

INTERNATIONAL SYMPOSIUM MICROBE-ASSISTED CROP PRODUCTION – OPPORTUNITIES, CHALLENGES & NEEDS DEC. 2<sup>nd</sup> – 5<sup>th</sup>, 2019

SCHLOSS SCHÖNBRUNN - ORANGERIE, APOTHEKERTRAKT VIENNA, AUSTRIA







# MICROBE-ASSISTED CROP PRODUCTION 2019

# OPPORTUNITIES, CHALLENGES & NEEDS

DEC. 2 – 5, 2019 SCHLOSS SCHÖNBRUNN|ORANGERIE VIENNA, AUSTRIA

ORGANIZING COMMITTEE: Angela Sessitsch, Alexandra Khassidov, Günter Brader

SCIENTIFIC COMMITTEE: Karen L. Bailey, Gabriele Berg, Günter Brader, Trevor Charles, Kellye Eversole, Philipp Franken, Paolina Garbeva, Heribert Hirt, Michael Ionescu, Jenny Kao-Kniffin, Adam Schikora, Klaus Schlaeppi, Alga Zuccaro & Angela Sessitsch



GENERAL INFORMATION	3
INTRODUCTION	5
INVITED SPEAKERS	6
SCIENTIFIC PROGRAM	11
DAY 1   MON, 2. DEC.   1 <sup>P.M.</sup> - 8:30 <sup>P.M.</sup>	11
DAY 2   TUE, 3. DEC.   8:30 <sup>A.M.</sup> – 3:55 <sup>P.M.</sup>	13
DAY 3   WED, 4. DEC.   8:30 <sup>A.M.</sup> - 6:30 <sup>P.M.</sup>	15
DAY 4   THU, 5. DEC.   8:30 <sup>A.M.</sup> - 2:00 <sup>P.M.</sup>	18
POSTERS TABLE - POSTERSESSION I	20
POSTERS TABLE - POSTERSESSION II	21
LECTURES	22
OPENING & OPENING LECTURE	23
SUCCESSFUL MICROBIAL APPLICATIONS	24
MECHANISMS MEDIATING HOLOBIONT AND MULTIPARTITE INTERACTIONS	27
PLANT UNDERSTANDING OF INTERACTIONS WITH BENEFICIAL MICROBES	33
MICROBIOME UNDERSTANDING BEYOND PROFILING	40
Morning Keynote	48
MICROBIAL BIOCONTROL OF PESTS, PATHOGENS AND WEEDS	49
MICROBIAL APPLICATIONS FOR IMPROVING NUTRITION AND ABIOTIC STRESS TOLERANCE	61
REGULATORY ISSUES – SPECIAL SESSION	71
DISRUPTIVE APPROACHES FOR ENGINEERING THE PHYTOBIOME & MICROBIAL DELIVERY	73
Closing & Closing Keynote	76
POSTER PRESENTATIONS	77
Poster Session 1: Successful microbial applications	78
Poster Session 1: Mechanisms mediating holobiont and multipartite interactions	84
POSTER SESSION 1: PLANT UNDERSTANDING OF INTERACTIONS WITH BENEFICIAL MICROBES	91
POSTER SESSION 1: MICROBIOME UNDERSTANDING BEYOND PROFILING	105
Poster Session 1: Varia	109
Poster Session 2: Microbial biocontrol of pests, pathogens and weeds	113
POSTER SESSION 2: MICROBIAL APPLICATIONS FOR IMPROVING NUTRITION AND ABIOTIC STRESS TOLERANCE	126
POSTER SESSION 2: DISRUPTIVE APPROACHES FOR ENGINEERING THE PHYTOBIOME & MICROBIAL DELIVERY	138
AUTHOR INDEX	142
PARTICIPANT INDEX	150
NOTES	161
Imprint	161



# **General Information**

# TAXI:

+431 40100 or +431 31300

# **VENUE ADRESS:**

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

# miCROPe 2019 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

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

WLAN SSID: Meetings PW: Habsburg

Follow us on <u>Facebook</u> and <u>LinkedIn</u> for updates and further information on upcoming events. www.facebook.com/micrope.symposium/

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 usbstick. The program is very tight, so please take care of the prescribed talktime.

# **POSTER PITCHES:**

The uploaded Poster pitches from presenters will be preloaded on the presentation computer.

# **POSTER SESSIONS:**

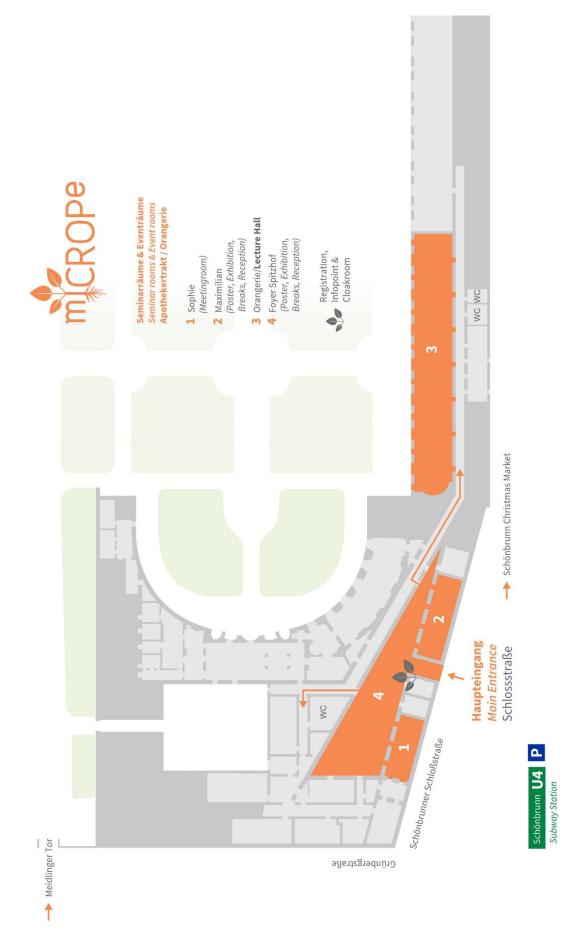
Posters are only accepted in A0 upright format and in English. The poster sessions are organized into poster sessions I & II and each poster has been assigned to a session. You can see the assignment within the poster table on page 20-21.

Postersession I Part A - Even poster numbers presenting: Monday 2. Dec., 18:30 – 20:30 Postersession I Part B - Odd poster numbers presenting: Tuesday 3. Dec., 12:25 – 14:30 Postersession II Part A - Even poster numbers presenting: Wednesday 4. Dec., 12:40 – 14:30 Postersession II Part B - Odd poster numbers presenting: Wednesday 4. Dec., 16:35 – 18:30

Posters from poster session I have to be removed until the evening of December 3rd.

Five posters will be awarded with Best Poster Awards.





#### **General Information**



## Introduction

Dear miCROPe attendees,

Our awareness on the importance of naturally occurring microorganisms, frequently referred as to microbiomes, has increased substantially. Plant microbiota are diverse and 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 increasing awareness of and interest in plant microbiota is linked to the urgent need to find solutions for current challenges in global crop production such as climate change and global demographic development. 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. Innovations based on the functioning of plant microbiota have the potential to contribute to combating these challenges.

The symposium "Microbe-assisted crop production – opportunities, challenges and needs" (miCROPe 2019) addresses basic and applied aspects of applying beneficial microorganisms in crop production. Scientific sessions will address mechanistic understanding of holobiont interactions, functional understanding of microbiomes, plant understanding, microbial control of pests, pathogens and weeds, microbial application to improve nutrition and abiotic stress as well as disruptive approaches in microbiome applications. Our aim is to promote innovation as well as implementation of new technologies and to enable discussions between academia and industry.

Around 190 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. 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



6

Invited Speakers

## **Invited Speakers**

### Karen L. Bailey, Agriculture & Agri-Food Canada, CA

Dr. Karen Bailey, Emeritus Research Scientist from Agriculture & Agri-Food Canada, trained as a plant pathologist and applied this expertise to improve plant health by finding solutions to reduce the impact of soil-borne plant diseases and by using fungi for biological control of Canada thistle and other broadleaved weeds. She has more than 300 publications, inventions disclosures and patents, and has received recognition from her peers with awards such as the Queen Elizabeth II Diamond Jubilee Medal, CPS Outstanding Research Award, and CPS Award for Achievements in Plant Pathology. Although having retired from AAFC, she continues to support commercialization activities related to her bioherbicide discoveries.

### Gabriele Berg, Graz University of Technology, 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, "ÖGUT Umweltpreis" (2011) and Fast Forward Award Styria (2015). She belongs to the most influential researchers world-wide (top 1, Clarivate Analytics for the category Cross Fields in 2018).

### Günter Brader, AIT Austrian Institute of Technology, AT

Dr. Günter Brader studied biology at the University of Vienna and obtained his PhD in plant biology 1997. After gaining post doc experience in the field of molecular biology and plantmicrobe interactions at the Institute of Biotechnology and the Department of Genetics of the University of Helsinki, Finland, he was appointed to be Docent in Plant Molecular Biology by the Helsinki University. He is now senior scientist at the Austrian Institute of Technology. Günter Brader is author of 60 peer reviewed publications. The research interests of Günter Brader are in the field of plant-microbe interactions and in understanding and characterizing plant diseases. His work focuses also on the exploitation of beneficial bacteria for biocontrol and nutrient solubilisation and description of the underlying mechanisms.

### Trevor Charles, University of Waterloo, CA

Trevor Charles is a bacterial geneticist with a research program in plant-microbe interactions, functional metagenomics, and bacterial genome engineering for bioproducts. Following B.Sc. Microbiology at University of British Columbia, he obtained his Ph.D. in Turlough Finan's lab at McMaster University (symbiotic nitrogen fixation) and did postdoctoral work in Gene Nester's lab at University of Washington (*Agrobacterium*). He held a faculty position at McGill University before moving to his current position at University of Waterloo in 1998, where he is currently director of Waterloo Centre for Microbial Research. He is also co-founder and CSO of the company Metagenom Bio, which applies metagenomic and microbial community analysis to challenges in the agriculture and environment sectors.









#### **Invited Speakers**

### Philipp Franken, University of Applied Sciences Erfurt, DE

Philipp Franken started to work on the arbuscular mycorrhizal (AM) symbiosis in 1991 at the MPI of Plant Breeding Research in Cologne and the INRA in Dijon, France. In 1995, Philipp Franken established a working group on AM molecular biology at the MPI of terrestrial Microbiology in Marburg. By the discovery of the plant growth-promoting fungus Piriformospora indica in 1998, he became also interested in other groups of beneficial rootcolonisers. After his habilitation in Applied Botany and Microbiology at Marburg University, Philipp Franken took over the head of the Plant Nutrition Department at the Leibniz-Institute of Vegetable and Ornamental Crops in 2002. Among other horticultural topics, he further worked mainly on the functions of root-fungus interactions. Since 2019, Philipp Franken is scientific

director of the Erfurt Research Centre for Horticultural Crops at the University of Applied Sciences Erfurt. In four research groups, the centre is working on questions of horticultural practice using current methods of biosciences. The scientific work is supported by the Friedrich Schiller University in Jena where Philipp Franken holds a chair of Molecular Phytopathology. The research in his group is aimed towards integrating knowledge about mycorrhizal fungi and other beneficial root colonisers in novel strategies for sustainable plant production systems.

### Paolina Garbeva, NIOO-KNAW, NL

Dr Paolina Garbeva is microbiologist by training with strong affinity for Microbial Chemical Ecology. She recieved her PhD degree from the Leiden University (Netherlands) in 2005. During her PhD, she investigated the significance of microbial diversity on disease suppression in agricultural soil.

After a post-doc at the University of Aberdeen in Scotland, she returned to the Netherlands to work on a personal VENI grant obtained from the Dutch Research Council (NWO). With the VENI project, she discovered that soil bacteria could distinguish among different competitors and fine-tune their strategies to survive. Between 2010 and 2013, Dr Garbeva

obtained three personal grants (MEERVOUD, VIDI and ASPASIA) that allowed her to establish a research group within the Department of Microbial Ecology at Netherlands Institute of Ecology. The focus of her research group is to understand the fundamental mechanisms of microbial interactions and communication with particular attention paid to the role of microbial volatiles. This is a novel and unique line of research in the field of Microbial Ecology. Using omicsbased tools and novel imaging techniques, her research group would like to further decipher and harness the communicating molecules used by plants and microbes in order to improve plant growth and health.

### Heribert Hirt, KAUST, SA

Hirt studied biochemistry at the Univ. of Cape Town and received his PhD from the Univ. of Vienna in 1987. After post-doctoral fellowships at the Univ. of Oxford and Wageningen, he became Professor of Genetics at the Univ. of Vienna. In 2007, he became Director of the INRA Plant Genomics Institute in Paris and in 2014 of the Center for Desert Agriculture at KAUST. His key biological questions are how plants can survive under stress conditions and how microbes contribute to these events by positively or negatively interacting with plants. In the DARWIN21 project (https://www.darwin21.org) he searches for beneficial microbes from desert plants with the aim to enhance stress tolerance of plants. A main focus of his research

is to identify the microbial and plant genes and pathways that provide stable stress tolerance to plants without interfering with growth and yield. His long term goal is to provide farmers with tailored microbial communities to enhance the performance and protection of crops to specific stress conditions.







### Michael Ionescu, Lavie-Bio, IL

Michael Ionescu is the VP Research of Lavie-Bio, a subsidiary of Evogene, focused on development of novel ag-biologicals products. For the last 6 years, Michael leads the research and optimization activity of novel microbiome-based Ag-biological to drive food quality and sustainability. His interdisciplinary research team is implementing a rationale biology driven design of microbiome-based products by leveraging a proprietary Computational Predictive Biology (CPB) Platform utilizing genomic and phenotypic big data and advanced informatics, focusing on the discovery and optimization of both Bio-Stimulants and Bio-Pesticides. Michael received his PhD degrees from the Hebrew University of Jerusalem in Life Sciences and Environmental Studies, researching stress

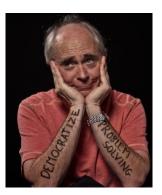


response mechanisms in enteric bacteria. He had conducted his postdoctoral research at the University of California at Berkeley in phytopathology, studying cell-cell communication system in plant pathogens with relation to interaction with host plant.

### Richard Jefferson, Cambia & QUT, AU

Richard Jefferson is a Professor of Biological Innovation at the Queensland University of Technology (QUT) and founder and CEO of Cambia & The Lens. Richard received a PhD in Molecular, Cellular and Development Biology from University of Colorado at Boulder in 1985, where he developed the glucuronidase (GUS) system as a molecular heuristic tool for transgenesis, developmental and ecosystem studies.

During his NIH postdoc at the Plant Breeding Institute in Cambridge, UK, Richard adapted GUS for agricultural biotechnology and pioneered an open source paradigm by distributing the toolkits to hundreds of labs around the world before publication. This enabled the genetic engineering of virtually all commercial crops, and is now the most cited molecular technology in agriculture. In 1987, with colleagues at PBI, Richard led the world's first field release of a biotech food crop. In 1989 Richard was appointed the first Molecular Biologist



for the United Nations FAO/IAEA in Vienna, and in 1991 founded Cambia, an autonomous global social enterprise to democratize science enabled problem solving. Besides Cambia's role in inventing and distributing open source enabling technologies it runs Lens.org, now the longest operating, largest and most comprehensive free, open and secure platform for scholarly and patent discovery, analytics and metrics.

Based on work done on diverse microbial GUS and arylsulfatases since 1980, and their essential role in modulating hormone action in the holobiont, Richard proposed the landmark hologenome theory of evolution in 1994 at Cold Spring Harbor. He was the first to describe the role of the microbiome as the driver of biological evolution, and its role in understanding and optimizing performance of biological systems. In 1997, Richard proposed the concept of ecotherapeutics as a strategy for modulating agriculture and health systems performance by adjusting population structures of microbial constituents. The hologenome theory has profound implications for how we think about ourselves, living systems, the origin of disease, the origins of social behavior and even social institutions in innovation. Richard is an 'Outstanding Social Entrepreneur' of the Schwab Foundation and a regular panelist at the World Economic Forum's (WEF) Davos annual meetings and Summits. Richard served on the WEF Global Agenda Council on Intellectual Property and the Global Agenda Council on the Economics of Innovation. He was named to Scientific American's list of the world's 50 Most Influential Technologists. His work has featured in countless media, including The Economist, New York Times, Newsweek, Red Herring, Nature, Science, Nature Biotechnology.

### Jenny Kao-Kniffin, Cornell University, US

Jenny Kao-Kniffin [pronunciation: GAOW-nif-IN] is an Associate Professor at Cornell University's School of Integrative Plant Science. She received her Ph.D. from the University of Wisconsin-Madison in Land Resources, with a specialization in Ecosystem Microbiology. She then served as a Postdoctoral Research Fellow with the National Science Foundation (NSF) investigating landscape-scale patterns of microbial composition near Barrow, Alaska. In 2019, she received the Presidential Early Career Award for Scientists and Engineers from the United States White House for her work on agricultural microbiomes. The research subjects range from crops and model plant species to invasive plants and weeds in agricultural and natural ecosystems, with a major focus on microbiome assembly, modification, and resilience impacting plant traits.





### Steven Lindow, University of California, Berkeley, US

The Lindow lab focuses on the ecology and management of plant-associated bacteria with a focus on both epiphytic and endophytic bacteria. A thrust of the lab has been on identification of traits that confer fitness and stress tolerance of bacteria on leaf surfaces and their regulation. The contribution of intra- and inter-species chemical communication that mediates expression of cell density-dependent traits in both Pseudomonas syringae and Xylella fastidiosa are being addressed with the aim of modifying their behaviors to achieve plant disease control. The emigration from and immigration to bacteria to plants via airborne transport is being studied to better understand processes determining the contextdependent assembly of epiphytic communities on leaves.

### Jos Raaijmakers, NIOO-KNAW, NL

Jos Raaijmakers received his MSc and PhD degrees from the University of Utrecht (Netherlands), where he studied phyllosphere and rhizosphere microbiology. His PhD work specifically focused on siderophore-mediated iron acquisition by rhizosphere bacteria. He undertook postdoctoral research at USDA and Washington State University (USA) on disease suppressive soils and the antifungal activity of secondary metabolites (phloroglucinols, phenazines) of root-associated bacteria. Upon returning to the Netherlands, he became an associate professor Plant Pathology at Wageningen University working on microbe-microbe/microbe-plant interactions and the diversity & functions of cyclic lipopeptides. Currently he is head of the Microbial Ecology department of the Netherlands Institute of Ecology (NIOO-KNAW) and a Professor at the Institute of Biology of Leiden University. His research program focuses on i) the impact of plant

domestication on microbiome assembly, and ii) the role of the plant microbiome in biotic stress tolerance.

### Adam Schikora, Julius Kühn-Institut, DE

Adam Schikora studied biology at the Universities of Warsaw, Poland and Göttingen, Germany and received his PhD in mineral plant nutrition. During the post-doctoral trainings in France and Austria, Adam focused on signaling pathways and the cellular responses to diverse stress stimuli, including the response to human pathogenic bacteria. Collaborative action between a host plant and associated bacteria is crucial for the establishment of an efficient interaction. In 2009 he became the leader of the Plant-Bacteria Interaction Group at the Institute of Phytopathology at JL University Giessen, Germany. His group investigates the stimulation of plant immune system by bacterial quorum sensing molecules on one hand, and on the other, how bacteria (e.g. the human pathogen Salmonella enterica) manipulate plant defense

mechanisms. In 2015 the group moved to Julius Kühn-Institut in Braunschweig, where he continues to study the interaction between crop plants and beneficial as well as human pathogenic bacteria. Currently Adam Schikora lectures plant physiology and microbiology at the TU Braunschweig.

### Klaus Schlaeppi, University of Bern, CH

Klaus Schlaeppi studied plant-microbe interactions and obtained his PhD from the University of Fribourg (Switzerland) based on work investigating plant defences against pathogens. As postdoctoral scientist at the MPI for Plant Breeding Research in Cologne (Germany) he contributed to method development and characterization of the commensal root model plant Arabidopsis thaliana and microbiota of the related Brassicaceae species. Back in Switzerland, as junior group leader he broadened his research interests to rhizosphere microbial ecology and how the root microbiota could be manipulated to improve agriculture. Today he is lecturer at the University of Bern

(Switzerland) and investigates with his team the contribution of the root microbiota to plant growth and how plants communicate to their root microbiota and take influence on their activities. The long-term ambition of his research program is to make use of plant microbiomes in smart and sustainable agriculture.











### Steven Vandenabeele, Aphea.Bio, BE

Steven Vandenabeele holds a PhD in biotechnology (University of Ghent) and has 20 years of experience in plant biotech (VIB Department of Plant Systems Biology, Rockefeller University, BASF Plant Science). Steven has worked in the ag-biotech industry for more than 10 years: he worked at BASF Plant Science as a group leader Technology Management, as the coordinator for the high-throughput plant phenotyping platform, and as global research manager of the rice yield project. Serving in these functions, he has gained strong experience in people, process and research project management. Three years ago, together with the VIB, he started building the business strategy to develop superior agricultural biologicals based on microbials. Aphea.Bio NV is currently operational since 2017, backed by venture capital. Steven is Aphea.Bio's Chief Scientific Officer and heads a team of 18 expert scientists.



### Alga Zuccaro, University of Cologne, DE

Alga studied marine biology at the University of Ancona, Italy before going on to do her PhD at the Technical Institute of Braunschweig, Germany in cooperation with the University of Portsmouth, UK. She held postdoctoral research positions in Braunschweig as well as at Oregon State University. After a period as Group Leader at the Institute of Phytopathology and Applied Zoology in Gießen, Germany and as Research Group Leader at the Max Planck Institute for Terrestrial Microbiology in Marburg, Germany, she became Professor in Ecological Genetics of Microbes at the University of Cologne, Germany in 2014. Research in Alga's group focuses on the mechanisms that enable symbiotic fungi to colonize plants successfully and on the processes accounting for variations in host preferences and fungal lifestyles. The prime models for her studies are the root endophyte *Serendipita indica* 



(Basidiomycota, Sebacinales), and the orchid mycorrhizal *Serendipita vermifera*, two beneficial symbionts that colonize the root epidermal and cortex cells of a broad range of plant species, including the dicot model plant *Arabidopsis thaliana* and the agriculturally important monocot *Hordeum vulgare*.



# **Scientific Program**

....

- - D M

11:00 - 13:00	Arrival, registration						
13:00 - 13:15	WELCOME						
13:15 - 14:00	OPENING LECTURE Supported by <b>NEW CONTERNATIONAL</b> Steven Lindow (University of California, Berkeley, US) Assembly of epiphytic bacterial communities on plants and their interactions with the plant host: insights for managing the plant microbiome						
<b>SESSION 1</b> 14:00 - 15:00	SUCCESSFUL MICROBIAL APPLICATIONS Session chairs: Kellye Eversole & Angela Sessitsch						
14:00 - 14:15	<b>Steven Vandenabeele (Aphea.Bio, BE)</b> An integrated technology pipeline for the development of superior agricultural biologicals						
14:15 - 14:30	Carolin Schneider (Inoq GmbH, DE) Concept for a reasonable use of mycorrhizal fungi in green business						
14:30 - 14:45	Fabricio Dario Cassan (Universidad Nacional de Rio Cuarto, AR) The successful history of <i>A. brasilense</i> Az39 in Agriculture. A metadata analysis						
14:45 - 15:00	<b>Robert Rotter (Multikraft, AT)</b> Market 2 Research 2 Market – The Multikraft Model						
15:00 - 15:30	Coffee break						
<b>SESSION 2</b> 15:30 - 17:35	MECHANISMS MEDIATING HOLOBIONT AND MULTIPARTITE INTERACTIONS Session chairs: Alga Zuccaro & Paolina Garbeva						
15:30 - 15:55	Alga Zuccaro (University of Cologne, DE) Molecular basis of plant-microbe interaction: know-how and tools for designing microbial communities with beneficial effects on plant growth						
15:55 - 16:20	Paolina Garbeva (NIOO-KNAW, NL) The importance of microbial chemical interactions for plant and soil health						
16:20 - 16:35	Luzia Stalder (University of Neuchâtel, CH) The functional ecology of plant microbiome interactions between the dominant fungal wheat pathogen <i>Zymoseptoria tritici</i> and Pseudomonas bacteria						
16:35 - 16:50	<ul> <li>Ahmed Elhady (Julius Kühn-Institut, DE)</li> <li>Modulation of rhizosphere microbiomes to suppress phytonematodes</li> </ul>						

### 16:50 - 17:05 POSTER PITCHES:

Rita Grosch (Leibniz Institute of Vegetable and Ornamental Crops, DE) Long-term organic and mineral fertilization strategies shape the rhizosphere microbiota and performance of lettuce Riitta Nissinen (University of Jyväskylä, FI) Soil glyphosate treatment impacts plant endophytic communities in plant species specific manner Kay Moisan (Wageningen University, NL) Can soil microbes enhance plant health without direct contact? Ana Bejarano Ramos (University of Trento, IT) Wanted: helper bacterial strains enhancing the biocontrol activity of Lysobacter capsici AZ78 17:05 - 17:20 Eva Baldassarre Svecova (Institute of Botany of the Czech Academy of Sciences, CZ) Effects of plant biostimulant treatments on the root-associated fungi of wheat and barley Ole Nybroe (University of Copenhagen, DK) 17:20 - 17:35 Bacterial communities associated with hyphae of plant beneficial fungal biofertilizers

- 17:35 20:30 Welcome Reception & Networking
- 18:30 20:30 **Poster Session I**



## DAY 2 | Tue, 3. Dec. | 8:30<sup>A.M.</sup> – 3:55<sup>P.M.</sup>

<b>SESSION 3</b> 08:30 - 10:10	PLANT UNDERSTANDING OF INTERACTIONS WITH BENEFICIAL MICROBES Session chairs: Heribert Hirt & Adam Schikora				
08:30 - 08:55	Heribert Hirt (KAUST, SA) Lessons from desert microbes to enable saline agriculture on arid lands				
08:55 - 09:10	Wu Xiong (Utrecht University, NL) Protists within rhizosphere microbiome determine plant health				
09:10 - 09:25	POSTER PITCHES:				
	<ul> <li>Robert R. Junker (Philipps-University Marburg, DE)</li> <li>Bacteria-flower interactions: bacterial modifications of floral sugar and scent composition result in changes in pollinator behavior and plant reproduction</li> <li>Beatriz Ramos-solano (University San Pablo Ceu, ES)</li> <li>F3H plays a pivotal role of on flavonoid metabolism improving adaptation to biotic stress in blackberry</li> <li>Martina Franchini (University of Nottingham, GB)</li> <li>Transcriptional response of tomato plants to the growth stimulation provided by Gluconacetobacter diazotrophicus</li> <li>Shree Pariyar (Forschungszentrum Jülich, DE)</li> <li>Plant growth promoting bacteria promotes germination and enhances early root traits</li> </ul>				
09:25 - 09:40	Shalini Kirthi Vasan (Teri-deakin Nanobiotechnology Centre, IN) Understanding molecular, metabolic and phylogenomic events underlying Arbuscular Mycorrhizal Symbiosis: Scope for improving crop productivity				
09:40 - 09:55	<b>Carmen Bianco (CNR, IT)</b> Co-inoculation of rice plants with nitrogen-fixing and indole-3-acetic acid (IAA)- producing endophytes: changes in physiological parameters of the host plant				
09:55 - 10:10	Sofie Goormachtig (VIB- UGent, BE) Streptomyces as a plant's best friend				
10:10 - 10:30	Coffee break				
<b>SESSION 3</b> 10:30 - 11:10	PLANT UNDERSTANDING OF INTERACTIONS WITH BENEFICIAL MICROBES Session chairs: Heribert Hirt & Adam Schikora				
10:30 - 10:55	Adam Schikora (Julius Kühn-Institut, DE) Genetic differences in barley govern the responsiveness to N-acyl homoserine lactone				
10:55 - 11:10	Astrid Forneck (University of Natural Resources and Life Sciences Vienna, AT) Role of Microbes in the Galler-Plant Interaction: <i>Pantoea agglomerans</i> affecting the compatible Grape Phylloxera ( <i>Daktulosphaira vitifoliae</i> ) - <i>Vitis</i> spp. Interaction				

<b>SESSION 4</b> 11:10 - 12:25	MICROBIOME UNDERSTANDING BEYOND PROFILING Session chairs: Jenny Kao-Kniffin & Klaus Schlaeppi						
11:10 - 11:35	Jenny Kao-Kniffin (Cornell University, US) Applying Concepts in Group-level Evolutionary Processes to Assemble Plant Beneficial Microbiomes						
11:35 - 11:50 <b>Rafael de Souza (University of Campinas, BR)</b> Synthetic microbial community from the sugarcane core microbiome i genetic features for successful plant colonization							
11:50 - 12:10	<ul> <li>POSTER PITCHES:</li> <li>Lukas Wille (FiBL &amp; ETHZ, CH)</li> <li>Genotype x soil interaction in the composition of root-rot pathogens of pea detected by quantitative PCR</li> <li>Romain Darriaut (INRA, FR)</li> <li>Contrasting soil microbial community profiles in healthy and declined vineyards</li> <li>Gorka Erice (Atens, ES)</li> <li>Changes in soil microbiome can alter peach tree physiology with implications in plant development and in the composition of secondary metabolites</li> <li>Arthur Goldstein (ESPCI Paris, FR)</li> <li>Functional analysis of soil microorganisms for agriculture using millifluidic droplets</li> <li>Birgit Mitter (AIT Austrian Institute of Technology, AT)</li> <li>The bacterial community in potato is recruited from soil and partly inherited across generations</li> <li>Yanyan Zhao (Université catholique de Louvain, BE)</li> <li>Root fungal community structure of <i>Alkanna tinctoria</i> differs with plant developmental stage</li> </ul>						
12:10 - 12:25	Mette Haubjerg Nicolaisen (University of Copenhagen, DK) A novel microcosm for recruiting phytate-degrading microbial communities under inherently competitive soil conditions						
12:25 - 14:30	Lunch break & Poster Session I						
14:30 - 14:45	Group Photo						
<b>SESSION 4</b> 14:45 - 15:55	MICROBIOME UNDERSTANDING BEYOND PROFILING Session chairs: Jenny Kao-Kniffin & Klaus Schlaeppi						
14:45 - 15:10	Klaus Schlaeppi (University of Bern, CH) Plant responsiveness to soil microbial feedbacks						
15:10 - 15:25	Hanna Faist (AIT Austrian Institute of Technology, AT) Evaluating the diversity and functional potential of plant microbiota to improve the selection of potato genotypes able to cope with combined water and nutrient limitations						
15:25 - 15:40	Matthieu Barret (INRA, FR)						
	Succession of microbial assemblages during seed development						
15:40- 15:55	Steffen Kolb (Leibniz Centre for Landscape Research - ZALF, DE) Volatilome of Wheat Microbiota System under Drought and Flooding: The VolCorn Consortium						
16:00	SOCIAL EVENT: Vienna tours (optional)						



## DAY 3 | Wed, 4. Dec. | 8:30<sup>A.M.</sup> - 6:30<sup>P.M.</sup>

08:30 - 09:00	KEYNOTE Bichard Lofferson (Cambia & OUT, AU)						
	Richard Jefferson (Cambia & QUT, AU)						
	Crops as merobionts: Regenerative agriculture, the microbiome and the climate crisis through the lens of the hologenome theory						
SESSION 5	MICROBIAL BIOCONTROL OF PESTS, PATHOGENS AND WEEDS						
09:00 - 10:25	Session chairs: Karen Bailey & Gabriele Berg						
09:00 - 09:25							
09.00 - 09.25	Gabriele Berg (Graz University of Technology, AT) Plant microbiome management for sustainable agriculture						
09:25 - 09:40	Michael Rothballer (Helmholtz Zentrum München, DE)						
	The functional relevance of microbe-plant-insect interaction in a cereal crop						
00.40 00.55	system POSTER PITCHES:						
09:40 - 09:55	Alejandro del Barrio Duque (AIT Austrian Institute of Technology, AT)						
	Mycolicibacterium strains interact positively with Serendipita (Piriformospora)						
	<i>indica</i> for crop enhancement and biocontrol of pathogens						
	Lara Reinbacher (Agroscope, CH)						
	Biological control of wireworms in cover crops						
	Mei Li (Nanjing Agricultural University, CN)						
	Facilitation promotes invasions in plant-associated microbial communities						
	Williams O. Anteyi (University of Hohenheim, DE)						
	In vivo localization and role of Fusarium oxysporum f.sp. strigae and Bacillus						
	subtilis against Striga hermonthica in an integrated biocontrol system						
09:55 - 10:10	Xingchen Zhao (Ghent University, BE)						
	Behaviour of Bt ABTS-1857 as a biological control agent on spinach plants, cut						
10.40 40.25	leaves and spinach juice						
10:10 - 10:25	Gary Felton (The Pennsylvania State University, US)						
	The leaky gut syndrome: insect gut bacteria exacerbate physical and chemical defenses of plants						
10:25 - 10:45	Coffee break						
SESSION 5	MICROBIAL BIOCONTROL OF PESTS, PATHOGENS AND WEEDS						
10:45 - 12:40	Session chairs: Karen Bailey & Gabriele Berg						
10:45 - 11:10	Karen L. Bailey (Agriculture & Agri-Food Canada, CA)						
10.45 11.10	Bioherbicides from creation to commercial success – What's the problem?						
11:10 - 11:25	Julia Friman (Wageningen University, NL)						
11.10 11.25	Contrasting effects of the rhizobacterium <i>Pseudomonas simiae</i> on above- and						
	belowground insect herbivores						
11:25 - 11:40	POSTER PITCHES:						
	Leone Olivieri (NIAB EMR, GB)						
	Microbial ecology of the European apple canker pathosystem ( <i>N. ditissima</i> )						
	Shivani Khatri (Indian Institute of Technology, Delhi, IN)						
	Microbiome-assisted management of plant diseases						
	Daria Rybakova (Graz University of Technology, AT)						
	A species-specific crosstalk via volatile exchange between a biocontrol agent						
	Serratia plymuthica HRO-C48 and fungal plant pathogens.						
	Valeska Villegas Escobar (EAFIT University, CO)						
	Effect of <i>Bacillus subtilis</i> EA-CB0575 on the microbiota, growth development and						
	health of banana plants						



11:40 - 11:55	Anne Muola (University of Turku, FI)
	The role of endophytic entomopathogens in modulating plant-microbe-insect interactions
11:55 - 12:10	Friederike Trognitz (AIT Austrian Institute of Technology, AT)
	Plant associated bacteria for the control of <i>Impatiens glandulifera</i>
12:10 - 12:25	Alejandro Gimeno (Agroscope and University of Zurich, CH)
	Suppressing <i>Fusarium graminearum</i> and mycotoxins by application of microbial
	antagonists on infected crop residues
12:25 - 12:40	Mohammadhossein Ravanbakhsh (Utrecht University, NL)
	Combining nanomaterials and phages for enhanced bacterial wilt control
12:40 - 14:30	Poster session II & Lunch break
SESSION 5	MICROBIAL BIOCONTROL OF PESTS, PATHOGENS AND WEEDS
14:30 - 15:15	Session chairs: Karen Bailey & Gabriele Berg
14:30 - 14:45	Christopher Dunlap (USDA-ARS, US)
14.30 - 14.43	Iturinic lipopeptide diversity of the <i>Bacillus subitlis</i> group
14:45 - 15:00	Jie Hu (Utrecht University, NL)
14.45 - 15.00	Microbial consortia effectively suppress and prevent infections of <i>Ralstonia</i>
	pseudosolanacearumin Rosa sp.
15:00 - 15:15	Sylwia Jafra (Intercollegiate Faculty of Biotechnology UG and MUG, University of
19.00 19.19	Gdansk, PL)
	The plant protecting and plant growth promoting abilities of the synthetic micro-
	consortium of antagonistic bacterial strains.
SESSION 6	MICROBIAL APPLICATIONS FOR IMPROVING NUTRITION AND ABIOTIC STRESS
15:15 - 16:35	TOLERANCE
10.00	Session chairs: Philipp Franken & Günter Brader
15:15 - 15:40	Philipp Franken (University of Applied Sciences Erfurt, DE)
10.10	How plants benefit from root-colonizing fungi: There's more than one way to crack
	an egg
15:40 - 16:05	POSTER PITCHES:
10.00	Mirjam Seeliger (INOQ GmbH, DE)
	Interactions of arbuscular mycorrhizal fungi and winter wheat in contrasting
	cropping systems
	Raphael Boussageon (Burgundy University, FR)
	Impact of beneficial microorganisms on strawberry growth, fruit production,
	nutritional quality and volatilome
	Annamaria Bevivino (Italian National Agency for New Technologies, Energy and
	Sustainable Economic Development, IT)
	SIMBA: Design, formulation and optimization of plant growth-promoting microbes
	for their use as microbial consortia inoculants
	Francisco Martin Usero (Arid Zones Experimental Station, CSIC, ES)
	Influence of soil microbial communities linked to organic matter addition on
	tomato (Solanum lycopersicum L.) plant growth under intensive farming
	Mohammed Antar (McGill University, CA)
	Microbial consortia: a way to enhance crop yield under both controlled
	environment and field conditions
	Shubhangi Sharma (Leibniz-Institut für Gemüse-und Zierpflanzenbau,
	Großbeeren, Germany, DE)
	Effect of coinoculation of Rhizoglomus irregulare, and hyphae attached phosphate
	solubilizing bacteria on Solanum lycopersicum



