production is a by-product of the methyl-group-transferring HOL enzyme that is involved in plant defence. We proved in our study that rapeseed emits CH<sub>3</sub>Cl. Thus, cultivation of rapeseed may be a substantial source of CH<sub>3</sub>Cl. Rapeseed is subjected to environmental stresses induced by climate change, such as salinization of soil and heat periods. Plant-associated methylotrophs (microorganisms that utilize one-carbon compounds) degrade CH<sub>3</sub>Cl and hence gain a selective advantage while colonizing plants and counteract the emission from the plant. Thus, the understanding of response of the holobiont (plant incl. its microbiome) is a prerequisite to understand changes in emissions. Beyond, CH<sub>3</sub>Cl-degrading methylotrophs also thrive in forest soils and in the phyllo- and rhizospheres of grassland plants. Our experimental study aims to resolve the rapeseed holobiont's response to the environmental stresses salt and heat in regard to CH<sub>3</sub>Cl emission and its microbiome. We addressed with the rapeseed summer cultivar MAKRO the following objectives: (i) Determination of CH<sub>3</sub>Cl emission rates from individual rapeseed holobionts in differing NaCl and temperature treatments (pot-based experiments in phytotrons) and (ii) assessment of the rapeseed microbiome and the CH<sub>3</sub>Cl-degrading members through amplicon sequencing of bacterial 16S rRNA genes and functional gene markers. We identified methylotrophs that responded to salt and temperature stress in the phyllo- and rhizosphere of rapeseed and correlated those to the net emission rates. In addition, the dominant CH<sub>3</sub>Cl-degrading microbial community associated with forest soil and the phyllo- and rhizospheres of abundant plant species of managed grassland assessed by nucleic acid stable isotope probing (SIP) is used as reference information to identify rapeseed-specific CH<sub>3</sub>Cl degraders.

### **PP-MU-04 Ecological patterns of seed microbiota diversity, transmission and assembly**

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Plants carry diverse microbial assemblages (known collectively as the plant microbiota) that can influence plant productivity through enhancement of biomass accumulation or disease resistance. Promotion of these plant traits by manipulation of microbial assemblages requires a fundamental knowledge on the processes that drive the assembly of the plant microbiota.

Seed-associated microbial assemblages are ecologically interesting because they both represent an endpoint and a starting point for community assembly of the plant microbiota. According to diversity surveys performed on seeds of various plant species, these assemblages are composed of bacterial and fungal taxa frequently observed in other plant habitats, which therefore suggest that the seed is the primary inoculum of the plant microbiota. Potential connections existing between seed and other plant habitats such as the anthosphere and the spermosphere will be examined in light of recent results obtained in our lab. Moreover, the ecological process (niche vs neutral) that drive the assembly of the seed microbiota will be discussed.

## PP-MU-05 Endophytes in tomato: understanding their structure and function

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Endophytes support their host plant to overcome abiotic and biotic stresses but their performance is less understood. In this study, endophytic microbiomes of two different cultivars of tomato (Moneymaker, Hildares F1) grown in two soil types (sandy loam, commercial tomato growing substrate) were examined by Illumina sequencing of 16S rRNA gene amplicons and processed by bioinformatics tools. Simultaneously, we tested 2,103 bacterial isolates for their antagonistic activity towards the plant pathogens *Fusarium oxysporum* and *Clavibacter michiganensis* (biotic stress) and for their potential to cope with high sodium chloride concentrations and desiccation (abiotic stress).

*Pseudomonadaceae* was the most dominant family among all plant compartments: up to 11.8% were found in seeds and 41.9% in the endorhiza, even if its abundance was below 2% in the soil. Uncultured bacteria composed 50.5% of the soil microbiomes with Anaerolineaceae (5.5%) representing the most abundant bacterial family. In the rhizosphere *Pseudomonadaceae* (7.0%), *Rheinheimera* (6.0%), *Cellvibrio* (5.0%), *Flavobacterium* (5.0%) were prevalent. Considering the number of unique OTUs, the differences among soil types and among seed cultivars were comparable (21.8% and 24.7%) when considering soil and seeds singularly. On the other hand, when considering how soil type and cultivar effect all plant compartments, unique OTUs differ by 13.5% among cultivars and by only 1.1% among soil types. A high proportion of bacterial antagonists was found within these microhabitats with 44.8% (939 isolates) activity towards *F. oxysporum* and 16.0% (150 isolates) towards *C. michiganensis*. Most antagonists tolerated high salt concentrations of up to 100% of the antagonists at 4.80% NaCl. Our results indicate a high potential of tomato endophytes as stress protecting agents.

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## PP-MU-06 The dynamics of microbial and environmental factors in shaping root microbiomes

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The root microbiota, consisting of diverse microbial communities in and around plant roots, can significantly influence plant development and stress tolerance. Understanding the communication between microbes in the rhizosphere and between plant roots and microbes is essential to decipher the beneficial potential of microbial communities. Interestingly, certain fungi such as members of the family Sebaciniales can be found in root microbiomes of different ecosystems worldwide and transfer various beneficial effects to plants. *Serendipita indica*, a member of the Sebaciniales, is a mutualistic fungal root endophyte that has been observed to improve plant performance and disease resistance to host plants. Considering the omnipresence of Sebaciniales, understanding the effect of *S. indica* on the composition of the rhizobiome of the model organism *Arabidopsis thaliana* and crop model organisms can reveal plant and/or microbe-derived communication patterns that contribute to the shaping of root microbiomes. Furthermore studying the effect of environmental factors is essential to understand the impact of the abiotic environment on those microbial interactions and inter-microbial communication. Being part of the Warwick Synthetic Biology Centre, the long-term aim of this study is to integrate molecular and environmental factors and use this knowledge to generate customized beneficial microbiomes that can be applied to sustain crop productivity.

## PP-MU-07 Impact of using different primer sets on abundance and diversity of native diazotrophic communities associated with irrigated rice plants

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Biological nitrogen fixation is a well-known process that has been extensively studied by its major role in sustainability of agricultural systems. Nitrogen-fixing bacteria comprise a diverse physiological group belonging to not only Bacteria but also Archaea. Consequently, many primer sets have been designed and used to address diversity and abundance of this microbial guild. According to *in silico* studies performed by other authors, many primer sets commonly used does not present a good diversity coverage, particularly for anaerobes. In Uruguay, rice is sown once per year in late spring over dry soil. After 45–60 days (tillering), the plots are flooded and remain in this condition until one week before harvest. Biological nitrogen fixation is an oxygen-sensitive process. A previous work showed a remarkable change in diversity and abundance of diazotrophs after flooding. A wide variety of aerotolerant and anaerobic diazotrophic bacteria with diverse metabolic traits were retrieved from flooded rice roots by *nifH* pyrosequencing analyses using primers PolF/PolR (Poly et al. 2001).

In this work, the primers PolF/ PolR and F2/R6 (Marusina et al., 2001) were evaluated as methodological alternatives to address community structure of diazotrophic communities from rhizosphere and rice roots of different agricultural rotation systems. Three different crop rotation systems from a long-term paddy rice-summer crops rotation experiment were studied (intensive rice, rice- pasture, rice- soybean). A significant difference in abundance of *nifH* genes was retrieved with the two primer sets employed. The *nifH* gene abundances retrieved with primers PolF/ PolR were significantly higher than those obtained with primers F2/R6 for rice roots and rhizospheric soils. In addition, *nifH* abundances were higher in flooding than in the dry stage (roots and rhizospheric soil). T-RFLP of *nifH* gene with *AluI* revealed diverse diazotrophic communities with both primer sets. According to these analyses, roots and rhizospheric communities presented distinct communities, grouped in clustering analysis by type of sample and crop stage. According to the diversity indices obtained, primers F2/R6 retrieved a greater diversity of endophytic diazotrophic communities from rice in the dry stage of crop.

## PP-MU-08 Pan-Domain microbial community profiling using targeted capture of *cpn60* gene fragments

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Molecular profiling of complex microbial communities has become the basis for examining the relationship between microbiome composition and structure and metabolic functions of those communities. At present, microbial community structure is assessed using PCR targeting taxonomic or functional gene markers, or increasingly, shotgun metagenomic DNA sequencing. We present a novel hybridization method (capture-seq) for profiling complex microbial communities using capture probes based on the chaperonin-60 universal target that provides the ability to generate an in-depth, pan-Domain community profile with significantly less expenditure and sequencing effort than a shotgun metagenomic sequencing approach. Molecular microbial profiles were generated for both a synthetic bacterial community and antibiotic-amended soil samples using universal target amplification, shotgun metagenomic sequencing, and capture-seq hybridization protocols. The capture-seq method generated a dataset of chaperonin-enriched sequences that provided a microbial community profile with much greater depth and sensitivity than shotgun metagenomic sequencing while minimizing the bias effects associated with universal target amplification. The resulting community profile encompassed bacterial, archaeal and eukaryotic Domains while requiring significantly less sequencing effort than shotgun metagenomic sequencing. Chaperonin-based capture-seq microbiome profiling generated a sensitive, pan-Domain microbial community profile that balanced the sampling depth of PCR with the unbiased output of shotgun metagenomic DNA sequencing.

## PP-MU-09 Understanding the microbiome of potato tubers during storage

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All plants and plant tissues are colonized by more or less complex microbial communities. Plant microbiomes are known for their importance for healthy growth and development of the host. Strong effort has been made to understand the microbial community composition and dynamics in crop plants during vegetative growth. However, there is hardly any information on the microbiome (composition and dynamics) of crops during crop storage.

The potato is the world's number four food crop and number one non-grain food commodity, with production reaching a record 325 million tons in 2007. Europe was 2007 second major potato producing region with 7.5 million hectares dedicated to potato production and 130 million tons production. In 2009 calculated post-harvest crop loss of roots and tuber was about 55% in developed countries. This was supported by recently published data for Switzerland showing that about 53% - 55% of the initial fresh potato productions and 41–46% of the initial processing potato production are lost across the whole potato value chain mostly due to pathogen infection, scarification, water loss of tubers, and early sprouting during storage.

The post-harvest life of potato tubers ranges up to 270 days. Storage stability of tubers is known to depend on the potato variety but also on storage conditions like temperature and sprout suppressants. However, the influence of the tuber microbiome on storage stability of potatoes is entirely unknown and this prompted us to study the bacterial community composition and dynamics in tuber of selected potato varieties with good and poor storage stability (i.e. Ditta, Agata, Hermes, Lady Claire and Fabiola) during storage. Potatoes were grown in agricultural soils from different regions in lower Austria. After harvesting, samples were taken at defined time points from dormancy stage until sprouting. Potato tuber associated microbiomes were analyzed by next generation sequencing of 16S ribosomal RNA gene amplicons. The reproducible occurring microbiome was determined and statistical analysis revealed a strong influence of the cultivation soil on the microbiome composition and changing communities in tubers over time.

