Plant Biologicals Network

SYMPOSIUM

2022

About PBN

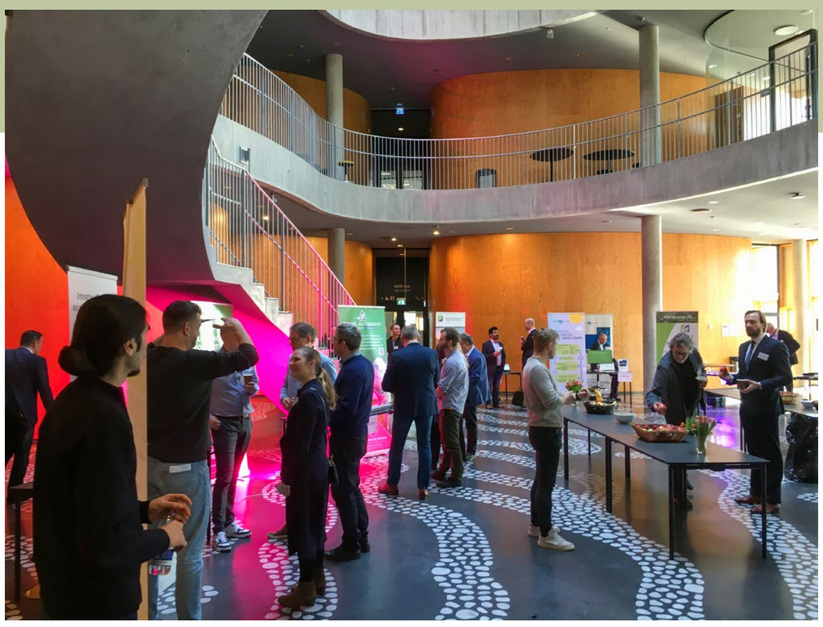
The Plant Biologicals Network has been formed by a number of key players in Southern Scandinavia with the purpose of facilitating knowledge exchange on research, innovation, regulation, and education within the field of plant biologicals. Additionally, the network aims at creating awareness of the future for these promising new technologies.

Currently the network has 24 members from Denmark and Sweden that range from large, multinational companies to small one-person companies and also includes 5 research/ educational institutions and a number of business associations.

The network is organized as a membership organization and is open for application from new members.

Contact person: Lene Rasmussen, lras@plen.ku.dk

Practical information



Venue

The Copenhagen Plant Science Centre auditorium
Department of Plant and Environmental Sciences
University of Copenhagen
Bülowsvej 21A
1870 Frederiksberg

Parking

It is possible to park at Frederiksberg Campus. You can get a parking permit at the registration desk. See the university parking areas near the venue.

Symposion dinner

After the Symposium, there will be a dinner at a restaurant nearby. Bus transport will be organized from the venue to the restaurant and back.

Public transport

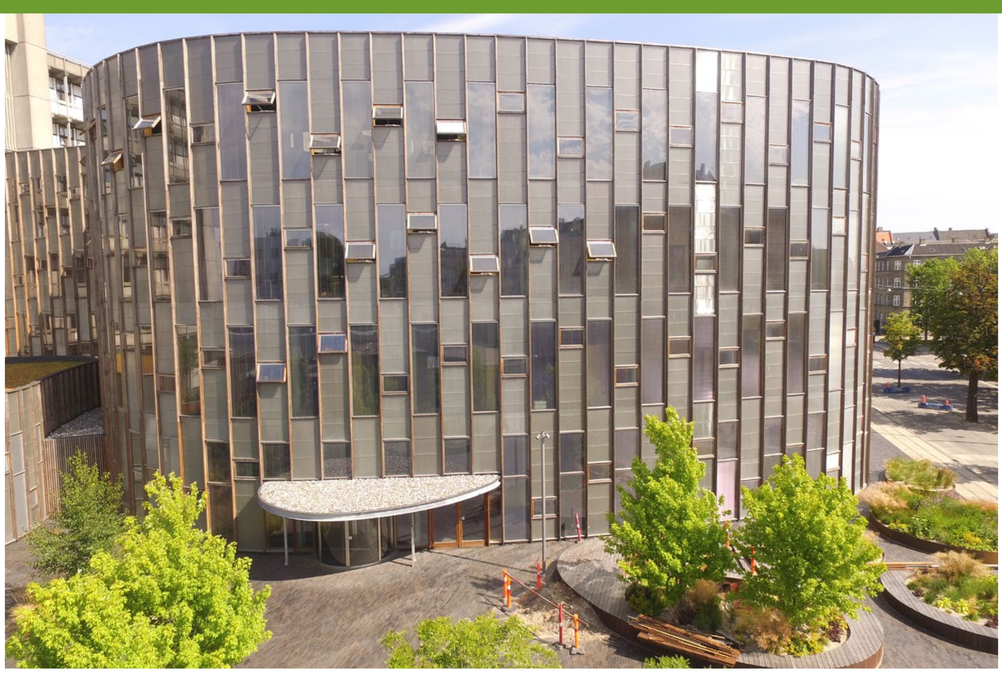
It is possible to go by metro or bus from CPH Airport or Copenhagen Central Station to the venue:

The **M2 Metro** runs diretly from the airport to the 'Forum' station which is a short walk from the venue.