16:05 - 16:20 **Tania Galindo (The Pennsylvania State University, US)** Matching root anatomical and architectural phenotypes with soil microorganisms to improve nutrient and water uptake efficiency: a new perspective in plant microbiome research

16:20 - 16.35 Shubham Dubey (IIT Delhi, IN) Combating salinity stress with Rhizosphere Engineering: A next-generation approach

- 16:35 18:30 Coffee Break & Poster session II
- 19:00 23:00 Conference Dinner (optional)



## DAY 4 | Thu, 5. Dec. | 8:30<sup>A.M.</sup> - 2:00<sup>P.M.</sup>

SESSION 6	MICROBIAL APPLICATIONS FOR IMPROVING NUTRITION AND ABIOTIC STRESS							
08:30 - 10:25	TOLERANCE							
	Session chairs: Philipp Franken & Günter Brader							
08:30 - 08:55	Günter Brader (AIT Austrian Institute of Technology, AT)							
	Phosphate fertilization in crops – the contribution of bacteria and fungi							
08:55 - 09:10	Klára Bradáčová (University of Hohenheim, DE)							
	Maize inoculation with microbial consortia: contrasting effects on rhizosphere							
	activities, nutrient acquisition and early growth in different soils							
09:10 - 09:25	Borjana Arsova (Forschungszentrum Jülich, DE)							
	The impact of beneficial microbes on <i>Brachypodium</i> nutrient uptake under limiting							
	supplies of nitrogen and phosphorus, monitored with non-invasive phenotyping							
	and molecular approaches							
09:25 - 09:40	Chanz Robbins (Université de Lausanne, CH)							
	Does genetic variation in single spore progeny of an arbuscular mycorrhizal fungus							
	impact cassava yield							
09:40 - 09:55	Sarah Symanczik (Forschungsinstitut für biologischen Landbau, CH)							
	Fertiledatepalm – a transdisciplinary collaboration project to ameliorate date palm							
	cultivation via microbial inoculation, organic matter management and mixed							
09:55 - 10:10	cropping using nurse plants Jaderson Armanhi (University of Campinas, BR)							
09.55 - 10.10	Unraveling plant physiological behavior modulated by a synthetic microbial							
	community using a non-invasive and continuous medium-scale phenotyping							
	platform							
10:10 - 10:25	Narges Moradtalab (Universität Hohenheim, DE)							
	Synergistic contribution of microbial consortia, micronutrients, and ammonium							
	fertilization to cold tolerance in maize by regulating phytohormone homeostasis							
	and oxidative stress defence							
10:25 - 10:45	Coffee break							
10:45 - 11:15	SPECIAL SESSION - REGULATORY ISSUES							
10:45 - 11:00 Gianpiero Gueli Alletti (APIS Applied Insect Science GmbH, DE)								
	Registration of biopesticides in the European Union							
11:00 - 11:15	Faina Kamilova (Knoell NL BV, NL)							
	Proposal for the application of microbiomes in industry: regulatory challenges and							
	opportunities							
SESSION 7	DISRUPTIVE APPROACHES FOR ENGINEERING THE PHYTOBIOME & MICROBIAL							
11:15 - 12:35	DELIVERY							
	Session chairs: Trevor Charles & Michael Ionesco							
11:15 - 11:40	Trevor Charles (University of Waterloo, CA)							
	Can we tune the microbiome in controlled environment agriculture?							
11:40 - 12:05	Michael Ionescu (Lavie-Bio, IL)							
	Harnessing the power of computational genomics to optimize next generation ag-							
	biologicals							
12:05 - 12:20	Sascha Patz (University of Tübingen, DE)							
	Lifting the veil of virulence and benefits of plant-associated bacteria by							
	metagenomics approaches							



### Scientific Program

12:20 - 12:35				
	Opportunities and challenges of microbial seed application			
12:35 - 13:00	Refreshment Break			
13:00 - 13:40	CLOSING LECTURE			
	Jos Raaijmakers (NIOO-KNAW, NL)			
	Towards new road MAPs to engineer plant microbiomes			
13:40 - 14:00	AWARDS & CLOSURE			



### **Posters Table - Postersession I**

### Postersession I Monday 2. Dec., 18:30 - 20:30 (Even no.) & Tuesday 3. Dec., 12:25 – 14:30 (Odd no.)

Poster #	Successful microbial applications	Poster #	Plant understanding of interactions with beneficial microbes	PF-PU-03	Martina Franchini
PP1-SA-01	Enrique Gutiérrez Albanchez	PP1-PU-01	Florian Schindler	PF-PU-04	Shree Pariyar
PP1-SA-02	Natacha Bodenhausen	PP1-PU-02	Tetard-Jones Catherine		
PP1-SA-03	Cintia Csorba	PP1-PU-03	Karolin Pohl	Poster #	Poster Session 1: Microbiome understanding beyond profiling
PP1-SA-04	Michele Pallucchini	PP1-PU-04	Jin-Soo Son	PP1-MU-01	Silvia D. Schrey
PP1-SA-05	Andreea Cosoveanu	PP1-PU-05	Johan Meijer	PP1-MU-02	Anurag Chaturvedi
PP1-SA-06	Ryan Sebring	PP1-PU-06	Jemma Roberts	PP1-MU-03	Dagmara Sirová
PP1-SA-07	Isabelle Caugant	PP1-PU-07	Oleg A. Kharchuk	PP1-MU-04	Annika Hoffmann
PP1-SA-08	Katharina Kraxberger	PP1-PU-08	Maximilian Hanusch	PP1-MU-05	Marie Legein
PP1-SA-09	Lisa-Maria Ohler	PP1-PU-09	Michelle K. Carkner		
		PP1-PU-10	Marjo Helander	PF-MU-01	Lukas Wille
Poster #	Mechanisms mediating holobiont and multipartite interactions	PP1-PU-11	Ankita Chopra	PF-MU-02	Romain Darriaut
PP1-MI-01	Pierre-Emmanuel Courty	PP1-PU-12	Frank Waller	PF-MU-03	Gorka Erice
PP1-MI-02	Kari Saikkonen	PP1-PU-13	Barbara Bort Biazotti	PF-MU-04	Arthur Goldstein
PP1-MI-03	Dorota Magdalena Krzyzanowska	PP1-PU-14	Nikoleta Galambos	PF-MU-05	Birgit Mitter
PP1-MI-04	Mason Kamalani Chock	PP1-PU-15	Sa-Youl Ghim	PF-MU-06	Yanyan Zhao
PP1-MI-05	Angelique Rat	PP1-PU-16	Abhishek Shrestha		
PP1-MI-06	Henry Müller	PP1-PU-17	Christoph Lehnen		
PP1-MI-07	Suni Anie Mathew	PP1-PU-18	Stephan Wawra		
PP1-MI-08	Lena Fragner	PP1-PU-19	Romy Moukarzel		
PP1-MI-09	Boyoung Lee	PP1-PU-20	Soo-yeong Lee		
PP1-MI-10	Shruti Pavagadhi	PP1-PU-21	Michael Opitz		
PP1-MI-11	Aditi Buch	PP1-PU-22	Daniela Sangiorgio		
PP1-MI-12	Henry David Naranjo Benavides	PP1-PU-23	Anna Marie Hallasgo		
PF-MI-01	Rita Grosch	PP1-PU-24	Dongmei Lyu		
PF-MI-02	Riitta Nissinen	PP1-PU-25	Muhammad Ahmad		
PF-MI-03	Kay Moisan	PF-PU-01	Robert R. Junker		
PF-MI-04	Ana Bejarano Ramos	PF-PU-02	Beatriz Ramos-solano		

20 micRope

### **Posters Table - Postersession II**

## Postersession II Wednesday 4. Dec., 12:40 - 14:30 (Even no.) & Wednesday 4. Dec., 16:35 - 18:30 (Odd no.)

Poster #	Microbial biocontrol of pests, pathogens and weeds	PF-MB-03	Mei Li	PP2-MA-20	Donald Smith
PP2-MB-01	Pierre-Antoine Noceto	PF-MB-04	Williams O. Anteyi	PP2-MA-21	Beatriz R. Vazquez-de-Aldana
PP2-MB-02	Thure Pavlo Hauser	PF-MB-05	Leone Olivieri	PF-MA-01	Mirjam Seeliger
PP2-MB-03	Tomasz Maciag	PF-MB-06	Shivani Khatri	PF-MA-02	Daniel Wipf
PP2-MB-04	Louisa Robinson Boyer	PF-MB-07	Daria Rybakova	PF-MA-03	Annamaria Bevivino
PP2-MB-05	Maria Isabella Prigigallo	PF-MB-08	Valeska Villegas Escobar	PF-MA-04	Francisco Martin Usero
PP2-MB-06	Franz Stocker			PF-MA-05	Mohammed Antar
PP2-MB-07	Gwendolin Wehner	Poster #	Microbial applications for improving nutrition and abiotic stress tolerance	PF-MA-06	Shubhangi Sharma
PP2-MB-08	Hadis Jayanti	PP2-MA-01	Francisco Javier Gutierrez-mañero		
PP2-MB-09	Alessandro Passera	PP2-MA-02	Antoine Persyn	Poster #	Disruptive approaches for engineering the phytobiome & microbial delivery
PP2-MB-10	Lisa Kappel	PP2-MA-03	Allene A. Macabuhay	PP2-DA-01	Lilach Iasur-Kruh
PP2-MB-11	Daniel Uribe	PP2-MA-04	Daniel Buchvaldt Amby	PP2-DA-02	David L. Hallahan
PP2-MB-12	Marco Saracchi	PP2-MA-05	Markus Weinmann	PP2-DA-03	Soon-Kyeong Kwon
PP2-MB-13	Wolfgang Hinterdobler	PP2-MA-06	Yoshinari Ohwaki	PP2-DA-04	Randy Martin
PP2-MB-14	Abhishek Anand	PP2-MA-07	Julian Preiner		
PP2-MB-15	Birgit Jensen	PP2-MA-08	Zhichun Yan	Poster #	Varia
PP2-MB-16	Marta Streminska	PP2-MA-09	Juliya Thomas	PP2-V-01	Jasper Schierstaedt
PP2-MB-17	Rachel Backer	PP2-MA-10	Agnieszka Domka	PP2-V-02	Martina Sauert
PP2-MB-18	Sabine Gruber	PP2-MA-11	Patricia Dorr de Quadros	PP2-V-03	Johannes Ben Herpell
PP2-MB-19	David B. Collinge	PP2-MA-12	Annapurna Kannepalli	PP2-V-04	Tanja Kostic
PP2-MB-20	Leandro Astarita	PP2-MA-13	Md Mohibul Alam Khan	PP2-V-05	Humberto Castillo Gonzalez
PP2-MB-21	Kumar Aundy	PP2-MA-14	Sowmyalakshmi Subramanian	PP2-V-06	Prashantee Singh
PP2-MB-22	Anthi Vlassi	PP2-MA-15	Bong-Nam Chung		
PP2-MB-23	Giovanni Bubici	PP2-MA-16	Xu Cheng		
PP2-MB-24	Eliane R. Santarém	PP2-MA-17	Jakub Jez		
PF-MB-01	Alejandro del Barrio Duque	PP2-MA-18	Przemysław Bernat		
PF-MB-02	Lara Reinbacher	PP2-MA-19	Kerrie Farrar		

21

npe

# LECTURES



## miCROPe 2019 - Microbe-assisted crop production opportunities, challenges & needs Vienna, Austria, December 2 – 5, 2019

### **Opening & Opening Lecture**

**Chair: Angela Sessitsch** 



# O-01 Assembly of epiphytic bacterial communities on plants and their interactions with the plant host: insights for managing the plant microbiome

#### Steven Lindow

Plant and Microbial Biology, University of California, Berkeley, United States of America

Arial plant surfaces often harbor large epiphytic bacterial populations. The size and composition of these communities however are determined by both small-scale interactions of bacteria with each other and with their plant host that determine growth and survival, as well as large-scale features such as the proximity and abundance of other plant species that contribute immigrant inoculum. The maximum population size of epiphytic bacteria is limited by Carbon availability on the plant surface and differs among plant species due to the differing amounts of exudates. These Carbon sources and therefore sites of bacterial colonization on plants are spatially heterogeneous, with the majority of bacteria residing in localized sites harboring relatively large, mixed species cellular aggregates. Cell density-dependent behaviors, often modulated by so-called quorum sensing signal molecules facilitate preferential survival of bacteria at such sites during stressful desiccation conditions. Bacteria also modify the local environment on plant surfaces by their production of hygroscopic biosurfactants that make liquid water more available. Many bacteria also produce compounds such as 3-indole acetic acid (IAA) that apparently facilitate the plant conversion of sucrose to fructose, thus facilitating the growth of epiphytes that typically can consume such monosaccharides at the relatively low concentrations made available by exhibition from plants, but which cannot consume disaccharides at such low concentrations. The composition of epiphytic bacterial communities is only moderately plant species-specific, apparently driven by yet to be determined morphological and chemical features of plant surfaces. Epiphytic bacteria readily escape from the surface of plants and strongly influence the composition of airborne bacteria nearby. Such airborne bacteria are a primary source of immigrant bacteria for the establishment of epiphytic communities on plants that typically harbor few or no resident bacteria early in their development. Because of the differing amounts and types of surrounding vegetation present during the development of new tissues of a given plant species, the composition and size of epiphytic communities on crop species is very context-dependent, and can be strongly influenced by management practices that influence the agro-ecological context of a given crop plant and can be strongly influenced by inoculation of immigrationlimited crops by beneficial bacteria.



### miCROPe 2019 - Microbe-assisted crop production opportunities, challenges & needs Vienna, Austria, December 2 – 5, 2019

## Successful microbial applications

Chairs: Kellye Eversole & Angela Sessitsch



# SA-01 An integrated technology pipeline for the development of superior agricultural biologicals.

### Steven Vandenabeele

#### Aphea.Bio, Belgium

Agricultural biologicals is the broad term for naturally occurring materials such as microorganisms and natural extracts that have the potential to improve the health status and yield of crops. Biologicals can complement or substitute agricultural chemical products and form a cornerstone in the path towards an urgently needed sustainable, integrated agriculture.

Aphea.Bio's mission is 'Applied Nature for Better Agriculture' and develops novel and superior agricultural biologicals. Aphea.Bio focusses on microorganism-based products that help reducing fertilizer application and controlling fungal diseases for maize and wheat and is in a unique position to deliver novel and powerful solutions to the market because of its innovative research platform. Therefore, it studies and exploits the *in nature* occurring beneficial interactions between plants and soil microorganisms. By identifying amongst the hundred thousands of microorganisms present in the soil those that closely and actively interact with the plant, Aphea.Bio is able to boost plant growth and health by applying the beneficial microorganisms as a seed coating or a sprayable.

Aphea.Bio has built a collection of wheat/maize biostimulant candidate products that are being validated in field trials across the EU. The biostimulant R&D pipeline comprises different steps: a vast microbiome mapping approach across based on wheat and maize rhizo- and endospheres grown in ~100 different low nutrient soils, the proprietary microbial culturing technologies that allows to tap into the 'unculturable' microbial strain pool, the high-throughput phenotypic *in planta* screening in the greenhouse and the initial field trial results will be presented. Besides, in the biocontrol program, the screening of ten thousands of microbial extracts against *Fusarium graminearum, Zymoseptoria tritici* and *Puccinia striiformis* forms the basis for a portfolio of microbial strains that significantly reduce disease symptoms *in planta* in the climate chambers. Screening of the lead strains against other major fungal diseases in other crops such as vegetables are performed to test their broad efficacy. In this presentation, an overview of the biocontrol and biostimulant technologies will be discussed.

### SA-02 Concept for a reasonable use of mycorrhizal fungi in green business

**Carolin Schneider<sup>1</sup>**, Louis Mercy<sup>1</sup>, Eva Lucic<sup>1</sup>, Alberico Bedini<sup>1</sup>, Alicia Varela Alonso<sup>2</sup>, Philipp Rödel<sup>2</sup>, Stéphane Declerck<sup>3</sup>, Philipp Franken<sup>4</sup>

<sup>1</sup> Inoq GmbH, Germany

<sup>2</sup> Institut für Pflanzenkultur, Solkau 2, 29465 Schnega, Germany

<sup>3</sup> Université catholique de Louvain, B-1348 Louvain-la-Neuve, Belgium

<sup>4</sup> Erfurt Research Centre for Horticultural Crops, University of Applied Sciences Erfurt and Friedrich Schiller University Jena, Kühnhäuser Straße 101, D-99090 Erfurt, Germany

There is a discussion ongoing about the management of arbuscular mycorrhizal (AM) fungi in large-scale agriculture. Recent papers reviewed management practices of industrial agriculture (without additional inoculation of AM fungi), their impact on abundance and diversity of the symbionts and on crop yield, but concluded little evidence that mycorrhizal fungi need to be a target of management, at least in wheat. Others found that given the need to feed more people in the world, the yield is not the only parameter to consider, but long-term sustainability and especially yield stability of agroecosystems will become even more important: AM fungi and other soil biota make important contributions to soil aggregation and many other ecosystem functions, e.g. yield stability under changing environmental conditions. Manifold studied improvements in nutrient uptake through AM fungi may reduce the need for fertilizer, whilst achieving an equal yield. Even after harvest, AM fungi can enhance food storage properties. New results of biotisation with mycorrhiza and bacteria in phytopharmaceutical drug production will be highlighted in the presentation.

If everything would be simple and positive, the use of AM fungi inoculum and the awareness of farmers to manage AM fungi and other soil biota would be routine, but this is only the case for a limited number of crops and environments. For sure there are still important questions to answer before we will be able to master the application and predict the effect of mycorrhizal fungi in industrial agriculture, among them the factor implementing plant-fungi interactions in plant breeding. Here new results of the concept of training of AM fungi to subsequent environmental conditions will be presented. This includes the use of root organ cultures for acclimatization to high Pi, and the response of the acclimatized AM fungal strain to different stimuli (strigolactones and different Pi levels) during the pre-symbiotic and the symbiotic phase.



### SA-03 The successful history of *A. brasilense* Az39 in Agriculture. A metadata analysis

Belén Rodriguez, Sofía Nievas, Gastón López, Romina Molina, Anahí Coniglio, Verónica Mora, Fabricio Cassán

Laboratorio de Fisiología Vegetal y de la Interacción Planta-Microorganismo, Universidad Nacional de Rio Cuarto, Argentina

Azospirillum is one of the most studied bacterial genera in the last 60 years; However, the history of the appearance of biological products formulated with this bacterium began in the 1980s, but intensified in the last 20 years in Argentina, Brazil and the rest of South America. In the case of Argentina, A. brasilense Az39 is the strain that has been recommended for more than 40 years for the production of biofertilizers for wheat, sorghum, corn and soybean (coinoculation). This strain has demonstrated a great capacity to promote plant growth with average yield increases greater than 10.0% and a success rate higher than 70% in different crops in thousand experiments. Despite the immense amount of information available at the agronomic level, until a few years ago very little was known about the molecular basis that determined the ability of this strain to promote plant growth. In 2012, the Laboratorio de Fisiología Vegetal y de la Interacción Planta-Microorganismo of the Universidad Nacional de Río Cuarto conformed an international consortium with the aim to analyze the genome sequence of A. brasilense Az39 and B. japonicum E109, two of the most used strains for biofertilizer production in South America. Using a combined sequencing strategy, it was established that the Az39 genome has a size of 7.39 Mpb distributed in 6 replicons [1 chromosome, 3 chromides and 2 plasmids]. Through the use of comparative bioinformatics tools, numerous genes and putative proteins involved in the expression of plant growth promotion mechanisms and other related with the rhizosphere lifestyle were identified. The decoding of this information has provided a solid basis for the elucidation of new mechanisms of interaction and growth promotion, as well as some specific components that would determine the agronomic success of this microorganism. In this presentation we will address some of the new biological models recently identified for this bacterium and how they affect their rhizosphere lifestyle.

\* BR and SN equally contributed to this work

Financial Support: This work was supported by Consejo Nacional de Investigación Científico-Tecnológica de Argentina (CONICET) and FONCyT through your projects PICT 2012, 2015 and 2017.

### SA-04 Market 2 Research 2 Market – The Multikraft Model

#### **Robert Rotter**

#### Multikraft, Austria

How a former stock food company started to use an unknown microorganisms-mix 22 years ago and how this started a new field of successful household and professional animal and plant applications.

How basic EM (Effective Microorganisms)-Technology was transferred into Research based Multikraft-Technology, the main microbial isolates and connecting ingredients of different applications.

In-depth R&D to improve inner properties, quality and effects of this technology AND how research is transferred to market.

How our professional customers learn to act preventative using microbial applications rather than reacting on their problems in conventional ways with less positive effects.



### miCROPe 2019 - Microbe-assisted crop production opportunities, challenges & needs Vienna, Austria, December 2 – 5, 2019

Mechanisms mediating holobiont and multipartite interactions

Chairs: Alga Zuccaro & Paolina Garbeva



# MI-01 Molecular basis of plant-microbe interaction: know-how and tools for designing microbial communities with beneficial effects on plant growth

### Alga Zuccaro

#### University of Cologne, Germany

Progress achieved in multipartite interactions allows us now to characterize the mechanisms underlying microbe-plant symbioses in a community context and thus achieve a step change in understanding the functional interconnections between soil, microbiota and plants. Here we address how interaction between the beneficial root endophyte *Serendipita vermifera* and the pathogen *Bipolaris sorokiniana* affects fungal behavior and barley host responses in a microbial community context.

### MI-02 The importance of microbial chemical interactions for plant and soil health

#### Paolina Garbeva

Microbial Ecology, NIOO-KNAW, Netherlands

Microorganisms produce a vast array of secondary metabolites, both soluble and volatile, which have diverse and important biological functions.

The production of microbial metabolites is often triggered by intra- and inter-specific microbial interactions. For example, the antimicrobial volatile compound 2,5-bis(1-methylethyl)-pyrazine is produced as a result of the interaction between the Gram-positive *Paenibacillus* sp. and the Gram-negative *Burkholderia* sp..

In my talk, I will focus on belowground interactions and discuss some of the microbial metabolites involved in microbemicrobe and plant-microbe interactions. I will highlight the ecological importance of microbial chemical interactions for plant and soil health.



# MI-03 The functional ecology of plant microbiome interactions between the dominant fungal wheat pathogen *Zymoseptoria tritici* and Pseudomonas bacteria

Luzia Stalder<sup>1</sup>, Monika Maurhofer<sup>2</sup>, Daniel Croll<sup>1</sup>

<sup>1</sup> Biology, University of Neuchâtel, Switzerland

<sup>2</sup> Group of Plant Pathology, Institute of Integrative Biology, ETH Zurich, Zurich, Switzerland.

Plants are exposed to a wide range of pathogenic fungi and bacteria. It has been shown that the outcome of individual interactions between pathogen and plant cannot be understood in isolation, as the presence of other microorganisms can act synergistically or antagonistically in the disease progression. Yet, factors governing complex (i.e. at least tripartite) interactions are largely unknown. Here, we establish a new microbiome interaction model using tripartite interactions of bacteria, fungi and plants. For this, we focus on wheat, *Zymoseptoria tritici*, the major fungal pathogen of wheat, and the bacteria *Pseudomonas*, a dominant member of the phyllosphere. We characterize how intra-specific variation in a fungal pathogen determines microbial activities in the phyllosphere using genome-wide association mapping. In addition, we will characterize how differential gene expression of the fungus and the bacteria influences the outcome of bacterial-fungal competition. Our results will provide insights into the mechanism of competitive exclusion in the phyllosphere microbiome. We will generate knowledge of the exact loci that fungi evolved as defenses against *Pseudomonas*. The identification of such previously unknown loci will likely reveal previously unknown antimicrobial compounds that could be assessed for agricultural and even human applications.

### MI-04 Modulation of rhizosphere microbiomes to suppress phytonematodes

Ahmed Elhady, Johannes Hallmann, Holger Heuer

Institute for Epidemiology and Pathogen Diagnostics, Julius Kühn-Institut (JKI), Germany

In soil, beneficial and pathogenic biota simultaneously colonize the plant rhizosphere. Among the harmful organisms are plant-parasitic nematodes that are economic threats worldwide. They migrate through the rhizosphere to their host plants and live on the cytoplasm of living root cells. Plants influence the microbiome in their rhizosphere and thereby pass a modified microbiome on to the plant subsequently growing in the same soil. In this study, we investigated the effect of plant-soil feedback of different pre-crops rotated with soybean in order to suppress root lesion nematodes (RLN). Transplanting the rhizosphere microbiome from different crops resulted in different degrees of suppressiveness against RLN on soybean roots. The inoculated microbiomes from soybean, Ethiopian mustard and maize significantly reduced the invasion of RLN compared to the microbiomes from bulk soil or tomato rhizosphere. In the analogous experiment with tomato plants and either RLN (Pratylenchus penetrans) or root-knot nematodes (Meloidogyne incognita), the microbiomes from maize and tomato reduced root invasion of both nematodes compared to the microbiomes from soybean or bulk soil. In a split-root experiment, the suppressive effect of the microbiome on P. penetrans was mediated by the plant and depended on the plant species from which the microbiome was transplanted. The DGGE fingerprints of the fungal and bacterial communities of the donor rhizospheres significantly differed among the treatments, as well as the fungal and bacterial communities attached to the surface of RLN that were recovered from those rhizospheres. This implied that attached microbes might antagonized the RLN, directly and/or by signals to the plant. Engineering the plant associated microbiome through pre-, cover- or inter-crops may lead to eco-friendly crop protection.



# **PF-MI-01** Long-term organic and mineral fertilization strategies shape the rhizosphere microbiota and performance of lettuce

Doreen Babin<sup>1</sup>, Soumitra Paul Chowdhury<sup>2</sup>, Loreen Sommermann<sup>3</sup>, Samuel Jacquiod<sup>4</sup>, Søren J. Sørensen<sup>5</sup>, Jörg Geistlinger<sup>3</sup>, Michael Rothballer<sup>6</sup>, Kornelia Smalla<sup>1</sup>, **Rita Grosch<sup>7</sup>** 

<sup>1</sup> Julius Kühn-Institut, Federal Research Centre for Cultivated Plants (JKI), Institute for Epidemiology and Pathogen Diagnostics, Braunschweig, Germany

<sup>3</sup> Anhalt University of Applied Sciences, Bernburg, Germany

<sup>4</sup> Agroécologie, AgroSup Dijon, INRA, Univ. Bourgogne Franche-Comté, France

<sup>5</sup> University of Copenhagen, Department of Biology, Section of Microbiology, Copenhagen, Denmark

<sup>6</sup> Helmholtz Zentrum München, German Research Center for Environmental Health, Germany

<sup>7</sup> Plant-microbe systems, Leibniz Institute of Vegetable and Ornamental Crops, Germany

Belowground plant-microbe interactions are crucial for plant development and health. Although previous studies have shown that soil microbial communities are influenced by fertilization strategies, less is known about the aboveground plant response to the rhizosphere microbiota assemblage shaped by agricultural management strategies. In our study, we aimed to investigate the effects of long-term fertilization strategies across field sites on the rhizosphere prokaryotic (Bacteria and Archaea) community composition and plant performance. We conducted growth chamber experiments with lettuce (Lactuca sativa L.) cultivated in soils from two long-term field experiments situated in Therwil, Switzerland and Thyrow, Germany, each of which compared organic vs. mineral fertilization strategies. High-throughput sequencing of bacterial 16S rRNA genes amplified from total community DNA showed a rhizosphere core microbiota shared in all lettuce plants across soils, going beyond differences in community composition depending on field site and fertilization strategies. Firmicutes were enriched irrespective of the field site in the rhizosphere of lettuce grown in organically fertilized soils. When cultivated in organically fertilized soils, a higher expression of several stress-related genes was observed by RT-qPCR analysis in lettuce leaves although plants were visibly free of disease symptoms. Another experiment showed that in presence of the soil-borne model pathogen Rhizoctonia solani AG1-IB, the plant productivity (dry biomass) decreased in soils from Thyrow with both long-term organic and mineral fertilization strategies. Moreover, we observed that the expression of genes like BGlu42 (β Glucosidase), OPT3 (Iron transporter) and MYB15 (Transcription factor) were significantly higher in the plants grown in organically fertilized soils in presence of R. solani. This could indicate an ISR response via iron-mobilizing phenolics, simulating root iron-deficiency response and changes in ironhomeostasis mechanisms in the rhizosphere, which can be expressed systemically throughout the plant. The ongoing analysis of the rhizosphere microbiome would reveal more information about the suggested mechanism. Taken together, besides effects of fertilization strategy and field site, results of our study under controlled conditions demonstrate the crucial role of the lettuce plant in driving the rhizosphere microbiota assemblage.

# **PF-MI-02** Soil glyphosate treatment impacts plant endophytic communities in plant species specific manner

Riitta Nissinen<sup>0</sup>, Miia Rainio<sup>1</sup>, Suni Mathew<sup>1</sup>, Irma Saloniemi<sup>1</sup>, Kari Saikkonen<sup>1</sup>, Marjo Helander<sup>1</sup>

<sup>1</sup> University of Turku, Finland

<sup>0</sup> Biological and Environmental Science, University of Jyväskylä, Finland

Glyphosate (N-phosphonomethylglycine) is a broad spectrum herbicide, used in weed killing products over four decades, and currently the most commonly used herbicides in agricultural systems and landscaping in EU. Glyphosate is applied before crops are sown, and facilitate better growth of crops by eliminating competing weeds. In soil, glyphosate is quickly adsorbed to soil particles and degraded by soil microbes. Currently, limited information is available on impact of glyphosate on structure and functioning of non target microbial communities, and no information is at hand on impact on plant microbiomes, especially in cold climate agrosystems, where glyphosate degradation might be impacted by cold climate.

In this study, we investigated the impact of soil glyphosate treatment on microbial communities of five different agricultural plant species: faba bean, meadow fescue, oat, potato and hemp. We treated the sample plots (12 replicate plots per treatment) with glyphosate salt or control solution (no glyphosate) twice a year (spring and autumn) for several years. The plants were sown two weeks after glyphosate (or control) treatment, and were harvested in August. Microbial communities were analysed by 16S rRNA gene (bacteria) or ribosomal ITS (fungi) targeted sequencing from community DNA isolated from plant leaves, roots and from bulk soils.

At the time of sampling, no glyphosate was detected in soil or in plant tissue samples, except in potato roots. In agreement with previous studies, we saw no impact of glyphosate treatment on diversity or structure of soil bacterial communities. Plant bacterial communities were primarily impacted by plant species and tissue (leaf, root, nodule). Species richness of faba bean and potato bacterial communities were lower than in other plant species. Glyphosate treatment had no impact on species richness of leaf or root microbial communities. However, bacterial community



<sup>&</sup>lt;sup>2</sup> Institute of Network Biology, Helmholtz Zentrum München, German Research Center for Environmental Health, Germany

structures were impacted in most of the plant species. The community structures in potato roots, and in the leaves of meadow fescue, hemp (OTU level) and oat (family level) were significantly different in plants from glyphosate treated and non treated plots. These differences were driven by significantly lower abundances of several OTUs representing bacterial genus *Pseudomonas* and families *Xanthomonadaceae* and *Rhizobiaceae* in plants grown in glyphosate treated soils.

### PF-MI-03 Can soil microbes enhance plant health without direct contact?

Kay Moisan<sup>1</sup>, Viviane Cordovez<sup>2</sup>, Dani Lucas-Barbosa<sup>1</sup>, Jos M. Raaijmakers<sup>2</sup>, Marcel Dicke<sup>1</sup>

<sup>1</sup> Laboratory of Entomology, Wageningen University, Netherlands

<sup>2</sup> Netherlands Institute of Ecology (NIOO-KNAW), Droevendaalsesteeg 10, 6708PB Wageningen, The Netherlands

To cope with the different biotic and abiotic stresses that they encounter during their growing phase, plants have evolved different defense strategies that include the recruitment of beneficial soil-borne microorganisms. These microbial partnerships comprise direct colonization and direct protection of the plant tissues, but interestingly also priming of plant defense and growth promotion. Remarkably, it was recently found that in the absence of direct physical contact, plants can still perceive soil microbes *via* volatiles, and respond to these odour cues. Volatiles are commonly produced by microbes, yet the specificity of plant responses to volatiles emitted by microbes of different lifestyles has been overlooked. In this presentation, I will address the effects of volatiles emitted by a range of soil-borne fungi, including plant pathogens and plant mutualists, on plant growth, flowering and resistance. I will discuss how fungal volatiles can influence plant interactions with insect pests, both aboveground and belowground, and affect plant performance. Addressing microbial volatiles as part of the phytobiome opens up new potential applications for crop protection.

# **PF-MI-04** Wanted: helper bacterial strains enhancing the biocontrol activity of *Lysobacter capsici* AZ78

Ana Bejarano<sup>1</sup>, Chiara Gasparetto<sup>2</sup>, Gerardo Puopolo<sup>2</sup>, Ilaria Pertot<sup>1</sup>

<sup>1</sup> Centre of Agriculture, Food and Environment, University of Trento, Italy

<sup>2</sup> Department of Sustainable Ecosystems & Bioresources, Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Italy

Bacterial biocontrol agents (BBCAs) represent a promising strategy for the sustainable management of soilborne plant pathogens. Nonetheless, their efficacy in field often differs from the one achieved in controlled conditions. This might be related to the biological complexity of agricultural soils. Indeed, BBCAs might be considered as invaders that must compete with and integrate in resident soil microbial communities. At the same time, occurring microbial interactions can positively influence the establishment and functioning of a given BBCA. Based on this assumption, the aim of the work herein was to select helper bacterial strains (HBS) boosting the biocontrol activity of the model BBCA *Lysobacter capsici* AZ78 (AZ78) and understand the mechanisms regulating the interaction. A collection of 36 bacterial isolates deriving from tomato rhizosphere was screened for evaluating their impact on the biocontrol activity of AZ78 and further characterized. 16S rRNA gene sequence analysis showed high microbial diversity among the isolates. Out of the 36 isolates, 15 enhanced the activity of AZ78 against *Pythium ultimum in vitro*.

The enhancement of AZ78 biocontrol activity might be associated to the establishment of inter-specific interactions based on the release and perception of quorum-sensing (QS) signal molecules between the HBS and AZ78. Interestingly, *Ensifer adherans* strains producing long chain (C6-C12) N-acyl-homoserine lactones (AHLs) QS molecules boosted the production of antimicrobial compounds active against *P. ultimum*. In contrast, a similar positive effect on AZ78 inhibitory activity was not observed in one *E. adherans* strain producing C4 AHLs. However, other bacterial strains belonging to *Achromobacter deleyi, Stenotrophomonas tumilicola, Variovorax boronicumulans* boosted AZ78 inhibitory activity even if they were unable to produce AHLs. Based on these results, further investigations will be dedicated to understanding how QS signals modulate AZ78 biocontrol activity.



# MI-05 Effects of plant biostimulant treatments on the root-associated fungi of wheat and barley

**Eva Baldassarre Švecová**<sup>1</sup>, Libor Mrnka<sup>1</sup>, Tomáš Frantík<sup>1</sup>, Christoph Stephan Schmidt<sup>1</sup>, Martin Bárnet<sup>2</sup>, Miroslav Vosátka<sup>1</sup>

<sup>1</sup> Department of Mycorrhizal Symbioses, Institute of Botany of the Czech Academy of Sciences, Czech Republic

<sup>2</sup> AGRA GROUP a.s., Tovární 201, 387 15 Střelské Hoštice, Czech Republic

One of the great challenges the society faces today is to reduce negative impacts of agriculture on the environment while maintaining high crop yields. A promising strategy is to use environmentally friendly plant biostimulants which can enhance crop growth performance, for example, by affecting plant metabolism. However, there has been a lack of knowledge on the effects of biostimulants on the interaction of plants with root-associated fungi which represent important drivers of soil and plant functioning.

In this work, the effects of protein hydrolysate-based biostimulants on field-grown wheat and barley and their rootassociated fungi were investigated. Plant biostimulants were foliar-applied in the stem elongation stage (BBCH31) and the plants were sampled 14 and 56 days after the treatments. We aimed to determine putative changes in the rootassociated fungal symbiotic communities over time, along with plant growth responses to the biostimulants. Leaf/root dry weight, leaf chlorophyll content and plant height were positively affected by the biostimulant treatments depending on crop species and a type of biostimulating product.