## PP-MU-10 Deciphering composition and function of the root microbiome of the desert legume plant *Indigofera argentea*

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Microbial communities in the rhizosphere play a prominent role in plant fitness under diverse environmental conditions. We hypothesize that the relationship between plant and microbiome is particularly important in case of desert plants that have to cope with multiple and critical threats such as nutrient deficiency, drought, salinity, high temperature. However, only a limited number of studies have been conducted to characterize microbial communities associated with desert plants. Therefore the contribution of the microbiome to plant fitness in deserted regions remains elusive. To obtain such insights we exploited salinified agricultural fields in Jizan, Saudi Arabia, which are scarcely populated with a single dominant species, the legume *Indigofera argentea*. By using a Meta-16S rDNA amplicon (V4 region) sequencing approach, the bacterial composition of *I. argentea* rhizosphere and root endophytic compartment was characterized. This revealed <1,000 operational taxonomic units (OTUs) that associate with roots of *I. argentea*. Of these root associated OTUs, only ~100 were enriched (fold change > 2,  $p < 0.001$ ) when compared to soil samples. This shows that *I. argentea* grown in Jizan desert soil has fairly simple bacterial microbiome. Representative strains of the predominant OTUs of the *I. argentea* rhizosphere and endophytic compartment have been isolated. Effects of these bacterial strains on growth of *I. argentea*, the model plant *Arabidopsis thaliana* and tomato (*Solanum lycopersicum*) is currently investigated.

## PP-MU-11 Isolation, functional characterisation and genomics-enabled discovery of bacterial catabolic adaptations for degradation of nitroaromatic contaminants in polluted soils

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Using plants and bacteria to improve the degradation of pollutants is an attractive solution for soil clean-up. However, enriching catabolically active microbes in soils has not always shown effective results, emphasising the need for additional research at this knowledge frontier. For this reason, we take a multidisciplinary approach to unearth rhizospheric bacterial catabolic and plant host modulation features using genome sequencing and bacterial community profiling of a native *Acer pseudoplatanus* in a deciduous forest on a military site. To begin, a metagenomic inventory was used to identify the most abundant genera that were markedly enriched in the rhizosphere to guide pure culture isolation. Isolates (n=400) were tested for their ability to degrade nitroaromatics like 2,4,6-trinitrotoluene (TNT) and nitrophenol, and their potential to promote plant growth under pollution conditions. One isolate, *Raoultella ornithinolytica* strain TNT, was able to significantly increase plant biomass, and concomitantly detoxify TNT via nitro-group reduction and ring-reduction whereby nitrite released from TNT was used for growth. Draft genome sequencing confirmed the presence of nitroreductases including Old Yellow Enzyme homologues. Another isolate and novel species, *Novosphingobium rhizosphericus* ST904, was able to completely mineralise 4-nitrophenol. The presence of a 4-nitrophenol monooxygenase gene cluster (*pnpA-D*) was confirmed. Draft genome sequencing also revealed the presence of multiple plant growth-affiliated traits, including plant hormone production, plant-microbe communication, and root colonisation. In all, we show that an integrated approach promotes cultivation of catabolically important strains, and furthers insights in their degradative potential and physiology, which is crucial for effective inoculum design for phytoremediation.

## PP-MU-12 The rhizosphere microbiome as possible inducer of priming in barley

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Priming is a mechanism which leads to a unique physiological state that allows plants to enhance their protection against diverse pathogens. The response to biotic and abiotic stresses of primed plants appears to be faster and stronger than of unprimed plants. The strategy of resistance enhanced via priming was so far mainly investigated on the model plant *Arabidopsis*. Therefore, the knowledge of priming and the influence of inducing factors in barley is still scarce. The rhizosphere microbial community of plants is known to harbor several beneficial microbes which also might show the ability to induce priming by release of specific compounds, such as N-acyl homoserine lactones (AHLs). Cultivation-dependent as well as cultivation-independent methods allow a first insight into bacterial populations which are enriched in the rhizosphere of barley grown in different soils. Further studies using next generation sequencing techniques will include identification of rhizosphere bacterial community members with high abundance. Rhizosphere bacterial communities of two barley cultivars grown in different soils from known long-term field experiments were extracted and inoculated into the rhizosphere of barley plants grown in a substrate. The priming capacity of the different rhizosphere bacterial communities is quantified by their induced resistance towards the powdery mildew causing fungus *Blumeria graminis*. Furthermore, promising isolates will be collected and tested for priming-inducing compounds. The ability of specific rhizosphere microbial communities in barley to induce priming offers great potential for new plant breeding strategies.

### **PP-MU-13 Microbial communities of the roots of unique soybean (*Glycine max.*) germplasm in alkaline and non-alkaline soils**

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Plant roots are essential for nutrient and water uptake. The microbial communities present within the endosphere, rhizosphere, and soil near the plant roots play a vital role in plant development, nutrient acquisition and in some cases promote growth. This research aims to discover and characterize functional interactions between soil microorganisms and soybean roots. Due to limited iron availability in alkaline soils, iron deficiency causes decreased plant growth and a decline in yield. To study the microbial communities in a field setting, the endosphere, rhizosphere, and bulk soil of eight genotypes from an a soybean population segregating for iron deficiency chlorosis tolerance were grown in Valley, Nebraska. Genotypes were selected based on sensitivity and tolerance scores to alkaline soil. The genotypes consisted of two parent lines and six recombinant inbred lines that showed more extreme phenotypes than the parent lines when grown in alkaline soils. The field in Valley has naturally occurring streaks of higher pH with lower available iron and areas with more neutral pH. This provides an ideal study site to make comparisons between the microbial communities due to pH and the sensitive and tolerant soybean genotypes. The endosphere, rhizosphere, and bulk soil of soybean plants were collected at three growth stages. The V4 region of the 16s rRNA gene was sequenced using Illumina MiSeq and multiple bioinformatics analyses were conducted to provide data on the presence, absence, and abundance of microbial taxa. These data provide insight into the identity and composition of microbial communities associated with the endosphere, rhizosphere, and bulk soil of soybean plants. As part of these analyses, we are searching for microorganisms that aid plant growth in alkaline soils and have established culture collections to test microbes. In the future, testing of microorganisms that protect plants against the effects of alkaline soil in a controlled environment will allow for a deeper understanding of how microorganisms help plants acquire nutrients in these naturally occurring environments.

### **PP-MU-14 Response of soil microbiomes to alterations in soil phosphorus levels and consequences for alfalfa (*Medicago sativa*) growth**

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Sudden shifts in soil phosphorus (P) levels are common in agricultural systems relying on P fertilizer applications. However, little is known of the resiliency of soil microorganisms to changes in P pools and the effects on their plant hosts. In this experiment, we investigated how P amendments affected soil microbial composition in reference to *Medicago sativa* growth. A common starting microbiome was selected for the emergent phenotype of high *Medicago sativa* biomass under four different soil P amendment treatments: control (no P amendment), organic P (compost amendment), low inorganic P (moderate triple superphosphate (TSP) amendment), and high inorganic P (high TSP amendment). Following four generations of planting and selection, microbiome composition and hydrolytic enzyme activity was distinct in each treatment. Microbiomes from each nutrient regime were then reciprocally transplanted across all nutrient conditions, which caused most microbiomes to converge and resemble each other in the same contemporary soil conditions. The exception was microbiomes from the high TSP treatment, which maintained a unique compositional footprint in other soil treatments. Correspondingly, *Medicago sativa* grown with the high TSP microbiome had lower biomass, fewer nodules, and lower %N than plants grown in the same soil with other microbiomes. These findings suggest that chronic or excessive inorganic P fertilization may have a legacy effect on soil microbiomes that inhibits their ability to support plant growth.

## PP-MU-15 Factors affecting pathogenicity in the microbial ecology of *Pseudomonas syringae* pv. *actinidiae*

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The bacterial canker of kiwifruit, caused by *Pseudomonas syringae* pv. *actinidiae* (Psa), is the most severe disease of commercially cultivated kiwifruit species, since its pandemic outbreak in 2008. Although the infection may lead the host plants to death within one season, the pathogen is frequently found in the epiphytic microflora even in absence of serious plant symptoms. The infection with Psa leads to a dramatic rearrangement of the plant microbiome. In particular, other *Pseudomonas* spp. (notably, *P. viridiflava* and *P. syringae* pv. *syringae*) were found to grow epiphytically in association with Psa in the diseased tissues. Therefore, the present work investigates the role of the epiphytic microbiome in the transition from the epiphytic to the pathogenetic stage of Psa.

To investigate the influences of the microbial community, Psa was exposed to spent media and compounds derived from several bacteria populating the epiphytic niche, resulting in the promotion of genes related to exploration and virulence, as a consequence of an increased competition in the phyllosphere and, possibly, of a cooperative network with other pathogens.

Moreover, the epiphytic biocoenosis was screened to identify putative biological control agents. Among them, the occurrence of some species (*Bacillus subtilis*, *Pantoea agglomerans*) was strongly reduced by infection, whereas *Lactobacillus plantarum* populations were only moderately affected.

This work evidenced some factors contributing to Psa virulence, and determined by the biological contour of the pathogen. The control of such factors may mitigate the spread and/or severity of Psa. In addition, criteria for the selection of prospective biological control agents are proposed.

## PP-MU-16 Insights into endophytic bacterial community structures of seeds amongst various *Oryza sativa* L. rice genotypes

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This study aimed to investigate the endophytic bacterial communities amongst various rice seed genotypes and to further reveal the correlation between rice genotypes and their endophytic bacterial flora. Simple sequence repeats (SSR) molecular marker technology was used to investigate the genetic polymorphism in five different genotypes of rice. Endophytic bacterial communities in rice seeds were investigated using the Illumina based 16S rRNA gene. The results showed that there was high correlation between endophytic bacterial communities of seeds and their rice phylogeny. However, rice genotype had little impact on the diversity and richness of endophytic bacteria in seeds. The various rice genotypes do not have significantly different communities of endophytic bacterial in seeds, but the endophyte abundance distributions are obviously different, especially the dominant endophytic genera. Some phyla, such as Acidobacteria, Fusobacteria, Chlamydiae, and Gemmatimonadetes, were first detected in rice seeds using high-throughput sequencing technique. As expected, five different rice genotypes were found to have a shared microbiome. At the genus level, *Pantoea* (28.33%-72.77%, average=50.99%), *Acinetobacter* (0.16%-34.23%, average=16.72%) and *Xanthomonas* (3.20%-13.51%, average=9.40%), which are probably the core microflora in *indica* rice seeds, served as the dominant genera that coexisted in all rice seeds tested.

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## PP-MU-17 Fungal endophyte diversity of the tropical forage grass *Brachiaria*.

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Microbial endophytes can be beneficial to the host plant, providing or promoting resistance against biotic and abiotic stresses. Beneficial fungal endophytes have been well characterised in temperate forage grasses, in particular systemic asexual *Epichloë* species; strains of which have been commercialised to provide resistance against insect pests in pastoral systems. Knowledge about fungal endophytes of tropical forages is more limited in comparison to temperate grasses. Of particular interest is the forage grass *Brachiaria*, a native to Africa and highly cultivated throughout tropical regions of the world. Fungal endophytes in the genus *Acremonium* have been found to form endophytic associations with *Brachiaria* species. A strain of *A. implicatum* has been shown to improve *Brachiaria* resistance to leaf spot disease caused by fungal pathogen *Drechslera/Bipolaris* complex. Here, we have investigated the diversity of fungal endophytes associated with *Brachiaria*, and assessed their potential ability to provide defence against pathogens and pests found in Eastern Africa. A combination of methods have been used to identify and characterise fungal endophytes associated with *Brachiaria* species. Our collection of fungal strains isolated from the asymptomatic tiller material have been observed to form non-systemic associations with host plants. We also detected multiple strains of *Acremonium implicatum* and showed by molecular and morphological characterisation that this species is actually *Sarocladium terricola* and that the original *A. implicatum* strain reported is identical to our new strains. We have also found no indication that *S. terricola* is seed transmitted as was originally described. Future work will more intensively investigate via inoculation trials the mode of transmission of selected endophytes and evaluate the potential abiotic and/or biotic benefit(s) of these endophytes to commercial cultivars of *Brachiaria*.

## PP-MU-18 Maize-microbiome interactions under silver nanoparticle exposure

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Understanding the reaction of plants to changes in their microbiome can identify important microbial players and their role in plant growth and health. In agricultural phytotoxicity studies, silver nanoparticles are a likely cause of such changes in the microbiome as these nanoparticles are applied to agricultural fields through agrochemicals, biosolids and other sources.

Because of the complexity of plant-microbe interactions, they are often left out of the equation in phytotoxicity studies with environmental pollutants. This is also the case for the assessment of nanosilver toxicity. Silver nanoparticles are toxic to plants, as is shown by growth inhibition in multiple hydroponics studies, but are even more toxic to microorganisms. This toxicity to microorganisms is reflected in the discrepancy of phytotoxicity tests in soil and these in hydroponics or other artificial media, as we have found that nanosilver exposure in soil can increase, decrease and be neutral to plant growth in natural soil. This suggests the existence of a link between shifts in microbial community structure caused by nanosilver exposure, and plant growth.

In this work, we investigate the link between microbial community shifts and maize growth changes, caused by nanosilver exposure. Multiple exposure experiments are conducted where plant growth evaluation is combined with assessment of the rhizosphere and endophytic microbial community in terms of composition, activity and metabolic diversity. A detailed look into the possible mechanisms behind the effects is generated by a metatranscriptomics study. Overall, this work sheds more light on the role of the plant microbiome and highlights the importance of plant-microbe interactions in phytotoxicology studies and in maize cultivation.

## PP-MU-19 Simplified bacterial endophytic microbiome of rice

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Rice is currently the most important food crop in the world and we are only just beginning to study the bacterial associated microbiome, its diversity and potential role of these microorganisms in providing growth and fitness advantage to this crop. It is of importance to develop new plant-microbe models as well as simplified microbiomes for increasing our understanding about the formation and function of plant microbiomes. In order to begin to address this aspect, we have performed the isolation of 102 bacterial isolates obtained from endorhizosphere of two rice cultivars from Venezuela. The validation of plant-growth promoting (PGP) bacterial activities *in vitro* has led us to select and characterize 15 isolates for *in planta* studies such as germination test, endophytism ability and PGP. Consequently, a set of 10 isolates was selected for the set-up of two endophytic consortia as simplified models of the natural rice bacterial endomicrobiome. Consortium A was composed by the aforementioned 10 isolates and consortium B by the same 10 isolates plus 22 bacterial isolates from our collection of endophytes of rice grown in Italy. Upon inoculation, the colonization and abundance of each strain within the rice roots was tracked by a culture-independent technique in gnotobiotic conditions in a period of 30 days. Strains belonging to *Pseudomonas*, *Agrobacterium*, *Chryseobacterium*, *Herbaspirillum* and *Ensifer* genera have shown a promising capacity for colonizing and coexistence in root tissues. Other results of *in vitro*, *in planta* and consortia tracking will be presented, analyzed and discussed. This study is part of a growing body of research on synthetic microbiomes which strengthens the formation process of the endophytic community leading to a better understanding of the rice microbiome aimed at providing practical solutions for a more sustainable agriculture.

## PP-MU-20 Taxonomic characterisation of bacteria communities from water of diversified aquaponic systems

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In 2030, the world's population should reach 8.3 billion people and 60% of them will live in urban areas. It is thus necessary to develop intensive yet sustainable urban production systems in order to increase cities' resilience such as aquaponics which appears to be adapted for the cities. Aquaponics is a combination of hydroponic and aquaculture techniques and functions with plants, fish and microorganisms which play an important role in nitrification and mineralization of fish wastes into nutrients absorbable by plants. Herein we aim at characterizing the non-nitrifying bacteria present in diversified aquaponics systems to better understand the composition and role of these communities. To this end, 9 diversified aquaponic systems were sampled. The DNA from each bacteria community was extracted and sequenced with Illumina MiSeq technology by targeting the V1-V3 16S rDNA region. The sequences were analysed with the QIIME bioIT software. Results show that *Proteobacteria* and *Bacteroidetes* are the dominant phyla for all the aquaponic systems. Depending on the system, different proportions of *Verrucomicrobia*, *Actinobacteria*, *Planctomycetes*, *Fusobacteria* and *Nitrospirae* phyla are also present among the bacterial community. The genera which compose all the identified phyla are more diverse and an important proportion of them are usually found in soils and rhizosphere. One of the roles that could be linked to these genera is the breaking down of complex organic compounds which could be related to the mineralization phenomenon observed in aquaponic systems. Studies are ongoing to identify the relevant taxa or group of taxa that fit well to enhance the productivity of the aquaponic system.

Keywords: aquaponics, bacteria communities, bacteria's functions, NGS, 16S rDNA

## PP-MU-21 Comparison of root and panicle microbiota of *Setaria* spp. suggest vertical transmission and the contribution of insects to endophytic assemblages

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Virtually all studied plant tissues have been found to be internally inhabited by endophytes, which are investigated due to their implications in the growth, health and development of their hosts. While the drivers shaping root bacterial assemblages have been broadly described, communities colonizing plant reproductive organs still demand investigation. In this work, we dissected the bacterial assemblages of roots and ripe grain-harboring panicles of *Setaria viridis* and *Setaria pumila* collected from 15 different locations by 16S rRNA gene-based Illumina sequencing and furthermore genotyped plant populations. Bacterial assemblages were composed mainly by Gammaproteobacteria and were shaped primarily by the plant compartment, followed by the sampling site and lastly, by the plant species and genotype. Panicle-specific communities were governed by *Enterobacteriaceae* and included OTUs classified as insect endosymbionts (*Buchnera* and *Sodalis*), whereas root-specific assemblages showed higher diversity of bacterial taxa. Furthermore, 59 core OTUs were identified among roots and panicles of all sampling sites, from which 23 showed homologies to sequences described as uncultured seed microbiota members of rice, among other plant species. Additionally, this core microbiome included sequences with low homologies to existing entries in public databases and showed high abundances, suggesting that an important portion of the conserved *Setaria* microbiota may represent novel, thus uncharacterized taxa, which are potentially adapted to a life *in planta*. Altogether, these results suggest the contribution of plant-insect interactions to endophytic assemblages and point towards the existence of a highly conserved microbiota in *Setaria*, which persevere within individual plants irrespective of species and geographical location.

## PT-MU-22 Legacy effects of long-term organic or conventional farming practices on the rhizosphere microbiome and health of the next plant generation measured under controlled conditions.

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There is an increasing world-wide demand for sustainable food supply and the current challenge is to enhance the agricultural productivity, while avoiding long-term negative consequences for the quality of soils and the soil microbiome. To understand how long-term fertilization strategies affect the microbiome and plant health, soils were collected from two long-term field experiments (LTEs) established in 1978 (DOK, Therwil, Switzerland), and 2006 (HUB, Thyrow, Germany) having a documented history of conventional or organic farming. Lettuce (*Lactuca sativa*) plants were grown for 6 weeks in these soils under controlled conditions and leaves and rhizosphere sampled. The expression of 21 putative genes involved in the plant response to stress conditions was monitored using a RT-qPCR based system. Although, no clear effect of organic vs. mineral fertilization on plant growth was observed, comparing gene expression, similar patterns were observed in plants grown on organically managed soils despite different types and origins of soils. The expressions of several genes with documented roles in oxidative and biotic stress were up-regulated in plants grown in soils with long-term organic management. This might be triggered by the rhizosphere microbiome. Results from denaturing gradient gel electrophoresis and Illumina sequencing of 16S rRNA gene amplicons showed significant differences in bacterial and archaeal communities between soils that originated from different sites and different long-term fertilization. Interestingly, organic fertilization resulted in a higher rhizosphere bacterial diversity compared to mineral fertilization in both LTEs. A higher relative abundance of *Firmicutes* (mostly belonging to genera *Bacillus* and *Paenibacillus*) in rhizosphere of both organically fertilized soils was also observed. It is well known that a number of beneficial bacteria belong to this phylum and that may positively influence plant defense priming. Our results strongly support the hypothesis that soil properties are characterized by the legacy of agricultural management. Additionally, there seems to be a soil memory effect on the rhizosphere bacteria that might result in altered performance and health of the next plant generation.

## PT-MU-23 Engineering the plant microbiome to improve crop quality and yield

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New approaches are required to meet the demand for an increased global food supply that do not harm the environment and are produced in a sustainable fashion. This study proposes to examine how the microorganisms associated with plants, the plant microbiome can be engineered to improve crop quality and yield using synthetic biology approaches. The overall aim of the project is to contribute to an alternative and more sustainable future for agriculture.

The methodology used in this study is based on the CRISPR-Cas9 genome editing approach, which is increasingly being adopted in microbiology due to its ability to allow the genetic manipulation of previously genetically intractable organisms. We are applying the technique to modify the genomes of plant growth-promoting rhizobacteria and also the metagenome of the rhizosphere community in order to obtain a synthetic bacterial consortium with improved capabilities of promotion of disease resistance or resource utilization in the partner crop. As a result, we also hope to gain a broader understanding of the interactions occurring between bacteria and plants in this complex environment.

## PT-MU-24 Unravelling the key players of grassland plant microbiomes behind methanol consumption

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Managed grasslands are global sources of atmospheric methanol. Methanol is one of three most abundant reactive volatile organic compounds (VOCs) and contributes to the overall oxidative capacity of the troposphere and also to ozone depletion. Grasslands exhibit higher methanol emissions than forests and may be net sinks, i.e. they are globally affecting the methanol budget. Often the emitted methanol is of plant origin (~80-90%). The phyllosphere can be regarded as a favoured habitat of methanol-utilizing microorganisms being as abundant as up to 17% of the total phyllosphere microbiome. But the information regarding rhizosphere methanol utilizers is still scarce. Few studies suggested that the estimated global emission rate of methanol is considerably higher than the observed emission rates of terrestrial ecosystems. Thus, methanol utilizers of the plant microbiome are crucial in mitigating the emission rates through methanol consumption. The current study aims to identify the key methanol utilizers of a managed grassland in Germany. Hence, a central motivation of our study was to test the hypothesis if specific plant species are associated with specific methanol utilizers. We address this objective by the identification of active methanol utilizers of four grassland plant species (i.e. *Poa trivialis*, *Festuca arundinacea*, *Trifolium repens*, *Taraxacum officinale*) by DNA and RNA stable isotope probing (SIP) and metatranscriptomics at different growth stages (germination and flowering). We used gas-tight plant growth chambers to investigate the phyllosphere, endosphere, and rhizosphere of complete and intact grassland plants to avoid physical harms, which may lead to change of methanol fluxes. We measured the phyllospheric and rhizospheric methanol production and will identify those methanol utilizers that will have incorporated  $^{13}\text{C}$  from supplemented [ $^{13}\text{C}_1$ ]-methanol. Additional to the taxonomic identities, the metatranscriptome data of the labelled microbiome will deliver insights (i) in preferred metabolic pathways for methanol assimilation and dissimilation and (ii) in further metabolic interactions with the host plants.