Biostimulants did not affect the root colonization by mycorrhizal fungi in either crop but they significantly increased the abundance of vesicles/spores in comparison with controls in wheat. The metabarcoding of root fungal biodiversity revealed that the community composition was significantly affected by the sampling time, but not by the treatments. Few dominant fungal species differed in the abundance among the treatments. The abundance of *Glomerales* was highest in wheat treated with protein hydrolysates but not statistically different from the controls. Based on our results, protein hydrolysate treatments that promote plant growth do not significantly influence the composition of root-associated fungal communities, though their side contribution to the biostimulants' effect cannot be excluded. More extensive research is necessary to decipher mechanisms of plant-mediated effects of protein hydrolysates on root-associated fungi.

### MI-06 Bacterial communities associated with hyphae of plant beneficial fungal biofertilizers

Xiuli Hao<sup>1</sup>, Behnoush Ghodsalavi<sup>4</sup>, Stefan Olsson<sup>2</sup>, Yong-Guan Zhu<sup>3</sup>, Mette H Nicolaisen<sup>4</sup>, **Ole Nybroe<sup>4</sup>** 

<sup>1</sup> State Key Laboratory of Agricultural Microbiology & Key Laboratory of Arable Land Conservation, Huazhong Agricultural University, Wuhan, PR China

<sup>2</sup> College of Plant Protection, Fujian Agriculture and Forestry University, Fuzhou City, Fujian Province, PR China

<sup>3</sup> Key Lab of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen, PR China

<sup>4</sup> Plant and Environmental Sciences, University of Copenhagen, Denmark

Fungi from the genus *Penicillium* colonize the rhizosphere and solubilize inorganic phosphate (P), thereby potentially increasing P availability to plants. We have isolated beneficial bacteria from *Penicillum* hyphae that promote fungal growth and P solubilization. Exploiting this positive interaction has high potential for development of biofertilizer consortia for increased plant nutrient use efficiency. However, the main drivers for assembly of hyphae-associated bacterial communities and for their abilities to solubilize or mobilize P in soil remain elusive. We developed a novel baiting type microcosm to study bacterial colonization of hyphae in soil. The approach was used to investigate the impact of soil type on bacterial communities associating with hyphae of two Penicillium species. 16S rRNA gene targeted sequencing analysis showed that hyphae-associated communities had lower diversity and less variation in taxonomic structure than soil communities. Besides the hyphosphere effect, the soil type had a large impact on hyphae-associated communities. In particular, soil properties as pH, total carbon, concentrations of P and Mg, as well as soil texture significantly affected the relative abundance of several higher taxa. In contrast, the effect of fungal species was visible only for few discriminative taxa and specific enriched OTUs. qPCR analysis revealed increased abundance of genes involved in inorganic or organic P cycling in several hyphae-associated communities. Taken together, the Penicillium hyphosphere represents a unique niche, where soil type and fungal species together orchestrate microbiome assemblage and where fungal as well as bacterial activities may create a hot spot for P turnover. The current study provides a knowledge base important for future development of robust biofertilizer consortia.



### miCROPe 2019 - Microbe-assisted crop production opportunities, challenges & needs Vienna, Austria, December 2 – 5, 2019

## Plant understanding of interactions with beneficial microbes

Chairs: Heribert Hirt & Adam Schikora



### PU-01 Lessons from desert microbes to enable saline agriculture on arid lands

Axel de Zelicourt<sup>1</sup>, Cristina Andres-Barrao<sup>2</sup>, Abdul Aziz Eida<sup>2</sup>, Hanin Alzubaidy<sup>2</sup>, Rewaa Jalal<sup>2</sup>, Marilia Almeida-Trapp<sup>3</sup>, Kiruthiga Mariappan<sup>2</sup>, Olga Artyukh<sup>2</sup>, Axel Mithoefer<sup>3</sup>, Maged Saad<sup>2</sup>, **Heribert Hirt<sup>2</sup>** 

<sup>1</sup> IPS2, Gif-sur-Yvette, F <sup>2</sup> KAUST, Saudi Arabia

<sup>3</sup> MPI, Jena, D

A major global challenge of any country in this century is to achieve food security. This is largely hindered by excessive heat, salinity and a lack of water, making up for 60% of annual yield losses. Moreover, 20% of Earth's land surface are made up of desert regions, which are currently considered unfit for agriculture. A simple solution to the above challenge would be to expand agriculture to so far unused land and use abundantly available saline water. Since most crops lack the ability to cope with salinity, major plant breeding efforts are underway to enhance crop tolerance to salt stress. However, these costly and long-term approaches so far provided mostly disappointing results. In contrast, rhizosphere microbes from desert plants showed that various crops can be grown on marginal lands using saline irrigation, making a big step forward towards food security in the future. We show that the certain desert bacterial endophytes can enhance salt tolerance of crops by favourably reducing salt uptake into the shoots. A molecular analysis of the plantmicrobial interaction in Arabidopsis unravelled a major role of the sulfur pathways in both organisms and a coordinate regulatory role of plant ethylene signalling in this process. These findings open new possibilities for breeding salt-adapted crops and tailoring functional synthetic communities to complement deficiencies in soil, crop and disease resistance.

### PU-02 Protists within rhizosphere microbiome determine plant health

**Wu Xiong**<sup>1</sup>, Alexandre Jousset<sup>1</sup>, George Kowalchuk<sup>1</sup>, Zhong Wei<sup>2</sup>, Yuqi Song<sup>2</sup>, Yian Gu<sup>2</sup>, Tianjie Yang<sup>2</sup>, Yangchun Xu<sup>2</sup>, Qirong Shen<sup>2</sup>, Stefan Geisen<sup>3</sup>

<sup>1</sup> Department of Biology, Utrecht University, Netherlands

<sup>2</sup> Nanjing Agricultural University

<sup>3</sup> Netherlands Institute for Ecology (NIOO-KNAW)

Plant health is intimately controlled by the associated rhizosphere microbiome. Most microorganisms interact positively or neutrally with plants, thereby mitigating negative pathogen-induced effects. However, most microbiome research is focusing on bacteria. As such, it remains unknown if other microbial components, especially the main bacterial predators – protists – are linked to pathogen suppression. In a field setting, we monitored the rhizosphere microbiome throughout the growth of tomato plants, that either developed disease symptoms induced by the bacterial pathogen Ralstonia solanacearum or remained healthy. To explore potential underlying mechanism that determine plant performance, we investigated the taxonomic and functional structure of protist communities and linked those with fungi, bacteria, pathogen and bacterial-produced secondary metabolites biosynthesis genes. We show that pathogen development is best predicted by the community structure of protists. In line with bacteria, the community structure of protist consumers (*i.e.*, phagotrophs) at the start of plant differ between later diseased and healthy plants could serve as important indicator of plant health. The relative abundance of phagotrophs negatively correlated with pathogen abundance, suggesting direct predator-prey interactions of protists leading to pathogen declines. In addition, protist community composition was linked with distinct bacterial communities in healthy compared with later diseased plants; this link led to increased expression of secondary metabolite genes that are linked with disease suppression. Therefore, we highlight that protists integrate rhizosphere microbiome structure and functioning throughout plant growth, determining plant performance. This illustrates the potential to both predict plant performance based on initial screening of protist communities and for targeted application of protists to steer microbiome structure and functioning to increase plant performance.



# **PF-PU-01** Bacteria-flower interactions: bacterial modifications of floral sugar and scent composition result in changes in pollinator behavior and plant reproduction

**Robert R. Junker<sup>1</sup>**, Polina Marchenko<sup>1</sup>, Gerard Farré-Armengol<sup>1</sup>, Paul Gaube<sup>2</sup>, Alexander Keller<sup>2</sup>, Ricarda Scheiner<sup>3</sup>, Sina Strauß<sup>3</sup>, Raimund Tenhaken<sup>1</sup>

<sup>1</sup> Evolutionary Ecology of Plants, Philipps-University Marburg, Germany

<sup>2</sup> University of Würzburg, Animal Ecology and Tropical Biology, Am Hubland, 97074 Würzburg, Germany

<sup>3</sup> University of Würzburg, Behavioral Ecology and Sociobiology, Am Hubland, 97074 Würzburg, Germany

Bacteria play crucial roles in plant growth, development, and health and we are slowly beginning to acknowledge their involvement in floral ecology. We focused on two bacterial strains (*Pseudomonas syringae* and *Pantoea agglomerans*) that commonly colonize above-ground plant surfaces including flowers to investigate their effects on floral phenotypes, pollinator behavior and plant reproduction. The genera *Pseudomonas* and *Pantoea* were commonly associated with several plant species along a land-use gradient. Sugar composition of plant surfaces was found to be plant organ and species specific. In lab-experiments we found that *P. syringae* and *P. agglomerans* differently respond to sucrose, glucose and fructose and selectively remove sugars from media containing all three sugars. Thus, flowers may be able to control bacterial community composition by the sugars provided as carbon sources. In turn, bacteria may alter availability of sugars on flower surfaces and nectar. In further experiments, we found similar effects in interactions between bacteria and floral scent compounds. Sugar and floral scent compounds are key in mediating flower-pollinator interactions. Accordingly, we found that *P. syringae P. agglomerans* affect the behavior of honeybees and bumblebees in the lab, and whole flower visitor communities in the field. These effects on pollinator behavior resulted in increased plant reproduction. Our study reveals mechanisms how flowers may control bacterial community composition and how bacteria affect plant reproduction with implications for field applications to increase crop yield.

# **PF-PU-02 F3H** plays a pivotal role of on flavonoid metabolism improving adaptation to biotic stress in blackberry

Enrique Gutierrez Albanchez<sup>1,2</sup>, Ana García-Villaraco<sup>1</sup>, José Antonio Lucas<sup>2</sup>, JA Lucas, F. Javier Gutierrez-Mañero<sup>1</sup>, **Beatriz Ramos-Solano**<sup>1</sup>

<sup>1</sup> Universidad San Pablo-CEU Universities. Facultadad de Farmacia. Ctra Boadilla del Monte Km 5.3, Boadilla del Monte 28668 Madrid, Spain.

 $^{\rm 2}$  BIOBAB R&D. Bajada a Vargas 1. Agüimes, 35260 Las Palmas, Spain

The aim of this study is to determine the involvement of the flavonol-anthocyanin pathway on plant adaptation to biotic stress using the *B.amyloliquefaciens* QV15 to trigger blackberry metabolism and identify target genes to improve plant fitness and fruit quality. To achieve this goal, field-grown blackberries were root-inoculated with QV15 along its growth cycle. At fruiting, a transcriptomic analysis by RNA-Seq was performed on leaves and fruits of treated and non-treated field-grown blackberries after a sustained mildew outbreak; expression of the regulating and core genes of the Flavonol-Anthocyanin pathway were analysed by qPCR and metabolomic profiles by UHPLC/ESI-qTOF-MS; plant protection was found to be up to 88%. Overexpression of step-controlling genes in leaves and fruits, associated to lower concentration of flavonols and anthocyanins in QV15-treated plants, together with a higher protection suggest a phytoanticipin role for flavonols in blackberry; kempferol-3-rutinoside concentration was strikingly high. Overexpression of *RuF3H* (Flavonol-3-hidroxylase) suggests a pivotal role in the coordination of committing steps in this pathway, controlling carbon flux towards the different sinks. Furthermore, this C demand is supported by an activation of the photosynthetic machinery, boosted by a coordinated control of ROS into a sub-lethal range, and associated to enhanced protection to biotic stress



# **PF-PU-03** Transcriptional response of tomato plants to the growth stimulation provided by *Gluconacetobacter diazotrophicus*

**Martina Franchini<sup>1</sup>**, Michele Pallucchini<sup>1</sup>, Nathalie Narraidoo<sup>2</sup>, Marco Moretto<sup>3</sup>, Phil Hill<sup>4</sup>, Michele Perazzolli<sup>3</sup>, Rupert Fray<sup>4</sup>

<sup>1</sup> Plant Sciences, University of Nottingham, United Kingdom

<sup>2</sup> Azotic Technologies Ltd., Biocity, Pennyfoot St, Nottingham NG1 1GF, UK

<sup>3</sup> Fondazione Edmund Mach, Via Edmund Mach, 1, 38010 San Michele All'adige (TN), Italy

<sup>4</sup> University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, Leicestershire, UK

*Gluconacetobacter diazotrophicus* (Gd) is a non-nodulating endophytic nitrogen-fixing bacterium able to colonise a wide range of crops and to provide beneficial effects to the plant. Besides the production of phytohormones, Gd ability of fixing nitrogen for the plants consumption makes it a good candidate for applications as biofertiliser. The interaction mechanism between Gd and the plant has been partly characterised in sugar cane, but little information is available on interaction mechanisms with other plants.

The aim of this project was to evaluate the effect of Gd inoculation on tomato plants and to elucidate the molecular mechanism of this interaction.

In this study, a wild type (WT) and a nitrogen fixation-impaired strain (nifD<sup>-</sup>) of Gd were employed. Tomato seedlings were grown at two different nitrogen levels (0 mM and 2 mM KNO<sub>3</sub>) for 10 days and the Gd bacterial suspension was then applied to tomato roots. Four days after incubation, the bacterial suspension was removed and plants were incubated for 14 days before phenotypical evaluations. At 0 mM KNO<sub>3</sub>, WT-inoculated plants showed an increase in chlorophyll content and shoot length in comparison to uninoculated (U.T.) and nifD<sup>-</sup>-inoculated plants. Comparison between U.T. and nifD<sup>-</sup>-inoculated plants showed no difference in chlorophyll content and shoot length. At 2 mM KNO<sub>3</sub>, WT-inoculated plants presented an increase in chlorophyll content and shoot length was positively affected by nifD<sup>-</sup> inoculation in comparison to U.T.. The positive effect provided by nifD<sup>-</sup> inoculated plants displayed no difference in terms of chlorophyll content.

Root samples were collected at one and 14 days after inoculation from U.T., WT- and nifD<sup>-</sup>-treated plants and subjected to RNA-Seq analysis. Functional annotation of differentially expressed genes is currently in progress in order to identify transcriptional regulations responsible for the early and late response of tomato plants to Gd.

# PF-PU-04 Plant growth promoting bacteria promotes germination and enhances early root traits

**Shree R. Pariyar<sup>1</sup>**, Kerstin A. Nagel<sup>1</sup>, Jonas Lentz<sup>1</sup>, Robert Koller<sup>1</sup>, Daniel Pflugfelder<sup>1</sup>, Varghese Thomas<sup>2</sup>, Michael Klueken<sup>2</sup>, Isolde Haeuser-Hahn<sup>2</sup>, Lakshmi P. Manavalan<sup>2</sup>, Michelle Watt<sup>1</sup>

<sup>1</sup> IBG-2: Plant Science,, Forschungszentrum Jülich, Germany

<sup>2</sup> Bayer Crop Science Division

Holistic understanding of the relationship of plant trait functionalities (roots and shoots) with beneficial microbes requires advanced technological platforms. In past, various, mostly destructive methods have been used to measure plant microbe interaction, which are not able to quantify the dynamic reaction of plants to microbes. Our major goal was to validate if non-invasive high throughput phenotyping platforms such as *GrowScreen-PaGe* (germination paper based; Gioia et al., 2016) and *GrowScreen-Rhizo* (soil-filled rhizotrons; Nagel et al., 2012) enable quantification of the dynamic effects of plant microbes on root and shoot traits. We monitored the effect of plant growth promoting bacteria (PGPB) on Soybean roots and shoots and quantified the associated microbial abundance on roots and growth media (paper and soil) at three depth layers. Our results show that PGPB promotes early germination and increases root traits on germination paper as well as in soil-filled rhizotrons. The results indicate that both phenotyping approaches were efficient to quantify responses of root and shoot traits during plant-microbe interaction. The results will shed new insights into the dynamics of plant microbe interactions and novel application options for utilizing biologicals for crop improvement.



# PU-03 Understanding molecular, metabolic and phylogenomic events underlying Arbuscular Mycorrhizal Symbiosis: Scope for improving crop productivity

**Shalini K. Vasan<sup>0</sup>**, Divya Srivastava<sup>0</sup>, Xavier Conlan<sup>1</sup>, Leena Johny<sup>0</sup>, Pushplata Singh<sup>0</sup>, Sangram Lenka<sup>0</sup>, David Cahill<sup>1</sup>, Alok Adholeya<sup>0</sup>

<sup>1</sup> Deakin University, Waurn Ponds, geelong, Australia

<sup>o</sup> Sustainable Agriculture Division, Teri-deakin Nanobiotechnology Centre, India

Arbuscular Mycorrhizal Fungi (AMF) are mutualists that colonize more than 80% terrestrial plants. This study uses in vitro hairy root cultures to analyze the regulatory control exerted over AMF by the plant hosts and non-hosts by integrating transcriptome, metabolome and phylogenomic analysis. We have screened 21 hairy root cultures using bright field microscopy and ink vinegar staining approach to identify a host (Tomato Roma) and a non-host (Tomato Grafter) of AMF Rhizophagus irregularis. Bi-compartmental studies comprising of a mycorrhiza established host (Daucus carota) was used to re-confirm non-host status of Tomato Grafter. Comparative transcriptomics of mycorrhized host, blank host and blank non- host revealed >2000 differentially expressed genes (DEGs, FDR 0.01). KEGG pathway analysis of DEGs was used as a reference for complete metabolomic profiling aimed at analyzing AMF-specific early signalling patterns distinguishing a host from a non-host tomato root culture. Top 50 DEG hits were functionally characterized in silico and among these DEGs most relevant 12 gene-targets were subjected to phylogenetic analysis in 7 hosts and 4 non- host plant species for identifying the pattern of gene convergence and/or divergence which could trace the evolutionary molecular patterns for adaptations favouring AM symbiosis in hosts. Further, we have proposed the concept of conditional non- host (Tomato Grafter) versus absolute Non- host (Arabidopsis thaliana, Brassica rapa, Beta vulgaris and Nelumbo nucifera). Understanding the molecular basis of AM symbiosis distinguishing a host from nonhost might provide scope for introducing crops that are modified to attain better nutrient uptake, crop productivity, drought and pathogen tolerance. This study paves the way for application of mycorrhization in agriculturally relevant host plants.

# PU-04 Co-inoculation of rice plants with nitrogen-fixing and indole-3-acetic acid (IAA)producing endophytes: changes in physiological parameters of the host plant.

Anna Andreozzi<sup>5</sup>, Pilar Prieto<sup>1</sup>, Jesus Mercado-Blanco<sup>2</sup>, Stefano Monaco<sup>3</sup>, Elisa Zampieri<sup>3</sup>, Silvia Romano<sup>5</sup>, Gianpiero Valè<sup>4</sup>, Roberto Defez<sup>5</sup>, **Carmen Bianco<sup>5</sup>** 

<sup>1</sup> Departamento de Mejora Genética, Instituto de Agricultura Sostenible (IAS), Consejo Superior de Investigaciones Científicas (CSIC), Campus 'Alameda del Obispo', Avd. Menéndez Pidal s/n, 14004 Córdoba, Spain

<sup>2</sup> Departamento de Protección de Cultivos, Instituto de Agricultura Sostenible (IAS), Consejo Superior de Investigaciones Científicas (CSIC), Campus 'Alameda del Obispo', Avd. Menéndez Pidal s/n, 14004 Córdoba, Spain

<sup>3</sup> CREA – CI, Research Centre for Cereal and Industrial Crops, 13100, Vercelli, Italy

<sup>4</sup> DISIT, Dipartimento di Scienze e Innovazione Tecnologica, Università del Piemonte Orientale, Piazza San Eusebio 5, I-13100 Vercelli, Italy <sup>5</sup> IBBR, CNR, Italy

To cope with the growing world population an increase in the production of the main crops for human nutrition, including rice, is now urgently needed. To achieve this goal, the expensive and polluting chemical fertilizers have already been overused. Nitrogen (N) is one of the primary nutrients limiting plant growth in agriculture. Biological nitrogen fixation (BNF) by diazotrophic bacteria, which reduce atmospheric N to ammonium using nitrogenase enzyme systems, accounts for 30-50% of the total N in crop fields. The area of BNF research has been expanded by the discovery of Nfixing bacterial endophytes in non-nodulating plants. In the last few years a wide diversity of bacteria associated with cereals have shown to possess the nifH gene coding for dinitrogenase reductase. This gene is genetically conserved and thus traditionally used as a marker gene to study the genetic diversity of diazotrophs in nature. To improve plant growth and yield the use of genetically modified diazotrophs or the co-inoculation with nitrogen-fixing and plant growth promoting bacteria has been proposed. We have previously reported that the strain Enterobacter cloacae RCA25-64, engineered to produce and release 36-fold more indole-3-acetic acid (IAA) than the wild type E. cloacae RCA25, showed increased nifH gene expression and nitrogenase activity in liquid cultures and inoculated rice plants. In the present study we analysed the effect of purified IAA on the nitrogen-fixing ability of E. cloacae RCA25. Co-inoculation studies were also carried out to test the ability of different wild type IAA-producing endophytes to enhance the nifH gene expression and nitrogenase activity in E. cloacae RCA25, preventing the use of engineered strains. Our results showed that Herbaspirillum huttiense RCA24 performed best. Improvements in nitrogen-fixation and changes in physiological parameters such as chlorophyll, nitrogen content and shoot dry weight were observed for rice plants (Oryza sativa L. cv. Baldo) co-inoculated with strains RCA25 and RCA24 in a 10:1 ratio. Based on confocal laser scanning microscopy analysis, strain RCA24 was the best colonizer of the root interior and the only IAA producer located in the same root niche occupied by RCA25 cells. Our data highlight that the assessment of location and distribution of the individual microbial components within the host plant tissues is fundamental to select bio-inoculants containing IAA-producer strains able to enhance nitrogen-fixation.



## PU-05 Streptomyces as a plant's best friend

Sarah Langendries<sup>2</sup>, Emilie Froussart<sup>2</sup>, Tom Viaene<sup>2</sup>, Paulo Cortesi<sup>1</sup>, Sofie Goormachtig<sup>2</sup>

<sup>1</sup> DeFENS - Department of Food, Environmental and Nutritional Sciences Università degli Studi di Milano Via Celoria, 2 - 20133 Milano - Italy <sup>2</sup> VIB- UGent, Belgium

Plant roots release diverse compounds to create a unique environment, the rhizosphere, in which a vast amount of microorganisms find their niche for growth. A subset of these microorganisms, commonly referred to as PLANT GROWTH-PROMOTING RHIZOBACTERIA (PGPR), greatly contributes to plant health and productivity in various manners. Microbiome based studies revealed that *Streptomyces sp.* belong to the root microbiome of many different plant species and that their relative abundances change when plants are grown in changing environments. Indeed, by performing 16S amplicon sequencing and using wheat and maize as examples, we observed that drought causes an increase in the relative abundances of *Streptomyces sp.* of the root microbiome while cold treatment caused a depletion indicating that various abiotic factors cause different changes in the root microbial composition.

These observations triggered us to unravel the molecular interaction between plant roots and *Streptomyces sp.* and to get insights into how the latter can support plant growth. Using wheat but also *Arabidopsis thaliana* as model plants, a detailed phenotypical and molecular insight will be presented into how *Streptomyces sp.* colonize plants and how this colonization provokes plant growth promotion.

# PU-06 Genetic differences in barley govern the responsiveness to N-acyl homoserine lactone

### Adam Schikora

Institute for Epidemiology and Pathogen Diagnostics, Julius Kühn-Institut, Germany

Priming crop plants for enhanced resistance using biocontrol agents is an efficient disease management strategy. Enhanced resistance in barley (*Hordeum vulgare L.*) against pathogens, such as the powdery mildew-causing fungus *Blumeria graminis* f.sp. *hordei* (*Bgh*), is of high importance. The beneficial effects of bacterial quorum sensing molecules on resistance and plant growth have been shown in different plant species, includig barley. Here, we present the effects of the *N*-3-oxotetradecanoyl-L-homoserine lactone (oxo-C14-HSL) on the resistance of different barley genotypes. Genetically diverse accessions of barley were identified and exposed to either the beneficial, oxo-C14-HSL-producing bacterium *Ensifer meliloti* or the pure *N*-acyl homoserine lactone (AHL) molecule. Metabolic profiling along with expression analysis of selected genes and physiological assays revealed that the capacity to react varies among different barley genotypes. We demonstrate that upon pretreatment with the AHL molecule, *AHL-primable* barley genotype expresses enhanced resistance against *Bgh*. We further show that pretreatment with AHL correlates with stronger activation of barley MAP kinases and regulation of defense-related *PR1* and *PR17b* genes after a subsequent treatment with chitin. Noticeable was the stronger accumulation of lignin. Our results suggest that appropriate genetic background is required for AHL-induced priming. At the same time, they bear potential to use these genetic features for new breeding and plant protection approaches.



# PU-07 Role of Microbes in the Galler-Plant Interaction: *Pantoea agglomerans* affecting the compatible Grape Phylloxera (*Daktulosphaira vitifoliae*) - *Vitis* spp. Interaction

Markus Eitle<sup>2</sup>, Magdalena Walch<sup>2</sup>, Urska Vrhovsek<sup>1</sup>, Astrid Forneck<sup>2</sup>

<sup>1</sup> Food Quality and Nutrition Department, Fondazione Edmund Mach, San Michele all'Adige, Italy

<sup>2</sup> Department of Crop Sciences, University of Natural Resources and Life Sciences Vienna, Austria

Grape phylloxera (*Daktulosphaira vitifoliae* Fitch) induces histoid leaf galls on susceptible *Vitis* spp. leading to severe host damage symptoms and economic losses in commercial vineyards. In the last years the reports of canopy infestations with leaf-galling *D. vitifoliae* were increasing with particularly high insect populations observed on the foliage of interspecific fungi resistant hybrids (European *V. vinifera* x American *Vitisspp.*). The mutualistic bacterium *Pantoea agglomerans* was detected in leaf galls and attached to the larval integuments of grape phylloxera. In previous work we showed that grape phylloxera associated Pantoea ssp. are not maternally transmitted. So far *Pantoea* species were shown to play diverse roles for the compatibility of the host-parasite interactions by e.g. breakdown of toxic substance or affecting systemic host plant defences by altering the biosynthesis of volatile metabolites.

Here we aim to assess and compare the volatile metabolomes of grapevine leaves of different hosts under grape phylloxera attack. We hypothise that *V. vinifera*L. leaves release quantitatively more host defence associated volatiles than interspecific hybrids under grape phylloxera attack. Secondly we hypothise that the presence of mutualistic *P. agglomerans* results in a decrease of host defence associated secondary metabolites thereby favouring insect development and gall formation.

In total twenty-one single eye cuttings of either V. viniferaL. Riesling and Vitisspp. Muscaris (Solaris× Gelber Muskateller) are rooted and cultivated in isolated quarantine cages located in climate chambers ( $26\pm3^{\circ}$ C, 60% rH, 16 h pp) After 2.5 months of incubation insect demographic and host response parameters per plant are determined. Leaf galls, differentiated by 4 insect larval stages vs. not infested leaf tissues are sampled. Subsequent semi-quantitative GC-MS analyses screen the sampled tissues for released host associated defensive VOCs such as MeJA, MeSA, terpenes, aromatic compounds, alcohols and *n*-alkanes.

We expect that the comparative analysis of the generated volatile metabolomes reveals potentially effective secondary volatiles against leaf infesting grape phylloxera, whose biosynthetic and transport pathways are already conserved within the grapevine genome. Furthermore we hope to gain deeper insights in the so far elusive tritrophic interaction between grape phylloxera, *P. agglomerans* and *Vitis* spp.



# miCROPe 2019 - Microbe-assisted crop production opportunities, challenges & needs Vienna, Austria, December 2 – 5, 2019

# Microbiome understanding beyond profiling

Chairs: Klaus Schlaeppi & Jenny Kao-Kniffin



# MU-01 Applying Concepts in Group-level Evolutionary Processes to Assemble Plant Beneficial Microbiomes

### Jenny Kao-Kniffin

### Plant Science, Cornell University, United States of America

Group-level processes dominate rhizosphere interactions impacting plant growth and development; however, empirical studies of plant beneficial microbiomes largely focus on single species or strains of microorganisms. In a series of experiments on directed evolution of the rhizosphere microbiome, we aimed to develop microbial communities associated with enhanced seed yield in rapidly cycling *Brassica rapa* and altered flowering time in *Arabidopsis thaliana* using a multi-generation experimental evolution system. We hypothesized that phenotypic plasticity can be modified through selective pressure on the plant trait, while the agents of selection are microorganisms associated with the modified plant traits. Microbiomes were collected from the rhizosphere soil of a subset of plants to be used as inoculants for the subsequent planting generation. After multiple generations of selection for modified plant traits, the composition and function of the rhizosphere microbiome shifted away from the control microbiomes. The microbiomes assembled from a specific trait selection pressure showed the ability to alter the plant traits of novel plant host genotypes or species. The results of the experiments suggest that directed evolution of rhizosphere microbiomes impact the plasticity of plant phenotypes, which could play an important role in commercial plant production systems.

# MU-02 Synthetic microbial community from the sugarcane core microbiome reveals genetic features for successful plant colonization

Rafael de Souza<sup>1</sup>, Jaderson Armanhi<sup>1</sup>, Natalia Damasceno<sup>1</sup>, Juan Imperial<sup>2</sup>, Paulo Arruda<sup>1</sup>

<sup>1</sup> Genomics for Climate Change Research Center (GCCRC), University of Campinas, Brazil

<sup>2</sup> Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid, Spain

Recent advances in microbial studies have shown that the microbiome has a profound impact on plant health and development. However, the genetic and molecular mechanisms involved in plant-microbe communication that are responsible for the establishment of the microbial community in plants are largely understudied. Unraveling microbial traits responsible for a successful host colonization is an imperative step towards building biotechnological tools based on microbiome to benefit economically relevant plants. Here we explore these traits by investigating the colonization profile and genome sequence of a synthetic microbial community (SynCom) comprised of representatives from the sugarcane core microbiome that show robust colonization with different plant models. By using culture-independent techniques, we found that the sugarcane is inhabited by a core microbial community comprised of less than 20% of the total microbial richness and that sum up for over 90% of the total microbial relative abundance in plant organs. We created a microbial culture collection comprised of over 5 thousand isolates and selected bacterial representatives to design a SynCom comprised of naturally dominant groups from the sugarcane core microbiome. Inoculation assays and microbial profiling revealed that the SynCom robustly colonized, stimulated root development and tripled maize plant biomass. Curiously, genome sequencing showed that robust colonizers lack commonly investigated plant growthpromoting features such auxin production, nitrogen fixation, phosphate acquisition and ACC-deaminase activity, which might indicate that these features are not deterministic for a successful host colonization. Although robust and nonrobust bacterial colonizers showed substantial functional overlaps, we show that significant differences may explain their divergent colonization lifestyle.



# **PF-MU-01** Genotype x soil interaction in the composition of root-rot pathogens of pea detected by quantitative PCR

Lukas Wille<sup>1</sup>, Mario Kurmann<sup>2</sup>, Monika M. Messmer<sup>3</sup>, Bruno Studer<sup>2</sup>, Hohmann Pierre<sup>3</sup>

<sup>1</sup> Crop Sciences (FiBL) / Inst. of Agronomy (ETHZ), FiBL & ETHZ, Switzerland

<sup>2</sup> Molecular Plant Breeding, Institute of Agricultural Sciences, ETH Zurich, Zurich, Switzerland

<sup>3</sup> Department of Crop Sciences, Research Institute of Organic Agriculture (FiBL), Frick, Switzerland

Disease resistance encompasses the mechanisms that allow a plant to withstand or ward off a pathogen. The molecular responses of plants under pathogen attack and the underlying genetics have been extensively studied. However, resistance is not only a trait defined by the warfare between pathogen and host. In fact, resistance is an emergent phenotype of the interactions between the microbial community and the host. Fungal root diseases threaten pea (Pisum sativum L.) cultivation, and therefore a valuable protein source and important crop in low-input farming systems. Resistance in current pea varieties against multiple root pathogens is lacking. In order to acknowledge the rhizosphere microbiome as an integral part of the environment, 261 pea genotypes were screened for resistance on naturally infested field soil in a pot-based experiment. Thereof, eight lines with contrasting disease levels were selected and tested on four soils with different disease pressure in a follow-up pot experiment. Along root rot assessments, pea pathogens (F. solani, F. oxysporum, F. avenaceum, A. euteiches, P. ultimum and D. pinodella) and arbuscular mycorrhizal fungi were quantified in diseased roots using qPCR assays. The amount of fungal DNA detected in the roots differed among the pea genotypes and the four soils and a significant pea genotype x soil interaction was evidenced for several pathogen species. For example, the quantity of *F. avenaceum* in the roots mostly depends on the soil (two-way ANOVA, p < 0.01) and differs significantly between pea genotypes (p = 0.013). F. oxysporum and F. solani quantities showed significant pea genotype x soil interactions (p < 0.01 for both species). Significant correlations were found between F. avenaceum and F. solani quantity and root rot index ( $r_s = 0.38$ , p < 0.01 and  $r_s = 0.56$ , p < 0.01, respectively). On the other hand, F. oxysporum quantity shows no relationship with root rot ( $r_s = 0.007$ , p = 0.95). These results suggest differential roles of the microbes in root rot and highlight the importance of incorporating the complexity of the soil microbiome at early stages of resistance screenings and breeding efforts. Resistance breeding against root rot will be challenged by the fact that soil microbes interact with each other and the plant and that their composition varies between different soils. Further insights into plant-microbe interactions and emerging molecular plant breeding tools will fuel future plant breeding.

## **PF-MU-02** Contrasting soil microbial community profiles in healthy and declined vineyards

**Romain Darriaut<sup>1</sup>**, Jules Wastin<sup>2</sup>, Guilherme Martins<sup>3</sup>, Patricia Bellastra<sup>3</sup>, Coralie Dewasme<sup>1</sup>, Séverine Mary<sup>4</sup>, Guillaume Darrieutort<sup>4</sup>, Isabelle Masneuf-Pomarède<sup>2</sup>, Virginie Lauvergeat<sup>1</sup>

<sup>1</sup> (EGFV) Ecophysiology and Functional Genomic of Vine, INRA, France

<sup>2</sup> Université de Bordeaux, ISVV, Unité de recherche Œnologie EA 4577, USC 1366 INRA, Bordeaux INP, 33140 Villenave d'Ornon, France

<sup>3</sup> Université de Bordeaux, ISVV, Unité de recherche Œnologie EA 4577, USC 1366 INRA, Bordeaux INP, 33140 Villenave d'Ornon, France

<sup>4</sup> Université de Bordeaux, Vitinnov, Bordeaux Sciences Agro, ISVV, 1 cours du Général de Gaulle, 33170 Gradignan, France

Grapevine dieback can be defined as a multiannual crop yield loss due to the sudden or progressive vine death which could be associated with biotic or abiotic factors, resulting in a worldwide issue. Grape trunk diseases or viruses are one of the most frequent identified causes of vine dieback. However, a decline is sometimes observed while no disease symptoms or pathogenic causes could be identified. The microbiome at the interface with the root system (rhizobiome) impacts the physicochemical parameters of the soil and consequently influences the adaptation of the vine plant to its environment. Moreover, grapevine associated microbiota is known to be influenced by soil microbiome, therefore microbes from bulk and rhizospheric soils are good bio-indicators for vineyard health status displaying disease asymptomatic decline. VITIRHIZOBIOME project aims to assess the hypothesis that rhizobiome plays a role in the vine development and its impact can explain the contrasting vineyard dieback stages in different soils.

The microbial diversity and activity of 4 different vineyard plots of Bordeaux region during winter and spring periods were investigated. Those plots were selected due to their lack of disease symptoms even though defined dieback areas were detected and unexplained. Subsequently, one relevant plot has been selected, and its healthy and dieback soils were sampled for greenhouse experiment for phenotypical analysis of Cabernet Sauvignon (CS) scion grafted onto low vigour rootstock, *i.e* Riparia Gloire de Montpellier (RGM) and highly vigour one, *i.e* 1103 Paulsen (1103P). Plant Growth Promoting Rhizobacteria (PGPR) isolation and screening for further inoculation experiment has been done in parallel.

Significant differences in microbial biomass and activity were found among soils even if those present similar physicochemical characteristics. The results of enzymatic assays distinguished patterns from winter and spring periods with an overall greater activity for healthy soil. However, microbial activity seems to be higher in decline soils compared to healthy ones during spring period regardless the lower quantity of microbial biomass. MALDI-TOF mass spectrometry



allows us to identify the most predominant cultivable strains in both soils of the selected plot, and PGPR screening permit to select the most relevant strain for growth stimulation.

\*This project has received funding from the 'Plan National Dépérissement du Vignoble', FranceAgrimer/CNIV.

# PF-MU-03 Changes in soil microbiome can alter peach tree physiology with implications in plant development and in the composition of secondary metabolites

Gorka Erice<sup>0</sup>, Veronica Cirino<sup>0</sup>, Amelia Camprubi<sup>1</sup>, Cinta Calvet<sup>1</sup>, Paolo Bonini<sup>0</sup>

 $^{\rm 1}\,{\rm IRTA}$  Institute of Agrifood Research and Technology, Cabrils, Spain

<sup>0</sup> NGAlab, Atens, Spain

As global population grows in the transition between the current and the goal for future agricultural systems it is mandatory to maximize the food security goals for 2050. We simultaneously need to cope with environmental goals which include the reduction of greenhouse gas emissions, soil biodiversity loss and water pollution. In this new model the management of soil microorganisms can be used as an essential tool to improve plant productivity and fruit quality increasing soil microbiome diversity and water and nutrient utilization efficiency. Within the European Union countries Spain is the first producer of peaches and nectarines reaching more than 40% of the fruit production. The objectives of the high yielding regions include the increase in production with less disease incidence while ensuring the sustainability of the agricultural system. The interaction between plants and soil microorganisms increases the efficiency in nutrient uptake. These soil populations include arbuscular mycorrhizal fungi (AMF) among other fungi and plant growth promoting rhizobacteria. The application of the AMF *Rhizoglomus irregulare* has been demonstrated to help plants to face abiotic environmental stresses and Trichoderma can act both as plant biostimulant and as mycoparasite often used as biocontrol agent of many soilborne plant pathogens. The aim of the study is to reveal the mechanisms of plant metabolism regulation modulated by AMF in the presence of Trichoderma related to changes in soil microbial populations. Peach (Prunus persica L.) plants were grown in 2 I containers and were inoculated with Rhizoglomus irregulare BEG72 or simultaneously with R. irregulare and Trichoderma koningii TK7. Control plants were noninoculated. After 8 months plant growth was measured. Microbial communities have been analyzed through Next Generation Sequencing technologies. Leaf samples were collected to perform metabolomic analysis through ultra-highpressure liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry.

## PF-MU-04 Functional analysis of soil microorganisms for agriculture using millifluidic droplets.

Arthur Goldstein<sup>1</sup>, Hicham Ferhout<sup>2</sup>, Denis Cottinet<sup>3</sup>, Jérôme Bibette<sup>1</sup>, Jean Baudry<sup>1</sup>

<sup>1</sup> CBI (Chemistry, Biology, Innovation), ESPCI Paris, France

<sup>2</sup> AgroNutrition, France

<sup>3</sup> MilliDrop, France

**Introduction :** Soil is home to numerous and varied microorganisms. They are useful to crops in many ways: protection from disease, nutrient acquisition, drought resistance... However, while it is every day easier to examine the genome and transcriptome of these microbiotes, it is still laborious to conduct culture experiments on them. Culture provides essential pieces of information on their agronomic functions, though.

Thanks to millifluidics, it becomes possible to conduct automated, high-throughput and quantitative growth experiments. We aim to take advantage of this to assess the qualities and flaws of any given soil's microbiote. We are currently focussing on two functions:

Phosphorus solubilization: we aim to count microorganisms able to make soil phosphorus available to plant. Biocontrol: we are conducting co-culture experiments of pathogens and soil microbes, in order to assess their ability to protect crops from diseases.

**Materials and methods** We revisit ancient tests and adapt them to Millifluidics, using MilliDrop devises - microorganism culture automatons. They allow us to generate and optically monitor 1000-droplet trains for 48 hours, in a 20 meter long tube. Each droplet is 400nl and can contain either a unique cell or a community.

Microbes are extracted from the soil, and incubated in culture media. We add different reactants to reveal functions of interest.

In the Biocontrol test, the media is co-inoculated with a soil suspension and a pathogen strain.

**Results and projections** We are able to count viable cells per gram of soil, and to know how many of them are able to solubilize mineral phosphate.

We will soon start applying our protocol to various soil types in France, while continuing to develop tests on biocontrol.

In the future, our method could be applied to other functions such as dinitrogen fixation, or carbon sequestration.



# PF-MU-05 The bacterial community in potato is recruited from soil and partly inherited across generations

Franziska Buchholz<sup>0</sup>, Robert R Junker<sup>1</sup>, Livio Antonielli<sup>0</sup>, Tanja Kostić<sup>0</sup>, Angela Sessitsch<sup>0</sup>, **Birgit Mitter<sup>0</sup>** 

<sup>1</sup> Universität Salzburg, Fachbereich Biowissenschaften, 5020 Salzburg

<sup>0</sup> Health & Bioresources, AIT Austrian Institute of Technology GmbH, Austria

Strong efforts have been made to understand the bacterial communities in potato plants and the rhizosphere. Research has focused on the effect of the environment and plant genotype on bacterial community structures and dynamics, while little is known about the origin and assembly of the bacterial community, especially in potato tubers. The tuber microbiota, however, may be of special interest as it could play an important role in crop quality, such as storage stability. Here, we used 16S rRNA gene amplicon sequencing to study the bacterial communities that colonize tubers of different potato cultivars commonly used in Austrian potato production over three generations and grown in different soils. Statistical analysis of sequencing data showed that the bacterial communities in different tubers has changed over generations and has become more similar to the soil bacterial community, while the impact of the potato cultivar on the bacterial assemblage has lost significance over time. However, the communities in different tuber parts did not differ significantly, while the soil bacterial community showed significant differences to the tuber microbiota composition. Additionally, the presence of OTUs in subsequent tuber generation points to vertical transmission of a subset of the tuber microbiota. In summary, we conclude that the bacterial assemblage in potato tubers consists of bacteria transmitted from one tuber generation to the next and bacteria recruited from the soil.

# **PF-MU-06** Root fungal community structure of *Alkanna tinctoria* differs with plant developmental stage

**Yanyan Zhao**<sup>1</sup>, Ismahen Lalaymia<sup>1</sup>, Bryan Vincent<sup>1</sup>, Annalisa Cartabia<sup>1</sup>, Cintia Csorba<sup>2</sup>, Markus Gorfer<sup>2</sup>, Livio Antonielli<sup>2</sup>, Katerina Grigoriadou<sup>3</sup>, Nikos Krigas<sup>3</sup>, Eleni Maloupa<sup>3</sup>, Günter Brader<sup>2</sup>, Angela Sessitsch<sup>2</sup>, Stephane Declerck<sup>1</sup>

<sup>1</sup> Earth and life institute, Université catholique de Louvain, Belgium

<sup>2</sup> AIT Austrian Institute of Technology GmbH, Department of Health and Environment, Bioresources, Unit, Konrad-Lorenz Straße 24, A-3430 Tulln, Austria

<sup>3</sup> Laboratory for Conservation and Evaluation of Native and Floricultural Species-Balkan Botanic Garden of Kroussia, Institute of Plant Breeding and Genetic Resources, Hellenic Agricultural Organization Demeter, P.O. Box 60458, GR-57001 Thermi, Thessalo

Alkanna tinctoria produces alkannin/shikonin (pharmaceutical substances with a wide spectrum of biological properties) and growing evidence suggest to date that endophytes (i.e. bacteria and fungi) are beneficial to plant growth and secondary metabolites (SM) production. Since almost nothing is known about A. tinctoria root's fungal community structure, there is a need for a thorough analysis of its fungal community structure per developmental stage, allowing identification and isolation of promising microorganisms for future applications in SM production systems. We characterized the fungal community structure of A. tinctoria with Amplicon Sequence Variant (ASV) and diversity (Simpson) index by Illumina MiSeq sequencing based on the ribosomal ITS region. The plants were grown under controlled greenhouse conditions, in a mixture of sterilized substrate (peat moss and perlite) and natural (non-sterilized) soil from two locations in Greece (soil A and B). A control that only comprised the sterile substrate was included. The plants were harvested at four developmental stages (I, II, III and IV), corresponding to peak of growth, flowering, fruiting and dormancy, respectively. Based on ASV data, the fungal community diversity of the control plants was significantly lower and different from the plants grown in the two Greek soils, whatever the developmental stage. Similarly, the total fungal diversity in soil B was significantly higher than in soil A, regardless of stages. Finally, differences were noticed between stages and soils. The fungal communities of plants grown in soil A and B were similar at the stage I and IV, while different at stage II and III. In each stage, more than 30% of the fungi were shared between plants grown in soil A and B. A stable core microbiome (i.e. present at all developmental stages) was identified. In soil A, a total of 45 ASVs (16%) were present at the four stages examined and in soil B, 51 (18%). By merging these two results, 31 ASVs were always occurring in the roots of A. tinctoria, regardless the soil and developmental stage. This study reports for the first time the root fungal community of A. tinctoria. A wide diversity of fungi was detected in the root system along the plant developmental stages with a stable core microbiome identified throughout the stages. These results open the door to the isolation and testing of promising fungal endophytes to be applied in SM production systems aiming at high yields.



# MU-03 A novel microcosm for recruiting phytate-degrading microbial communities under inherently competitive soil conditions

Sabrina M Pittroff<sup>1</sup>, Courtney Giles<sup>2</sup>, Ashlea Doolette<sup>3</sup>, Timothy S George<sup>4</sup>, Ole Nybroe<sup>0</sup>, Ralf Greiner<sup>5</sup>, Alan E Richardson<sup>6</sup>, **Mette H Nicolaisen<sup>0</sup>** 

<sup>1</sup> Department of Plant and Environmental Sciences, University of Copenhagen, 1871 Frederiksberg C, Denmark

<sup>2</sup> University of Vermont, College of Engineering and Mathematical Sciences, 33 Colchester Avenue, Burlington VT 05405 USA

<sup>3</sup> School of Agriculture, Food and Wine, Waite Research Institute, University of Adelaide, Urrbrae, Australia

<sup>5</sup> Department of Food Technology and Bioprocess Engineering, Max Rubner-Institut, Karlsruhe, Germany

<sup>6</sup> CSIRO Agriculture and Food, PO Box 1700, Canberra, ACT, 2601, Australia

<sup>0</sup> Department of Plant and Environmental Science, University of Copenhagen, Denmark

Rock phosphorus (P), a core component of applied mineral fertilizer, is a finite resource, thus necessitating the development of innovative solutions to maintain and improve the efficiency of P fertilizer use to sustain optimal P nutrition in plants. Myo-inositol hexa-kis-phosphate (phytate) and its lower order derivatives constitute the majority of identified organic P in many soils and in some cases accumulates in soil with continuous application of P fertilizer. Phytate however is poorly available to plants and in alkaline soils may be precipitated as calcium (Ca)-phytate. Incorporating phytase-producing biofertilizers (i.e., microbial products with capacity to mineralize phytate) into soil for improved plant P nutrition presents a viable and environmentally acceptable way of utilizing P from phytate, whilst reducing the need for mineral P application. A baiting microcosm system consisting of Ca-phytate hotspots placed in low P availability soils was developed and used to recruit microorganisms with distinct taxonomic identities and functional capacities in relation to phytate degradation under natural soil conditions. Treatments containing Ca-phytate showed both direct and indirect evidence for Ca-phytate mineralization in vitro and in vivo, as well as an increased abundance of phoX and phoD genes that relate to organic P mineralization. In contrast, genes coding for the well-studied beta-propeller phytase, normally associated with phytase activity in soil, were not enriched in the Ca-phytate hotspots. The microcosms recruited communities with increased proportions of Actinobacteria, Firmicutes, and Proteobacteria, and the genus Streptomyces was specifically enriched in the presence of Ca-phytate. Hence, the current baiting microcosm represents a promising approach to isolate and characterize novel phytate degrading microorganisms that are inherently competitive in the soil environment.

# MU-04 Plant responsiveness to soil microbial feedbacks

### Klaus Schlaeppi

### Institute of Plant Sciences, University of Bern, Switzerland

The root microbiota has important direct functions for plant growth and health as wells as indirect impacts as apparent from plant-soil feedbacks or in disease suppressive soils. In indirect health functions, the selective recruitment of beneficial microbiota members to plant roots result in a form of 'soil-borne immune memory' at the benefit of the next plant generation. There is evidence that specific compositions of the complex soil microbiota can prime a 'state of alert' in plants, induce systemic resistance and thereby improve plant health. We found that benzoxazinoids (BX), a class of defensive secondary metabolites that are released by roots of cereals, alter the maize rhizosphere microbial communities. Such a BX-conditioned microbiota impacts the growth of a next generation of maize by increasing jasmonate signaling, plant defenses, and suppressing herbivore performance compared to a non-BX-conditioned microbiota and we can now make use of this genetic variation to obtain insights how plants respond to soil microbial feedbacks. Identifying plant loci for positive responsiveness to microbiota feedbacks will open new opportunities to integrate beneficial plant-microbiome interactions into crop breeding programs, which ultimately will enhance sustainability of agriculture.



<sup>&</sup>lt;sup>4</sup> James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK

# MU-05 Evaluating the diversity and functional potential of plant microbiota to improve the selection of potato genotypes able to cope with combined water and nutrient limitations

Hanna Faist<sup>1</sup>, Friederike Trognitz<sup>1</sup>, Sarah Symanczik<sup>2</sup>, Livio Antonielli<sup>1</sup>, Paul Mäder<sup>2</sup>, Philip White<sup>3</sup>, Angela Sessitsch<sup>1</sup>

<sup>1</sup> Bioresources, AIT Austrian Institute of Technology, Austria

- <sup>2</sup> Department of Soil Sciences, Research Institute of Organic Agriculture, Ackerstrasse 113, 5070, Frick, Switzerland
- <sup>3</sup> The James Hutton Institute, Dundee, DD2 5DA, UK

The H2020 project "SolACE - Solutions for improving Agroecosystem and Crop Efficiency for water and nutrient use" aims to identify agricultural practices, including the use of microbial inoculants, and plant genotypes of potato and wheat to cope better with water and nutrient limitations. Among 24 European partners, the AIT Austrian Institute of Technology and the Research Institute of Organic Agriculture are assessing how agronomic practices affect soil microbiota and their functions. We evaluated the performance of ten potato genotypes grown with or without the combined stresses of nutrient and water deficit in a field trial at the James Hutton Institute in Scotland. Using amplicon sequencing of phylogenetic marker genes and shotgun metagenomics, we analyzed how plant genotype, plant phenotype and the different stress scenarios affect bacterial and fungal microbiota in the root environment. Generally, Actinobacteria and Sordariales were increased while Proteobacteria, Olpidiomycota, Shannon diversity and richness were reduced in the rhizosphere of potatoes grown under stressed conditions. In the root endosphere under stress, the bacterial, but not the fungal, community changed greatly. Shared, unique and differently abundant microbial amplicon sequencing variants indicated a stress- and genotype-specific recruitment of microbes by potato plants. Metagenome analysis and the analysis of selected bacterial isolates will reveal information on plant growth-promoting potential and functional properties of potato microbiota. Results of this trial will help to identify below-ground traits and select efficient potato genotypes which are best suited to coping with combined stress scenarios while simultaneously supporting beneficial functions of soil and plant-associated microbiota.

# MU-06 Succession of microbial assemblages during seed development

Guillaume Chesneau<sup>2</sup>, Gloria Torres-Cortes<sup>2</sup>, Armelle Darrasse<sup>2</sup>, Ashley Shade<sup>1</sup>, Matthieu Barret<sup>2</sup>

<sup>1</sup> Department of Microbiology and Molecular Genetics; Program in Ecology, Evolutionary Biology, and Behavior; The DOE Great Lakes Bioenergy Research Center; and The Plant Resilience Institute, Michigan State University, East Lansing MI 48824 <sup>2</sup> IRHS. INRA. France

Seeds are involved in the transmission of microorganisms from one plant generation to another and consequently act as a starting point for assembly of the plant microbiome. In the current work, ecological succession of seed microbial assemblages was assessed during key seed development stages including seed filling and seed maturation. Common bean (*Phaseolus vulgaris*) and radish (*Raphanus sativus*) were selected as working models since these two plant species differ in their pollination modes. According to barcoding datasets, pioneer species associated to bean seeds were mostly derived from the vascular system, while primary colonists of radish seeds were either acquired through the vascular system or the floral pathway. In addition, a significant increase in phylogenetic diversity was observed during seed maturation for both plant species probably as a result of external transmission of micro-organisms from fruits. Culturebased collection and subsequent comparative genomics of representative bacterial strains isolated at different seed developmental stages provided some insights on determinants involved in successful seed colonization and persistance. Data generated through this work increase our basic understanding of the governing processes that drive assembly of the seed microbiota. This fundamental knowledge is a first step towards the design of efficient seed microbial inoculants.



# MU-07 Volatilome of Wheat Microbiota System under Drought and Flooding: The VolCorn Consortium

**Steffen Kolb**<sup>1</sup>, Saranya Kanukollu<sup>2</sup>, Andreas Ulrich<sup>2</sup>, Marina Müller<sup>2</sup>, Philipp Franken<sup>3</sup>, Michael Bitterlich<sup>4</sup>, Richard Pauwels<sup>4</sup>, Silke Ruppel<sup>4</sup>, Andreas Schedl<sup>5</sup>, Alexander Weinhold<sup>5</sup>, Nicole M. van Dam<sup>6</sup>, Geeisy A. Cid<sup>7</sup>, Mohammad-Reza Hajirezaei<sup>7</sup>

<sup>1</sup> Microbial Biogeochemistry, Leibniz Centre for Landscape Research - ZALF, Germany

- <sup>2</sup> ZALF Müncheberg, Germany
- <sup>3</sup> FH Erfurt, Germany
- <sup>4</sup> IGZ Großbeeren, Germany
- <sup>5</sup> iDiv, Leipzig-Jena, Germany
- <sup>6</sup> iDiv, Leipzig-Jena, FSU Jena, Germany
- <sup>7</sup> IPK Gatersleben, Germany

Climate change will increase vulnerability of global food production in Europe by drought and flooding as by associated and frequent weather extremes. The abiotic stressors flooding and drought diminish crop yield and make crop plants more susceptible to pathogens and herbivores. Crop health and yield are stabilized by their beneficial microbiota, especially by mycorrhizal fungi. As a crop plant-microbiota system, both biological components respond jointly to environmental challenges. Crop plant and microbiota emit a complex mixture of volatile organic compounds, the volatilome, which is important in plant-plant communication and plays a role in plant defense to herbivores. Wheat is one of the top three globally produced crops. Hence, we chose it as an experimental model for greenhouse and field manipulation experiments. We consider the volatilome as an integrated signal reflecting key metabolic changes of wheat and its microbiota in response to environmental stresses, and thus their functional interactions. We hypothesize that this volatile-based communication enhances the wheat-microbiota system capability to withstand combined biotic and abiotic threats, such as fungal pathogens or herbivores under drought and flooding. The Leibniz Competition 2019funded consortium "VolCorn" will reveal abiotic stress-induced volatiles of mycorrhizal or non-mycorrhizal wheat plants and correlate them with functional traits of its microbiota. The central mission of VolCorn is the identification of volatilome components (single or mixtures) that enhance the beneficial microbiota and in turn increase wheat fitness under environmental stress.



# miCROPe 2019 - Microbe-assisted crop production opportunities, challenges & needs Vienna, Austria, December 2 – 5, 2019

# Morning Keynote

# MK-01 Crops as merobionts: Regenerative agriculture, the microbiome and the climate crisis through the lens of the hologenome theory

### **Richard Jefferson**

Cambia & QUT, Australia

The climate crisis presents an existential threat to human society and ecosystem resilience. Agriculture is responsible for much of this impact. Systemic, diverse and effective interventions are urgently needed to mitigate the catastrophe.

Hologenome theory asserts that virtually all plants and animals comprise both a scaffold (or 'host') and myriad populations of microbial constituents: its microbiome. The composite organism can be considered a holobiont, in which diverse functions needed to flourish and for the information content to persist over evolutionary time are distributed amongst its genetic contributors.

However, I propose that the development of agriculture has *collapsed the hologenome* in plants, domestic animals and humans to create metastable *merobionts*, through massive inbreeding depression of the microbiome due to recurrent and homogeneous planting and concomitant sedentary lives of associated animals and humans.

In this model, *functional* microbial diversity both *in planta* and in cultivated soils has declined, both through human practice intervention and through a compensatory breeding of the plant 'genome' (typically nuclear and cytoplasmic genomes) at the expense of more agile trait contribution from the microbiome. This leaves the soil reservoir in cultivated areas impoverished of founder population diversity, and rendered the crop vulnerable, and the production system fragile. Free exchange of microbes by the macrobiota involves recruitment, amplification and dissemination of populations. This is not a neutral process in that the composition of sampled microbes does not match that of disseminated microbes. Rather we know that microbes are selectively recruited, differentially amplified and variably disseminated, and thus will have a disruptive, recursive effect on the microbial population structure in the environment, from which the next cycle of recruitment and amplification occurs, beginning a treadmill of population structural change and constriction.

Does this lead inexorably to such apparently disparate phenomena as disease and ecosystem fragility, and a decline in soil microbial diversity and carbon sequestration. I speculate that many of the lessons of resilience that we need to apply to the imperatives of agriculture will be gleaned from studying microbiome and holobiont relationships in minimally perturbed natural systems, not from merobionts in agricultural systems – whether industrial or artisanal.



# miCROPe 2019 - Microbe-assisted crop production opportunities, challenges & needs Vienna, Austria, December 2 – 5, 2019

# Microbial biocontrol of pests, pathogens and weeds

Chairs: Karen L. Bailey & Gabriele Berg



### MB-01 Plant microbiome management for sustainable agriculture

### Gabriele Berg, Henry Müller, Birgit Wassermann, Tomislav Cernava

Environmental Biotechnology, Graz University of Technology, Austria

The plant microbiome is crucial for growth and health (Berg *et al.* FEMS Microb. Ecol., 2017). Intense agriculture and overuse of chemicals leads to biodiversity loss and resistant pathogens, which are difficult to suppress but cause enormous yield losses. The plant microbiome will be the key to the second green revolution because it provides solutions for sustainable agriculture. To manage or exploit the plant microbiome require a deep understanding of its functioning. The microbiome can be managed indirectly by changing abiotic parameters or directly by microbial treatments or transplants. Seeds are ideal carriers for the latter (Berg & Raaijmakers ISME J, 2018). However, recent studies reveal an unexpected microbial diversity and abundance within seeds, and showed a vertical transmission of an indigenous core microbiome (Adam *et al.* Plant and Soil, 2017). Soil type, climate, geography and plant genotype were identified as main drivers of the seed microbiota. Within millennia of domestication, crops and their seeds underwent traceably many different adaptive trends, allowing rapid speciation and divergence that lead to phenotypic and genotypic distinction to their wild ancestors. During those dynamics, also the microbiomes have secretly co-evolved with the host plants. Interestingly, bacterial endophytes represent the symbiotic component within seeds; in native seeds they form a beneficial network with archaea, while fungi represent an antagonistic component (Wassermann et al. Microbiome 2019). To restore microbial diversity important for *one* health issues, tailored bacterial seed treatments can be composed based on the rich diversity of seeds of wild ancestors or other native plants.

# MB-02 The functional relevance of microbe-plant-insect interaction in a cereal crop system

Michael Rothballer<sup>1</sup>, Sophia M. Klink<sup>1</sup>, Sharon E. Zytynska<sup>2</sup>

<sup>1</sup> Institute of Network Biology, Helmholtz Zentrum München, Germany

<sup>2</sup> Technical University of Munich, Terrestrial Ecology, School of Life Sciences Weihenstephan, Freising, Germany

Beneficial rhizobacteria bear a high potential to improve the plant's resistance against biotic stress. However, it is not well-understood how microbial signaling influences the response of the plant against economically important pests. *Acidovorax radicis* N35 and *Rhizobium radiobacter* F4 are a N-acyl homoserine lactone (AHL) producing plant growth promoting rhizobacteria (PGPR), which were tested for their interaction with barley and foliar-feeding aphids within this study. Available AHL negative mutants of these strains will allow to elucidate the role of AHLs in these interactions. We performed several green-house experiments with barley cv. Grace, Chevalier, Scarlett, and Barke growing in commercial gardener's soil. Inoculation of barley seedlings (*Hordeum vulgare*) with *A. radicis* N35 was shown to clearly influence the growth rate of barley positively while the load of foliar-feeding aphids (*Sitobion avenae*) was significantly reduced. However, variations of this effect were observed depending on the cultivar with Barke being the least responsive. NGS Amplicon sequencing analyses of the root associated microbiome revealed a significantly positive correlation between the abundance of reads allocated to *Acidovorax* and plant growth, while a negative correlation was observed for aphid load. Inoculations with *R. radiobacter* F4 showed the same tendency in reducing aphid loads and improving plant growth although not as clear.

Ongoing experiments aim to find out whether indirect signaling via the plant or direct interaction (i.e. antagonism) of the endophytic microbes with aphids are responsible for the current findings. Understanding this inter-kingdom communication provides a promising basis to improve agricultural systems by enhancing crop resistance against herbivorous insects.



# PF-MB-01 *Mycolicibacterium* strains interact positively with *Serendipita* (*Piriformospora*) *indica* for crop enhancement and biocontrol of pathogens

### Alejandro del Barrio Duque, Livio Antonielli, Angela Sessitsch, Abdul Samad, Stéphane Compant

### AIT Austrian Institute of Technology, Austria

Serendipita (=Piriformospora) indica is a root-colonizing fungus with the capabilities to enhance plant growth and confer biotic resistance. However, the application of this fungus in the field has led to inconsistent results, perhaps due to antagonisms with other microbes. Here, we studied the impact of single bacteria from the endophytic bacterial community on the *in vitro* growth of *S. indica*. Furthermore, we investigated how combined inoculum of *S. indica* and bacteria influence plant growth and protection against *Fusarium oxysporum* and *Rhizoctonia solani*.

Data showed that, among other taxa, bacterial strains of the genera *Burkholderia*, *Enterobacter* and *Bacillus* negatively affect *S. indica* growth, whereas several strains of *Mycolicibacterium*, *Rhizobium* and *Paenibacillus* stimulate fungal growth. To further exploit the potential of the beneficial interaction, additional experiments were performed with *Mycolicibacterium* strains, as it was the most abundant genus showing positive effects on *S. indica* growth. Some dual inoculations of *S. indica* and *Mycolicibacterium* strains boosted the beneficial effect triggered by *S. indica*, further enhancing the growth of tomato plants, and alleviating the symptoms caused by the pathogens *F. oxysporum* and *R. solani*. However, some combinations of fungus and bacteria were sometimes less effective than microbes inoculated singly.

Genome analysis of four *Mycolicibacterium* strains revealed that these bacteria encode several genes predicted to be involved in the stimulation of *S. indica* growth, plant development and tolerance to biotic and abiotic stresses. Particularly, a high number of genes related to vitamin and nitrogen metabolism were detected. Taking into consideration multiple interactions on and inside plants, we showed in this study that some bacterial strains may induce beneficial effects on *S. indica* and this mutualistic relationship of microbial partners could be an approach to enhance plant growth promotion and tolerance to various biotic and abiotic stresses.

# PF-MB-02 Biological control of wireworms in cover crops

### Lara Reinbacher, Fionna Knecht, Christian Schweizer, Giselher Grabenweger

Ecological Plant Protection in Arable Crops, Agroscope, Switzerland

Wireworms cause substantial losses in marketable yield of potatoes but control options are limited, creating a demand for new alternatives. Laboratory and semi-field trials revealed the potential of the entomopathogenic fungus *Metarhizium brunneum* isolate ART2825 against *Agriotes obscurus* and *A.lineatus*, two of the most detrimental wireworm species. In this study we integrate the fungus in the agricultural crop rotation and try to adapt the application method to its ecological and environmental requirements. Application precedes sowing of cover crops in late summer in order to enhance disease development through higher soil temperatures and extend effect duration by the absence of soil disturbance.

During a two-year field trial, we were able to establish the fungus on site and demonstrate the infectivity of the treated soils in laboratory assays. Tendencies to lower potato damages were seen in the first season but damage levels did not significantly differ from the control. To improve plant protection efficacy the application rate was increased in the second year and application time precisely selected. Reasons for the yet pending success were further investigated and potential synergies with selected cover crop species explored.



## PF-MB-03 Facilitation promotes invasions in plant-associated microbial communities

**Mei Li<sup>0</sup>**, Zhong Wei<sup>0</sup>, Jianing Wang<sup>0</sup>, Alexandre Jousset<sup>1</sup>, Ville-Petri Friman<sup>2</sup>, Yangchun Xu<sup>0</sup>, Qirong Shen<sup>0</sup>, Thomas Pommier<sup>3</sup>

<sup>1</sup> Institute for Environmental Biology, Ecology & Biodiversity, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

<sup>2</sup> Department of Biology, Wentworth Way, YO10 5DD, University of York, York, UK

<sup>3</sup> Ecologie Microbienne, UMR1418, French National Institute for Agricultural Research (INRA), University Lyon I. F-69622 Villeurbanne, France <sup>0</sup> Resources and Environmental Science, Nanjing Agricultural University, China

While several studies have established a positive correlation between community diversity and invasion resistance, it is less clear how species interactions within resident communities shape this process. Here we experimentally tested how antagonistic and facilitative pairwise interactions within resident model microbial communities predict invasion by the plant-pathogenic bacterium *Ralstonia solanacearum*. We found that facilitative resident community interactions promoted and antagonistic interactions suppressed invasions both in the lab and in the tomato plant rhizosphere. Crucially, pairwise interactions reliably explained observed invasion outcomes also in multispecies communities, and mechanistically, this was linked to direct inhibition of the invader by antagonistic communities (antibiosis), and to a lesser degree by resource competition between members of the resident community and the invader. Together our findings suggest that the type and strength of pairwise interactions can reliably predict the outcome of invasions in more complex multispecies communities.

# PF-MB-04 In vivo localization and role of Fusarium oxysporum f.sp. strigae and Bacillus subtilis against Striga hermonthica in an integrated biocontrol system

#### Williams Oyifioda Anteyi, Frank Rasche

Institute of Agricultural Sciences in the Tropics, Agroecology section (490F), University of Hohenheim, Germany

In view of the variable susceptibility of Striga hermonthica to Fusarium oxysporum f.sp. strigae (Fos) isolates, the combined application of the mycoherbicide Fusarium oxysporum f.sp. strigae (Fos) and Bacillus subtilis (a plant growth promoting rhizobacterium (PGPR)), as an integrated Striga hermonthica biocontrol approach was examined. This was to understand the ecological niche (position and role) of Fos and PGPR in the integrated biosystem, in identifying a more effective biocontrol strategy to combat S. hermonthica. Localization of Fos isolates (Foxy-2, FK3) and B. subtilis (GBO3 strain) within infected S. hermonthica was monitored by fluorescent gene expression of transformed Fos and GBO3. Also, Foxy-2, FK3, GBO3, including Trichoderma viride (IMB12098 strain) as check, were applied as fungi-bacteria assemblages, and single treatments, to S. hermonthica infested rhizoboxes containing sorghum as host crop. Both Fos and GBO3 infected and co-localized diseased S. hermonthica shoot. Also, Fos penetration of Striga through trichome entry was revealed. Combined treatments of FK3 + GBO3, and Foxy-2 + GBO3, increased sorghum aboveground dry biomass (P < 0.05), but not IMB12098 + GBO3. None of the combined fungi-bacteria assemblages significantly suppressed S. hermonthica emergence. Single treatments of FK3 and GBO3 increased sorghum aboveground dry biomass (P < 0.05), but Foxy-2 and IMB12098 did not. Only FK3 suppressed S. hermonthica emergence (P < 0.05), but neither GBO3, Foxy-2 nor IMB12098. GBO3 counteracted Fos suppressive activity against Striga emergence. Despite GBO3 ineffectiveness in suppressing S. hermonthica emergence, it significantly promoted sorghum yield, either when applied alone or in combination with Fos isolates. In the given set-up, the combined application of Fos and GBO3 presented no added advantage in suppressing the emergence of the sampled S. hermonthica.

Keywords: Striga hermonthica, Fusarium oxysporum f.sp. strigae, Foxy-2, FK3, Bacillus subtilis GBO3 strain, fluorescent gene expression, ecological niche..



# MB-03 Behaviour of Bt ABTS-1857 as a biological control agent on spinach plants, cut leaves and spinach juice

**Xingchen Zhao**<sup>0</sup>, Marcelo Belchior Rosendo da Silva<sup>1</sup>, Inge Van der Linden<sup>0</sup>, Bernadette D.G.M. Franco<sup>1</sup>, Mieke Uyttendaele<sup>0</sup>

<sup>1</sup> University of São Paulo

<sup>0</sup> Department of Food technology, Safety and Health, Ghent University, Belgium

Bt ssp. aizawais strain ABTS-1857 is the active substance of Xentari, which is one of the most widely used commercial BCA in Europe. EFSA document in 2016 reported that dose-response and behavioural characteristics of Bt and filed studies after application of Bt biopesticides were quite scarce. Therefore, we studied the behaviour of Bt ABTS-1857 inoculated on plants (pre-harvest), cut leaves (post-harvest) of spinach and spinach juice (food). Spinach plants were growing indoors at ambient temperature and spray inoculated with Bt ABTS-1857 either spores or vegetative cells. Plants were analysed for Bt at day 0 and after 5, 10, 15 and 20 days by plating on MYP agar. Cut spinach leaves were spray inoculated with Bt ABTS-1857 vegetative cells. These leaves were stored at 12 °C for 5 days and daily analysed for Bt by plating on MYP agar. Spinach juice was made from spinach leaves by grinding using an immersion blender, centrifuged and filter sterilized before inoculation. The behaviour of Bt ABTS-1857 was monitored in 20% spinach juice on a daily basis up to 5 days storage at 12 °C and 22 °C. Non-inoculated controls of spinach plants, cut leaves or spinach juice were also plated on MYP agar. At pre-harvest simulation, results showed that the reduction of Bt spores on plants was only 0.48 log after 20 days, while Bt vegetative cells reduced 3.53 log after 20 days. The results indicated that the spores of Bt ABTS-1857 remained present in quite high numbers but did not germinate and grow on the spinach plants and were thus less impacted by the plant's ecosystem than the Bt vegetative cells populations. However, at post-harvest simulation, the inoculated Bt vegetative cells maintained stable on cut leaves and counts on MYP-agar kept at ca. 4 log throughout the 5 days storage. Thus, results showed that the Bt vegetative cells did not grow out on cut spinach leaves during 5-days storage at 12 °C (whereas in BHI Bt ABTS-1857 growth at 12°C occurred). Furthermore, results of growth assessment of Bt ABTS-1857 in 20% spinach juice showed that vegetative cells died from 3.6 to 1.2 log after 5 days at 12 °C, although growth was observed at 22°C in the spinach juice: from 3.6 to 6.95 log. The behaviour of Bt ABTS-1857 is thus very different depending whether spore or vegetative cell but also the physiology, nutrients or competing microbiota of plant, leave, juice (or BHI) and thus case by case experimental data needed to assess behaviour in a particular context.

# MB-04 The leaky gut syndrome: insect gut bacteria exacerbate physical and chemical defenses of plants

Charles Mason<sup>1</sup>, Swayamjit Ray<sup>0</sup>, Ikei Shikano<sup>0</sup>, Michelle Peiffer<sup>0</sup>, Asher Jones<sup>0</sup>, Dawn Luthe<sup>0</sup>, Kelli Hoover<sup>0</sup>, **Gary** Felton<sup>0</sup>

<sup>1</sup> Department of Entomology, Penn State University, USA

<sup>0</sup> Entomology, Pennsylvania State University, United States of America

Many plant defenses that deter insect herbivory target the attacker's digestive system. We found that plant defenses against the fall armyworm created opportunities for resident gut microbes to penetrate protective gut barriers, invading the body cavity and exacerbating the negative impacts of plant defenses on the insect. These interactions triggered insect immune responses and collectively overwhelmed the insect's ability to cope with multiple stressors. However, the effects varied between bacterial taxa, indicating that variation in the caterpillar microbiome can alter their phenotype. Our results reveal a previously unrecognized, and likely widespread, mechanism allowing the plant to use the insect's gut microbiota against it in collaboration with the plant's own defenses. These results are important for not only understanding the ecological function of plant defenses but also in the rational design and engineering of pest resistance in crops.



## MB-05 Bioherbicides from creation to commercial success – What's the problem?

### **Karen Bailey**

### Agriculture & Agri-Food Canada, Canada

The concepts of biological weed control have been around since the turn of the 20<sup>th</sup>century, but it was not until the 1970s when the thought of employing plant pathogens as biological herbicides emerged. It took until the early 1980s to release the first commercial products, DeVine® and Collego®. Since then, intensive efforts by researchers around the world have isolated many microbial species that show potential to reduce weed populations (the discovery and proofof-concept stage). But turning these discoveries into commercial bioherbicide products has been elusive. Since the registration of DeVine® and Collego®, there are only 10 bioherbicides registered by government regulatory agencies worldwide and few have sustained commercial sales for more than a few years. So, why do we find examples of commercial success with other biopesticides, such as those for disease and insect control, but so few examples for weed control? What are the key criteria that must be addressed to allow companies to commercialize future bioherbicide discoveries? The journey from discovery to commercialization of a bioherbicide is a 10-15 years process, so it makes sense to have a clear idea of the stages and steps to logically and swiftly move through the process. This presentation will identify the unique opportunities and challenges encountered with the business model template used to develop the fungus Phoma macrostoma as a bioherbicide for broadleaved weed control. Exploring the specific traits of this fungus and the features of this bioherbicide, we will demonstrate the role that science, industry, regulation, consumers and market forces have played in achieving success in urban, horticultural, forestry, and agricultural environments. We also reassess whether the business model template is the most appropriate model for developing bioherbicides and consider alternative approaches to getting bioherbicides into the hands of consumers.