## **PT-MU-25 Asymmetric dependencies – a novel statistic to reveal non-reciprocal relationships between plant associated microbes**

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Bacterial communities associated to plants are highly diverse assemblages that are formed by plant-based factors as well as by interactions between bacterial species. The latter have the effect that species' abundances often do not vary independently of each other. Abundances of mutualistic partners can be expected to exhibit positive correlation, whereas the abundances of competitors may be negatively correlated. Such linear or at least monotone associations are well quantified by Pearson's *R* or Spearman's *rho*. Additional known dependence measures allow for an identification of non-linear relationships in community data. Unfortunately, all of these dependence measures are symmetric (i.e. mutual), implying that the influence of species *X* on species *Y* coincides with the influence of *Y* on *X*. However, in many types of interactions one species may be more dependent on another species than *vice versa*, for instance if one bacterial species depends on a by-product of another species' metabolism. In order to facilitate detection of such non-mutual relationships in large datasets, we implemented an asymmetric copula-based dependence measure called *acdm*. With this measure it is possible to quantify dependence for any functional relationship in an asymmetric manner.

We will discuss the theoretical and ecological relevance of asymmetry in dependence measures and, using data on bacteria associated with plants, provide fundamentally new insights into the nature of associations between bacteria.

## **PT-MU-26 Biostimul'eau: crop quality improvement in (vertical) hydroponics through transplantation of a soil rhizosphere microbial community**

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Today, soilless cultivation systems are the most intensive production systems in the horticulture industry. Because there is an increasing demand for more tasteful and quality agricultural products with limited impact on the environment and on food quality further down the chain (e.g. presence of chemical residues), there is an opportunity to improve crop quality in hydroponics. The quality and taste of fruit and vegetables is to a large extent determined by the soil in which they are grown. This is due, in part, to the soil type, but also to soil life. Although there is a connection between a rich soil life and crop quality, it has not yet been introduced as a concept in hydroponics. If we transplant the microbial rhizosphere community from a soil grown crop to its hydroponics counterpart, this could have a significant impact on promoting the quality of the final product. This hypothesis was tested in a preliminary experiment on hydroponic lettuce (*Lactuca sativa* L.) in a vertical farm. The rhizosphere microbial communities of soil grown lettuce plants were extracted. Hydroponic lettuce seedlings were inoculated with the rhizosphere extract. A second inoculation took place two weeks later. To determine the effect of transplantation on the nutritional quality of the crop, several quality parameters were determined after harvest. Transplantation caused an increase in total phenolics (+15%), Fe (+14%), Ca (+24%), Mg (+17%) and Zn (+35%) content, and a decrease in nitrate content (-46%). This preliminary experiment shows the potential of transplantation of soil rhizosphere communities to hydroponics. With the Biostimul'eau project we aim to find the conditions that can optimize and stabilize the positive functioning of transplanted microbial communities in hydroponics. Further research will be focused on optimizing substrate composition next to identifying and isolating bacteria with quality-improving properties.

## **PT-MU-27 Root microbiome dynamics in response to phosphate**

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Plants require phosphorus (P) for growth. A small fraction of soil P is directly available for plant growth while the larger fraction is held to minerals and inaccessible for plants. Farmers compensate the lack of phosphate with fertilizers; however, the world reserves for phosphate fertilizers will be depleted within the next 50 to 100 years. On the other hand, microbes are able to solubilize the inorganic P fraction. Understanding how plants depend on microbes for P uptake is crucial for a more sustainable agriculture. In this study, we investigated the effect of P availability on the bacterial and fungal communities associated with *Petunia* roots using 16S rRNA and ITS gene sequencing. We found that the phosphate gradient affected both richness and community composition. We identified differentially abundant OTUs under high and low P conditions. Interestingly, the response to P availability differed between *Petunia*, which is colonized by arbuscular mycorrhizal fungi (AMF), and *Arabidopsis*, a plant that does not form symbiosis with AMF. Finally, through network analysis, we identified fungal-bacterial microbial hubs affected by the P gradient. Together these results suggest that the root microbiome responds dynamically to P availability in soil and that this response differs between mycorrhizal and non-mycorrhizal plants. P-dependent OTUs/hubs identified in this study could be used as target to develop new biofertilizers.

**Microbe-assisted crop production, opportunities, challenges & needs**

**Vienna, Austria, 4- 7 December 2017**

***Poster Session 3: Application technologies & formulations***

## **PP-ATF-01 Bio-inoculation of plant growth promoting bacteria as endospores: a promising strategy towards sustainable agriculture**

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In conventional agriculture, inappropriate management practices and use of agrochemicals has led to major impacts on both soil and water. For these reasons, the search for sustainable alternatives in agriculture has become a priority. In the mycorrhizosphere, microbes are considered as crucial actors of soil functioning for their involvement in beneficial activities supporting both below and above-ground ecosystems. Plant growth promoting rhizobacteria (PGPR) are well-known to enhance plant growth and health through biocontrol and biofertilization activities. Among PGPR, Bacilli, that are endospore forming bacteria (EFB), are common plant growth promoters. EFB have the ability to form dormant structures called endospores, allowing them to survive under harsh environmental conditions. This represents an interesting ability to enhance the survival of bio-inoculants before field application. As a result, using Bacilli as PGPR allows to combine both, their plant-growth promoting activity and their application in the field as spores. In this study we selected three *Bacillus* spp. with plant growth promoting abilities to investigate their effect on plant growth and their interactions with soil fungi. For this, a field experiment using oat (*Avena sativa*) was set using a mixed inoculant of the three mesophilic Bacilli strains. Bio-inoculation was carried out in the form of either vegetative cells or endospores onto the seeds. The results obtained suggest that bio-inoculation of both vegetative cells and endospores lead to positive effect on plant growth and fitness as compared to non-inoculated seeds. Inoculated seeds observed under a scanning electron microscopy confirmed the presence of the bio-inoculated bacteria on the surface of treated seeds as compared to the untreated seeds. With next generation sequencing the effect of the bio-inoculated strains on the total bacterial and fungal soil communities could be assessed. Since the survival of bacteria prior to inoculation is often a critical issue, the inoculation of bacteria in the form of endospores as a delivery system might represent an effective way to supply bio inoculants in the soil. In the future, this could provide an ecofriendly alternative to limit the use of agrochemicals.

## **PP-ATF-02 Microorganisms evaluation by Phosphorus Use efficiency Bio-Assay *in planta***

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The Phosphorus essential element is often the most limiting nutrient in agro-ecosystems although most agricultural soils in Europe have large amounts of inorganic and organic phosphorus. Unfortunately, these forms of Phosphorus are immobilized, with only a very low concentration of phosphorus available to plants. Thus, numerous developed plant Biostimulant products aim at helping to face the limitation of Phosphorus in soil by using microorganism strains as bio-effectors to increase the phosphorus-use efficiency of major non-leguminous crops.

In this context, Staphyt in collaboration with Celesta lab, has developed an *in planta* bio-assay to assess the putative effect of new microorganism bio-effectors to facilitate availability or assimilation of Phosphorus in controlled conditions. Our system is optimized for the usual Phosphorus input form in agriculture (P<sub>2</sub>O<sub>5</sub>) and provide key results about the Phosphorus exportation from the soil to the plant. This information is crucial to the transition from the lab to the field.

Our aim is to use this bio-assay as a screening test to search new effective solutions (microorganism strains and/or active substances combination) or to validate the efficacy of the newly developed products for a future field application.

### PP-ATF-03 Microbial metabolite bioprospection for sustainable biocontrol of plant pathogens

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Phytopathogens, in specific the *Xanthomonas* genus members continue to diminish the crop productivity while the chemical control approaches are not effective. These *Xanthomonads* have gained resistance and moreover, the chemical control strategies led to hazardous impacts to the environment, wildlife and human health. With this regard, we are exploring for sustainable microbial metabolites that can be safe, eco-friendly and economically profitable which is the need of the hour. In the course of this investigation, we isolated a new and promising bacterial strain from Chumathang Hot Spring, Ladakh, India, which was identified as *Pseudomonas aeruginosa* strain CGK-KS-1 (GenBank Accession No. KY203649). The bioactive metabolite produced by CGK-KS-1 was structurally elucidated as phenazine-1-carboxamide based on <sup>1</sup>H and <sup>13</sup>C NMR, FT-IR, EI-HR-MS and 2D NMR spectroscopic techniques. PCN potentially inhibited different strains of genus *Xanthomonas* where the MIC values ranged between 1.9-3.9 µg/ml against various test human pathogens and *Xanthomonas* strains. Interestingly, for the first time, we found that PCN also exhibited potent anti-biofilm properties against various human pathogens and *Xanthomonas* strains. *In vitro* cytotoxicity studies revealed that PCN was non-toxic to CHO cell line. The *in silico* docking studies showed that PCN strongly interacted with various target proteins of different *Xanthomonas* strains with high binding energies. Further, the strain CGK-KS-1 also produced two more effective bioactive compounds that inhibited *Xanthomonas* strains whose structural characterization is in progress and the details will be discussed during the presentation. These studies demonstrate that the extrolites from *P. aeruginosa* strain CGK-KS-1 as promising candidates for application in various biocontrol strategies.

### PP-ATF-04 Transgenic *Pseudomonas synxantha* 2-79 transformed with genes for the biosynthesis of pyrrolnitrin have improved biocontrol activity against soilborne diseases of wheat and canola

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The four-gene operon (*prnABCD*) from *Pseudomonas protegens* Pf-5 encoding the biosynthesis of the antibiotic pyrrolnitrin (PRN) was stably introduced into *P. synxantha* (formerly *P. fluorescens*) 2-79, an aggressive colonizer of wheat roots that naturally produces the antibiotic phenazine-1-carboxylic acid (PCA) and suppresses take-all of wheat. Recombinant strains produced both antibiotics and maintained population sizes in the rhizosphere of wheat that were comparable to those of the parental strain 2-79. Recombinant strains ZHW15 and ZHW25 inhibited *in vitro* the wheat pathogens *R. solani* AG-8 and AG-2-1, *Gaeumannomyces graminis* var. *tritici*, *Fusarium culmorum*, and *F. pseudograminearum*, and the canola pathogen *Sclerotinia sclerotiorum* significantly more than the wild-type strain 2-79. Both wild-type and recombinant strains were equally inhibitory of *Pythium ultimum*. When applied as a seed treatment the recombinant strains suppressed both take-all and Rhizoctonia root rot of wheat and Rhizoctonia root and stem rot of canola significantly better than the wild-type strain 2-79.

## **PT-ATF-05 Two-way protection of Gram-negative beneficial soil bacteria *Paraburkholderia phytofirmans* in dry alginate formulations: inducing the cell adaptation in tolerance to desiccation**

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The major drawback of Gram-negative bacteria to be applied as plant-growth promoters or biocontrol agents is their sensitivity to desiccation. Therefore, the aim of this study was to significantly improve desiccation tolerance of Gram-negative bacteria, such as *Paraburkholderia phytofirmans* PsJN and to develop functional alginate beads providing not only protection to the bacterial cells but also a gradual release of PsJN upon rehydration.