# MB-06 Contrasting effects of the rhizobacterium *Pseudomonas simiae* on above- and belowground insect herbivores

Julia Friman<sup>1</sup>, Ana Pineda<sup>2</sup>, Marcel Dicke<sup>1</sup>, Joop J.A. van Loon<sup>1</sup>

<sup>1</sup> Laboratory of Entomology, Wageningen University, Netherlands

<sup>2</sup> Department of Terrestrial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), P.O. Box 50, 6700 AB, Wageningen, The Netherlands

Plant-growth promoting rhizobacteria (PGPR) can enhance plant growth and reinforce plant defense. PGPR, via plantmediated effects, are generally regarded to have negative impact on the performance of leaf chewing insects. However, few studies include belowground herbivores and the effect of rhizobacteria on their performance is still unclear. To use these bacteria in sustainable agriculture, we need understanding the effects on pest insects interacting with aboveand belowground plant tissues.

The aim of this study was to investigate the effects of a PGPR on the performance of an aboveground and a belowground insect pest species. In a greenhouse experiment, we grew white cabbage (*Brassica oleracea* var. Christmas Drumhead) in a sterilized perlite-soil mix, together with the model bacterium in studies of rhizobacterial induced plant defense, *Pseudomonas simiae* WSC417r. After 5 weeks, we infested the plants with larvae of the diamondback moth *Plutella xylostella*, cabbage moth *Mamestra brassicae* or the cabbage root fly *Delia radicum*. Insect weight and plant biomass was recorded. Bacterial inoculation had contrasting effects on the aboveground and belowground herbivores: inoculation reduced the performance of *Plutella xylostella* caterpillars, did not influence *Mamestra brassicae* caterpillars, and enhanced the body mass of adult *Delia radicum*. Thus, the application of rhizobacteria differentially affected insect herbivores and may not result in a uniform protection against insect pests.



# **PF-MB-05** Microbial ecology of the European apple canker pathosystem (*N. ditissima*)

Leone Olivieri<sup>1</sup>, Xiangming Xu<sup>2</sup>, Alan Gange<sup>3</sup>

<sup>1</sup> NIAB EMR, United Kingdom

<sup>2</sup> NIAB EMR, East Malling, UK

<sup>3</sup> Royal Holloway, University of London, Egham, UK

European apple canker, caused by the fungus *Neonectria ditissima*, is one of the most important diseases of the apple tree and fruit globally, and is especially destructive in North-Western Europe. Disease management can be problematic because *N. ditissima* is able to produce asymptomatic infections which can last for months or even years. Additionally, as an increasing number of agrochemicals active against European apple canker are being banned in the EU, effective control measures are limited.

*N. ditissima* has been speculated to initially grow in plant tissues as a component of the endophytic microflora, and later switch to its pathogenic phase. The fungus' interactions with other apple tree endophytes, and their potential action as pathogen facilitators or antagonists, have never been investigated. Together with the host genetics, apple tree endophytes might account for the different levels of disease resistance observed across the different cultivars, and represent an untapped reservoir of biocontrol agents.

In this current project, we are investigating the role of apple tree fungal and bacterial endophytes in the European apple canker pathosystem. We have initially localized the pathogen *in planta* during the asymptomatic phase of the disease. Using high-throughput next generation sequencing techniques (meta-barcoding), we are now carrying out an extensive profiling of the fungal and bacterial endophytic species that co-localize with the *N. ditissima* asymptomatic infection, across different apple cultivars. Our goal is to analyze the correlation between the endophyte profiles characterizing each cultivar and its respective disease resistance level. This will help understand the components of European apple canker field resistance and will inform further research into the development of innovative management strategies, such as the exploitation of endophytes for biological control.

# PF-MB-06 Microbiome-assisted management of plant diseases

### Shivani Khatri

Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology, Delhi, India

Due to ever increasing global food demand, various advancements in agriculture practices are being adopted. The major challenge to maximize crop productivity is the incidence of a variety of plant diseases. Different chemical-based formulations are being used to control them. Although these conventional approaches have enhanced the output, they exert a deleterious impact on environment. An attractive sustainable alternative to this problem is organic farming which, avoids the use of chemicals and is completely based on natural resources. Soils treated with organic manure have enriched microbial diversity which subsequently leads to increased diseases suppressive ability against a number of plant pathogens, thus conferring general suppressiveness. The mechanism underlying general suppression of plant pathogens, which is an inherent property of some soil and is due to multitrophic and collective interaction between whole microbial community and diseases causing organisms in the soil ecosystem, is of key interest. In the present study long-term and short-term field experiments of rice-wheat cropping systems under organic and conventional farming practices were chosen to map suppressiveness against a number of common soil-borne pathogen in Indian arable land. The study aims to determine the impact of farming practice on disease suppressiveness. Further, diversity analysis and isolation of potent antagonistic strains along with other desirable PGP properties to suppress the pathogenesis of the plant pathogens in fields. Culture-independent techniques were employed to study the structural and functional diversity in the disease-suppressive soil. Further studies are directed towards employing metabolomic approach to understand the mechanisms and metabolites responsible for suppression of soil-borne pathogens in the soil. The soil treated organically was found to be more suppressive than conventionally treated and bulk soil against all the selected fungal and bacterial plant pathogens. Strains belonging to genera such as Pseudomonas, Bacillus and Actinomycetes having antagonistic potential that were isolated from soil treated with organic manure showed significant disease suppressive ability against selected bacterial and fungal pathogens. Strategies to design synthetic microbial community using these selected potent antagonistic strains is the next step of our study. This will prove to be an economical and sustainable solution to control plant diseases.



# **PF-MB-07** A species-specific crosstalk via volatile exchange between a biocontrol agent *Serratia plymuthica* HRO-C48 and fungal plant pathogens.

## Daria Rybakova

Institute of Environmental Biotechnology, Graz University of Technology, Austria

The rhizosphere-associated bacterium Serratia plymuthica HRO-C48, commercially used as a biocontrol agent (RhizoStar®, E-nema GmbH Raisdorf, Germany), is able to suppress symptoms caused by soil-borne fungal pathogens. The interaction of HRO-C48 with each of the three tested plant pathogenic fungi Rhizoctonia solani AG2 Kühn, Verticillium longisporum ELV25 Stark and Leptosphaeria maculans MB 158 was assessed by a volatile organic substance (VOCs) assay coupled with a transcriptomic analysis. The selected soil-borne fungal pathogens cause high yield losses in a range of plant crops all over the world. After 72 h of exposure to fungal VOCs, 233 differentially expressed genes were identified among S. plymuthica transcripts (94 up- and 139 downregulated). The predicted functions of the common genes that were downregulated due to the exposure of S. plymuthica to all three fungal VOCs suggest an unspecific stress response of Serratia to the fungal volatiles, as well as an enhanced biofilm formation. The later may be associated with a reduction of cellular motility. The interaction of S. plymutica with the V. longisporum VOCs resulted in a significant downregulation of 40 transcripts, while only 20 transcripts were upregulated. The strongest difference in the regulation of genes was found between the treatments of S. plymuthica with the VOCs of R. solani and L. maculans. R. solani, which growth was inhibited by bacterial volatiles at strongest, produced in its turn VOCs that resulted in the strongest upregulation of the bacterial transcripts. L. maculans VOCs, on the other hand, had exactly the opposite effect on the bacterial gene expression pattern with the majority of the genes being downregulated. Additionally, we found several transcripts putatively involved in antagonistic activity in the HRO-C48 transcriptome. Some of them were upregulated upon the contact with R. solani VOCs, and downregulated due to L. maculans volatiles. Thus, our data suggests an ongoing strongly species-specific crosstalk via volatile exchange between the fungal and bacterial cells, that may contribute significantly to the antifungal mode of action of the biocontrol agents such as S. plymuthica HRO-C48.

# PF-MB-08 Effect of *Bacillus subtilis* EA-CB0575 on the microbiota, growth development and health of banana plants

Gisell García-Giraldo<sup>1</sup>, Luisa F. Posada<sup>2</sup>, J.C. Alvarez<sup>2</sup>, Valeska Villegas-Escobar<sup>2</sup>

<sup>2</sup> Department of Biological Sciences, EAFIT University, Colombia

The artificial introduction of microorganisms has been a widely studied practice to modulate the desired plant responses that emerged as an alternative for biocontrol and handling in agriculture. The inoculation of micropropagated plant tissues has been one of the research fields that has shown improvements in different features of the treated plantlets. This research evaluates the effect of in vitro bacterial inoculation on the microbiota of banana plants during their growth stage under greenhouse conditions focusing on plants that showed a reduction in the severity of foliar disease caused by Pseudocercospora fijiensis. To this end, a bacterial community characterization associated with the roots of in vitro banana plants was initially carried out using a culture-independent approach to identify the main taxonomic groups located in this organ at an early development stage where results indicated that the genus Stenotrophomonas was dominant, exceeding 80% abundance in most samples. Following a screening in planta carried out with single bacteria and in combinations to determine the effect on plant growth speed and the severity of Black Sigatoka disease after 180 days of in vitro bacterial inoculation. The strain B. subtilis EA-CB0575 reduced necrotic area by 54% and 33% compared to the control in two experiments carried out at different times. The reproducibility effect obtained with rhizobacteria B. subtilis EA-CB0575 led to the third stage of this research focused on evaluating the impact of the inoculation of this strain on the root/rhizosphere microbiota 150 dai, where results indicated a differential enrichment of low abundance taxa between 0.01% and 0.8% out of the total bacterial community with a greater sequence number in the plants treated 24 h before infection with P. fijiensis and that was considerably reduced 28 h after infection with the pathogen. Lastly, a comparison of the bacterial community associated with the roots between banana plants in vitro with 9 months of development and 14 months old greenhouse grown plants was carried out, confirming that the development stage significantly influences the conformation of the bacterial profile.



<sup>&</sup>lt;sup>1</sup> Universidad Nacional de Colombia, Medellín - Colombia

# MB-07 The role of endophytic entomopathogens in modulating plant-microbe-insect interactions

### Anne Muola<sup>0</sup>, Marjo Helander<sup>1</sup>, Kari Saikkonen<sup>0</sup>

 $^{\rm 1}$  Section of Ecology, Department of Biology, University of Turku, Finland  $^{\rm 0}$  Biodiversity Unit, University of Turku, Finland

During recent years the endophytic entomopathogens, such as *Beauveria bassiana* and *Metarhizium anisopliae*, have been acknowledged to show biocontrol potential against insect herbivores in several different crops. Despite the reported negative effects of endophytic entomopathogens on insect herbivores the exact mechanisms responsible for these effects are still largely unknown. Furthermore, while the harmful side-effects of chemical pesticides on the natural enemies of the pests and pollinators are well known, very little is known about the potential role of endophytic entomopathogens in steering higher trophic level ecosystem services, such as those conducted by the natural enemies of pests or pollinators. Given this, the aim of our ongoing project is to test how endophytic entomopathogens modulate plant-microbe-insect interactions. As a model plant-herbivore-microbe system, we use oilseed *Brassica rapa*, its most important pests (pollen beetle, *Meligethes aeneus* and flea beetles, *Phyllotreta* spp.), and entomopathogenic fungi (*B. bassiana* and *M. anisopliae*) which are known to be able to colonize tissues of oilseed rape plants as asymptomatic endophytes. We are currently running the first set of replicated greenhouse experiments to test the biocontrol potential of the fungi directly as entomopathogens and indirectly via phytohormone signaling pathways of its host plant. Furthermore, at the later stages of the project we will determine if endophytic entomopathogens have bottom-up effects on natural enemies of pests as well as on pollinators.

This project is part of the European Union funded Horizon2020-project 'EcoStack'. The overall objective of EcoStack is to develop and support ecologically, economically and socially sustainable crop production via stacking and protection of functional biodiversity. By designing and implementing integrated systems for ecostacking, the project will contribute to a long-term sustainability of agriculture and food production.

# MB-08 Plant associated bacteria for the control of Impatiens glandulifera

### Friederike Trognitz, Lisa Lutz, Angeala Sessitsch

Center for Health and Environment, AIT Austrian Institute of Technology, Austria

*Impatiens glandulifera* is a highly invasive annual species, which has spread rapidly in many parts of Europe. It was introduced into Europe as ornamental plant in 1839 and later on planted as bee pasture and escaped into nature. Since the introduction in the UK as garden ornamental plant, *I. glandulifera* spreads rapidly. The plant is the tallest annual plant in Europe and reaches 50 to 250 cm in height.

Since 2016 *I. glandulifera* is listed in the EU regulation 1143/2014. Control measures have to be developed to mitigate this plant and reduce the spread. Currently contaminated sites are mostly mowed or mulched. The use of herbicides is mostly prohibited because of the neighboring water resources or destruction of the native vegetation, which makes the site prune for new infestation.

Therefore, a measure targeting only *I. glandulifera* without any negative effect to the environment is needed. The use of biological control agents is one measure to reduce the growth of the invasive plant.

We isolated plant associated bacteria and tested them for herbicidal effects against *I. glandulifera* and other weeds. In in vitro tests most of the bacteria reduced the growth of the weeds depending on the applied concentration. Some bacteria only reduced the growth of certain plant species, but others showed a growth reduction in broadleaved and monocot plants. Further tests are required to test for herbicidal effects in the presence of native soil microorganisms and under more realistic conditions.



# MB-09 Suppressing *Fusarium graminearum* and mycotoxins by application of microbial antagonists on infected crop residues

**Alejandro Gimeno<sup>1</sup>**, Irene Bänziger<sup>1</sup>, Andreas Kägi<sup>1</sup>, Eveline Jenny<sup>1</sup>, Dimitrios Drakopoulos<sup>1</sup>, Hans-Rudolf Forrer<sup>1</sup>, Beat Keller<sup>2</sup>, Susanne Vogelgsang<sup>1</sup>

<sup>1</sup> Ecological Plant Protection in Arable Crops, Agroscope and University of Zurich, Switzerland

The orientation towards sustainable agricultural systems requires innovative and integrated methods for control of the fungal disease Fusarium Head Blight (FHB) in wheat to reduce the risk of mycotoxins that contaminate food and feed. Preventive actions against the dominating pathogen *Fusarium graminearum* using biological control agents (BCA) on infected crop residues could contribute to reduced applications of synthetic fungicides. The efforts must focus on microbes with a proven activity against mycotoxin accumulation and a saprophytic lifestyle that is adapted to the environment. Within the Horizon 2020 project MycoKey, we investigated the ability of the fungal species *Clonostachys rosea* and *Trichoderma atrobrunneum* to suppress *F. graminearum* on maize residues and thus to reduce mycotoxins.

At first, we explored the antagonistic activity of *C. rosea* strain 016 on maize stalk pieces infected with *F. graminearum*, either 48 hours before, simultaneously or 48 hours after the treatment. In contrast to other fungal candidates, *C. rosea* strain 016 completely inhibited the formation of perithecia as well as the discharge of ascospores. Investigations on the cellular level using a novel microfluidic platform, the "Fungal-Fungal Interaction device", suggest parasitism behind the observed activity of *C. rosea* against *F. graminearum*. Subsequently, field experiments were carried out in 2016/17 and 2017/18 to compare the efficacy of formulations of *C. rosea* strain 016 and *T. atrobrunneum* strain ITEM908. The collected data included *Fusarium* spore dispersal during the infection period, disease symptoms, mycotoxin content as well as the incidence of *Fusarium* species and *F. graminearum* DNA in harvested grains. The treatments with *C. rosea* strain 016 resulted in significantly lower FHB symptoms and reduced the deoxynivalenol (DON) content in harvested grains by up to 82% in the first and by up to 90% in the second year. Likewise, zearalenone (ZEN) was reduced by up to 80% in the first and by up to 90% in the first year, DON and ZEN were reduced by up to 80 and 90% in the second year. The great potential of *C. rosea* to reduce FHB will be further investigated in on-farm experiments.

# MB-10 Combining nanomaterials and phages for enhanced bacterial wilt control

### Mohammadhossein Ravanbakhsh, George Kowalchuk, Alexandre Jousset

### Microbial Ecology, Utrecht University, Netherlands

*Ralstonia solanacearum* is plant-pathogenic bacterium caused bacterial wilt disease in a wide range of plant hosts. We proposed bacteriophage therapy as one of the most promising approaches to control this bacterium in our previous experiments. However, the application of the bacteriophage trophy for plant disease control is currently hindered by pathogen resistance development to bacteriophages and the limitation of movement within plant tissues. The goal of our study is to produce an enhanced virulence synthetic bacteriophage consortium against *Ralstonia solanacearum*, by combining phage trophy and nano-technology. Our results showed that the combination of bacteriophage and silicon nano-particle decreased the ability of bacteria evolving resistant to bacteriophage during the course of evolution, in vitro. We have tested this combination in roses plants under greenhouse condition and showed higher resistance and less disease severity in host plants. We concluded that the combination of nanomaterials and phages can be an effective biocontrol agent to protect the crop plants against phytopathogenic bacteria in agriculture and horticulture.



<sup>&</sup>lt;sup>2</sup> Zollikerstrasse 107, 8008 Zurich, Switzerland

# MB-11 Iturinic lipopeptide diversity of the *Bacillus subitlis* group

### **Christopher Dunlap**

### Crop Bioprotection, USDA-ARS, United States of America

Iturins and closely related lipopeptides constitute a family of antifungal compounds known as iturinic lipopetides that are produced by species in the *Bacillus subtilis* group. The compounds that comprise the family are: iturin, bacillomycin D, bacillomycin F, bacillomycin L, mycosubtilin and mojavensin. These lipopeptides are prominent in many *Bacillus* strains that have been commercialized as biological control agents against fungal plant pathogens and as plant growth promoters. The compounds are cyclic heptapeptides with a variable length alkyl sidechain, which confers surface activity properties resulting in an affinity for fungal membranes. This study identified 330 iturinic lipopeptide clusters in publicly available genomes from the *Bacillus subtilis* species group. The clusters were subsequently assigned into distinguishable types on the basis of their unique amino acid sequences. The results show some lipopeptides are only produced by one species, whereas certain others can produce up to three. In addition, four species previously not known to produce iturinic lipopetides were identified. The distribution of these compounds among the *B. subtilis* group species suggests that they play an important role in their speciation and evolution.

# MB-12 Microbial consortia effectively suppress and prevent infections of *Ralstonia pseudosolanacearum*in Rosa sp.

### Jie Hu, George Kowalchuk, Alexandre Alexandre

Department of Biology, Utrecht University, Netherlands

Bacterial wilt caused by *Ralstonia pseudosolanacearum* is one of the most destructive bacterial diseases in plants. This pathogen has evolved the ability to infect roses, causing a huge economic loss since its discovery in the EU in 2015. In this work, we seek to increase plant resistance to bacterial wilt by improving the rose microbiome. We discovered that roughly 10% of the cultivable naturally-occurring rose endophytic bacteria can suppress *R. pseudosolanacearum in vitro*. We further show that plant inoculation with a consortium of seven pathogen-suppressive strains could prevent bacterial wilt incidence by 50% amount of wilted rose plants compared with the control treatment. Further, the introduced consortia sharply reduced inside rose shoots and roots in asymptomatic plants. We conclude that the plant endophytic microbiome may be a promising target to prevent diseases outbreaks.



# MB-13 The plant protecting and plant growth promoting abilities of the synthetic microconsortium of antagonistic bacterial strains.

Tomasz Maciąg, Agata Turska, Magdalena Jabłońska, Dorota M. Krzyżanowska, Robert Czajkowski, Sylwia Jafra

Laboratory of Biological Plant Protection, Intercollegiate Faculty of Biotechnology UG and MUG, University of Gdansk, Poland

Plant associated microorganisms comprises bacteria possessing plant-growth promoting and plant protecting abilities. Activity of these bacteria results from different mechanisms including the production of a wide range of antimicrobials, e.g. volatile organic compounds (VOCs). These compounds may also influence plant growth. Application of plantbeneficial bacteria is potentially a promising strategy to protect plants against pathogens both during the plant growth and in storage. The mixture of the selected plant-associated bacteria and the individual strain were investigated as potential plant growth promoting and plant protecting rhizobacteria (PGPR). For this, the ability to colonize plant roots and to promote plant growth by the mixture of strains and an individual strain was verified using tomato and Arabidopsis thaliana seedlings. The capacity of inhibiting the growth of fungal pathogens (Rhizoctonia solani and Fusarium culmorum) was analyzed in dual culture of the selected fungus and the individual strain or the mixture. The influence of VOCs produced by the single strain and the mixture on the growth of fungal pathogens was analyzed using the topbottom culture approach. As the results, we observed that, the strains tested singly are equally effective in tomato roots colonization as the mixture of strains. Three individual strains affected negatively tomato growth after seedling colonization, while this effect was not observed in case of the mixture of strains. Yet, the effect of VOCs on A. thaliana growth indicated that VOCs of two strains increased the growth of the rosette area. This effect was not observed in case of the mixture of strains. For fungal growth inhibition, the individual strains were more effective in suppression of fungal growth than the mixture in dual cultures. Yet, the mixture was equally in inhibition of R. solani via VOCs, as the individual strains but this effect was not observed in case of F. culmorum. In conclusion, the use of the mixtures of the strains for plant protection and plant growth promotion has advantages over the use of individual biocontrol strain.



# miCROPe 2019 - Microbe-assisted crop production opportunities, challenges & needs Vienna, Austria, December 2 – 5, 2019

Microbial applications for improving nutrition and abiotic stress tolerance

Chairs: Philipp Franken & Günter Brader



# MA-01 How plants benefit from root-colonizing fungi: There's more than one way to crack an egg.

**Philipp Franken<sup>1</sup>**, Charlotte Berthelot<sup>2</sup>, Michael Bitterlich<sup>3</sup>, Damien Blaudez<sup>2</sup>, Van Cuong Bui<sup>3</sup>, Dalia Gaber<sup>3</sup>, Gábor M. Kovács<sup>4</sup>, Susanne Kreßner<sup>3</sup>, Richard Pauwels<sup>3</sup>, Hassan Shariyari<sup>3</sup>, Shubhangi Sharma<sup>3</sup>, Wael Yakti<sup>3</sup>

<sup>1</sup> Erfurt Research Centre for Horticultural Crops, University of Applied Sciences Erfurt, Germany

<sup>3</sup> Leibniz-Institute of Vegetable and Ornamental Crops, Grossbeeren, Germany

<sup>4</sup> Eötvös Loránd University, Institute of Biology, Department of Plant Anatomy, Budapest, Hungary

Different ecosystem services are provided by root-colonizing fungi, whereof their contribution to nutrient cycling plays an important role for plants. Fungi with saprophytic abilities like sebacinaceous *Serendipita indica* and Dark Septate Endophytes (DSEs) release plant-available phosphate from inorganic and organic compounds. Arbuscular mycorrhizal (AM) fungi are able to transport nutrients along their coenocytic hyphae towards the plant forming a continuum from the source in the soil to the sink inside the root. In case of phosphate, this is reflected by the regulation of nutrient transporters.

Plants also benefit from root-colonizing fungi if environmental conditions are detrimental for their functions. Under drought, many fungi increase the activity of tolerance mechanisms of the plant like the scavenging of reactive oxygen species or the osmotic balance of cells. Due to their hyphal spread outside the roots, AM fungi also impact soil structure providing favorable conditions for plant water uptake.

In order to increase the abilities of fungi to provide ecosystem services, different strategies have been followed. In one approach, an AM fungus was acclimatized to high heavy metal contamination and this increased the tolerance not only of the fungus, but also of the colonized plant. In a second approach, *S. indica* was combined with a bacterium forming biofilms on the hyphae. This led to higher biomasses of the fungus and of the co-inoculated plant under growth-limiting conditions. DSEs are characterized by their high melanin content in their hyphae which has been thought to confer tolerance to different abiotic stressors. The hypothesis concerning the role of melanin in abiotic stress tolerance was tested by molecular, biochemical and genetic approaches.

# **PF-MA-01** Interactions of arbuscular mycorrhizal fungi and winter wheat in contrasting cropping systems

Mirjam Seeliger<sup>1</sup>, Louis Mercy<sup>1</sup>, Leonidas Rempelos<sup>2</sup>, Neil Gray<sup>2</sup>, Carolin Schneider<sup>1</sup>, Paul Bilsborrow<sup>2</sup>

<sup>1</sup> INOQ GmbH, Germany

<sup>2</sup> Newcastle University, School of Natural and Environmental Sciences

The benefits of arbuscular mycorrhizal fungi (AMF) as plant performance improving symbionts with a broad range of plant species have been well documented in research and are more and more recognised in plant producing industry. However, the importance of AMF in agricultural systems has not been defined yet as a beneficial outcome of the sensitive symbiosis of plant and fungi depends on multiple environmental factors, but also the host genotype. This study investigates the interactions between AMF (native vs commercial inoculant), varieties (long vs short straw), fertiliser types (Biogas Digestate, Cow Manure, mineral fertiliser, no fertiliser) and crop protection (conventional vs organic) by using a multifactorial split plot field experiment over two years. In both growing seasons, shoots and roots are harvested for biomass and root colonisation assessment at five key growth stages. First year results show low impact of inoculation on grain yield, plant growth and health, but major effects of fertiliser and host genotype on AMF colonisation of roots. Highest colonisation rates were reached at flowering in non-fertilised wheat plants of the conventionally bred variety while all fertiliser applications decreased AMF abundance significantly. Ongoing experiments analyse the AMF species composition in the harvested wheat roots by distinguishing between native and exogenous AMF strains.



<sup>&</sup>lt;sup>2</sup> Université de Lorraine, CNRS, LIEC, Nancy, France

# **PF-MA-02** Impact of beneficial microorganisms on strawberry growth, fruit production, nutritional quality and volatilome

Guido Lingua<sup>1</sup>, Valeria Todeschini<sup>2</sup>, Nassima Ait Lahmidi<sup>3</sup>, Eleonora Mazzucco<sup>1</sup>, Francesco Marsano<sup>1</sup>, Fabio Gosetti<sup>1</sup>, Elisa Robotti<sup>1</sup>, Elisa Bona<sup>2</sup>, Nadia Massa<sup>1</sup>, Laurent Bonneau<sup>3</sup>, Emilio Marengo<sup>1</sup>, Graziella Berta<sup>1</sup>, Pierre Emmanuel Courty<sup>3</sup>, **Daniel Wipf<sup>3</sup>** 

<sup>1</sup> Dipartimento di Scienze ed Innovazione Tecnologica, Università del Piemonte Orientale, Viale Michel 11 – 15121, Alessandria, Italy

<sup>2</sup> Dipartimento di Scienze ed Innovazione Tecnologica, Università del Piemonte Orientale, P.zza S. Eusebio 5 – 13100, Vercelli, Italy <sup>3</sup> UMR Agroecology, Burgundy University, France

Arbuscular mycorrhizal fungi (AMF) colonize the roots of most terrestrial plant species, improving plant growth, nutrient uptake and biotic/abiotic stress tolerance. Similarly, plant growth promoting bacteria (PGPB) enhance plant fitness and production. In our study three different AMF (Funneliformis mosseae, Septoglomus viscosum and Rhizophagus irregularis) were used in combination with three different strains of Pseudomonas sp. (19Fv1t, 5vm1K and Pf4) to inoculate plantlets of Fragaria x ananassa Duch var. Eliana F1.The effects of the different fungus/bacterium combinations were assessed on plant growth parameters, fruit production and quality, including health-promoting compounds. Uninoculated plants were kept as controls. At harvest, fresh and dry weights of roots and shoots, mycorrhizal colonization and concentration of leaf photosynthetic pigments were measured in each plant. Many fruit parameters were recorded: pH, titratable acids, concentration of organic acids, soluble sugars, ascorbic acids and anthocyanidins; volatile and elemental composition were also evaluated. Data were analyzed with standard statistical methods (ANOVA) and the data obtained from all analyzed parameters were subjected to multivariate statistical methods (PCA and PCA-DA). In general, AMF mostly affected the parameters associated with the vegetative portion of the plant, while the PGPB were especially relevant for fruit yield and quality. The plant physiological status was differentially affected by inoculations, resulting in enhanced root and shoot biomass. Inoculations affected fruit nutritional quality, increasing sugar and anthocyanin concentrations, and modulated pH, malic acid, volatile compounds and elements. In our study, we show for the first time that strawberry fruit concentration of some elements and/or volatiles can be affected by the presence of specific beneficial soil microorganisms. In addition, our results indicated that it is possible to select the best plant-microorganism combination for field applications, reducing chemical inputs, and improving fruit production and quality, also in terms of health promoting properties.

Todeschini V., Ait Lahmidi N., Mazzucco E., Marsano F., Gosetti F., Robotti E., Bona E., Massa N., Bonneau L., Marengo E., Wipf D., Berta B. and G. Lingua (2018) Impact of beneficial microorganisms on strawberry growth, fruit production, nutritional quality and volatilome. Frontiers in Plant Science, https://doi.org/10.3389/fpls.2018.01611

# PF-MA-03 SIMBA: Design, formulation and optimization of plant growth-promoting microbes for their use as microbial consortia inoculants

**Annamaria Bevivino**<sup>1</sup>, Silvia Tabacchioni<sup>1</sup>, Patrizia Ambrosino<sup>2</sup>, Stefania Passato<sup>2</sup>, Giusto Giovannetti<sup>3</sup>, Daniel Neuhoff<sup>4</sup>, Marina Caldara<sup>5</sup>, Nelson Marmiroli<sup>5</sup>, Soren Sørensen<sup>6</sup>, Joseph Nesme<sup>6</sup>, Alex Sczyrba<sup>7</sup>, Andreas Schlüter<sup>7</sup>, Andrea Brunori<sup>1</sup>, Anne Pihlanto<sup>8</sup>

<sup>1</sup> Sustainability Dept., Biotechnologies and Agroindustry Division, Italian National Agency for New Technologies, Energy and Sustainable Economic Development, Italy

<sup>2</sup> AGRIGES srl, San Salvatore Telesino (BN), Italy

<sup>3</sup> CCS AOSTA srl, Italy

<sup>4</sup> Institute of Crop Science and Resource Conservation, Dept. Agroecology & Organic Farming Rheinische Friedrich-Wilhelms-Universität Bonn, Germany

<sup>5</sup> SITEIA.PARMA, Interdepartmental Centre for Food Safety, Technologies and Innovation for Agri-food - Department of Chemistry, Life Sciences and Environmental Sustainability

<sup>6</sup> Department of Biology, University of Copenhagen, Universitetsparken 15 Bldg 1, Copenhagen, Denmark

<sup>7</sup> Center for Biotechnology – CeBiTec, Bielefeld University, Universitätsstraße 25, Germany

<sup>8</sup> Natural Resources Institute Finland (Luke), Myllytie 1, FI31600 Jokioinen, Helsinki, Finland

The interactions between plant-roots and the surrounding soil, including the resident microbial populations, play an essential role on crop yield. A growing body of evidence demonstrates the potential of various microbes to enhance plant productivity in cropping systems although their successful field application may be impaired by several biotic and abiotic factors. In this context, the activities of Work Package 2 of SIMBA (Sustainable Innovation of MicroBiome Applications in the food system) project were dedicated to exploit the full potential of Plant Growth-Promoting Microorganisms (PGPMs) for sustainable crop production by optimising the efficacy and reproducibility of field applications. In order to identify the PGPMs to be applied as bioinoculants on different crop plants (wheat, maize, potato and tomato) in Italy and Germany, a comprensive literature survey was performed by examining peer-reviewed articles and results from European related projects. The following functional groups of microorganisms were considered; i.e., phosphate solubilizing microbial strains, nitrogen-fixing bacteria, biocontrol strains, endophytic bacteria. To guarantee the development of compatible microbial consortia, selected PGPMswere preliminary screened *in vitro* for their ability



to coexist and exhert a PGP activity. Supported by funding from the EU Horizon 2020 research and innovation programme under grant agreement No. 818431 - SIMBA (Sustainable Innovation of Microbiome Applications in the Food System, https://simbaproject.eu.).

# PF-MA-04 Influence of soil microbial communities linked to organic matter addition on tomato (*Solanum lycopersicum* L.) plant growth under intensive farming

**Francisco Martin Usero**<sup>1</sup>, Armas Cristina<sup>1</sup>, Morillo José A.<sup>1</sup>, Gallardo Marisa<sup>2</sup>, Thompson Rodney B.<sup>2</sup>, Pugnaire Francisco I.<sup>1</sup>

<sup>1</sup>Functional and Evolutionary Ecology, Arid Zones Experimental Station, CSIC, Spain <sup>2</sup>Department of Agronomy (University of Almería)

Intensive greenhouse crop production is one of the most important economic activities in south-east Spain. Intensification has limited the application of organic matter (OM) as a fertilizer in favor of chemical fertilizers, leading to altered soil microbial communities and production loss. We addressed the effects of different greenhouse OM managements on soil microbial communities and their effect on productivity. Greenhouse managements were i) conventional (with addition of chemical fertilizers) without OM application; ii) conventional with OM application, and iii) organic with yearly OM application (without addition of chemical fertilizers). We extracted soil microbial communities from five greenhouses per management type, added the inocula to pots with sterile substrate, and seeded disinfected tomato seeds. Plants grew for 2 months, and at harvest we measured photosynthetic rate, plant growth, and leaf functional traits. Soil microbial diversity and abundance in the original soils did not differ across greenhouse management type. At harvest, plants grew more and had greater photosynthetic rate in pots inoculated with extracts from organic greenhouses than from greenhouses under conventional management without OM application, and potting substrate in the former showed higher bacterial abundance and phylogenetic diversity than the latter. NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> content differed across treatments at harvest, but not at the onset of the experiment, showing differences in microbial activity between treatments. Results suggest that soil microbial communities from organic greenhouses had an overall positive effect on crop productivity.

# PF-MA-05 Microbial consortia: a way to enhance crop yield under both controlled environment and field conditions

Mohammed Antar<sup>1</sup>, Selvakumari Arunachalam<sup>1</sup>, Pierre Page<sup>2</sup>, Donald Smith<sup>1</sup>

<sup>1</sup> Plant Science (Plant-Microbe Interactions Laboratory), McGill University, Canada

<sup>2</sup> SynAgri Company - Saint-Hyacinthe Quebec J2R 2B4 Canada

Root-rhizosphere microbiome associated microbial communities play a key role in the establishment of a plant and contribute to the plant's health and development; plants control this community through root exudates and signal compounds.

This study mainly focuses on the evaluation of specific microbial consortia as microbe coatings for fertilizers, as plant growth promoting agents.

The research evaluates i) field performance and efficacy of the microbial consortia in different soil types and fertility, and ii) how the consortia interact with drought stress under greenhouse conditions.

Corn and potato were grown under field conditions where two different microbial consortia, at various concentrations, were applied at two seeding dates (early and late dates). During the growing season, data were collected for growth variables (plant height, leaf area and biomass) while at the end of the growing season, cobs and tubers were harvested for yield components.

Some of the first year's results show both microbial consortia increased yield in potatoes by 20-23% over untreated plants. The response of corn to the consortia was varied as clay soil resulted in the highest yield, 10-14% greater than the control, while sandy loam showed only a 2.5% increase. On the other hand, the consortia provided potato with tolerance to mild and severe drought stress conditions. Particularly, the consortia promoted root growth and prolonged the shoot growth under greenhouse conditions.



# PF-MA-06 Effect of coinoculation of *Rhizoglomus irregulare*, and hyphae attached phosphate solubilizing bacteria on *Solanum lycopersicum*

Shubhangi Sharma<sup>1</sup>, Stéphane Compant<sup>2</sup>, Max Bernhard Ballhausen<sup>3</sup>, Silke Ruppel<sup>1</sup>, Philipp Franken<sup>4</sup>

<sup>1</sup> Plant microbe interaction, Leibniz-Institut für Gemüse-und Zierpflanzenbau, Großbeeren, Germany, Germany

<sup>3</sup> Freie Universität, Berlin Altensteinstraße 6, 14195 Berlin, Germany.95

<sup>4</sup> Erfurt Research Centre for Horticultural Crops, University of Applied Sciences Erfurt, Kühnhäuser Straße 101, D-99090 Erfurt.

Sustainable agricultural practices are needed to improve plant yield and to solve the global crisis of optimal food production in the coming years without further detrimental impact on the environment. Arbuscular mycorrhizal fungi share a symbiotic relationship with the majority of terrestrial plants, playing a key role in improving availability of nutrients and water uptake of plants. AM fungi are barely able to solubilize phosphate in significant amounts but can aid in the transfer of P from the soil to the plants. AM fungal hyphae have been shown to be colonized by a diverse bacterial community. It is important to study the interaction between P-solubilizing bacteria and AM fungi, to unravel if the bacteria can further improve plant P nutrition when AMF are present. It is further important to decipher these interactions in order to exploit the full potential of these microorganisms as bioinoculants. One approach for enhancing the effects of such bioinoculants could be co-formulations.

The aim of the present study was to isolate P-solubilizing bacteria strongly attached to the hyphae of *Rhizoglomus irregulare* using a two compartment pot system (a root compartment and a hyphal compartment), separated by a 30  $\mu$ m nylon mesh through which AMF hyphae could pass but not the plant roots. *Allium ampeloprasum* (Leek) was used as the host plant inoculated with *R. irregulare*.

A total of 128 hyphae-associated bacteria were isolated, whereof 12 showed stable phosphate-solubilizing activity. Finally, three bacteria belonging to the Pseudomonas family, namely PSB1, PSB11 and PSB18 showed highest potential for inorganic and organic phosphate mobilization.

The three bacteria were further evaluated for their functional characteristics, for interaction with the AM fungus and for their impact as single or co-inoculants on plant growth promotion. We tested the effect of co-inoculation of the bacterial-fungal consortia on *Solanum lycopersicum* and found that plants inoculated with the combination of fungus and bacteria had significantly higher root biomass and improved P uptake compared to the single inoculations.

We conclude that co-formulations of AM fungi and functionally important hyphal colonizers such as P-solubilizing bacteria can be a way to significantly enhance AMF inoculum benefits.

# MA-02 Matching root anatomical and architectural phenotypes with soil microorganisms to improve nutrient and water uptake efficiency: a new perspective in plant microbiome research

Tania Galindo-Castañeda<sup>1</sup>, Martin Hartmann<sup>2</sup>, Jonathan Lynch<sup>1</sup>

<sup>1</sup> Plant Science, The Pennsylvania State University, United States of America

<sup>2</sup> Department of Environmental Systems Science, ETH Zürich, Zürich, 8092 Switzerland

Root phenotypes are highly diverse at the architectural and anatomical levels of organization. Specific root phenotypes are associated with better plant growth under low nutrient and water availability. Therefore, root ideotypes have been proposed as breeding targets for crops growing under nutrient and/or water scarcity. For example, roots with phenotypes that correspond to the ideotype Topsoil foraging are associated with better plant growth under lowphosphorus stress, and the ideotype Steep, Cheap and Deep is linked to low nitrogen/water stress tolerance. We propose that the natural variation in root phenotypes translates into a diversity of different niches for microbial associations in the rhizosphere, rhizoplane and root cortex, and that microbial traits could be synergistic with the beneficial effect of specific root phenotypes. Oxygen and water content, carbon rhizodeposition, nutrient availability, and root surface area are all factors that are modified by root anatomy and architecture and determine structure and functioning of the associated microbial communities. Therefore, the selection of root phenotypes linked to better plant growth under specific edaphic conditions should be accompanied by investigating and selecting the microbial partners better adapted to each set of conditions created by the corresponding root system. Microbial traits such as nitrogen transformation or phosphorus solubilization could have a synergistic effect when correctly matched with promising plant root ideotypes for improved nutrient and water capture. Recent results indicate that root traits that may modify the microbial communities associated with maize are aerenchyma and rooting angle, and root hairs and root class have been studied in other plant species. We will present examples of the effects of anatomical and architectural root phenotypes linked to differential microbial associations as obtained by our research and from recently published results, and propose a model to test hypotheses about the interactive effects of root phenotypes and microbial functions on plant nutrient and water uptake.



<sup>&</sup>lt;sup>2</sup> Austrian Institute of Technology, Konrad Lorenz Strasse 24, 3430 Tulln, Austria.

### MA-03 Combating salinity stress with Rhizosphere Engineering: A next-generation approach

### SHUBHAM DUBEY, SHILPI SHARMA

### Department of Biochemical Engineering and Biotechnology, IIT Delhi, India

Plant growth is drastically affected by the salinization of soil. With the ever-increasing mean global temperature and the poor quality of water being used for irrigation, this problem of soil salinization is expected to grow further and will lead to several socio-economic problems. Approximately 50% of the total land under irrigation is adversely affected by salinity. A major push in crop productivity is required to feed the exponentially growing population. The conventional strategy of cultivating salt-tolerant plant varieties has often failed to address this problem effectively. Due to many notable impacts of microorganisms on crops, the use of various microorganisms harboured by crops has gained attention. The area in the immediate vicinity of the root is known as "Rhizosphere" and has been well characterised for its intense microbial activity under the influence of rhizodeposits. Single strains of microbes in the form of inoculants are often ineffective growth of plant and stress adaptability, mainly owing to the competition with the indigenous rhizospheric microbial community and restricted colonization effectiveness. The plant along with its associated microbiome is considered as meta-organism and is known as *holobiome*. We have used the approach of plant-assisted multigeneration approach for acclimatizing the microbiome in which the host plant is allowed to select a microbiome that is beneficial for the growth of the plant. The model system used for the study was Vigna radiata (mung bean) owing to its short life cycle. The adapted microbiome helped the plant to better withstand the salinity stress. The plant growth promotion ability and mitigation of salt stress was validated by the enhancement of plant biometric parameters and reduction of plant stress markers. The long-term aim of our research is to develop a synthetic microbial community with the ability to help plant ameliorate salt stress. These innovations will open fresh avenues to capitalize on the rhizosphere microbiome in order to reinforce the tolerance of a plant to salt stress and thus refine agricultural practice under saline circumstances.

# MA-04 Phosphate fertilization in crops – the contribution of bacteria and fungi

### Günter Brader

### Center for Health & Bioresources, AIT Austrian Institute of Technology, Austria

Modern agriculture depends on resource-intense fertilization for optimal nutrition of crops to meet the demand for food and feed. The three main macronutrient components of fertilizers are phosphorus (P), nitrogen (N) and potassium (K). P and K are mined resources and especially mined P (in the form of rock phosphate) is a critical resource for the European Union, where 90% of is imported. In mineral fertilizers P is supplied in form of processed superphosphates. P is often not rare in soils, but not available for many crop plants as it becomes fixed to soil mineral complexes and depending on soil parameters such as pH only a small fraction of P from fertilizers is available for plant uptake.

Bacteria such as Bacilli and Pseudomonads and certain fungi (*Penicillium* and *Aspergillus* spp.) have been long described for P-solubilizing effects and could be applied to substantially reduce the need for application of the limited and energy intense resource P in agriculture. The knowledge on the mechanisms and the contribution of different microorganism on P-uptake in plants and the ability to survive in agricultural soils are crucial factors for improvement of agricultural applications. This and the limitations and the complexity of solutions with living microorganisms for broader applications as P-fertilizers in agriculture will be discussed.



# MA-05 Maize inoculation with microbial consortia: contrasting effects on rhizosphere activities, nutrient acquisition and early growth in different soils

**Klára Bradáčová**<sup>1</sup>, Maximilian Sittinger<sup>2</sup>, Katharina Tietz<sup>1</sup>, Benjamin Neuhäuser<sup>1</sup>, Ellen Kandeler<sup>3</sup>, Nils Berger<sup>4</sup>, Uwe Ludewig<sup>1</sup>, Günter Neumann<sup>1</sup>

<sup>1</sup> Institut of Fertilisation and Soil Matter Dynamics, University of Hohenheim, Germany

<sup>2</sup> Institute for Biological Control, Julius Kühn- Institut Heinrichstraße 243, 64287 Darmstadt

<sup>3</sup> Institute of Soil Biology (310b), Universität Hohenheim, Emil-Wolff-Straße 27, 70593 Stuttgart, Germany

<sup>4</sup> EuroChem Agro GmbH, 8165 Mannheim, Germany

The benefit of plant growth-promoting microorganisms (PGPMs) as plant inoculants is influenced by a wide range of environmental factors. Therefore, microbial consortia products (MCPs) based on multiple PGPM strains with complementary functions, have been proposed as superior, particularly under challenging environmental conditions and for restoration of beneficial microbial communities in disturbed soil environments. To test this hypothesis, the performance of a commercial MCP inoculant based on 17 PGPM strains and seaweed extracts, was investigated in greenhouse experiments with maize on three soils with contrasting pH, organic matter content and microbial activity, under different P and N fertilization regimes. Interestingly, the MCP inoculant stimulated root and shoot growth and improved the acquisition of macronutrients only on a freshly collected field soil with high organic matter content and high background microbial activity, exclusively in combination with stabilized ammonium fertilization. This was associated with transiently increased expression of AuxIAA5 in the root tissue, a gene responsive to exogenous auxin supply, suggesting root growth promotion by microbial auxin production as a major mode of action of the MCP inoculant. High microbial activity was indicated by intense expression of soil enzyme activities involved in C, N and P cycling in the rhizosphere (cellulase, leucine peptidase, alkaline and acid phosphatases) without detectable effects induced by MCP inoculation. Contrastingly, the MCP inoculation did neither affect maize biomass production, nor nutrient acquisition on soils with very little C-org and low microbial activity, although a moderate stimulation of rhizosphere enzymes involved in N and P cycling was recorded. There was also no indication for direct MCP-induced solubilization of Ca-phosphates on a highly buffered calcareous sub-soil supplied with rock-phosphate. The results demonstrate that the MCP strategy, combining large numbers of PGPM strains with complementary properties, not necessarily translates into plant benefits under challenging environmental conditions. Soil properties, such as organic matter content, pH buffering and paticle size distribution but also the fertilization regime may crucially influence the plant-microbial interactions. Thus, a better characterization of the conditions determining successful MCP application is mandatory.

# MA-06 The impact of beneficial microbes on *Brachypodium* nutrient uptake under limiting supplies of nitrogen and phosphorus, monitored with non-invasive phenotyping and molecular approaches

Borjana Arsova<sup>0</sup>, Stefan Sanow<sup>0</sup>, Martino Schillaci<sup>1</sup>, Weiqi Kuang<sup>0</sup>, Pitter Huesgen<sup>2</sup>, Ute Roessner<sup>1</sup>, Michelle Watt<sup>0</sup>

<sup>1</sup> School of BioSciences, The University of Melbourne, 3010 Victoria, Australia

<sup>2</sup> Plant Degradomics Group, ZEA3 – Analytics, Forschungszentrum Jülich, 52425, Jülich, Germany

<sup>0</sup> IBG-2 – Plant Sciences, Forschungszentrum Jülich, Germany

In times of increasing global population and decreasing arable land per capita, the understanding of plant nutrient uptake and novel strategies to improve nutrient uptake are of utmost importance. Our work focuses on nitrogen (N) – the second most abundant nutrient in plants and phosphorus (P) – a finite global resource. We present studies where use of plant growth promoting rhizobacteria (PGPR) resulted in improved plant performance under limited N or P in *Brachypodium*- a model plant for cereals. Plant roots were analyzed with the non-invasive root phenotyping platform GrowScreen Page (Gioia *et al.*, 2017), or with the 3D printed EcoFab microcosms (Sasse *et al.*, 2019). The latter was adapted and used in combination with Plant Screen Mobile (Muller-Linow *et al.*, 2019), for non-invasive shoot area estimation, in conjunction with root scanning, over time.

In the case of P limitation, plant biomass was higher in plants inoculated with a PGPR. Time series image-analysis of root phenotype allowed visualization of increased root length and changes in root architecture, pin-pointing the timewindow when growth promotion took effect after inoculation. A sand experiment similarly resulted in increased biomass in inoculated plants. Study of the molecular mechanisms behind this whole plant, dynamic phenotype is ongoing and involves metabolomics and lipidomics.

In the case where plants with limiting N supply were inoculated with N-fixing PGPR, an end-point harvest showed that ratio of lateral to primary root length increases. More importantly, N concentration in root and shoot tissue increased, along with greater shoot biomass and leaf area. We complemented this destructive harvest with proteomics to investigate the systemic response of *Brachypodium* constitutively grown under limiting N, to the interaction with the



PGPR. Data analysis revealed that these N-fixing bacteria impact central nitrogen metabolism in *Brachypodium*, and indicate a mode of action that upregulates specific N transporters on the root plasma membrane.

The grass model can thus clearly benefit from PGPR, however the time points, tissue responses and molecular mechanisms were different for organisms and nutrient conditions. Efforts are needed to elucidate plant responses to the microorganisms, addressing molecular and tissue architecture, while taking in context plant developmental stage (Arsova *et al.*, 2019) and time since application.

# MA-07 Does genetic variation in single spore progeny of an arbuscular mycorrhizal fungus impact cassava yield

**Chanz Robbins**<sup>1</sup>, Consolée Aletti<sup>1</sup>, Réjane Seiler<sup>1</sup>, Diego Camilo Peña-Quemba<sup>2</sup>, Paweł Rosikiewicz<sup>1</sup>, Jérémy Bonvin<sup>1</sup>, Joaquim Cruz-Corella<sup>1</sup>, Frédéric Masclaux<sup>1</sup>, Alia Rodriguez<sup>2</sup>, Ian Sanders<sup>1</sup>

<sup>1</sup> Département de Écologie et Évolution, Université de Lausanne, Switzerland

<sup>2</sup> Universidad Nacional de Colombia, Bogotá, Colombia

Arbuscular mycorrhiza fungi (AMF) are prolific soil microbes forming symbiotic relationships with the majority of plant species, including most crops. While crops have witnessed intense genomic modifications through centuries of selective breeding, little has been achieved to exploit desirable traits of the AMF symbiosis, even though it is known that they can increase plant productivity. Recent studies using the model AMF species, *Rhizophagus irregularis*, demonstrated that both mono- and dikaryotic states exist in this mostly asexually propagating species. Being coenocytic in nature, nuclei packaged into spores is posited to be a random process, creating disproportional numbers of nuclei in individual spores and leading to allele frequency changes between single spore progeny. Single spores can be taken from individuals and be used to produce new AMF cultures displaying distinct genetic identities. These 'novel' individuals can then be systematically tested with host plants to begin elucidating dependencies of observed plant traits on AMF genetics. To address these questions, we generated more than 40 single spore cultures originating from one of six parental isolates, consisting of the two karyotic states across the *R. irregularis* phylogeny. A reduced representation ddRADseq protocol was performed and obtained reads were mapped to their parent. We further tested these isolates in cassava cultivation in Colombia where inoculation with different progeny lines caused differences in cassava yield. We report allele frequency changes in progeny and address their potential to increase cassava production.



# MA-08 Fertiledatepalm – a transdisciplinary collaboration project to ameliorate date palm cultivation via microbial inoculation, organic matter management and mixed cropping using nurse plants

Rania El Hilali<sup>1</sup>, Mohamed Ouzine<sup>1</sup>, Asma Chebaane<sup>2</sup>, Said Kinany<sup>3</sup>, El Hassan Achbani<sup>3</sup>, Rachid Bouamri<sup>1</sup>, Lotfi Fki<sup>4</sup>, Ahmed Mliki<sup>5</sup>, Gian Nicolay<sup>0</sup>, Paul Mäder<sup>0</sup>, **Sarah Symanczik<sup>0</sup>** 

<sup>1</sup> Department of Plant and Environment Protection, National School of Agriculture of Meknes, 50001 Meknes, Morocco

<sup>3</sup> Laboratory of Bacteriology and Biological Control, Research Unit of Plant Protection, CRRA, INRA Meknes, 50001 Meknes, Morocco

<sup>4</sup> 2Department of Biology, Faculty of Sciences of Sfax, Road of Soukra BP 802, 3038 Sfax, Tunisia

<sup>5</sup> 2Department of Biology, Faculty of Sciences of Laboratoire de Physiologie Moléculaire des Plantes, Centre de Biotechnologie de Borj Cedria, BP 901 2050 Hammam-Lif, Tunisia

<sup>0</sup> Forschungsinstitut für biologischen Landbau, Switzerland

Date palm is an important crop in Morocco, Tunisia and other drylands with a high agricultural, economic and cultural value. Harsh environmental conditions of those areas, further accelerated by climate change and the spread of root diseases, threaten date palm cultivation. To overcome limitations in productivity, high inputs of mineral fertilizers and pesticides are applied. However, these external inputs strongly affect the environment and livelihoods.

The project aims at establishing an integrated microbe-assisted fertilization approach, combining the inoculation of native soil microbes, namely arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) during the different date palm growth stages, with adapted agricultural management practices using organic amendments and mixed-cropping in Morocco and Tunisia.

As initial step, we established a culture collection of native microbes, isolated from date palm roots and rhizosphere composed of 24 AMF isolates including eight species from six genera, twelve bacterial endophyte isolates composed of *Paenibacillus, Mycobacterium*, and *Achromobacter* species and 34 PGPR isolates. Functional characterization of PGPRs revealed that around 50 % can solubilize phosphorus and potassium and between 9 % and 68 % have the ability to produce siderophores, hydrogen cyanid, chitinase, cellulase, amylase and protease. Consortia of microbes were formed and used for inoculations.

Experiments under nursery conditions revealed that inoculation with AMF and PGPR combined with compost significantly increased growth of date palms as compared to non-amended controls enabling farmers to decrease the time prior to field transplantation. On-farm trials performed in productive date palm groves have shown that PGPR inoculation with or without mixed-cropping with sorghum as nurse plants significantly increase fruit characteristics such as fruit flesh weight as well as fruit length and diameter for up to 14 % and leaf macronutrient concentrations for up to 200 % while in addition enhancing the mycorrhizal potential of the soil.

Our integrated fertilization approach is developed in a participatory approach with key stakeholders in so-called innovation platforms, working at laboratory, on-station and on-farm scale to best tackle farmers' needs in order to facilitate adoption and implementation.

# MA-09 Unraveling plant physiological behavior modulated by a synthetic microbial community using a non-invasive and continuous medium-scale phenotyping platform

### Jaderson Armanhi, Rafael de Souza, Paulo Arruda

Genomics for Climate Change Research Center, University of Campinas, Brazil

The intimate association of plants with bacterial and fungal communities directedly or indirectly modulates physiological behavior and helps plants to acquire nutrients, adapt to drought, heat and salinity stresses as well as facing pathogen attack. Despite multiple studies showing microbiome modulation of plant physiological behavior, our knowledge about the mechanisms governing plant response to beneficial plant-microbe interaction is still limited. Thus, aiming to deeply understand how microorganisms modulate plant phenotype, we designed a non-invasive and low-cost phenotyping platform capable to continuously measure several physiological traits every five minutes. This platform was used to assess the effect of an abundance-based synthetic bacterial community (SynCom) from the sugarcane core microbiome inoculated in maize. The SynCom dramatically increased plant biomass and enhance plant tolerance to drought stress. Observations of mature plants of three different commercial hybrids of maize showed that the SynCom positively impact plant physiology by delaying plants' response to drought stress. Severe water deficit led plants to bend over the ground, a symptom firstly observed in uninoculated plants. We also found that in rehydration inoculated plants showed a faster recovery compared to uninoculated ones, indicating that the SynCom optimizes water usage in the recovering process. The continuous phenotyping revealed that the SynCom positively affected leaf temperature as uninoculated plants strikingly presented a higher temperature compared to those in the presence of the SynCom, may indicating an effective water usage as the increase of plant temperature might be particularly harmful. In a condition of water deficit,



<sup>&</sup>lt;sup>2</sup> 2Department of Biology, Faculty of Sciences of Sfax, Road of Soukra BP 802, 3038 Sfax, Tunisia; Laboratoire de Physiologie Moléculaire des Plantes, Centre de Biotechnologie de Borj Cedria, BP 901 2050 Hammam-Lif, Tunisia

inoculated plants also presented a remarkable increase in yield. Altogether, our findings point to a significant potential of our SynCom in maize development under stressful condition, thus leading to further investigation on key molecular mechanisms involved.

# MA-10 Synergistic contribution of microbial consortia, micronutrients, and ammonium fertilization to cold tolerance in maize by regulating phytohormone homeostasis and oxidative stress defence

**Narges Moradtalab**<sup>1</sup>, Aneesh Ahmed<sup>1</sup>, Jörg Geistlinger<sup>1</sup>, Frank Walker<sup>1</sup>, Birgit Höglinger<sup>1</sup>, Uwe Ludewig<sup>1</sup>, Günter Neumann<sup>2</sup>

<sup>1</sup> Institut für Kulturpflanzenwissenschaften (340), Universität Hohenheim, Germany

<sup>2</sup> gd.neumann@t-online.de

Low soil temperature in spring is a major constraint for the cultivation of tropical crops in temperate climates. In this study, we describe the exploitation of synergistic interactions by combined application of micronutrients, consortia of plant growth-promoting microorganisms (PGPM) and the form of nitrogen fertilization (nitrate versus stabilized ammonium supply) on recovery and early growth of maize after two-weeks exposure to low root zone temperatures at 12 °C on a silty loam field soil (pH 6.8). In maize plants with nitrate fertilization, the cold stress increased the necrotic and chlorotic leaves by 133% and impaired acquisition of Zn, and Mn. A pre-selection trial with fungal and bacterial PGPM strains revealed superior cold-protective performance for a combined formulation of Trichoderma harzianum OMG16 and Bacillus spp. with Zn/Mn supplementation (CombiA), particularly in combination with ammonium fertilization. Compared with nitrate fertilization, stabilized ammonium supply improved Zn and Mn related with moderately increased enzymatic and non-enzymatic detoxification of reactive oxygen species (ROS). The shoot concentration of abscisic acid (ABA) as a key regulator for the adaptive cold stress responses was increased by 33 %. Moreover, ammonium fertilization also increased the root auxin (IAA) concentration (+176 %), associated with increased expression of auxin-responsive genes involved in IAA synthesis (ZmTSA), transport (ZmPIN1a) and perception (ZmARF12). Additional inoculation with the microbial consortium promoted root colonization with the inoculant strain T. harzianum OMG16 in combination with ammonium fertilization. Further increased IAA concentrations in the root (+121 %) and shoot tissues (+51 %) and increased ZmPIN1a and ZmARF12 expression resulted in a doubling of root length. An increased ABA/cytokinin ratio and increased concentrations of jasmonic (JA) and salicylic acids (SA) were associated with a further increase in enzymatic and non-enzymatic ROS detoxification in the shoot tissue. Additional supplementation with Zn and Mn further increased shoot IAA, root length and total antioxidants, associated with the highest shoot biomass production (+53 %) and the lowest proportion of oxidative leaf damage. These effects suggest a perspective for synergistic mitigation of cold stress symptoms and induction of stress priming effects by a strategic combination of stress-protective nutrients and selected microbial biostimulants.



# miCROPe 2019 - Microbe-assisted crop production opportunities, challenges & needs Vienna, Austria, December 2 – 5, 2019

Regulatory Issues – Special Session



#### Lectures

#### RI-01 Registration of biopesticides in the European Union

Gianpiero Gueli Alletti<sup>1</sup>, Agata K. Jakubowska<sup>2</sup>, Rüdiger Hauschild<sup>3</sup>

<sup>1</sup> APIS Applied Insect Science GmbH, Germany

Biopesticides, comprising microorganisms, plant extracts (botanicals) and semiochemicals (pheromones) are highly increasing in importance and attention on the market of plant protection products. Noteworthy, the reduction in the number of chemical active substances from ca. 1000 to 250 but also the request for sustainable and environmental-friendly agricultural alternatives are fueling the demand for biopesticides. Generally, most biopesticides have little to no effects to human health, non-target organisms and the environment. However, applicants have to overcome several regulatory hurdles for the registration of biocontrol products.

Registration of plant protection products in European Union has increasingly strengthened over the past years. The review program under the previous Directive 91/414 governing the registration process already resulted in fewer active substances. Since 2011 the active substances and products are evaluated according to Regulation (EC)1107/2009. Historically, data requirements for microorganisms are derived from those for chemicals. This is critical, since some data requirements which can be easily covered for synthetic chemicals cannot be applied to microorganisms for technical reasons. In contrast, the major advantage of most biopesticides is that their active ingredients are scientifically well-studied, and humans are familiar with those either through direct use or environmental exposure for a long time. The key information for the selection of experimental data is information on the biology of the organism itself or its compounds, in particular on taxonomy and the mode of action. Here, the process of registration will be presented and data requirements crucial for the evaluation of biopesticides will be critically reviewed based on our experience.

# **RI-02** Proposal for the application of microbiomes in industry: regulatory challenges and opportunities

#### Faina Kamilova<sup>1</sup>, José João Dias Carvalho<sup>2</sup>

<sup>1</sup> Regulatory Affairs, Knoell NL BV, Netherlands

<sup>2</sup> knoell Germany GmbH, Charlottenstr. 80 10117 Berlin Germany

The increasing understanding of synergistic effects between the various microbial components to the benefit of plants suggests that it is attractive to use the microbiome, or a combination of several of its constituents, in agricultural applications. When a promising positive effect of a mixture of a number of microbiome's microorganisms has been spotted, the major constituent(s) responsible for that effect as well as other helping players have to be identified. Today, the industrial-scale manufacturing processes of commercial microbiological product are based on the isolation and production of individual strains, which can then be used as a sole active ingredient or as a mixture of several microbes. Moreover, the mode of action of an individual strain, or a mixture of different strains, does play an important role when deciding on the market-access positioning of a commercial product thus setting the trail for which regulatory approach would be most appropriate.

According to the current EU regulations, each microorganism – used either in plant protection or biostimulant products – must be identified at the strain level ahead of regulatory approval. In addition, its safety for humans and the environment must be demonstrated by means of an individual dossier, i.e. per each microorganism, to be submitted for evaluation at the EU level. Hence, it becomes clear that a registration of commercial products containing a mixture of several microbes is extremely costly, under today's rules, and it will prevent microbiome technologies to reach the market unless new regulatory ideas are put in place quickly.

During this communication, we will be addressing some ideas to improve the present regulatory framework in order to embrace scientific innovations arising from new discoveries in plant-microbe interactions. To this end, we propose to look at the current regulation rules and guidance being used for plant extracts and other complex mixtures. The European Union regulations consider a plant extract as a single active ingredient and the whole extract is evaluated during the review process, even if components are to be identified. We will be exploring the idea to regulate a mixture of microbes in a similar way, by assessing the mixture of microbes as a whole even if the safety dossier will still include the taxonomical profiling at the strain level to exclude potential pathogens.



<sup>&</sup>lt;sup>2</sup> APIS Applied Insect Science S.L., Pza. Alfonso el Magnánimo nº3, 1º-A, 46003 Valencia, Spain

<sup>&</sup>lt;sup>3</sup> APIS Applied Insect Science GmbH, Kurze Straße 3, 21682 Stade, Germany

Lectures

### miCROPe 2019 - Microbe-assisted crop production opportunities, challenges & needs Vienna, Austria, December 2 – 5, 2019

Disruptive approaches for engineering the phytobiome & microbial delivery

Chairs: Trevor Charles & Michael Ionescu



#### Lectures

#### DA-01 Can we tune the microbiome in controlled environment agriculture?

Trevor Charles<sup>1</sup>, Michael Lynch<sup>2</sup>, Elena Zaikova<sup>2</sup>, Jiujun Cheng<sup>2</sup>, John Heil<sup>2</sup>

<sup>1</sup> Waterloo Centre for Microbial Research, University of Waterloo, Canada

<sup>2</sup> Metagenom Bio

Controlled environment systems, such as hydroponic greenhouses and vertical farms, offer unprecedented opportunities for ensuring favourable microbiome composition for plant growth, product yield and quality, and resilience against disease-causing microbes. Microbiomic and metagenomic surveys of the recirculating nutrient delivery systems of commercial vegetable greenhouses demonstrate clear, crop-specific effects on microbial community structure. The conditions of the rhizosphere are expected to have microbial community enrichment effects. The ability to degrade 1-aminocyclopropane-1-carboxylic acid (ACC), the immediate precursor to ethylene, is associated with many rhizospheric and endophytic bacteria that have plant beneficial effects. The key enzyme is ACC deaminase, which catalyzes the conversion of ACC to ammonia and  $\alpha$ -ketobutyrate. As a result, ACC can serve as nitrogen and carbon source, and the resulting reduction of ACC levels reduces stress ethylene. Of interest is the role that ACC plays in shaping the phytobiome, and how this in turn may influence crop health and productivity. As an initial step towards understanding, we used 16S rRNA gene sequence analysis and shotgun metagenomics to investigate the community dynamics of soil and hydroponic nutrient solution enrichment cultures with ACC as nitrogen source, compared to ammonia. We found that the community became much more constrained on ACC, consistent with ACC metabolism being more of a specialized trait. The ACC-enriched cultures were able to promote plant growth. Metagenomeassembled genomes (MAGs) and genomes of pure culture isolates confirmed the presence of acdS, encoding ACC deaminase, and provided insight into the nature and diversity of ACC metabolizing strains in recirculating nutrient delivery systems. These enrichment experiments lay the groundwork to guide strategies for microbiome optimization in operating hydroponic systems.

# DA-02 Harnessing the power of computational genomics to optimize next generation agbiologicals

#### Michael Ionescu, Galit Kuzntz

#### Research, Lavie-Bio, Israel

Lavie Bio, a subsidiary of Evogene, focus on discovery, optimization and development of microbiome-based agbiologicals to improve food quality, health and agriculture sustainability. Product challenges include achieving efficacy, stability (consistency) and commercial viability. Lavie Bio utilizes Computational Predictive Biology (CPB) platform to pre-design and optimize product candidates that can overcome product challenges utilizing the genomic-based prism. By breaking down product challenges to biological challenges (e.g. achieving sufficient shelf life, prolonged colonization of the microbiome), Lavie Bio identify limiting biologies (bottlenecks) that impose challenge on the road to product. Behind each limiting biology are biological functions that are associated with strains that can overcome each challenge. These functions are key tools for *ab initio* design of product candidates. In one example, gram-negative strains with short shelf life are matched based on their genomic content with personalized fermentation and formulation protocols that are predicted to significantly protect them during formulation and stabilize their viability during storage. By utilizing this approach, we already succeeded extending the shelf life of gram-negative product candidates by many folds. Such predictors are enabled by harnessing data from gene level through to the phenotypic level and by genome analyses technologies combining machine learning and comparative genomics. Lavie bio's 'biology driven design' approach and its implementation through the CPB platform for the discovery and optimization of microbiome based ag-biologicals products will be described in the presentation.



## DA-03 Lifting the veil of virulence and benefits of plant-associated bacteria by metagenomics approaches

**Sascha Patz<sup>0</sup>**, Caner Bağcı<sup>0</sup>, Gylaine Vanissa Tchuisseu Tchakounte<sup>1</sup>, Benjamin Albrecht<sup>0</sup>, Matthias Becker<sup>2</sup>, Silke Ruppel<sup>1</sup>, Daniel Hamlet Huson<sup>0</sup>

<sup>1</sup> Leibniz Institute of Vegetable and Ornamental Crops, 14979 Grossbeeren, Germany

<sup>2</sup> Institute for National and International Plant Health, Julius Kühn-Institute – Federal Research Centre for Cultivated Plants, 38104 Braunschweig,

Germany

<sup>0</sup> Center for Bioinformatics, University of Tübingen, Germany

In the century of climate change and environmental pollution agricultural application of microbes to soil and plants aims to improve plant vitality, resistance and yield. The challenge is to define microbes that provide desired effects under a distinct or multiple different states, such as nutrient deficiency and/or pathogen infestation. Recently emerged innovative high-throughput technologies in molecular biology and informatics allow us to explore microbial interactions in all their detailed perspectives and dependencies. In particular, metagenomics discloses microbial community structures, and gives us the chance to investigate their genomic information and to identify site- and host-specific microbial hubs.

**Our approach** targets at currently known bacterial genomes stored in publicly available databases. In three major steps we (i) filter single nucleotide and structural genomic variations, (ii) associate specific patterns with their metadata, and (iii) distinguish between plant growth-promoting (PGP) and virulent (human pathogenic or phytopathogenic) genomic variants. We further apply the obtained information to screen metagenomes and respective bacterial strains for those patterns.

**Recently**, we have established a new ontology for PGP to facilitate the detection of respective functions in genomes and metagenomes, available as implementation in MEGAN6. Hence, it allows to predict PGP potential in metagenomic samples, ideally originating from plants or soil. Noteworthy, the combination with the detection of virulence factors (MEGAN6), implies the potential to differentiate PGP bacteria (PGPBs) from pathogenic strains.

**Our great achievement** is a tool for mobile screening of PGPBs and pathogens, using long read sequences, that are generated by e.g. a MinION device directly connected to your personal computer. Additionally, we try to predict *in silico* strains that may have the potential (i) to survive in the current microbiome by vacant niche occupation or by revealing versatile competitive traits, (ii) to reduce pathogen abundance, and/or (iii) to improve plant growth by carrying multiple PGP traits.

**In conclusion**, the novel approach and tool tenders a bunch of application scenarios, like identification of pathogens during pre-/post-harvesting processes, estimation of plant's metagenome virulence and beneficial potential or prediction of applicable PGPBs.

You are interested in my research? I encourage you to talk to me and discuss your remarks.

### DA-04 Opportunities and challenges of microbial seed application

#### **Carola Peters**

#### Research & Development, Incotec Europe B.V., Netherlands

Seeds and seedlings face many challenges in the field, either biotic or abiotic. Both stresses have major impact on germination, (early) plant development and yield. Microorganisms have been shown to aid seed and plant to cope with these challenges by improving nutrient availability, relieving drought stress and fighting off hostile microorganisms and insects.

Currently, soil pathogens and pest insects are controlled by chemical plant protection products. The use of many of these products is under review. Therefore, alternative approaches or techniques to fend against environmental challenges are needed.

Nowadays, most microbial products for plants are applied as a soil drench or granulates. The benefit of adding microorganisms already to the seed is that the microbials can grow with the development of the seedling, ensuring rapid colonization of the roots and thus providing nutrients and/or protection at a very early stage of plant development.

Several application methods, such as film coating or pelleting can be used for microbial seed application. For optimal effectivity, a tailor-made solution is needed dependent on the microbial-crop combination. Many microbial species are drought-sensitive and assuring a prolonged microbial survival on seed has been proven challenging. Additionally, seed companies request an integrated approach were the microorganisms, fungicides and insecticides need to be applied to the same seed. Examples will be given on the seed application of several microorganisms.



### miCROPe 2019 - Microbe-assisted crop production opportunities, challenges & needs Vienna, Austria, December 2 – 5, 2019

### **Closing & Closing Lecture**

**Chair: Angela Sessitsch** 

### C-01 Towards new road MAPs to engineer plant microbiomes

Ben Oyserman<sup>1</sup>, Viviane Cordovez<sup>2</sup>, Victor Carrion<sup>1</sup>, Marnix Medema<sup>3</sup>, Jos M. Raaijmakers<sup>1</sup>

<sup>1</sup> Microbial Ecology, NIOO-KNAW, Netherlands

<sup>2</sup> Institute of Biology, Leiden University, Leiden, Netherlands

<sup>3</sup> Bioinformatics Group, Wageningen University, Wageningen, Netherlands

Ben Oyserman<sup>1</sup>, Viviane Cordovez<sup>1,2</sup>, Victor Carrion<sup>1,2</sup>, Marnix Medema<sup>3</sup>, Jos M. Raaijmakers<sup>1,2</sup>

<sup>1</sup> Department of Microbial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, Netherlands; <sup>2</sup> Institute of Biology, Leiden University, Leiden, Netherlands; <sup>3</sup> Bioinformatics Group, Wageningen University, Wageningen, Netherlands

Microbial interactions with plants contribute directly or indirectly to plant health and fitness. Of particular interest are interactions that result in the emergence of beneficial plant phenotypes such as disease suppression, improved nutrient acquisition, or drought tolerance. Engineering plant-associated microbiomes to optimize these emergent *m*icrobiome *a*ssociated *p*henotypes (MAPs) is expected to transform the agricultural industry and to lead to more sustainable food production by replacing current unsustainable practices. To achieve this, numerous bottlenecks must be addressed. First, a key challenge will be to develop a quantitative and systematic platform for identifying and prioritizing MAPs. Once prioritized, the second challenge is to unravel the molecular mechanisms for both host and microbiome. Due to the complexity and endless possibilities of confounding ecological interactions, developing an appropriate model to test the importance or particular interactions is essential to develop robust MAPs. In this presentation, a theoretical framework will be presented that can tackle these bottlenecks in this nascent field of engineering MAPs. Next I will present on our BackToRoots work to link the rhizosphere microbiome to host genotype and to discover the 'missing' plant microbes and functional traits in the plant microbiome.



### **POSTER PRESENTATIONS**



Poster Presentations

### miCROPe 2019 - Microbe-assisted crop production opportunities, challenges & needs Vienna, Austria, December 2 – 5, 2019

Poster Session 1: Successful microbial applications



# PP1-SA-01 A new formulation with bacillus and pseudomonas increases plant fitness, net photosynthesis, yield and precocity in blackberry.

**Enrique Gutierrez Albanchez<sup>1</sup>**, Ignacio Horche<sup>1</sup>, Ana García-Villaraco<sup>2</sup>, José Antonio Lucas<sup>2</sup>, Beatriz Ramos-Solano<sup>2</sup>, Javier Gutierrez-Mañero<sup>2</sup>

<sup>1</sup> Microbiology, Biobab R&D, Spain

<sup>2</sup> Universidad San Pablo-CEU Universities. Facultadad de Farmacia. Ctra Boadilla del Monte Km 5.3, Boadilla del Monte 28668 Madrid, Spain.

A plant biostimulant is any substance or microorganism applied to plants with the aim of enhancing nutrition, efficiency, tolerance to abiotic and biotic stress, or improving crop quality. By extension, plant biostimulants also designate commercial products containing mixtures of such substances and/or microorganisms. Biobab R&D S.L. is currently developing effective biostimulants with strain with a solid background. The present study reports effects of 6 potential products in blackberry, a type of crop characterized by their benefits over human health derived from the secondary metabolites present in their fruits.