Two different approaches were considered for improvement of desiccation tolerance and storage stability at ambient conditions:

First, growing PsJN under mild stress conditions induced by osmotically active solutes such as glycerol, sucrose, NaCl and polyethylene glycol 600 with water activity values of 0.95, 0.96, 0.97 and 0.98 to allow the bacteria to adapt and secondly, to add protective compounds to the alginate prior to encapsulation. Using osmotic treatment with either glycerol or sucrose storage stability of encapsulated PsJN was enhanced by 10 times compared with PsJN cultured in basic LB medium. This was observed for beads of various size (150 µm to 450 µm). The second approach was based on addition of protective compounds to alginate prior to encapsulation. For this purpose, skimmed-milk, trehalose, betaine and molasses were tested. Only addition of skimmed milk and/or molasses led to a substantial improvement in cell viability: cell counts of PsJN in alginate beads decreased by only two orders of magnitude in comparison with six orders in pure alginate beads. The formulations remained stable for at least 70 days at ambient conditions with log 4.5 CFU PsJN/mL alginate. Finally, both approaches were combined in order to preserve cells, both from the “inside and outside”. Furthermore, different types of starch were added to alginate in order to modify the bead porosity and tune the time course of bacterial release upon rehydration. Release studies were performed in 100% humidity in presence of saline solution. Performance of beads was evaluated with respect to storage stability and release profile at ambient conditions in comparison with Gram-positive bacteria (*Paenibacillus* sp.).

In conclusion, applying a two-way approach for PsJN protection, we were able to maintain cell viability after air-drying: The storage time of alginate formulations was prolonged from 4 days in pure alginate beads to up to 70 days.

## **PT-ATF-06 Microbial nanoclay and nanocellulose composite formulations using Gram negative endophytic bacteria for maize**

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Formulation granting prolonged survival on storage and both survival and sufficient level of bio-activity in the field represents the bottleneck in the broad scale implementation of biological means in plant protection urged by the EU directive on Integrated Pest Management 2009/128/EG. A major reason is the fact that bacteria-loaded water-based biopolymer coatings are of low mechanical stability and are prone to increased permeability of small molecules like water vapor and oxygen including reactive oxygen species that dramatically reduce bacterial viability and functionality and furthermore shelf life of the formulated seed.

We therefore evaluate the integration of high performance composites based on nanoclay and nanocellulose to meet the requirements for a stable and high quality microbial formulation to be used as seed coating for maize. We present new material designs comprising biobased polymer composites on well-defined structure/property relationships to enable substantial improvement of microbial viability, mechanical performance and water vapor and O<sub>2</sub> barrier properties of microbial formulations. Biopolymers, such as alginate, methoxy pectin, starch, arabic gum and gelatin will be used as binders. Better mechanical performance is expected to drastically reduce abrasion of coated seeds, while enhanced water vapor and O<sub>2</sub> barrier properties shall significantly improve bacterial survival and storage stability of formulations and formulated seeds at ambient conditions.

We demonstrate using the Gram negative bacteria *Paraburkholderia phytofirmans* PsJN and *Pseudomonas* sp. that the integration of new biobased filler materials (hemicellulose, nanoclay and nanocellulose) to develop fully biobased composites for application in biofertilizer products is significantly improving microbial viability and shelf life of microbial formulations.

## PT-ATF-07 The project "SaatMaisPlus": Development of non-chemical seed treatments for maize

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Maize is one of the most important crops worldwide. In Germany it is grown on 2.5 Mill. Hectar, of which 84 % are silage maize and 16 % grain maize. Especially under conditions of cool and wet soils, seed germination and crop establishment of maize are often affected by pathogenic fungi like *Fusarium*, *Pythium* and *Rhizoctonia*. Maize seed is therefore routinely treated with chemicals, commonly TMTD (thiram). So far, non-chemical seed treatments for maize are not available. Based on previous positive results with electron seed treatment of small-grain cereals and vegetables the project "SaatMaisPlus" aims at developing the application of low-energy electrons in combination with micro-organisms as a routine seed treatment method for maize. The project consortium includes two research institutions (JKI, CAU), the company EVONTA specialized in electron seed treatment, a specialist for production of microorganisms for sustainable agriculture (ABiTEP) as well as a supplier of seeds of arable and forage crops (DSV).

Because seed treatment with electrons is expected to be primarily effective against seed-borne pathogens (e.g. fusaria), microorganisms will be employed to protect seedlings against soil-borne attack. For this purpose, bacteria and fungi from maize roots and other sources will be screened in bioassays for activity against soil-borne *Fusarium*, *Pythium* and *Rhizoctonia*. The method of electron seed treatment will be adapted and optimized for maize regarding safety of the treatment and efficacy against pathogens and combined with the most effective micro-organisms. The efficacy of the single and combined treatments will be evaluated in greenhouse and field experiments in relation to the standard seed treatment TMTD.

The project is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support programme.

**Microbe-assisted crop production, opportunities, challenges & needs**

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***Poster Session 3: Phytobiomes Alliance***

## PP-PA-01 Microbial Bio-Effectors for alternative Plant Nutrition Strategies: technical, legal and economic Challenges

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For many bacterial (e.g. *Pseudomonas*, *Bacillus* spp.) and fungal (e.g. *Penicillium* spp.) strains mobilization of mineral nutrients, stimulation of root growth, improved stress resistance, synergistic interactions with other helpful microorganisms, as well as general or specific effects against pathogens are proven modes of action how they can promote plant growth. Yet, an increasingly stringent legislation in the European Union concerning the registration of active agents, including microorganisms, that are regulated either under the plant protection or fertilizer law led to a far-reaching loss of microbial products from the market and deters companies from launching innovative products. Niche applications and small companies are suffering particularly whereas the interest of chemical industries to commercialize microbial agents as biological alternatives or supplements to conventional pesticides and fertilizers is increasing. For 2016 the value of the global market for agricultural "biologicals", comprising "bio-pesticides", "bio-fertilizers" and "bio-stimulants", has been estimated to be worth 15 billion US \$ and is projected to grow with a compound annual growth rate of 6 % to reach 20 billion US \$ by 2021, with bio-pesticides having the largest share (Market Data Forecast, 2017).

Aware of this trend, the EU project "BioFactor" aimed to develop comprehensive biological approaches to integrate the versatile actions of plant growth-promoting bacteria and fungi for implementation in sustainable fertilization strategies. The results showed that *Pseudomonas*, *Bacillus* or *Penicillium* products could significantly improve the yield and profitability of tomato production with organic fertilization under greenhouse conditions. In combination with stabilized ammonium and alternative phosphorus fertilizers diverse microbial agents could improve the mineral nutrient acquisition and growth of wheat. Positive effects were also observed in maize, where stimulation of root growth by a *Pseudomonas* strain could increase the rooting density for the efficient exploitation of fertilizer depots in soil.

A one-sided functional classification of these microorganisms as bio-pesticides would disregard many of their other beneficial traits that could be reasonably used in integrated fertilization strategies for sustainable plant nutrition. The practical relevance of bio-fertilizer applications is discussed with respect to technical, legal and economic aspects.

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## PP-PA-02 Cotton fungal endophytes: From discovery to commercial application

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Beneficial fungal endophytes can confer protection to plants against a variety of stressors and improve yields in major agricultural crops. We have characterized fungal endophytes originally isolated from cultivated cotton (*Gossypium hirsutum*) by genomic studies, experiments in the laboratory, glasshouse, and other pre-commercial trials prior to launching them as commercial products. Using seed treatment protocols, individual cotton plants can be inoculated with natively occurring fungi to deliver useful agronomic phenotypes throughout the growing season. We show that the targeted addition of selected fungal endophytes to cotton can mediate plant tolerance to multiple stressors including drought, pests, and nematodes, with significant positive impacts on plant performance and yield under field conditions.

## PP-PA-03 Artificial selection of root microbiota associated to plant phenotype changes

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Artificial selection applied at community level is an important, but still growing topic in the field of ecology and experimental evolution [1-3]. Its recent implementation to microbial communities holds not only appealing promises in terms of fundamental knowledge about selection itself [3], but also in terms of relevant applications to our society, including bioremediation [4] and plant traits enhancement [5]. Here we transposed the concept of artificial selection of communities to perform experimental evolution of root microbiota inducing relevant phenotypic changes in plants. We grew ten successive generations of four weeks old *Brachypodium distachyon* inoculated with artificially selected root microbiota from the previous generation, corresponding to ~3700 plants. Depending on experiment goals, selection was applied based on specific plant phenotypic traits of interest such as aboveground biomass or leaves color nuances as a proxy of nitrogen content, using an automated high-throughput plant phenotyping platform. We orientated evolution in different directions by respectively selecting plants within several lineages displaying the lowest and the highest values for targeted traits against random selection controls. Root microbiota were characterized during the selection experiment by means of 16S rRNA gene amplicon sequencing. Despite challenges associated with ensuring efficient heredity of selected communities and plant phenotypic changes, results obtained showed rapid response of plants to artificial root microbiota selection, with significant divergence for targeted traits after few generations. Our results support the fundamental notion that plant phenotypic changes may be rapidly acquired via artificial selection of root microbiota, which could potentially contribute to rethink the way we select for desired plant functions.

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## PP-PA-04 PGPRs to alleviate cold stress in maize

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Due to its South American origin, maize growth suffers from low temperature conditions especially during juvenile growth stages. As a result, farmers in colder climates wait for sowing resulting in premature crop harvesting and increased prices due to post-harvest treatments. We investigate whether microorganisms residing in the rhizosphere and endosphere of maize roots can support growth in the cold by combining microbiome analyses, microbial isolation and dedicated mode of action studies.

Bacteria have been isolated from maize endospheres and screened for growth promoting effects in both maize and *Arabidopsis*. So far, several positive bacteria have been isolated from which one *Caulobacter* strain is of particular interest. We aim at understanding the plant signaling networks on which these bacteria impinge to provoke the growth promoting traits and use maize and *Arabidopsis* as model plants. We will report on the in-depth investigation of the phenotypic effects and on the molecular networks that are modulated by these PGPRs.

## PP-PA-05 Bacterial endophytes assemblages in seeds of a gymnosperm

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Since Bernheim and Lehmann disputed the existence of bacterial associations with seeds in 1889, it has been acknowledged that seeds are inhabited by several microorganisms. Some of them contribute to plant growth and health, while others display detrimental or neutral effects on their hosts. Seed endophytes have been mainly studied in Angiosperms. Down to the present day the microbiomes of dry seeds derived from gymnosperms have not been investigated. Despite the broad range of members of this group, which are of silvicultural interests, no effort has been invested into clarification of microbiomes of these seeds and the possible importance for thereof developing plants. It is well known, that the microbiome of plants, which is affected by the seed microbiome, plays an important role in germination, plant performance, survival and pathogen resistance. Already in 1996, several members of *Burkholderia*, *Pseudomonas*, and *Streptomyces* were isolated from root seedlings of Douglas fir (*Pseudotsuga menziesii*) and showed biocontrol activity against root pathogens. However, the presence, characterization, and location of the seed microbiome of this tree were not yet investigated.

Within this study, we focused on the microbiome of the seeds of Douglas fir using Illumina 16S rRNA gene amplicon sequencing. Variability of the microbial composition was obtained between seeds. However, a conserved microbiome was found among the samples consisting of Alpha-, Beta-, and Gammaproteobacteria as well as Firmicutes and Actinobacteria. A core microbiome was also obtained at genus level showing abundance of *Burkholderia/Paraburkholderia*, *Pseudomonas*, *Rhizobium*, *Delftia*, and *Acinetobacter* as the top 5 dominant genera. Bacterial taxa were further visualized in different seed parts such as the root embryo, cotyledon and the seed coat using DOPE-FISH/CSLM microscopy. Alphaproteobacteria, Gammaproteobacteria, Firmicutes and Actinobacteria were present in all seed parts, while Betaproteobacteria were restricted mainly to the seed coat and cotyledon, describing different niches of bacteria taxa within the seeds. Taken altogether, the results describe for the first time the assemblages of seed endophytes that are associated with a gymnosperm member, as well as their location within seed tissues, contributing to the exploration of the broad range of unknown seed microbiomes for a better understanding of plant-microbe associations.

## PP-PA-06 Effect of endophyte on drought tolerance of tall fescue (*Festuca arundinacea* Schreb.)

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Tall fescue (*Festuca arundinacea* Schreb.), a cool-season perennial grass used as forage and turf, harbors a fungal endophyte (*Epichloë coenophiala*). The plants infected with endophyte (E+) provides enhanced resistance to biotic and abiotic stresses compared to those without endophyte (E-). Selected endophytes are in use that retain the agronomic benefits to the grass but do not produce alkaloids that are harmful to grazing livestock. A tall fescue mapping population (n = 208) was developed by crossing between a drought tolerant (T348) and a susceptible (S947) genotype. Inherent endophyte of each genotype was killed through fungicide treatment to develop E+ and E- clonal pairs of each parent and progenies. The entire population was evaluated in greenhouse and field experiments to study the influence of endophyte on drought tolerance and to identify genetic loci and genomic locations influencing the host and endophyte interactions. Both genotype and endophyte influenced plant height, plant biomass, chlorophyll content, relative water content (RWC), osmolal concentration (OC), electrolytic conductivity (EC), and root penetration. Significant genotype x endophyte interactions were also observed for most of the studied traits. The E+ plants showed higher RWC than those of E- under drought, while the effect was masked under well-watered condition. Endophyte enhanced the OC increment as a response of drought tolerance. Concomitantly EC, which represents plasma membrane damage due to stress, was also low with endophyte presence under drought condition. Endophyte helped tall fescue plants for better root penetration. Identification of genetic loci associated with important traits is in progress.

## **PP-PA-07 Visualization and quantification of wounds as ports for intrusion of microbial food safety hazards in leafy vegetables**

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Contamination of leafy vegetables with human pathogens is a food safety concern, due to increased production and consumption of these commodities. Human pathogens can internalize plant tissue via natural openings such as stomata, but also through damaged plant tissue. Damaged tissues are nutrient rich habitats that can be exploited by phyllosphere microorganisms. In the present study we detected and quantified leaf lesion area that occurs on field grown spinach and Swiss chard during cultivation, induced by different biotic and abiotic factors. Dead and damaged cells of plant tissue were used as a marker for lesions. As undamaged plant tissue cells are very selective concerning the compounds that pass through their membrane, trypan blue dye staining enabled the discrimination between intact cells and cells with damaged membranes, visualizing the lesion area in distinctive blue color. After staining, images of individual, stained leaves were taken, and digital image analysis methods were used for quantification of the total lesion area at the leaf scale. We developed an algorithm for quantification of damaged plant tissue and determined number, size and shape of individual lesions, as well as lesions spatial distribution. We conclude that it is important to thoroughly investigate and recognize the paths for pathogen internalization.

Keywords: lesions, leafy vegetables, human pathogens, internalization, food safety, image analysis

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### **PP-FR-01 Antimicrobial activities of actinomycetes isolated from *Sonchus crassifolius* and *Limonium majus* collected from south-eastern Spain (Granada)**

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Natural products produced by actinobacteria have demonstrated an important role in drug discovery and they still have a great potential for the discovery of new bioactive compounds. Actinomycetes are one of the most productive microbial communities in soil and they are especially rich in plant rhizospheres. In an attempt to isolate novel actinomycetes to discover new potential producers of antimicrobial compounds against human pathogens, we studied the rhizosphere microbial communities of *Sonchus crassifolius* and *Limonium majus*, two south-eastern Spain endemic plants from the area of El Margen (Granada). We assessed the diversity of the microbial community obtained following the different isolation approaches, and all the isolates were identified taxonomically on the basis of their macro and micromorphology, and their 16S rDNA gene sequences. The microbial population presented a wide diversity, not only within members of the genus *Streptomyces*, but also within other minor taxa of Actinobacteria as well as other groups of eubacteria. A selection of 215 strains including the most diverse and representative microorganisms were selected to be cultivated in 96-well plate microfermentations and extracted to be screened for the production of new antimicrobial agents active against a panel human pathogens (*Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *E. coli*, *Staphylococcus aureus* MRSA, *Candida albicans*). Our results confirm that rhizospheres are an extremely rich reservoir for the isolation of a wide diversity of actinobacteria, many of them still representing a rich untapped source of bioactive secondary metabolites with potential application in different therapeutic areas and biotechnological fields.

### **PP-FR-02 The fate of fungal biofertilizers - where do they go and how do they get there?**

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The use of fungal biofertilizers is common place, yet there are few data describing the spread of biofertilizers into the natural environment. Commercial inoculants may pose a threat to local fungal communities which may not be resistant to alien introductions. Unfortunately, long term data on the fate of commercial fungal inoculants are lacking.

We evaluated the establishment and spread of a commercial arbuscular mycorrhizal inoculant in a variety of agricultural and natural settings to evaluate its value as a biofertilizer and its threat to indigenous fungal communities, including two vineyards, two grain cropping systems and a natural grassland. We measured its abundance over 2-7 years and looked at corresponding changes in plant performance.

Our results show that while inoculant establishment occurs in some cases, it is not easily predictable. Inoculant establishment failed in certain scenarios but flourished in others. Inoculant spread beyond point of introduction was also difficult to predict and was influenced by both indigenous soil and plant communities.

Our data provides the longest dataset tracking a commercial inoculant in the field. Given the ubiquity of commercial inoculant use, and its ability to establish and spread from point of introduction, the use of fungal inoculants should be carefully evaluated.

# Author Index

## A

Adato, Avital .....	80
Adss, Shimaa .....	47
Ågren, Jon .....	58
Ait Abderrahim, Nawel .....	133
Akther, Adnan .....	135
Aldrighetti, Anna .....	121
Aleti, Gajender .....	139
Alonso-Blanco, Carlos .....	58
Alsanius, Beatrix .....	162
Alsharif, Wiam .....	103
Álvarez, J. C. ....	133
Alzubaidy, Hanin .....	32
Alzubaidy, Hanin S. ....	103
Ameloot, Nele .....	71, 151
Andres-Barrao, Cristina .....	32
Andrteozzi, Anna .....	51, 113, 124
Anjaiah, Vanamala .....	91
Antonielli, Livio .....	64, 98, 134, 139, 143, 149, 161
Asp, Torben .....	111
Aspray, Dr Thomas J .....	120
ATIENO, MARY .....	31
Audenaert, Kris .....	92, 105
Auer, Desmond .....	28
Aurelie, Gfeller .....	41
Aurelien, Bailly .....	41
Ávila, Paula .....	116

## B

Babin, Doreen .....	70, 149
Bachinger, Karl .....	128
Baert, Geert .....	92
Bahar, Ofir .....	45
Baier, Sebastian .....	98
Bakker, Peter .....	61, 97
Baldani, Jose Ivo .....	112
BALLESTER, JORDI .....	77
Ballhausen, Max-Bernhard .....	97, 141
Bandari, S. ....	159
Banerjee, Samiran .....	65
Barret, Matthieu .....	140
Barrio Duque, Alejandro del .....	134
Bassi, Dr. Albert .....	27
Baudson, Caroline .....	39, 108
Beaumont, Marco .....	72, 156
Becker, Y .....	115
Bedini, Alberico .....	43
Behrend, Anne .....	48
Beirinckx, Stien .....	160
Beitzen-Heineke, Elisa .....	89
Beitzen-Heineke, Wilhelm .....	89
Bejaoui, Bilel .....	33
Bejarano, Ana .....	75
Bell, Terrence .....	145

Bengtsson, Marie .....	162
Benichis, Marina .....	60
Berendsen, Roeland .....	61, 97
Berg, Gabriele .....	59, 97, 102, 127, 141
Berger, Nils .....	54, 136
Bergna, Alessandro .....	127, 141
Bernhard Ballhausen, Max .....	130
Beust, Andreas .....	44
Bianco, Carmen .....	51, 113, 124
Bianco, Piero A. ....	128
Bianka, Kästner .....	140
Biggot, Yvonne .....	64
Bindschedler, Saskia .....	65, 154
Birr, Tim .....	72, 157
Bisseling, Ton .....	143
Black, Jennifer .....	161
Blouin, Manuel .....	160
Bobusheva, Saykal .....	92
Bocacci, Paolo .....	113
Bodenhausen, Natacha .....	48, 70, 152
Boeckx, Pascal .....	92
Bokhari, Ameerah .....	32
Bonfante, Paola .....	41
Bonsall, Robert .....	66
Boon, Nico .....	71, 151
Bopape, Francina Lebogang .....	76
Borin, Sara .....	33
Bouffaud, Marie-Lara .....	63
Bowen, Benjamin P. ....	81
Bradáčová, K. ....	159
Bradacova, Klára .....	54, 136
Brader, Günter .....	53, 128, 136, 139
Brandt, Nathalie .....	91
Bravo, Daniel .....	36, 95
Bredenbruch, Sandra .....	37, 107
Breeuwsma, Suzanne .....	100
Brochetti, Matteo .....	33
Brodie, Eoin L. ....	81
Brown Miyara, Sigal .....	104
Buchholz, Franziska .....	143
Bucki, Patricia .....	104
Buriani, Giampaolo .....	119, 146
Bziuk, Nina .....	114, 144

## C

Cabrera, Raimundo .....	128
Cailleau, Guillaume .....	65
Calvo Alegre, Olga C. ....	52, 125
Camehl, Iris .....	48
Caradus, John .....	147
Cardozo, Marina .....	93
Carrión, Víctor J. ....	53, 137
Carvalho, Eric X. ....	116
Casati, Paola .....	128
Castillo Lopez, Diana .....	159
Castro, Paula .....	116, 117
Cellini, Antonio .....	119, 146

## Author Index

Cernava, Tomislav.....	97, 102, 127
Cesco, Stefano .....	121, 123
Chai, Yen Ning.....	145
Chang, Matteo.....	69, 150
Chaudhry, Vasvi.....	139
Chávez, Pamela.....	98
Chen, Yageng.....	143
Chen, Zhongqiang.....	67
Cheng, Xu.....	143
Cherif, Ameer.....	33
Cherif, Hanen.....	33
Chevalier, Mickaël.....	91
Chitarra, W. ....	131
Chitarra, Walter.....	113
Chlubek, Antonia.....	111
Choukr-Allah, Redouane.....	33
Ciuffo, M. ....	131
Cliff, John.....	66
Clocchiatti, Anna.....	38, 106
Coleman-Derr, Devin.....	89
Collavino, Mónica.....	93
Collinge, David.....	99
Collinge, David B.....	52, 103, 124
Collinge, David Brian.....	119
Colombo, Elena Maria.....	35, 94
Compant, Stéphane.....	134, 149, 161
Conlong, Des.....	93
Constanze, Hauser.....	129
Cortesi, Paolo.....	35, 94
Cortés-Patiño, Sandra.....	36, 95
Cosoveanu, Andreea.....	128
Coutos-Thévenot, Pierre.....	42
Crotti, Elena.....	33
Cuozzo, Danila.....	113
Czajkowski, Robert.....	60, 88, 135