Experiments were conducted from November 2018 to May 2019 in production greenhouses in Huelva (Spain). Six bacterial combinations made with two bacillus and 3 different pseudomonas strains were defined, two of them with 3 strains, and four with two; treatments were compared with the coformulant (algae) and the negative control. Sixty plants conformed each treatment (20 plants per replicate), treatments were root inoculated once a month (5 applications from transplant to the end of the production period). At flowering, photosynthesis (fluorescence of light reactions and carbon fixation) and leaf photosynthetic pigments were measured. Total phenols, flavonols, and anthocyanins, as well as, nutritional properties of fruits were measured at the peak of production. Yield was recorded along the whole cycle.

Production was significantly increased by 3 bacterial combinations (treatments 4, 5, and 6), and only two (4, 5) of them anticipated fruiting (precocity), an excellent asset for producers, as fruit reaches the market earlier. From these, treatments 5 and 6 significantly increase net carbon fixation and transpiration, while treatment 5, a combination of 3 strains, is the only one able to also increase chlorophylls and carotenes, supporting the active input of energy necessary for carbon fixation and enhanced yield. None of the treatments were able to improve fruit quality, either nutritional or bioactives. Coformulants did not improve any parameter against controls, which demonstrates that results obtained depend exclusively on microorganisms. Treatments performed differently depending on the bacterial combination, so effects depend not only on the effective strains but are improved by certain combinations that are strain specific.

### PP1-SA-02 Improving field inoculations with arbuscular mycorrhizal fungi

Natacha Bodenhausen<sup>1</sup>, Julia Hess<sup>2</sup>, Klaus Schlaeppi<sup>3</sup>, Marcel van der Heijden<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

<sup>3</sup> Institute of Plant Sciences, University of Bern, Switzerland

Arbuscular mycorrhizal fungi (AMF) form a symbiosis with 80% of plant species, where they receive carbohydrates in exchange for nutrients, in particular phosphate and nitrogen. In addition, AMF reduce nutrient leaching and nitrous oxide emission; therefore, inoculation with AMF can improves soil nutrient cycles and could participate in a more sustainable agriculture. However, field-inoculation with AMF is highly context dependent. Soil fertility, and in particular, N and P fertilization, play an important role. In this project, we performed on farm inoculation experiments in 18 fields with a diverse range of soil properties. Upon maize seeding, the AMF *Rhizoglomus irregulare* or a non-mycorrhizal control inoculum (carrier substrate) were inoculated. Fertilization was applied manually with all plots receiving standard levels of N and K, but only half of the plots receiving P. We confirmed that AMF inoculation success, as measured by maize biomass, was highly context dependent with enhanced yields being found in 1/3 of the fields. There is no evidence for an interaction effect of AMF inoculation and P fertilizer, suggesting that in the tested agricultural soils, fertilized over many years, phosphate levels were adequate for plant growth. We are currently modeling the relationships between soil properties and AMF establishment for a better understanding of the context dependency of successful AMF inoculations.



# PP1-SA-03 Correlation of the bacterial microbiome, genotypic variance and alkannin/shikonin content of wild *Echium vulgare* L., a plant with potential medicinal properties

**Cintia Csorba<sup>1</sup>**, Nebojsa Rodić<sup>2</sup>, Günter Brader<sup>1</sup>, Muhammad Ahmad<sup>1</sup>, Livio Antonielli<sup>1</sup>, Eva M. Sehr<sup>1</sup>, Andreana Assimopoulou<sup>2</sup>, Angela Sessitsch<sup>1</sup>

<sup>1</sup> Health and Bioresources, Austrian Institute of Technology, Austria

<sup>2</sup> Aristotle University of Thessaloniki (AUTh), School of Chemical Engineering, Thessaloniki, Greece

Several members of the plant family Boraginaceae are confirmed producers of the secondary metabolites alkannin and shikonin. These compounds bear several biological activities such as anti-inflammatory or antibacterial effects or acceleration of wound-healing. *Echium vulgare* L., a common Boraginaceae species native to Europe, produces alkannins, which are mainly found in the periderm of the root system. As microorganisms have been reported to interact with secondary metabolism of some plants, our aim was to see whether there is a potential link between plant microbiota and secondary metabolite production in *E. vulgare*.

We collected plants at two different growth stages of wild *E. vulgare* at six different sites in Austria to correlate bacterial community patterns, genotypic variance and the production of secondary metabolites. We analysed microbial community composition in different sections of the root system, in the surrounding rhizosphere and bulk soil by next generation sequencing of 16S rRNA genes. Furthermore, we genotyped 64 individuals of E. vulgare using 12 microsatellite markers and determined total alkannin/shikonin content of dried root samples by ultra-high-performance liquid chromatography-high-resolution mass spectrometry. The high variance of alkannin/shikonin content in the collected *E. vulgare* roots in our study suggests that factors like the genotypic variance or associated microbiota may influence secondary metabolite production. According to our analysis results we will discuss how microbiota composition and plant population genetic differences correlate with alkannin/shikonin production and which microorganisms might play a role in influencing secondary metabolite production. This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 721635.

# PP1-SA-04 Nitrogen-fixation and plant-growth promoting features of *Gluconacetobacter diazotrophicus* on tomato plants

Michele Pallucchini<sup>1</sup>, Martina Franchini<sup>1</sup>, Nathalie Narraidoo<sup>2</sup>, Phil Hill<sup>3</sup>, Michele Perazzolli<sup>4</sup>, Rupert Fray<sup>3</sup>

<sup>1</sup> University of Nottingham, United Kingdom

The endophyte *Gluconacetobacter diazotrophicus* (Gd) is a nitrogen-fixing, plant growth-promoting bacterium (PGPB) originally isolated from sugarcane. Gd colonises plant tissues and establishes close interactions within the inter/intracellular spaces. In contrast to rhizobia, Gd can fix nitrogen under aerobic conditions and establish nitrogen-fixing symbiosis with a wide range of crops.

In this study, the Gd strain AZ0019 was tested in tomato plants grown under hydroponic conditions. The use of fluorescent Gd tagged strains allowed to confirm the colonisation in plant tissues and to detect the plant-bacterial interaction during different colonisation stages. The RNA was extracted from roots at different time points in order to correlate the tomato growth promotion with the modulation of Gd gene markers of colonisation and nitrogen fixation activity.

These data allowed to build a model of Gd mode of action *in planta* throughout different colonisation stages in tomato. Integrating the plant-bacterial interaction imaging with the gene expression will provide a better understanding on how Gd establishes a functional symbiosis and positively affects the plant growth.



 $<sup>^{\</sup>rm 2}$  Azotic Technologies Ltd., Pennyfoot St, Biocity, Nottingham NG1 1GF, UK

<sup>&</sup>lt;sup>3</sup> University of Nottingham, Sutton Bonington, Loughborough LE12 5RD, UK

<sup>&</sup>lt;sup>4</sup> Fondazione Edmund Mach, Via Edmund Mach, San Michele All'adige, Province of Trento

#### PP1-SA-05 Action against Alternaria: does Fusarium become ally of tomato plants?

#### Andreea Cosoveanu<sup>1</sup>, Samuel Rodriguez Sabina<sup>2</sup>, Raimundo Cabrera<sup>2</sup>

<sup>1</sup> Laboratory For Useful Organisms, Research - Development Institute For Plant Protection, Romania

<sup>2</sup> Facultad de Ciencias—Sección Biología, Dept. Botanica, Ecologia & Fisiologia Vegetal, Universidad de La Laguna, Apdo. 456, 38200 La Laguna, Spain

The study pursues the success of fungal endophytic strain HRO8 inoculation (i.e. root apparatus bath and soil application) into tomato plants and its development as innocuous to the culture plants. Biometric measurements considering plants inoculated and not inoculated with HRO8 as well as plants inoculated and not inoculated with pathogenic strain Alternaria alternata were performed. Symptoms of alternariosis (i.e. necrosis) provoked by Alternaria were evaluated. Overall appreciation: no Fusarium wilt stem symptoms were observed when cutting the fragments of the selected samples to proceed with isolation of endophyte and pathogen. No symptoms of fusariosis were observed on leaves, stems or roots during the biometric measurements. The inoculation of the pathogenic strain of Alternaria alternata was performed at two timings (i.e. 30 days and 60 days). No significant difference was observed between the two groups of plants (p > 0.5) in terms of biometric measurements, pathogen and endophyte isolation and symptoms of alternariosis. When the pathogen was inoculated, the plants in which the endophyte was also inoculated through soil application had an average of 1.1. necrotic leaflets while the plants where the endophyte was inoculated through root bath had an average of 10.4 necrotic leaflets. However, this average of 10.4 is less than half of the average value of control plants inoculated with the pathogen without the endophyte. We might assume that the method of application of the endophyte through roots bath might cause radicular damages which further manifest through plant sensitivity (i.e. in this case higher grade of symptoms caused by the fungal pathogen). These preliminary results indicate that the fungal endophyte HRO8 caused no harm to the tomato plants when directly applied to the soil and also protected the host against Alternaria alternata.

#### PP1-SA-06 Bibb lettuce response to *G. diaz* inoculation under hydroponic growth conditions.

Ryan Sebring<sup>1</sup>, Ray Bryant<sup>2</sup>, Joshua Lambert<sup>1</sup>, John Regan<sup>1</sup>, Robert Berghage<sup>1</sup>, Sjoerd Duiker<sup>1</sup>

 $^{\rm 1}$  Horticulture, The Pennsylvania State University, United States of America  $^{\rm 2}$  USDA-ARS

The objective of this study was to evaluate the effect of bacterial seed inoculation by the nitrogen fixing endophyte *Gluconacetobacter diazotrophicus* over six levels of nitrogen fertilization on root growth and yield of Lettuce. The experiment was conducted in hydroponically managed Kratky jars in a growth chamber at the USDA-ARS Agricultural Research Building on the Penn State University Park campus, from February to April 2019. Treatments consisted of six levels of nitrogen fertilization (60, 82.5, 105, 127.5, 150 and 172.5 ppm N in 800 ml soln) and two levels of inoculation (seed soaked with endophyte, seed soaked without), arranged in a 6 x 2 factorial design with three or four repetitions. The 12 treatments were assigned in a 44 jar completely randomized design, with the highest and lowest nitrogen levels having three replications each and the rest four. The nitrogen source used in the study was nitrate biased to reduce bacterial nif gene suppression by ammoniacal sources, and was supplied by calcium nitrate. Plants were harvested at 31 days post sowing. Bacterial presence significantly influenced the total plant biomass, harvestable plant yield, and root biomass. Aerial tissue production (p<0.0337). Overall, total plant production was 12.5% greater (p<0.0042) under bacterial inoculation, and yield trends exhibited a nitrogen response plateau at lower applied N levels in the presence of the endophyte.

**Key words:** *Gluconacetobacter diazotrophicus*, endophyte, Kratky, hydroponics, nitrogen, calcium nitrate, *Lactuca sativa v. Bibb.* 



# PP1-SA-07 Phytobiomes Research for Enhancing the Sustainable Producon of Food, Feed and Fiber

**The Phytobiomes Alliance**<sup>1</sup>, Gwyn A. Beattie<sup>2</sup>, Natalie W. Breakfield<sup>3</sup>, Kellye Eversole<sup>1,4</sup>, Magalie Guilhabert<sup>5</sup>, Jan Leach<sup>6</sup>, Matthew J. Ryan<sup>7</sup>, Angela Sessitsch<sup>8</sup>

<sup>1</sup> International Alliance for Phytobiomes Research, United States of America

- <sup>2</sup> Iowa State University, Ames, IA 50011, US
- <sup>3</sup> NewLeaf Symbiotics, St. Louis, MO 63132, US
- <sup>4</sup> Eversole Associates, Bethesda, MD 20816, US
- <sup>5</sup> Bayer CropScience, West Sacramento, CA 95605, US <sup>6</sup> Colorado State University, Fort Collins, CO 80523, US
- <sup>7</sup> CABI, Egham, Surrey, TW209TY, UK
- <sup>8</sup> AIT Austrian Institute of Technology, 1210 Vienna, AT

A major paradigm shift in agricultural production is required to meet the demands of a global world population projected to reach 9.7 billion in 2050. We need to sustainably increase crop productivity, while preserving biodiversity, natural resources, and grower income in the context of climate change. To optimize sustainable productivity and profitability on farms, grasslands, and forests, scientists must embrace a holistic, systems-level approach and focus on the complexity within phytobiomes. The term "phytobiome" refers to a plant growing within a specific environment, or biome, and all of the micro-and macro-organisms living in, on, or around it—such as microbes, animals, insects, and other plants—as well as the geophysical environment, which includes soil, air, water, weather, and climate. By establishing a foundation of knowledge on how phytobiomesalliance.org) a non-profit alliance of industry, academic, and governmental partners created in 2016, aims at addressing today's agricultural challenges. The Alliance facilitates and coordinates international efforts toward expanding phytobiomes research in order to accelerate the sustainable production of food, feed, and fiber for food security.

Current priority areas of the Alliance include filling the gaps in our knowledge of how microbes interact with other phytobiome components in outdoor and controlled environments as well as building a regulatory science foundation to support rapid commercialization of sustainable, microbial based products that increase the productivity and viability of agricultural production systems.

# PP1-SA-08 The Multikraft technology & the ability of multi-microbe products to degrade pesticides

#### Katharina Kraxberger

University of Natural Resources and Life Sciences/Multikraft Produktions- und HandelsgmbH, Austria

Microorganism based products make an active contribution to climate and environmental protection for the sake of future generations, which forms the company's central philosophy. The Company works with nature as a role model, promotes it's regeneration and supports natural processes. For the intense research on multi-microbe products Multikraft hosts a modern microbiology laboratory, which includes convential and real-time PCR machines. Close collaborations with international partners such as Barworth Research Ltd in Lincolnshire, SASA (Science and Advice for Scottish Agriculture) in Edingburgh and national partners such as the University of Natural Resources and Life Sciences in Vienna exist. Multikraft is committed to developing and producing its multi-microbe products based on the latest scientific research. To name an example, the main product in the gardening segment for watering and spraying is EM Active, a soil additive containing amongst others *Lactobacillus casei*, *Lactobacillus plantarum*, *Rhodopseudomonas palustris*, and *Saccharomyces cerevisae*.

Due the fact that bacteria and fungi have been proven very powerful in degrading chemicals introduced into the environment multikraft performs intensive research on pesticide degrading bacteria. A study performed in 2016 (Mr. Frank Korting, State Education and Research Center of Viticulture, Horticulture and Rural Development, Rheinland-Pfalz, Germany) showed that by the use of multi-microbe products, accelerated degradation of many active ingredients occur. Actually, there is a project (supported by FFG) which aims at finding pesticide degrading strains and integrating them in the production process to explore if they can survive and multiply within the system. Also, the genomic basis underlying pesticide degradation should be explored within this "Multikraft degraders" project.



### PP1-SA-09 Microbe-assisted vegetation cover to reduce erosion in alpine environments – concept and first results

Lisa-Maria Ohler<sup>1</sup>, Sabine Kraushaar<sup>2</sup>, Stefan Haselberger<sup>2</sup>, Jan-Christoph Otto<sup>3</sup>, Robert R. Junker<sup>1</sup>

<sup>1</sup> Department of Biosciences, University of Salzburg, Austria

<sup>2</sup> University of Vienna, Department of Geography and Regional Research

<sup>3</sup> University of Salzburg, Department of Geography and Geology

Studies on crop species demonstrated the growth-promoting abilities of microbes and also their ability to alter plant traits. We adopted this approach in order to exploit beneficial plant-microbe interactions to reduce sediment remobilisation. Glaciers are facing ongoing and fast retreat due to global warming. The receding ice leaves unvegetated surfaces covered by unconsolidated deposits of sediment, so-called moraines. Sediments remobilised during extreme precipitation and flooding events may have negative effects on natural and anthropogenic structures downstream. It has been shown that high vegetation cover serves as effective protection against erosion, which is also supported by our findings. Apart from cover, our results indicate that plant communities with higher community weighted means in specific functional traits such as root mass and leaf area are more effective in slope protection than plant communities with other functional compositions. Therefore, we tested the effects of naturally occurring microbes on plant growth and trait expressions of the naturally occurring alpine plant species *Campanula barbata* (Campanulaceae) in order to enhance slope protecting abilities of this plant species. A screening of native bacteria collected in the test site identified those that significantly affected seed germination as well as functional trait characteristics in *C. barbata*. In the next steps, we will apply the microbe-assisted seed mixture to the field sites and monitor erosion from experimental plots in the Kaunertal Valley, Austria. Our results provide new insights into plant-microbe interactions in natural ecosystems with implications for a nature-based solution to reduce sediment erosion in high mountain areas.



### miCROPe 2019 - Microbe-assisted crop production opportunities, challenges & needs Vienna, Austria, December 2 – 5, 2019

Poster Session 1: Mechanisms mediating holobiont and multipartite interactions



## PP1-MI-01 Resource exchanges in leguminous-gramineous associations: impact of multitrophic associations with beneficial microbes

Raphaël Boussageon<sup>1</sup>, Leonardo Casieri<sup>1</sup>, Loïc Cusant<sup>2</sup>, Marine Forges<sup>1</sup>, Diederik van Tuinen<sup>1</sup>, Christophe Roux<sup>2</sup>, Daniel Wipf<sup>1</sup>, **Pierre-Emmanuel Courty<sup>1</sup>** 

<sup>1</sup> INRA Dijon, France

<sup>2</sup> Laboratoire de Recherche en Sciences Végétales, Université de Toulouse, UPS, CNRS, 24 Chemin de Borde Rouge-Auzeville, Castanet-Tolosan, France.

Arbuscular mycorrhizal fungi (AMF) and Nitrogen Fixing Bacteria (NFB) have gained an increasing interest as agroecosystem service providers capable of maintaining crop productivity and quality. They can affect the ecosystem productivity by (i) improving plant mineral nutrition, (ii) saving resources, and (iii) having a low environmental footprint. In agroecosystems, one of the objectives is to limit or avoid competition between cultivated plants and to favor "facilitation between plants" for a better productivity. Plant-plant facilitation refers to a "donor" plant that facilitates the growth and development of a "receivor" plant. It is essential to understand how these biotrophic interactions between plants, AMFs and NFBs are established; function, and influence plant growth. This would allow a reasoned soil resources in the context of a productive and sustainable agriculture.

Likewise the great majority of land plants, *Fabaceae* live in a symbiotic associations with AMF, connecting different plant species through common mycorrhizal networks (CMNs). They additionally form a symbiosis with NFBs. Although several studies unveiled the mechanisms of nutrients' exchange between plants and their symbionts in controlled conditions, understanding the terms of trade between the partners of CMNs and rhizobia involved in a complex environment might become experimentally challenging, but of prior importance in order to better picture the exchanges in a crop ecosystem.

To address this question, microcosms containing a pair of test plants (the C3 *Medicago truncatula*, and the C4 *Sorghum bicolor*), connected through a CMN of the AMF *Rhizophagus irregularis* or *Funneliformis mosseae*, with and without the rhizobial interaction with *Sinorhizobium meliloti* (on the leguminous plant), were used.

The differences in <sup>13</sup>C/<sup>12</sup>C isotope compositions of the photosynthates from the two plants and the administration of <sup>15</sup>N to the microcosm, coupled with RNAseq analysis, allowed us to assess the carbon and N exchanges between symbionts. More specifically we addressed two main questions: i) which is the specific C investments of the two plans species in the AM interaction when cultivated in monoculture (Sorghum-Sorghum and Medicago-Medicago) or mixed culture conditions (Medicago-Sorghum), and ii) how the C/N exchanges in the system might be modified/disrupted when a supplementary symbiotic partner, improving the N nutrition, was introduced.

### PP1-MI-02 Complexity of microbe-plant interactions

#### Kari Saikkonen

Biodiversity Unit, University of Turku, Finland

Microbes have driven eco-evolutionary adaptations of plants from the origin of life. Here I propose that to understand the importance of microbe-plant interactions to ecosystem functions and services requires understanding microbial versatility, and plants and their associated microbiome should be regarded as co-evolving ecosystems, holobionts. I contend that human perspective and conventional disciplines of life sciences might hamper and/or distract understanding the nature of microbe-plant interactions. For example, research on different microbial taxa and the related scientific disciplines have largely developed separately, and comprehensive community-level studies on bacterial and fungal interactions are lacking. Here, I use *Epichloë* species as examples to demonstrate that plant-microbe interactions are labile ranging from mutualistic to antagonistic, and how these keystone microbial species can modulate microbiota of their shared host plant. I suggest that the next step toward a better understanding of the microbe-plant interactions requires multidisciplinary approaches taking into account complexity and context dependency of these interactions.



### PP1-MI-03 Chemical analysis of root exudates to study the interactions between tomato, maize and the plant-associated *Pseudomonas donghuensis* P482

Dorota Magdalena Krzyzanowska<sup>1</sup>, Magdalena Jabłońska<sup>1</sup>, Małgorzata Czerwicka<sup>2</sup>, Zbigniew Kaczyński<sup>2</sup>, Sylwia Jafra<sup>1</sup>

<sup>1</sup> Laboratory of Biological Plant Protection, Intercollegiate Faculty of Biotechnology UG and GUMed, Poland

<sup>2</sup> Laboratory of Mass Spectrometry, Faculty of Chemistry, University of Gdansk, Wita Stwosza 63, 80-308 Gdańsk, Poland

Plant rhizosphere is inhabited by numerous microorganisms. Plants drive this abundance by secreting a blend of chemical compounds into their root zone. This provides microbes with nutrients the composition of which has a profound effect on the shape and the physiological activity of the microbial community. The profile of compounds present in the plant root exudates depends on many factors. Among other, it varies among different plant species. Despite more and more research is dedicated to deciphering the sophisticated interaction between plant-associated bacteria and their hosts, most studies do not go beyond testing a single bacterium-plant model. Therefore, it remains largely unknown how particular compounds, characteristic for the exudates of certain plant species, are responsible for the host-specific aspects of bacterial interactions with plants.

*Pseudomonas donghuensis* P482 is a plant-associated bacterium isolated from the rhizosphere of tomato (*Solanum lycopersicum*). Apart from tomato, P482 is also able to colonize other plant species including maize (*Zea mays*). In this study, we aimed to characterize the composition of root exudates of tomato (cv. St. Pierre) and maize (cv. Bejm) and to develop a gnotobiotic plant growth setup, applicable for both plant species, to further investigate differential response of *P. donghuensis* P482 to the compounds secreted by the two plants.

When collecting root exudates for biological assays or chemical analysis, one must take into account the bias introduced by a particular experimental setup. Although the composition of root exudates of tomato and maize has already been reported, the results cannot be easily extrapolated for different culture conditions. Here, tomato and maize were grown for 18 days in sterile conditions from surface-sterilized seeds. The procedure included seed germination on media plates with a composition allowing easy screening of seedlings for potential microbial contamination. Seedlings were transferred to sterile vessels containing ½ or ¼ Hoagland's medium, for tomato and maize, respectively, and sterile gravel as support. For chemical analysis, compounds secreted by 18-days-old plants were harvested into ultrapure water, lyophilized, and their composition was determined by GC-MS and NMR, revealing a set of plant-specific compounds.

# PP1-MI-04 Competitive traits of foliar yeasts and their effect on *Psuedomonas syringae* inhibition in tomato

#### Mason Chock, Isabella Muscettola, Britt Koskella

Botany, University of Hawaii, United States of America

Fungi living within and on leaf surfaces play an integral role in plant functional processes including resistance to pathogens. The microbial interactions occurring at these interfaces often mediate pathogen protection or facilitation within the plant host. Foliar yeasts are a particular group of unicellular fungi that have been extensively studied for their antagonistic nature towards foliar pathogens. Less studied, however, is the way these yeasts associate with other microbes and persist in the phyllosphere. This knowledge is integral to assessing their effectiveness and viability as biocontrol agents towards plant pathogens over diverse biotic and abiotic conditions. In this study, we characterize various competitive traits of foliar yeasts isolated from tomato, *Solanum lycopersicum*, leaves, including dispersal, *in vitro* antagonism (dual cultural assays), and *in vitro* secretion of volatile organic compounds (VOC). From these traits, we infer how these microbes affect leaf community assembly and ultimately resistance to the foliar pathogen *Pseudomonas syringae* within the plant host.



## PP1-MI-05 Activity of root endophytic bacteria isolated from an alkannin/shikonin producing plant: wild *Alkanna tinctoria*

**Angélique Rat**<sup>1</sup>, Nikos Krigas<sup>2</sup>, Katerina Grigoriadou<sup>2</sup>, Eleni Maloupa<sup>2</sup>, Vassilis Papageorgiou<sup>3</sup>, Andreana Assimopoulou<sup>3</sup>, Anne Willems<sup>1</sup>

<sup>1</sup> Biochemistry and Microbiology, UNIVERSITY OF GENT, Belgium

<sup>2</sup> Laboratory of conservation and evaluation of native and floricultural species, Institute of Plant Breeding and Genetic Resources, Hellenic Agricultural Organization Demeter, GR 57001, Thermi, Thessaloniki (Greece)

<sup>3</sup> cOrganic Chemistry Laboratory, School of Chemical Engineering, Aristotle University of Thessaloniki, Thessaloniki 54124 and Center of Interdisciplinary Research and Innovation of AUTh, Natural Products Research Centre of Excellence (NatPro-AUTH), The

Endophytes are defined as microorganisms colonizing the internal tissues of plants with no external sign of damage to their host. The colonization *in planta* of these organisms may influence plant secondary metabolite production. Alkannin and shikonin are enantiomeric naphthoquinones produced in the roots of *Alkanna tinctoria*, among other 150 species of the *Boraginaceae* family. These molecules have strong anti-microbial properties and are therefore a source of strong interest for researchers and pharmaceutical companies. Nevertheless, the influence the endophytic bacteria, is not known. To assess the relationship between the production of these compounds by the plant and the endophytic bacteria, the first step was to investigate the diversity of culturable endophytic bacteria in the root. Then, plant-microbes interactions were explored by assessing plant growth promoting activity of the endophytes and the antimicrobial properties of alkannin and shikonin.

Isolation of root endophytic bacteria of wild *Alkanna tinctoria* collected from regions nearby Athens and Thessaloniki, Greece, was performed. Representative strains identified by MALDI-TOF mass spectrometry were then characterized genetically: the 16S rRNA gene was amplified and partially sequenced. Two hundred and eight distinct phylotypes of endophytic bacteria were detected and the most abundant genera were *Pseudomonas, Xanthomonas, Variovorax, Bacillus, Inquilinus, Pantoea* and *Stenotrophomonas*. These genera were then tested *in vitro* for their plant growth promoting activity (phosphate solubilisation, siderophores production, IAA, ACC deaminase). Among them, *Pseudomonas, Pantoea, Bacillus* and *Inquilinus* showed interesting properties. Moreover, the interaction between alkannin and shikonin and the bacteria was investigated, especially through resistance and biodegradation assays.

#### PP1-MI-06 The methylome of plant-beneficial Stenotrophomonas and Serratia strains

#### Henry Müller, Manuel Reisinger, Gabriele Berg

Environmental Biotechnology, Graz University of Technology, Austria

Plants live in close association with microorganisms that provide beneficial functions. These plant-beneficial bacterial strains have long since been isolated by researchers and employed as inoculum to improve plant growth and health. Here, we shed light on the role of DNA methylation as a regulatory factor for host - and niche specific adaptation of plant-beneficial bacteria. We focused on phylogenetically closely related strains within the genera *Serratia* and *Stenotrophomonas*, which are already well known for their plant-growth promoting and stress protecting capabilities. Despite the high genotypic similarities of our sample strains, the colonization competence and the ability to exhibit beneficial effects *ad planta* were shown to be host-specific. Gene-by-gene comparison revealed that the present repertoire of genomic features alone cannot explain the strain-specificity of the plant-microbe interactions. Though, each strain possesses unique DNA methylases with specific recognition sites resulting in distinct DNA methylation patterns. To elucidate how methylotypes affect gene expression we analyzed the transcriptome of bacterial cultures in response to plant root exudates and nutrient composition. Our results indicate that DNA methylation contributes in controlling gene expression and may represent a significant factor accounting for host plant specificity. The results of this study will advance our understanding of the interplay between the genotype, methylotype and phenotype of plant-beneficial bacteria regarding their interaction with host plants.



# PP1-MI-07 Differential impact of winter on endophytic bacterial and fungal communities of same boreal host plants

Suni Anie Mathew<sup>1</sup>, Cindy Given<sup>2</sup>, Kati Heikkilä-Huhta<sup>3</sup>, Riitta Nissinen<sup>2</sup>

<sup>1</sup> Biodiversity Unit, University of Turku, Finland

- <sup>2</sup> Department of Biological and Environmental Science, University of Jyväskylä, FINLAND
- <sup>3</sup> Oulu Steiner School, Oulu, Finland

Long winters with little solar radiation, low temperatures and snow cover have strongly shaped arctic and subarctic vegetation, and the ability to survive cold winters largely determines plant climatic distribution. Arctic and subarctic plants have evolved various physiological and anatomical modifications to survive the cold winters. Virtually nothing is known about winter microbiomes in cold climate plants, although plant associated microbes, including microorganisms such as bacteria and fungi are known to contribute significantly to plant survival, growth and protection from environmental stresses. In this context, we studied the composition of endophytic bacterial and fungal communities in the key plant species of boreal biome - *Pinus sylvestris Picea abies, Vaccinium vitis-idaea* and *Vaccinium myrtillis* - in summer and winter at nine different geographical locations across Finland, with aim to detect putative seasonal fluctuation in the microbiomes. The project was conducted as a citizen science project in collaboration with nine high schools.

Using 16S rRNA gene (bacteria) and ribosomal ITS (fungi) targeted sequencing and sequence analysis, we analyzed the influence of various factors on the composition of bacterial and fungal communities. The bacterial community constituted of 421 OTUs assigned to 111 families. The fungal community constituted 382 OTUs categorised into 67 families. The permutational multiple analysis of variance (PERMANOVA) based on Bray-Curtis dissimilarity matrix of data showed that among the three factors – season, plant and location - plant species had the largest impact on the bacterial communities were evident from divergent abundances of several OTUs from bacterial genera *Sphingomonas, Pseudomonas* and *Granulicella* in all four plants. These season-specific bacterial OTUs were closely related to bacteria from other cold climate plants.

In contrast, fungal communities were mainly shaped by plant species and sampling site, and to clearly lesser extent, season. Further studies on the functions of season specific microbes are needed to unravel their role in plant seasonal fitness.

# PP1-MI-08 Metabolomics and machine learning techniques applied to investigate beneficial plant-bacteria interactions

Lena Fragner<sup>1</sup>, Florian Schindler<sup>1</sup>, Johannes Herpell<sup>1</sup>, Weimin Li<sup>1</sup>, Xiaoliang Sun<sup>2</sup>, Anke Bellaire<sup>3</sup>, Wolfram Weckwerth<sup>1</sup>

- <sup>1</sup> Dep.of Ecogenomics and Systems Biology, University of Vienna, Austria
- <sup>2</sup> Vienna Metabolomics Center, University of Vienna, Vienna, Austria

<sup>3</sup> Department of Structural Botany, University of Vienna, Vienna, Austria

Endophytic non-pathogenic colonization of plant tissue by bacteria is a well-known and wide spread phenomenon, even expected to be the case for all angiosperms. Symbiotic plant-bacteria interactions comprise various levels of obligations and ecological benefits to at least one of the partners. The beneficial effects and functions for plants are manifold, including enhanced stress resistance, plant growth promotion or capacity for controlling plant-pathogens. In the present work, we focus on obligatory and constant symbioses occurring in the plant families *Rubiaceae, Primulaceae* and *Dioscoreaceae*. Highly specialized bacterial symbionts are mainly host-specific, often not cultivable and their absence can lead to dwarf phenotypes of the host-plants. Endophytic bacteria of leaves can be evenly distributed between the mesophyll cells or accumulated in specific leaf areas or in specialized structures.

Most studies focused on genome and proteome analyses suggesting potential alterations of secondary metabolism caused by the presence of beneficial symbionts. However, detailed mechanisms and functions of these highly specialized mutualistic plant-bacteria symbioses are not yet fully understood.

In the present study we investigate alterations in the metabolome of colonized leaf tissue. Primary and secondary metabolites were analyzed by GC-MS and LC-MS respectively, complemented with physiological and morphological data, and analyzed with machine learning techniques. Results indicate distinctive mechanisms of the symbiosis in investigated beneficial plant-bacteria interactions and will be discussed in detail.



# PP1-MI-09 Road to reveal the genetic factors of the plant-probiotic rhizobacterium TRM1 contributing to bacterial wilt resistance in tomato

Boyoung Lee<sup>1</sup>, Hyein Park<sup>1</sup>, Min-Jung Kwak<sup>2</sup>, Soon-Kyeong Kwon<sup>3</sup>, Ju Yeon Song<sup>1</sup>, Jihyun F. Kim<sup>1</sup>

<sup>1</sup> Systems Biology, Yonsei University, Korea, Republic of

<sup>1</sup>Department of Systems Biology, Division of Life Sciences, and Institute for Life Science and Biotechnology, Yonsei University, 50 Yonsei-ro, Seodaemun-gu Seoul 03722, Republic of Korea; <sup>2</sup>ChunLab Inc., JW TOWER, 2477, Nambusunhwan-ro, Seocho-gu, Seoul 06725, Republic of Korea; <sup>3</sup>Division of Life Science, Gyeongsang National University, 501 Jinju-daero, Jinju, Gyeongsangnam-do 52828, Republic of Korea.

Tomato is one of the most in-demand vegetables in the world, and bacterial wilt caused by the soil-borne pathogen *Ralstonia solanacearum* is a devastating lethal disease in solanaceous crops. As reported in 2018, a plant-probiotic flavobacterium TRM1 was found to be enriched in the rhizosphere microbiome of the bacterial wilt-resistant tomato variety Hawaii 7996 relative to the susceptible cultivar Moneymaker and also be able to suppress disease development in the susceptible plant. Although TRM1 was shown to reduce bacterial wilt in tomato and its functions were deduced from the genome sequence information, which genetic factors of this plant probiotic are responsible for disease suppression or how the factors affect the plant host or the wilt pathogen remains elusive. As the first attempt to investigate how TRM1 could endow the tomato plant with disease resistance, we examined if TRM1-10 affects the growth of *R. solanacearum* SL341 by an *in vitro* co-cultivation method. The growth of SL341 was inhibited by TRM1-10, while that of TRM1-10 was not affected. This result suggested that inhibiting the growth of the pathogen by TRM1 might be one way of reducing plant disease. For the next step to figure out the genetic factors of TRM1 involved in reducing the tomato wilt disease, we employed transposon mutagenesis and massive sequencing (Tn-seq) expecting to disclose genes contributing to the molecular interactions between the plant-probiotic microbe and the wilt pathogen and also between the plant probiotic and the tomato plant in the rhizosphere, and the findings will be presented.

# PP1-MI-10 Live-Exudation Assisted Phytobiome Cultromics System (LEAP-CS) to characterize physiological, molecular and metabolic phenotypes

**Shruti Pavagadhi**, Gourvendu Saxena, Mohd Firdaus Abdul-Wahab, Miko Poh Ho Ching, Yoon Ting Yeap, Sanjay Swarup

#### National University of Singapore, Singapore

Healthy plants are associated with a rich diversity of microbes forming complex microbial consortia that impact their growth and productivity. These plant-associated microbiomes or phtyobiomes confer a multitude of benefits to their hosts, developing and engineering them can aid in cultivating climate-resilient, nutrient-efficient and sustainable food crops. In that view, characterizing model rhizosphere phytobiomes, which occupy the niche developed by the gradients of root exudates in the rhizosphere region is of particular interest due to its probable direct role in providing specific factors for plant growth and resilience. However, the lack of a model rhizosphere phytobiomes and its associated metabolic exchanges with the host has restricted the much needed mechanistic understanding of plant-microbe interactions in the rhizosphere. To this end, we have developed a novel Live-Exudation Assisted Phytobiome-Cultromics System (LEAP-CS) which supports development of complex phytobiomes and is suitable for characterizing live host and microbial phenotypes. The foundation of LEAP-CS rests on natural ecological processes underlying the complex and dynamic phytobiome relationships. Modularity of LEAP-CS supports (i) easy manipulation of the phytobome communities; (ii) chemical complementation assays; (iii) different biological and analytical platforms for phenotyping; and (iv) live, non-invasive plant growth profiling. Our system captures (i) plant-microbe and microbe-microbe interactions; (ii) metabolic exchanges and crosstalk; (iii) microbial community shifts through simultaneous multi-omics analyses at different levels for integrated biological outputs. Consequently, LEAP-CS can be utilized for both mechanistic and translational studies involving plant-microbial interactions and can be used a tool for developing synthetic phytobiomes and consortia for agricultural applications. Integrative omics data from LEAP-CS system for model species (Arabidopsis thaliana) will be presented and shared in this paper.