## D

Danzberger, Jasmin.....	64
Daur, Ihsanullah.....	32
David, Weller.....	155
de Boer, Wietse.....	38, 106, 127
de la Cruz, Mercedes.....	164
De Rocchis, Vincenzo.....	134
De Vrieze, Mout.....	41
De Zelicourt, Axel.....	32
De Zélicourt, Axel.....	103
de-Bashan, Luz E.....	133
Debode, Jane.....	160
Defez, Roberto.....	51, 113, 124
Delaplace, Pierre.....	39, 108
Delphine, Chinchilla.....	41
Deng, Siwen.....	89
Denise, Bachmann.....	140
Depuydt, Stephen.....	105
Desrut, Antoine.....	42
Devi, Indira.....	66
Dhed'a, Benoît.....	92
di Pietro, Antonio.....	43
Díaz, Caridad.....	164
Dietel, Kristin.....	72, 127, 157
Dill-Macky, Ruth.....	55, 137
Dinse, Theresa.....	44
Djonovic, Slavica.....	79

Donati, Irene.....	119, 146
Dong-Gyu, Hwang.....	109
Doolotkedlieva, Tinatin.....	92
Dopierala, Adam.....	101
Doppler, Sara.....	72, 75, 156
Döring, Matthias.....	161
Dror, Orit.....	45
Drouveli, Zoi.....	130
du Jardin, Patrick.....	39, 108
Dumas, Bernard.....	102
Dumonceaux, Tim.....	142
Dunlap, Christopher.....	76
Duran, Paloma.....	58

## E

Eck, Mathilde.....	148
Eckelmann, Dennis.....	51, 123
Edwards, Jacky.....	28
Edwards, Robert.....	69, 150
Ehaliotis, Costas.....	130
Eida, Abdul Aziz.....	32, 103
Eigner, Herbert.....	102
Eileen, Kröber.....	140
Ekandjo, LK.....	115
Ek-Ramos, Maria Julissa.....	159
El Fahl, Mustafa.....	33
Elhady, Ahmed.....	47
Escobar Rodríguez, Carolina.....	149
Escobar-Rodriguez, Carolina.....	64
Estévez, Mar.....	164
ESTÉVEZ, VIRGINIA.....	77
Etzioni, Adi.....	80
Eversole, Kellye.....	79
Ezra, David.....	60, 104

## F

Fangmeier, Andreas.....	52, 125
Farneti, Brian.....	119
Fernández Scavino, Ana.....	132
Ferrandino, Alessandra.....	113
Ferrando, Lucia.....	142
Ferrando, Lucía.....	132
Feuerstein, Ulf.....	72, 157
Firestone, Mary K.....	81
Flahaut, Christophe.....	91
Floriane, L'Haridon.....	41
Fluch, Silvia.....	98
Fomsgaard, Inge.....	66
Francis, Isolde.....	105
Franken, Carolien.....	143
Franken, Philipp.....	48, 130, 134
Freitas, Ana D.S.....	116
Freitas, Ana Dolores S.....	118
Furtado, Bliss.....	111

## G

Galdeano, Ernestina.....	93
Gambino, Giorgio.....	113

## Author Index

Gamliel, Abraham	60
GANESH KUMAR, CHITYAL	155
Gao, Jian	81
Garbeva, Paolina	42
Garcia, Emily	98
Garcia, Gisell	133
García-Oliva, Felipe	98
Garrido-Oter, Rubén	58
Gayraud, Damien	102
Geelen, Danny	71, 151
Geistlinger, Jörg	70, 149
Genilloud, Olga	129, 164
GERIN, Florence	105
Germida, Jim	80
Gerna, Davide	49
Geurts, Rene	143
Ghezel Sefloo, Negar	117
Ghiazza, Cecilia	142
Ghimire, Binod	104
Gianochetti, Fabrizia	148
Gifford, Miriam	141
Giongo, Lara	119
Giongo, Vanderlise	118
GIRAUD, Frédéric	154
Gołębiewski, Marcin	111
Gonçalves, Mariana	118
González, Ignacio	164
Gonzalez-Menendez, Victor	129
Goormachtig, Sofie	160
Gouesnard, Brigitte	63
Graef, George	145
Graf, Hannes	123
Greffe, Vincent	101
Gribaudo, Ivana	113
Grosch, Rita	59, 70, 97, 141, 149
Grundler, Florian M. W.	37, 107
Gstöttenmayr, Barbara	127
Gulati, Sneha	97, 141
Gutowski, Paweł	101

### H

Haas, Pia	100
Hackl, Evelyn	98, 112
Hacquard, Stéphane	58
Haesaert, Geert	92
Hage-Ahmed, Karin	117, 135
Hallahan, David	67
Hamberger, Bjørn	119
Hamel, Chantal	164
Han, Shengcai	112
Hannula, Emilia	38, 106
Harms, Karsten	102
Harpaintner, Rudolf	118
Harsh, James	66
Hart, Miranda	164
Hartman, Kyle	65
Hartmann, Anton	112
Hassen, Ahmed Idris	76
Hatrakova, Natalia	73, 156
Hbaish, Bahaa	45
Heller, Werner	112
Hemmingsen, Sean	142
Hengl, Julia	72, 75, 156

Henriksson, Tina	99
HERRMANN, LAETITIA	31
Heuer, Holger	47
Hirt, Heribert	32, 103
Ho, Tuan-Hua David	120
Hodkinson, Trevor R.	131
Hoeyer, Anna Kaja	131
Hofland-Zijlstra, Jantineke	100
Hofmann, Andreas	112
Hohmann, Pierre	48
Hossain, Mofakhar	28
Hryniewicz, Katarzyna	111
Hsu, Shu-Hua	121
Hulisz, Piotr	111
Hundscheid, Maria	38, 106
Hussain, Rana Muhammad Fraz	141
Hyun, Kim	109

### I

Iacomi-Vasilescu, Beatrice	128
Iasur-Kruh, Lilach	45
Ionescu, Michael	80

### J

Jack, Allison	159
Jackson, Ray	67
Jacques, Marie-Agnès	140
Jacques, Philippe	91
Jacquioud, Samuel	160
Jafra, Sylwia	88, 101, 135
Jahnke, Siegfried	111
Jalal, Rewaa	32
Jamil, Isha	154
Jansson, Janet	85
Jensen, Birgit	52, 99, 103, 119, 124
Jiang, Xun	44
Jijakli, Haïssam	133
Jijakli, M. Haïssam	148
Johnson, Linda	147
Jongbum, Jeon	109
Jørgensen, Hans	99
Jørgensen, Hans J. L.	103
Jørgensen, Hans Jørgen Lyngs	52, 119, 124
Junge, Helmut	72, 157
Junier, Pilar	65, 154
Junker, Robert R.	71, 151
Jürgen, Augustin	140

### K

Kallus, Katharina	48
Kaminsky, Laura	145
Kandeler, Ellen	54, 136
Kanukollu, Saranya	69, 150
Kao-Kniffin, Jenny	145
Karaoz, Ulas	81
Karchi, Hagai	80
Karin, Hage-Ahmed	129
Kema, Gert H.J.	53, 137

## Author Index

Kemen, Eric.....	58
Khanna, Rajnish .....	89
Kinkel, Linda L. ....	55, 137
Kiseok, Lee.....	109
Koch, Eckhard .....	72, 157
Koedam, Nico .....	91
Köhl, Jürgen .....	27
Kokkoris, Vasilis .....	164
Kolb, Steffen .....	69, 150
Koller, Robert.....	111
Korthals, Gerard .....	38, 106
Kostic, Tanja.....	143
Kotte, Mathias .....	72, 157
Kranner, Ilse.....	49
Krill, Christian .....	28
Krychowiak, Marta .....	101
Krzysztof, Wieczorek .....	129
Krzyzanowska, Dorota M.....	88
Krzżanowska, Dorota M.....	101
Kudjordjie, Enoch Narh.....	66
Kuipers, Oscar.....	104
Kuipers, Oscar P.....	37, 107
Kunova, Andrea .....	35, 94
Kusari, Souvik.....	51, 123
Kusstatscher, Peter.....	102

### L

La Maddalena, Nicola .....	33
Lafi, Feras F.....	103
Lagunas, Beatriz.....	141
Lamontanara, Antonella .....	146
Latz, Meike A. C. ....	103
Lauer, KF.....	159
Laure, Weisskopf .....	41
Lauterbach, Desirée.....	114, 144
Le Gouis, Jacques.....	63
Lehnen, Christoph .....	27
LESUEUR, DIDIER .....	31
Letourneau, Melissa .....	66
Li, Dan.....	112
Li, Guofen .....	43, 53, 136
Li, Honghong.....	122
Li, Zhibo .....	37, 107
Liarzi, Orna.....	60, 104
Lidor, Ofir.....	45
Lin, Chia-Wei.....	120
Linda, Thomashow.....	155
Lindblom, Tobias .....	162
Lindström, Kristina.....	122
Links, Matthew .....	142
Liu, Chi-Te .....	121
Llorens, Eugenio .....	88
Lo, Shuen-Fang .....	120
López Gastón, María Maura .....	93
Lopez, Sergio .....	114
Loque, Dominique .....	81
Louie, Katherine B. ....	81
Loznic, Brenda .....	28
Lucic-Mercy, Eva.....	43
Ludewig, U. ....	159
Ludewig, Uwe .....	54, 136
Luo, Dexian .....	160
Lur, Huu-Sheng .....	121

### M

Maciag, Tomasz .....	88
Maciąg, Tomasz .....	101, 135
Maes, Martine.....	160
Maier, Johann .....	102
Mann, Ross.....	28
Mannini, Franco .....	113
Manzotti, Andrea .....	52, 124
Mapelli, Francesca .....	33
Markus, Gorfer.....	129
Marquez, Luis.....	159
Marshall, Matthew .....	66
Marszałkowska, Marta .....	44
Martin, Jesus.....	129
Martínez-Romero, Esperanza .....	25
Martin-Sanchez, Lara .....	42
Marttila, Salla .....	162
Mashao, Khumbudzo .....	76
Massart, Sebastien.....	133
Massart, Sébastien.....	148
MATHU, SAM .....	31
Matthijs, Sandra .....	91
Mavordi, Dmitri.....	155
Mavrodi, Dmitri.....	66
Mavrodi, Olga .....	66
May, Robert .....	118
Mayer, Klaus, F.....	112
McCarthy, Luke .....	142
McPherson, Morgan .....	145
Melguizo, Ángeles .....	164
Memela, N. Sandra .....	93
Menezes, Rômulo S.C.....	116
Mercy, Louis.....	43
Messmer, Monika .....	48
Metzner, Ralf .....	111
Michiels, Jan.....	101
Migdal, Guy.....	80
Mimmo, Tanja.....	121, 123
Mitter, Birgit .....	49, 64, 143, 149, 161
Moango, Adrien .....	92
Moënné-Loccoz, Yvan .....	63
Mogren, Lars .....	162
Morad-Talab, N. ....	159
Moreira, Helena .....	116, 117
Morón, Alberto .....	98
Moronta, Felix.....	148
Morris, Dr Peter .....	120
Mpanga, IK. ....	159
Mueller, Jennifer.....	37, 107
Mulaosmanovic, Emina .....	162
Muller, Daniel .....	63
Müller, Marina .....	36, 108
Müller, T.....	159
Müller, Thomas .....	36, 108
Mülner, Pascal.....	127
Murphy, Brian .....	131