<sup>&</sup>lt;sup>2</sup> ChunLab Inc., JW TOWER, 2477, Nambusunhwan-ro, Seocho-gu, Seoul 06725, Republic of Korea

<sup>&</sup>lt;sup>3</sup> 3Division of Life Science, Gyeongsang National University, 501 Jinju-daero, Jinju, Gyeongsangnam-do 52828, Republic of Korea

# PP1-MI-11 Mining the bacterial inducers for plant defense and shikonin production in plants: an *in-silico* guided approach.

#### Henry Naranjo, Anne Willems

Biochemistry and Microbiology, Ghent University, Belgium

Several species from the Boraginaceae plant family are used since ancient times for their medicinal properties due to the presence of secondary metabolites such as shikonin (produced in the roots of the plant). Different induction systems for the production of shikonin in plants have been described and the metabolic pathway partially elucidated. However, the role of shikonin in nature has not been fully understood but it is suggested to be part of the plant defense against pathogens and some abiotic factors.

Different plant defense elicitors known as Microbe Associated Molecular Patterns (MAMPs) and some plant endogenous molecules referred as Damage Associated Molecular Patterns (DAMPs) are described in relation to a wide diversity of microorganisms associated with plants. MAMPs and DAMPs are not only related to pathogenic bacteria but also to non-pathogenic symbionts like endophytes.

In the present study we compared the genomes of several endophytic bacteria isolated from the roots of *Alkanna tinctoria* growing in wild conditions in order to mine for MAMPs or DAMPs related to bacteria that could be responsible for the plant defense and shikonin induction.

Based on the genomic comparison, well described MAMPs like flagellin (flg22) and EF-Tu factor are evenly present in the bacterial genomes. Besides, type II, IV and VI secretion systems are also represented in many isolates. Enzymes related with the degradation of pectins from plant cell wall (CAZy PL1, PL3, PL4, PL9, GH28, CE12), that potentially generate DAMP-like molecules known as oligogalacturonides are less represented among the genomes but found to be enriched in some bacterial groups like Chitinophagales, Burkholderiales, Sphingobacteriales and Pseudomonadales. Oligogalacturonides were previously recognized to induce shikonin production in the Boraginaceae plant *Lithospermum erythrorhizon*. In the future, the significance of the bacteria predicted to degrade pectins and other complex polysaccharides from plant origin will be tested *in-vitro* and *in-planta* for induction of shikonin production.



### miCROPe 2019 - Microbe-assisted crop production opportunities, challenges & needs Vienna, Austria, December 2 – 5, 2019

Poster Session 1: Plant understanding of interactions with beneficial microbes



# PP1-PU-01 Metabolomics of the leaf nodulated plant *Ardisia crenata* reveals novel putative cyclic-depsipeptides

**Florian Schindler**<sup>1</sup>, Lena Fragner<sup>1</sup>, Johannes Herpell<sup>1</sup>, Anke Bellaire<sup>1</sup>, Martin Brenner<sup>1</sup>, Sonja Tischler<sup>1</sup>, Andreas Berger<sup>2</sup>, Johann Schinnerl<sup>2</sup>, Lothar Brecker<sup>3</sup>, Xiaoliang Sun<sup>1</sup>, Wolfram Weckwerth<sup>1</sup>

<sup>1</sup> Molecular Systems Biology, University of Vienna, Austria

<sup>2</sup> Department of Botany and Biodiversity Research, University of Vienna, Vienna, Austria

<sup>3</sup> Department of Organic Chemistry, University of Vienna, Vienna, Austria

**Introduction** Ardisia crenata belongs to the ethno botanically used genus Ardisia in the family Primulaceae, which is rich in biologically active substances with great chemodiversity. The species also lives in obligate leaf endosymbiosis, wherein the plant harbours their bacterial endosymbionts in specialised glands at the leaf margin, referred to as leaf nodules. Expression of the nonribosomal peptid synthetase (frs) gene cluster in *Escherichia coli* leads to the synthesis of the cyclic-depsipeptide FR900359, which displays strong and selective inhibition of Gq proteins. This makes it not only a promising drug candidate but also an interesting lead structure for the development of novel pharmaceuticals. A non-targeted metabolomics approach shows substantial differences in secondary metabolite profiles of nodulated and non-nodulated tissues. Examination of MS data revealed novel putative derivatives of FR900359.

**Methods** Methanolic extracts of nodulated and non-nodulated leaf tissues of *A. crenata* leaves of different developmental stages are analysed by a metabolomics method tailored for secondary metabolites. The sample-components are separated by RP-HPLC and analysed by HRESIMS. The Dataset obtained is processed by the in-house software tool mzFun and subjected to multivariate statistical analysis (PCA) (ANOVA). MS2 fragmentation patterns are analysed to gain structural information of the m/z features.

**Results and Discussion** In the PCA a clear clustering of samples belonging to the same condition occurs, as well as a distinct separation between the clusters. A significant separation of old nodulated tissue from other tissues was found on principal component 1. The m/z features, which strongly contribute to this separation exhibit very similar MS2 fragmentation patterns. Besides FR900359, AC-1 and AC-SC, as well as other, previously reported putative cyclic-depsipeptides, 3 novel m/z features with similar fragmentation patterns were found. Due to the promising pharmaceutical effects of FR900359, ongoing work focuses on the isolation and structural elucidation of the novel putative cyclic-depsipeptides.

#### **Innovative aspects**

- New insights into the bacterial leaf nodule endosymbiosis of A. crenata on a metabolic level
- Discovery of novel putative cyclic-depsipeptides
- Convenient *m*/*z* feature extraction and annotation of LC MS/MS data by new software tool mzFun

### PP1-PU-02 Understanding plant glycan interactions with beneficial microbes in the rhizosphere

Catherine Jones<sup>0</sup>, Lionel Dupuy<sup>1</sup>, Nicola Holden<sup>1</sup>, Paul Knox<sup>2</sup>, William Willats<sup>3</sup>

<sup>1</sup> James Hutton Institute

<sup>2</sup> Leeds University

- <sup>3</sup> Newcastle University
- <sup>0</sup> Newcastle University, United Kingdom

Plant roots deposit structurally diverse polysaccharides into the surrounding rhizosphere via sloughed off border and epidermal cells and in the form of root exudates. Evidence from other terrestrial and marine ecosystems has shown that bacteria produce large numbers of carbohydrate active enzymes (CAZymes) with roles in metabolising polysaccharide substrates and thereby accessing energy and glycan building blocks for reprocessing. It has been shown in mammalian gut and algal-based ecosystems that complexity and diversity of polysaccharides drives evolution of diverse microbes with specialised CAZymes, but these processes are poorly understood in the rhizosphere and soil systems. We have developed a transparent soil system for *in-situ* live imaging of roots, polysaccharides and microbes. Our initial data is revealing complex multiscale patterning of polysaccharides in the rhizosphere and microbial responses to this. A deeper understanding of microbial utilisation of root polysaccharides could yield valuable insights into how we can manage a healthy, diverse microbial population to enhance plant production.



#### PP1-PU-03 Priming for enhanced defense in Hordeum vulgare

**Karolin Pohl<sup>1</sup>**, Torsten Will<sup>2</sup>, Nina Bziuk<sup>1</sup>, Desirée Lauterbach<sup>1</sup>, Gwendolin Wehner<sup>2</sup>, Johannes Krumwiede<sup>1</sup>, Maja Grimm<sup>1</sup>, Christine Hoppe<sup>2</sup>, Andrea Braun-Kiewnick<sup>1</sup>, Frank Ordon<sup>2</sup>, Kornelia Smalla<sup>1</sup>, Adam Schikora<sup>1</sup>

<sup>1</sup> Institute for Epidemiology and Pathogen Diagnostics, Julius Kühn-Institut (JKI), Germany

<sup>2</sup> Julius Kühn-Institut (JKI), Institute for Resistance Research and Stress Tolerance, Erwin-Baur-Str. 27, 06484 Quedlinburg, Germany

Plants can be exposed to one or several stresses, for example harsh environmental conditions and pathogens. Therefore, effective defense strategies are essential. *Priming* can enhance the natural plant resistance, it enables the plant to response faster and stronger upon challenge. Thus, a primed plant has a fitness benefit if compared to a naïve plant.

One of the inducers of the primed state in plants is the *N*-acyl homoserine lactone oxo-C14-HSL, which is naturally produced by the bacterium *Ensifer meliloti* as a quorum sensing molecule. The knowledge of priming as a part of induced resistance is currently mainly based on the model plant *Arabidopsis thaliana*. In this project we aim to expand the knowledge to crop plants and focused on barley (*Hordeum vulgare*) as one of the most important crop plants worldwide.

In different experimental settings we investigated the biology of priming and genetically-based differences in the priming capacity in a set of 7 diverse barley accessions. This set comprises two reference cultivars (Golden Promise and Morex) and five genetically distant cultivars from the spring barley GENOBAR collection (BCC768, BCC1589, BCC1415, BCC436 and HOR7985). We analyzed gene expression patterns in hydroponic system *via* MACE (massive analysis of cDNA ends) technique and real-time quantitative PCR of primed and unprimed barley before and three days after a challenge with chitin, which mimicked a fungal pathogen. In this context, we aimed to find new priming responsive marker genes and intend to gather new insights in the physiology of priming. Furthermore, we performed a field trial with the barley 7'set and *Ensifer meliloti* as a priming agent. We assessed the infected leaf area, did a qualitative scoring including leaf rusts, powdery mildews and aphids and determined the biomass and yield for primed and unprimed plants. Primed Golden Promise showed a reduced infected leaf area including reduction in chlorosis symptoms and aphid infestation while necrosis was slightly increased. In addition, primed and unprimed barley was challenged with aphids in greenhouse experiments. First results indicate a reduction of aphid biomass and less leaf damage for primed Morex.

In the future our results should help to understand the potential and biological background of priming, in order to improve resistance of barley and other economically relevant cereals and to identify promising breeding targets.

# PP1-PU-04 A beech mushroom-derived volatile, Linalool primes plant immunity against bacterial and insect pathogens

#### Jin-Soo Son, Soo-Yeong Lee, Yu-Xi He, Sa-Youl Ghim

School of Life Sciences, Kyungpook National University, Korea, Republic of

Plant are vulnerable to various biotic and abiotic stresses because they cannot move. Plants overcome these shortcomings by interacting with other living things around them. Specially, plant-associated microorganisms are able to promote plant health by airborne or underground signals. A beech mushroom is an edible mushroom that has been widely cultivated in East Asia for unique flavor and beneficial effects for human health. However, in nature, these mushroom are often founded on woods. In this study, we examined with whether the beech mushroom has positive interactions with plants. The wild type *H. marmoreus* Hm 3-10 was isolated from Duk-yu Mountain in Korea. *In vitro* I-plate system, tobacco exposed to fungal volatile blends from Hm 3-10 were significantly reduced disease severity compared with tobacco not exposed to fungal volatiles. Volatile organic compounds (VOCs) produced by Hm 3-10 were extracted and identified using gas chromatography mass spectrometry with solid phase micro-extraction. Total 15 volatile organic compounds (VOCs) was detected and composed of alcohols, aldehydes, and terpenes. Among these VOCs, linalool suppressed disease symptom by inducing jasmonate signaling in tobacco and pepper. Furthermore, It has been found that insects are less accessible to plants treated with linalool. This is first reported that plant-associated beach mushroom primed plant immunities by airborne signaling.



## PP1-PU-05 Studies of root traits that support rhizobacterial mediated growth stimulation and stress tolerance of oil crops

Kanita Orozovic<sup>2</sup>, Johannes Klint<sup>2</sup>, Philipp Rohmann<sup>2</sup>, Niklas Zeiner<sup>2</sup>, Thomas Moritz<sup>1</sup>, Johan Meijer<sup>2</sup>

<sup>1</sup> Department of Forest Genetics and Plant Physiology, Umeå Plant Science Centre, Swedish University of Agricultural Sciences, SE-901 87 Umeå, Sweden

<sup>2</sup> Plant Biology, Biocenter, Linnean Center for Plant Biology, Swedish University of Agricultural Sciences, Sweden

The aim of the study is to identify factors that favor root colonization of Plant Growth Promoting Rhizobacteria (PGPR) that result in improved growth and stress management of *Brassica* oil crops.

Use of PGPR is a promising tool to support more sustainable crop production. In addition to promote growth, many PGPR also prime abiotic stress tolerance and induced systemic resistance to pathogens. The PGPR *Bacillus velezensis* UCMB5113 has been shown to support plants such as oilseed rape (*Brassica napus*), wheat (*Triticum aestivum*) and *Arabidopsis thaliana*. Growth stimulation, improved tolerance to drought, cold and heat stress as well as protection to some pathogens have been shown. UCMB5113 produces phytohormones, lipopeptides and volatile compounds among other substances than the plant benefit from. However, information is poor in general with respect to host plant genotype variation and PGPR efficacy. Such knowledge is important if a PGPR is to be used successfully in agricultural practice.

For that reason we have initiated a screening of several commercial oilseed rape (*Brassica napus*) and rapeseed (*Brassica rapa*) winter and spring cultivars treated with UCMB5113 to study effects on growth, drought and cold tolerance. Sterilized seeds have been treated with different concentrations of *Bacillus* spores and cultivated in standard soil in controlled environment. Drought or freezing stress challenges are given to young plants being more sensitive and mimic cultivation conditions prevalent in middle Sweden. In addition, effects on root system architecture and UCMB5113 colonization efficacy will be analyzed as well as metabolite composition of root exudates. Finally root cell wall composition will be analysed. Using analysis of several variables we expect to find factors that can explain variation in response observed in preliminary experiments conducted in green house. Some results from the ongoing study will be presented.

Beneficial bacteria have great potential in agriculture to replace many chemicals currently used. Since PGPR effects are based on interaction between two very different organisms there will obviously be constraints to develop a successful interaction. Accordingly, identification of factors needed for establishment of beneficial plant-microbe interactions are needed to improve efficacy when applied in crop production. Such knowledge could also be used in breeding to generate plants that maximize the PGPR interaction potential.

### PP1-PU-06 Exploiting a mutualist toolkit: using effectors to activate beneficial plant pathways

Jemma Roberts<sup>0</sup>, Silke Lehmann<sup>0</sup>, Rory Osborne<sup>0</sup>, Julien Venail<sup>0</sup>, Thomas Nussbaumer<sup>1</sup>, Pascal Falter-Braun<sup>1</sup>, Patrick Schäfer<sup>0</sup>

<sup>1</sup> Institute of Network Biology, Helmholtz Zentrum Munich

<sup>o</sup> School of Life Sciences, University of Warwick, United Kingdom

The fungal symbiont *Serendipita indica* has shown to confer improved growth and stress resilience to a broad range of host plants, including important crops such as rice, wheat and barley. *S. indica* secretes specific proteins (termed 'effectors') to aid in plant root colonisation. Interestingly, effectors specific to *S.indica* can confer increased stress resilience in crops by targeting and thereby modifying, plant signaling in a highly specific manner. Determining the functions of *S.indica* effectors in increasing crop health, could aid in the development of crops with improved stress resilience and a reduction in the application of agro-chemicals in the future. We have shown increased growth and stress resistance in stably transformed *Arabidopsis thaliana* expressing individual *S. indica* effectors. By employing a high throughput screen of promoter activity, we have also determined how these effectors mediate specific hormonal and immune signaling. Yeast two hybrid screens have shown potential targets, these interactions have been confirmed with co-immunoprecipitation and *in planta* split luciferase assays, in preparation for functional experimentation. In resolving effector structure along with co-expression of target structure, the aim is to develop new stress protection strategies. In applying these methods to beneficial microorganism effectors, we hope to further elucidate how crop hosts perceive and process signals for increased biotic and abiotic resistance.



### PP1-PU-07 Soybean nodulation and moldavian bentonites

Leonid Onofrash<sup>1</sup>, **Oleg Kharchuk**<sup>2</sup>, Oleg Bolotin<sup>3</sup>, Aleksandru Budak<sup>2</sup>, Aleksandru Kirilov<sup>2</sup>, Vasili Toderash<sup>4</sup>, Nikolai Samokhvalov<sup>5</sup>

<sup>1</sup> 1Institute of Microbiology and Biotechnology, str. Academiei 1, Chișinău 2028, Republic of Moldova

<sup>2</sup> The Laboratory of Plant Mineral Nutrition and Water regime, Institute of Genetics, Physiology and Protection of Plants, Moldova, Republic of

<sup>3</sup> 3Institute of Geology and Seismology, str. Gh. Asachi 60/3, Chişinău 2028, Republic of Moldova

<sup>4</sup> Institute of Microbiology and Biotechnology, str. Academiei 1, Chișinău 2028, Republic of Moldova

<sup>5</sup> Institute of Geology and Seismology, str. Gh. Asachi 60/3, Chișinău 2028, Republic of Moldova

Rhizobia inoculants have contributed to increase N2 fixation and yield in legumes crops. However, most of the inoculants produced world-wide are of poor or suboptimal quality [1]. The nature of soil-borne populations of rhizobia (number, nitrogen-fixing capacity) is determined by factors closely related to physical features of the landscape and the rhizobial status of a soil can de predicted by reference to these physical features [2]. Soybean (*Glycine max* (L.) Merr.) can be inoculated by delivering inoculant mixed with mineral microgranules such as bentonit; effect of granules on soybean nodulation in field experiments was consisted in nodule number (per plant) at R4 20-30 [3]. Granular material must be taken into account to improve the efficiency of this inoculation process [4].

An essential component of bentonites is the layered silicates of the montmorillonite type. In the present work, finely dispersed montmorillonite was obtained from clays of the Prodanesti deposit of Moldova.We have shown that montmorillonite particles are tightly connected to the roots; moreover, the root growth path is determined by the location of montmorillonite microparticles in the soil. The use of aqueous suspensions of highly dispersed clay minerals (both bentonite and pure montmorillonite) together with a suspension of *Bradyrhizobium japonicum* made it possible to obtain a nodule number (per plant) at R4 no less 100.

**Conclusion**. Natural fine clay minerals obtained on the basis of clay deposits of the Republic of Moldova are a promising material for increasing soybean nodulation

**Acknowledgments**: This work was supported by project founded by the European Union, DevRAM: "Increasing the competitiveness of agri-food sector throught integration to domestic and global value chains, in particular in the soya sector".

Catroux G., Hartmann A., Revellin C. *Plant Soil*. 2001, **230**, 21. Brockwell J., Herridge D.F., Morthorpe L.J. and R.J. Roughley. In: Nitrogen Fixation by Legumes in Mediterranean Agriculture (eds. Beck D.P. and Materon A.), 1988, pp. 179. Fouilleux G., Revellin C., Hartmann A., Catroux G. *FEMS Microbiology Ecology*. 1996, **30**, 173. G. Revellin. C. and Catroux, G. *Can. J. Microbiol*. 1994, **40**, 322.

## PP1-PU-08 Successions of microbes associated with below and above ground plant parts in a glacier fore field

#### Maximilian Hanusch<sup>1</sup>, Xie He<sup>1</sup>, Victoria Ruiz<sup>1</sup>, Robert R. Junker<sup>2</sup>

<sup>1</sup> Functional Community Ecology Research Group, Universität Salzburg, Austria

<sup>2</sup> Philipps-University Marburg, Evolutionary Ecology of Plants, Department Biology, Karl-von-Frisch Str. 8, 35043 Marburg, Germany

Plant surfaces represent one of the largest habitat for bacteria and fungi where they occur in high diversities. Plant species are characterized by specific microbial communities, but the relative contributions of the plants' phenotype, biotic and abiotic environmental conditions, and dispersal from surrounding habitats to community diversity and composition remain poorly understood. Glacier forefields – large areas of deglaciated substrate, which can be colonized by microorganisms, plants, and animals – provide an excellent opportunity to study several decades of microbial succession over the distance of only a few hundred meters. In order to evaluate the importance of the structuring factors of microbial communities, bacterial and fungal communities of the plant phyllosphere and the soil microbiome, as well as vegetation cover and arthropod communities will be recorded along a temporal gradient spanning over 170 years of microbial primary succession. Additionally, we will test hypotheses generated from field data in microcosm in the lab in order to verify correlational findings in stringent experiments and to gain novel insights into the interdependencies of microorganisms with other taxonomic groups.



# PP1-PU-09 The ecology of P capture in organic wheat: Is selection under low P, organic conditions going to get us there sooner?

#### Michelle K. Carkner<sup>1</sup>, Jessica Nicksy<sup>2</sup>, Martin H. Entz<sup>1</sup>

<sup>1</sup> Plant Science, University of Manitoba, Canada

<sup>2</sup> Department of Soil Science, University of Manitoba, Canada

Canada's first organic participatory wheat breeding (PPB) program has produced 50 lines bred by organic farmers on their own farms for three years (F3-F6). Many of the lines were selected under environments that have been organic for 20+ years, therefore under low phosphorus (P) or sourced from biological P (manure) for many years. Greater understanding of the impact selection environment may have on AMF colonization and phosphorus uptake in breeding programs is essential as P is a finite, non-renewable, and geographically restricted resource. This work is part of a larger body of work examining phosphorus uptake strategies for farmer selected breeding lines under low P and alternative P sources.

Organic crop production systems create a soil environment very different from conventional systems, and in many cases, lower in P. Many studies have reported that organic farms exhibit greater biological activity and greater AMF colonization in host plants than conventionally managed land. In some cases, like on dryland organic farms in the Canadian Prairie Provinces, this was due to low inorganic P availability, most P was in the organic form unavailable to crops.

Preliminary yield trials comparing PPB breeding lines to conventional registered checks have been conducted at 5 organic sites across the Canadian prairies in 2017 and 2019. In 2017, as a group, farmer lines significantly out-yielded conventional checks by 266 kg ha<sup>-1</sup> in the low-yield site and 435 kg ha<sup>-1</sup> in the high yield site. The mechanisms resulting in greater yield performance by farmer-selected genotypes will be focus of future research. A preliminary study (Nicksy, unpublished) compared one farmer line "IG" with one conventional cultivar, "Brandon", under a low P environment with synthetic and biological fertilizers (monoammonium phosphate (MAP), compost, frass, and unfertilized). Biomass at Zadoks stage 31 exhibited a cultivar\*fertilizer interaction. The "IG" line was higher than "Brandon" in compost, frass, unfertilized by 251, 187, and 248 kg ha<sup>-1</sup> respectively, however, "Brandon" biomass was greater than "IG" in under MAP by 227 kg ha<sup>-1</sup>. Therefore, it appeared the farmer-selected line was better able to respond to biological P sources, while the conventional check line was better able to respond to synthetic fertilizer P. Future studies will investigate a wide range of farmer-selected lines and their interaction with different components and processes within the "soil-plant P ecosystem".

### PP1-PU-10 Glyphosate affects mycorrhizal colonization of plants

Marjo Helander<sup>1</sup>, Irma Saloniemi<sup>1</sup>, Marina Omacini<sup>2</sup>, Magdalena Druille<sup>2</sup>, Kari Saikkonen<sup>3</sup>

<sup>1</sup> Department of Biology, University of Turku, Finland

<sup>2</sup> IFEVA-CONICET, Cátedra de Ecología, Facultad de Agronomía, Universidad de Buenos Aires, Buenos Aires CPA 1417 DSE, Argentina

<sup>3</sup> Biodiversity Unit, University of Turku, 20014 Turku, Finland

Glyphosate, the world's most widely used pesticide, is controlling weeds in agriculture, horticulture, silviculture and urban landscapes. Glyphosate inhibits production of an enzyme in the shikimate pathway of plants and thereby production of some amino acids. Shikimate pathway is found also in microbes and therefore its effects on non-target microbiota important to the ecosystem functions and ecosystem services should not be ruled out. We experimentally studied if glyphosate can remain in the soil and accumulate in a weed grass, (*Elymus repens*) and forage grass (*Festuca* pratensis) in boreal climate. We observed mycorrhizal colonization in the grass roots, and studied if the possible effects of glyphosate on plants and associated mycorrhizal fungi are dependent on biotic and abiotic environmental factors. We detected residues in both target plants and non-target plants in the growing season following the glyphosate treatment. All the plants growing in no-till pots had higher glyphosate residues compared to conspecifics in tilled pots. The glyphosate application significantly reduced the total VA-mycorrhizal colonization and the number of arbuscules of the plants. These results demonstrate negative long-term effects of glyphosate on non-target organisms in agricultural environments and grassland ecosystems.



## PP1-PU-11 Plant growth promoting potential and quorum sensing of *Pseudomonas* sp. RTE4 isolated from the rhizosphere of Assam tea.

Ankita Chopra<sup>1</sup>, Praveen Rahi<sup>2</sup>, Surekha Satpute<sup>3</sup>, Pranab Behari Mazumder<sup>1</sup>

<sup>1</sup> Biotechnology, Assam University, India

- <sup>2</sup> National Centre for Microbial Resource (NCMR), National Centre for Cell Sciences (NCCS), Pune, India
- <sup>3</sup> Department of Microbiology, Savitribai Phule Pune Univeristy, Pune, India

Tea rhizosphere is dominated by members of Bacillus sp. and Pseudomonas sp. Majority of these rhizosphere bacteria are beneficial to plant and are referred as plant growth-promoting rhizobacteria (PGPR).PGPR are mainly responsible for production of phytohormone production, hydrolytic enzymes and antibiotics to combat against phytopathogens. PGPR not only colonise the roots of tea through the biofilm formation but also produce biosurfactants. All these traits are claimed to be regulated by bacterial communication which a density dependent phenomenon is called quorum sensing (QS) .QS phenomenon observed in Gram negative bacteria is due to the secretion of signalling molecules which are popularly known as acyl homoserine lactone (AHL). In this study, we isolated gram negative bacterium RTE4 from tea rhizosphere of Rosekandy tea garden, Assam, India. RTE4 induced violacein pigment production in biosensor Chromobacterium violaceum CV026. Additionally, RTE4also induced green fluorescent protein expression in biosensor E.coli MT20 (jBA132). AHL was extracted from early stationary phase of RTE4 by acidified ethyl acetate. Reverse phase TLC and LC-MS studies demonstrated the presence of C6-AHL by RTE4. The isolate also showed production of Indole acetic acid production (74.54 µg/ml), phosphate solubilisation (46µg/ml). The bacterium RTE4 also showed production of protease. Biocontrol studies demonstrated, RTE4 as a promising candidate against two foliar pathogenic fungi namely Corticium invisium and Fusarium solani. Also secretion of antibiotic phenazine was confirmed by LC-MS. RTE4 formed moderately adherent biofilm which was evident through confocal microscopic images. RTE4also produced biosurfactant in the presence of dextrose and fructose as carbon source where a sharp decrease in surface tension were observed. Metabolic profiling conducted by BIOLOG GEN III indicated abilities of RTE4 to grow in acidic pH which is ideal for structural stability of AHL molecules. Molecular identification by MALDI-TOF MS and phylogenetic analysis based on 16S rRNA gene sequence confirmed it relatedness with genus *Pseudomonas*. Overall the strain RTE4 showed multiple plant growth promoting activities and also QS molecule, which make it a suitable candidate for the application as biofertilizer.

# PP1-PU-12 An improved growth medium for enhanced inoculum production of the plant growth-promoting fungus *Serendipita indica*

Mohamed Osman, Christian Stigloher, Martin J. Müller, Frank Waller

Julius-von-Sachs-Institut, Biocenter, Julius-Maximilians-Universität Würzburg, Germany

Endophytic colonisation of plant roots can lead to improved growth and enhanced resistance of host plants against abiotic stress and against plant pathogens. Such positive effects have been shown in detailed studies for the basidiomycete fungus *Serendipita indica (Piriformospora indica) (Sebacinales)*, e.g. in Barley, Maize, Poplar, Wheat, Switchgrass, Tobacco, and Arabidopsis. Due to its ease of axenic cultivation and its broad host plant range, it is used as a model fungus to study beneficial fungus-root interactions. *S. indica* and closely related *Sebacinales* fungi were also suggested to be utilized for commercial applications, e.g. to enhance pathogen resistance and crop yield of barley.

Serendipita indica is currently mostly cultivated in a complex Hill-Käfer medium (CM medium) for inoculum preparation, however, growth in this medium is slow, and yield of chlamydospores which are often used for plant root inoculation are relatively low. We therefore tested and optimized growth media for enhanced yield of fungal inoculum. We propose here a vegetable juice-based medium (VJ medium) which was superior to the currently used CM medium with respect to biomass production in liquid medium and fungal growth on agar plates. Using VJ medium, chlamydospore production was more than 20 times higher within the shortened cultivation time of 8 days, compared with CM medium. Interestingly, VJ medium also supported growth and conidiation of other fungi, suggesting its utilization for the propagation of diverse fungi in both research and commercial applications.

The described VJ medium is composed of inexpensive components and is easy to prepare, and is therefore recommended for a streamlined and efficient inoculum production for the plant endophytic fungus *Serendipita indica*.



### PP1-PU-13 The diversity and dynamics of plant-associated microbial communities of droughttolerant *Vellozia* species under different precipitation regimes on *campos rupestres*

#### Barbara Biazotti, Rafael de Souza, Paulo Arruda

Genomics for Climate Change Research Center, University of Campinas, Brazil

Severe drought is among the most extreme climate events affecting natural and agricultural ecosystems. Natural ecosystems with recurrent periods of drought are considered a rich reservoir of microorganisms that can enhance drought tolerance in plants. This is the case of campos rupestres (rupestrian fields), a semi-arid ecosystem characterized by a prolonged dry season and high solar radiation. A dominant monocot plant family, the Velloziaceae, displays adaptive features for drought tolerance (desiccation-tolerant and non-desiccation-tolerant) that make them highly resistant to the seasonal availability of water. During the dry season, desiccation-tolerant species (resurrection plants) drift into a dissection state, with a dead-looking aspect, and return to a hydrated and photosynthetically active state in the rainy season. In contrast, non-desiccation-tolerant species resist dissection and remain evergreen by lowering photosynthetic and respiratory rates during prolonged drought periods. Exploring the microbiome associated with these species displaying different mechanisms of drought tolerance is crucial to understanding the molecular and functional bases of plant adaptation in resource-limited environments. Therefore, we aim to explore the diversity and dynamics of the microbiota associated with four Vellozia species, two desiccation-tolerant (V. nivea and V. tubiflora) and two nondesiccation-tolerant (V. intermedia and V. peripherica). These species are rocky outcrop vegetation of Serra da Canastra National Park in Brazil. Plants were collected during the dry and rainy seasons allowing us to correlate the microbial diversity with edaphic physicochemical characteristics, plant morphophysiological adaptations and colonization patterns. The microbial profiling is being performed by sequencing and analysis of molecular markers for prokaryotic (16S) and fungal (ITS) communities associated with the substrate and plant organs, accounting for 1,120 prokaryotic and fungal libraries for each species. Aspects of plant-microbe interaction, such as fungal colonization rate and community establishment, will be further investigated by microscopy. We believe that prospecting microorganisms associated with Velloziaceae species will reveal potential functions that can be translated into new biotechnological strategies to enhance yield and performance of agricultural crops under drought stress.

# PP1-PU-14 Transcriptomic changes of tomato plants in response to endophytic bacterial strains revealed key pathways of plant-growth promotion in presence of humic acid

Nikoletta Galambos, Marco Moretto, Carmela Sicher, Ilaria Pertot, Michele Perazzolli

Plant Pathology and Applied Microbiology, Fondazione Edmund Mach, Italy

Chemical fertilisers are widely used in conventional agriculture and they cause possible environmental impacts. Plant growth promoting endophytic bacteria can internally colonize plant tissues and promote plant growth without causing damage or eliciting defence responses. Some publications highlighted the synergistic effects of the combined application of endophytic bacteria and humic acid (HA) substances on plant growth. However, there is a lack of knowledge on the molecular mechanisms of their combined interaction with the plant host. The aim of this work was to get insight into the molecular basis of the interaction between endophytic bacteria and tomato plants in the presence of HA, in order to improve the understanding on the mechanism responsible for plant growth stimulation. Three bacterial strains that endophytically colonise tomato plants were selected and they were able to promote tomato shoot length in the presence of HA. Transcriptional changes activated in tomato leaves in response to the endophytic bacteria included the up-regulation of primary metabolic processes, defence responses, growth and development pathways. In tomato roots, genes responsible for defence reaction, transport and oxidative stress were up-regulated by the bacterial strains. Moreover, genes related to secondary metabolism, nitrogen metabolism and hormone signals were activated by the bacterial endophytes only in presence of HA. The presented transcriptome study highlighted also species-specific pathways activated by the three bacterial strains and provided new insights into endophytic bacteria/adjuvant/plant interactions in order to further develop efficient biofertilisers.



## PP1-PU-15 Plant growth promoting endophytic bacteria from *Sedum oryzifolium* Makino of Dokdo

Sa-Youl Ghim<sup>1</sup>, Soo-Yeong Lee<sup>1</sup>, Jin-Soo Son<sup>1</sup>, Yu-Xi He<sup>1</sup>, Ye-Ji Hwang<sup>2</sup>

<sup>1</sup> School of Life Sciences, Kyungpook National University, Korea, Republic of

<sup>2</sup> School of Life Sciences, Research Institutue for Dok-do & Ulleung-do Island, Kyungpook National University, 80 Daehakro, Bukgu, Daegu 41566, Republic of Korea

Plant growth-promoting bacteria (PGPB) live in the rhizosphere of plant that help plant growth through the synthesis of auxin, gibberellins and cytokinin like phytohormone, availability of limited plant nutrients and production of siderophore and plant beneficial volatile organic compounds (VOCs). Induced systemic resistance (ISR) was called that plant diseasesuppressed resistance mechanism by non-pathogenic rhizobacteria in the plant. ISR is indicated that systemic resistance induced by microorganisms other than pathogens. VOCs are being studied as a new microorganism determinant for enhancement of plant immunity. VOCs indirectly improve plant growth while reducing abiotic and biotic stress. Generally, the case of bacteria produce at least 30 different volatile organic compounds, and that have been reported to cause physiological changes in plant-bacterial, bacteria-bacterial and fungi-bacterial interactions with each other. Some VOCs such as 2,3-butanediol and tridecane were induced plant systemic resistance against pathogens. In this study, plant ISR by endophytic Erwinia spp. isolated from Sedum oryzifolium Makino has been examined. Erwinia is a genus of Enterobacteriaceae bacteria, which is closely related to a lot of plant disease. This plant pathogen is causing with a wide host range which carrot, potato, tomato, onion, etc. It is able to cause disease in almost plant tissue. Breaking the stereotype, in this study, induced-resistance by the genus *Erwinia* against *Xanthomonas axonopodis* pv. vesicatoria in pepper (Capsicum annum L.) plant and against Pectobacterium carotovorum pv. carotovorum in tobacco (Nicotiana benthamiana) has been determined. Also isolated PGPB were investigated the ability of plant growth promotion and induce resistance to plants. As a result, Erwinia spp. known as a plant pathogen were existed predominant and has ability of plant growth promoting ability, induced systemic resistance. This indicates that the genus Erwinia is in symbiosis with the plant. It was also confirmed that the volatile substances that 2,3-butanediol and acetoin produced by the strain KUDC3020, isolated bacteria of Sedum oryzifolium Makino, had a positive effect on the plants.

# PP1-PU-16 Specific responses to bacterial quorum sensing molecule are altered in complex interactions

Abhishek Shrestha, Ichie Ojiro, Maja Grimm, Johannes Krumwiede, Marek Schikora, Adam Schikora

Institute for Epidemiology and Pathogen Diagnostics, Julius Kuehn-Institut, Germany

Gram-negative bacteria primarily produce *N*-acyl-homoserine lactones (AHL) as quorum sensing (QS) molecules. These QS molecules help to coordinate the collective behavior within bacterial populations. In addition, AHL can modulate behavior of the eukaryotic neighbors. Plants, for example, perceive and react to AHL in a diverse manner; often activating specific physiological pathways resulting in augmented growth or/and resistance, phenomenon termed as AHL-priming. Today, AHL-priming for enhanced resistance seems to be an efficient disease management strategy. So far, most of the studies investigated bilateral relationship between a particular AHL molecule and the plant. However, this scenario is highly improbable in the rhizosphere since different bacteria produce different AHL molecules.

In order to examine deeper the impact of AHL on plants, we assessed the impact of five different AHL with varying length of the acyl side chain, from 6 to 14 carbons (oxo-C6-HSL, oxo-C8-HSL, oxo-C10-HSL, oxo-C12-HSL and oxo-C14-HSL) and all their possible combinations on the model plant *Arabidopsis thaliana*. We monitored phenotypic traits like root length and biomass and performed gene expression analyses of four defense-related marker genes to assess the impact on defense due to the potential AHL-priming. Our study confirmed previous results implying that short-chain AHL induce biomass and root growth, whereas the long-chain AHL enhance the immune response. Furthermore, we observed that although single AHL induced specific responses in the plant, the combinations triggered less specific responses. In addition, we observed that defense priming was more prominent in plants that were treated with a combination of more than two AHL molecules, regardless of their structure. Our results therefore indicate that the specific responses to single AHL molecules are altered in interactions with diverse AHL molecules.