### N

Naama, Alaa.....	45
Nagy, Istvan.....	111
Namtz, Yael .....	93

## Author Index

Naor, Vered .....	45
NARVÁEZ, JUAN JESÚS .....	77
Navarrete, Oscar .....	71, 151
Ndubuisi Chimelue, Nwabufu .....	52, 125
Nerva, L. ....	131
Neumann, G. ....	159
Neumann, Günter .....	54, 136
Nicolaisen, Mogens .....	66
Ninkovic, Velemir .....	162
Nkebiwe, PM. ....	159
Northen, Trent R. ....	81
Ntana, Fani .....	119
Nybroe, Ole .....	134

### O

Oburger, Eva .....	115
Olmedo-Álvarez, Gabriela .....	98
Ongena, Marc .....	91
Opitz, Michael .....	99
Oppenheim, Jacob .....	159
Ordon, Frank .....	114
Orrù, Luigi .....	146
Otto-Hanson, Lindsey K. ....	55, 137
Özkurt, Ezgi .....	132

### P

Paleskić, Caroline .....	128
Pallavicini, Alberto .....	148
Palmano, Sabrina .....	113
Pangesti, Nurmi .....	53, 137
Papadopoulou, Kalliope .....	130
Papathanasiou, Helena J. ....	59
Pardo-Díaz, Sergio .....	36, 95
Pasquali, Matias .....	35, 94
Passera, Alessandro .....	128
Patel, Anant .....	75, 89
Patil, Prabhu .....	139
Patz, S .....	115
Paul Chowdhury, Soumitra .....	70, 149
Penttinen, Petri .....	122
Pereira, Sofia .....	116, 117
Perneel, Maaïke .....	71, 151
Perrone, Irene .....	113
Pesek, Robert .....	159
Petric, Alexandra .....	64
Pfaffenbichler, Nikolaus .....	161
Pfeiffer, Stefan .....	64
Pflugfelder, Daniel .....	111
Philipp, Franken .....	43
Philippot, Laurent .....	160
Pierroz, Grady .....	89
Pieterse, Corne .....	61
Pieterse, Corné .....	47, 97
Pii, Youry .....	121, 123
Pizzatti, Cristina .....	35, 94
Pleyer, Lisa Marie .....	53, 136
Pohl, Karolin .....	114, 144
Porter, Ian .....	28
Posada, Luisa F. ....	133
Poschenrieder, Charlotte .....	118
Poșta, Gh. ....	159

Prasad, Vishal .....	94
Preiner, Julian .....	115
Preininger, Claudia .....	72, 73, 75, 156
Prigent-Combaret, Claire .....	63
Primo, Dário C. ....	116
Przyklenk, Michael .....	89

### Q

Queiroz, Rodrigo O. ....	116
--------------------------	-----

### R

Raab, Ted .....	89
Raaijmakers, Jos M. ....	53, 137
Radl, Viviane .....	64
Rajewska, Magdalena .....	101
Rändler, Manuela .....	97
Rariz, Gastón .....	132
Rashed, Ahmed .....	33
Rattei, Thomas .....	64
Raupp, M. ....	159
Ravensberg, Willem .....	83
Reinhold-Hurek, Barbara .....	44
Remus, R .....	115
Renoud, Sébastien .....	63
REY, Marjolaine .....	105
Rey, Thomas .....	102
Rezki, Samir .....	140
Riedle-Bauer, Monika .....	128
Riva, Valentina .....	33
Roach, Thomas .....	49
Rochfort, Simone .....	28
Röder, Olaf .....	72, 157
Rodríguez Torres, Ma. Dolores .....	98
Rodriguez, Alia .....	30
Rodriguez, Lorena .....	129
Rodriguez-Gil, Cristina .....	120
Roitsch, Thomas .....	134
Rojas, Edward .....	99
Romano, Silvia .....	51, 113, 124
Romero-Tabarez, Magally .....	133
Rothballer, Michael .....	70, 112, 149
Rouws, Luc F. M. ....	112
Roux, Fabrice .....	58
Ruiz, Sandra .....	164
Ruppel, S .....	115
Ruppel, Silke .....	130
Rutherford, Stuart .....	93
Ryan, Rebecca .....	159

### S

Saad, Maged M. ....	32, 103
Saha, Malay C. ....	161
SAINT-MACARY, Marie-Emmanuelle .....	154
SALDUCCI, Xavier .....	154
Sampaio, Everardo V.S.B. ....	116, 118
Samuels, Peter L. ....	55, 137
Sánchez, Isabel .....	164
Sánchez, Pilar .....	164

## Author Index

Sanders, Ian .....	30
Sandmann, Martin .....	70, 149
Sapkota, Rumakanta .....	66
Saracchi, Marco .....	35, 94
Sare, Abdoul R. ....	148
Sare, Abdoul Razack .....	133
Sarkar, Abhijit .....	44
Sauer, Ursula .....	75
Sauvêtre, Andres .....	118
Schachtman, Daniel .....	145
Schäfer, Martin .....	44
Schäfer, Patrick .....	141
Schierstaedt, Jasper .....	59
Schikora, Adam .....	47, 59, 114, 144
Schiro, Gabriele .....	36, 108
Schisler, David .....	76
Schlaepfi, Klaus .....	65, 70, 152
Schläpfer Sasse, Joelle .....	81
Schleker, Sylvia .....	37, 107
Schloter, Michael .....	64
Schmid, Michael .....	112
Schmidt, Stefan .....	93
Schmoll, Monika .....	43, 53, 136
Schneider, Carolin .....	43
Schneider, Sebastian .....	38, 106
Schneijderberg, Martinus .....	143
Schönhuber, Christina .....	128
Schröder, Peter .....	64, 118
Schulze-Lefert, Paul .....	58
Schwarz, Elisa .....	72, 157
Secchi, Francesca .....	113
Sehr, Eva Maria .....	98
Sergaki, Chrysi .....	141
Serrano, Rachel .....	129
Sessitsch, Angela .....	64, 98, 134, 143, 149, 161
Shade, Ashley .....	140
Shankar, Ajay .....	94
Sharma, Shubhangi .....	130
Sharon, Amir .....	88
Sharon, Or .....	88
Sheibani-Tezerji, Raheleh .....	64
Shoresh, Michal .....	80
Shrestha, Abhishek .....	47, 59, 114
Siegrid, Steinkellner .....	129
Sillen, Wouter .....	144, 147
Silva, Jessyca A.G.F. ....	118
Simmons, Tuesday .....	63
Simões Neto, Djalma E. ....	116
Simoneau, Philippe .....	128
SIRISHA, KANUGALA .....	155
Sittinger, Maximilian .....	54, 136
Siwinska, Joanna .....	88
Skorupa, Monika .....	111
Smalla, Kornelia .....	70, 114, 144, 149
Soja, Gerhard .....	135
Song, Chunxu .....	37, 42, 104, 107
Song, Yang .....	61, 97
Souza, Renata J.C. ....	118
Spangenberg, German .....	28
Spinelli, Francesco .....	119, 146
Spiteller, Michael .....	51, 123
Spor, Aymé .....	160
Stecova, Jana .....	35, 94
Steffen, Kolb .....	140
Steinkellner, Siegrid .....	100, 117, 135

Stöggl, Wolfgang .....	49
Studer, Bruno .....	48
Stukenbrock, Eva H. ....	132
Subandiyah, Siti .....	53, 137
Sutton, John C. ....	27
Swarup, Sanjay .....	69, 150
Sword, Gregory .....	159
Synek, Lukas .....	32
Synek, Lukáš .....	103

---

### T

Tabosa, José N. ....	116
Talukder, Shyamal K. ....	161
Tapia Torres, Yunuen .....	98
Tapia, Yunuen .....	114
Teasdale, Suliana .....	147
Tedford, Sherri .....	27
Tepper-Fobes, Eden .....	81
Tessnow, Ashley .....	159
Thiergart, Thorsten .....	58
Thijs, Sofie .....	144, 147
Thomashow, Linda .....	66
Thompson, Corrina .....	164
Thompson, Grant .....	145
Tormo, Jose R. ....	129
Tormo, José Rubén .....	164
Torres-Cortes, Gloria .....	140
Town, Jennifer .....	142
TRILLAS, MARIA ISABEL .....	77
Trognitz, Friederike .....	149
Trost, Eva .....	112
Trutschnig, Wolfgang .....	71, 151
Tsiknia, Myrto .....	130
Tsikou, Daniela .....	130
Turetschek, Reinhard .....	115
Turina, M. ....	131
Turra, David .....	43
Tyburski, Jarosław .....	111

---

### V

Valdespino, Natalia .....	114
Valencia, Cesar .....	159
Valente, Jordan .....	63
Valentinuzzi, Fabio .....	121, 123
VALETTE, Marine .....	105
Van Antwerpen, Pierre .....	91
van den Berg, Marlies .....	38, 106
van der Heijden, Marcel .....	65, 70, 152
van der Salm, Caroline .....	100
van der Wal, Annemieke .....	38, 100, 106
van Dusschoten, Dagmar .....	111
Van Gerrewey, Thijs .....	71, 151
van Hamme, Jonathan .....	144
van Overbeek, Leo .....	57
Vandecruys, Maarten .....	71, 151
Vangronsveld, Jaco .....	144, 147
Vaughan, Martha .....	76
Vega, Alberto .....	116, 117
Venneman, Jolien .....	92
Venturi, Vittorio .....	148
Vereecke, Danny .....	92, 105

## Author Index

Vergnes, Sophie .....	102
Verma, Vijay .....	159
Verrecchia, Eric.....	65
Verrone, Valeria.....	69, 150
Verwaeren, Jan .....	92
Viaene, Tom.....	160
Vidal, Stefan.....	89, 93
Villegas-Escobar, Valeska.....	133
Vismans, Gilles.....	61, 97
Vlaeminck, Lena.....	105
Vlot, Corinna.....	112
von Maltzanhn, Geoffrey.....	159
Vriet, Cécile .....	42
Vujanovic, Vladimir.....	80

---

### W

Wagner, Philipp .....	127
Walser, Jean-Claude .....	65
Wang, Peng.....	145
Weber, NF.....	159
Wehner, Gwendolin .....	114
Weinmann, M. ....	159
Weinmann, Markus .....	54, 136
Weller, David .....	66
Werbrouck, Stefaan.....	105
Weyens, Nele.....	144, 147
White, Jason .....	147
Wieczorek, Krzysztof .....	99, 100
Wienkoop, Stefanie .....	38, 106, 115
Wille, Lukas.....	48
Winter, Mark .....	55, 137
Wipat, Anil .....	69, 150
Wipf, Heidi.....	89
WISNIEWSKI-DYÉ, Florence .....	105
Wittwer, Raphaël.....	65

Witzel, K .....	115
Wood, Kent .....	67

---

### X

Xie, Yakun.....	32
-----------------	----

---

### Y

Yager, Julia .....	67
Yang, Jin .....	146
Yang, Li-Sen.....	120
Yang, Luhua.....	64
Yang, Mingming .....	155
Yong-Hwan, Lee .....	109
Yoonsung, Lee .....	109
Yoshioka, Miho .....	76
Yu, Ke .....	61
Yu, Su-May .....	120

---

### Z

Zachow, Christin.....	102, 141
Zchori Fein, Einat.....	45
Zhalnina, Kateryna .....	81
Zhang, Cai-Wen.....	146
Zhang, Jibin .....	155
Zhang, Jun .....	146
Zhang, Xiao-Xia .....	146
Zheng, Qi.....	143
Zhou, Lu .....	104
Zhou, Wenqing.....	159
Zimmermann, B. ....	159

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