From Copenhagen Central Station you can take the **M3 Metro** and get off at 'Frederiksberg Allé' which is a slightly longer walk. Alternatively, you can take **Bus 37**, get off at the stop 'Bülowsvej (Thorvaldsensvej)' or **Bus 2A** and get off at the stop 'Det Biovidenskabelige Fakultet (Rolighedsvej)'. Both bus stops are very close to the venue

Symposium Venue

The Copenhagen Plant Science Centre Auditorium, Department of Plant and Environmental Sciences University of Copenhagen, Bülowsvej 21A, 1870 Frederiksberg



Programme

9:00 - 9:30	**Registration**
9:30 - 9-40	**Welcome and opening adress**
9:40 - 11.00	**Session 1 - Regulation** **Chair: Jeanne Kjær, Novozymes** *Biopesticides: towards ambitious and fit-for-purpose EU policies* - **Rosa Criollo** EMEA Plant Health Regional Regulatory Manager, FMC Agricultural Solutions *RNA interference for crop protection and production* - **Jeremy Sweet,** Director, Sweet Environmental Consultants *Regulation of low risk active substances under Regulation 1107/2009 - a need-to-know approach* - **Eric Liegeois** Policy Officer Pesticides, European Commission, DG Sante, Health and Food Safety
11:00 - 11:30	**Coffee break**
11:30 - 12:50	**Session 2 - Testing biologicals** **Chair: Burghard Liebmann, FMC** *Development of methods for evaluation of plant biostimulants* - **Katja Ihrsén** Product Manager, Lantmännen Bioagri *Practical trials in potato fields* - **Carl Heiselberg** Farmer, The association for Danish potato farmers *Development of novel methods for assessing the impact of plant biologicals on crop productivity, climate, environment, and biodiversity* - **Svend Christensen & Philipp Trénel,** Danish Technological Institute/University of Copenhagen
12:50 - 13:50	**Lunch**
13:50 - 15:10	**Session 3 - Biostimulants** **Chair: Svend Christensen, University of Copenhagen** *'Bio-stimulating' our future* -**David King** Head of Technical UK, Eire and Nordics, Syngenta. *The plant microbiome: from rhizosphere to seeds and applications to improve crop performance* – **Angela Sessitsch,** Head of Bioresources Unit at AIT Austrian Institute of Technology *Long-term effect of chito-oligosaccharide application on strigolactone biosynthesis and AM fungal accommodation* - **Andrea Genre** Associate Professor, Department of Life Science and Systems Biology, University of Turin.
15:10 - 16:10	**Coffee + poster session**
16:10 - 17:40	**Session 4 - Biopesticides/Biological control** **Chair: Niels Bjerre, Bayer** *Notes from A Small Island – IPM in the UK* – **Aoife Dillon** Principal Scientist Crop Protection, Fera *Insect Sex Pheromone Precursors from Engineered Oilseed Plants for Sustainable Pest Control* - **Christer Löfstedt** Professor, Functional zoology, Lund University *Biologicals: The Market, Status, and Potential in Climate-Smart Agriculture* - **Pam Marrone**, PhD, CEO and Founder, Chestnut Bio Advisors (virtual)
17:40 - 18:10	**Reception + poster session**
18:10 - 18:30	**Transport to restaurant - Bus transportation will be organized**
18:30 - 21:30	**Symposium dinner (optional bus transport back to the venue at 21:00)**

Speakers

Session 1: Regulation

Biopesticides: towards ambitious and fit-for- purpose EU policies

Crop Life Europe (CLE) has a dedicated team on Biopesticides, the Biopesticide Expert Group (BioPPP EG), that advocates to support the development of a fit for purpose regulatory framework in the EU for Biopesticides.

Exciting innovative technologies such as peptides, antibodies, enzymes, RNAi, etc., are being developed in crop protection. But because of the lack of a clear regulatory pathway and expertise at authority level in Europe, those sustainable innovation products are not reaching the EU farmers.

The BioPPP EG has drafted a contribution document from the industry's perspective, trying to support regulators on a proposal in acceptable scientific approach for evaluation of novel biopesticides technologies under EC Regulation 1107/2009. As part of this contribution document, some examples of those innovative actives are intended to be presented as case-studies.

Rosa Criollo

EMEA Plant Health Regional Regulatory Manager

FMC Agricultural Solutions

I am Biologist with a master in applied ecology. Working on crop protection registrations and respective regulatory aspects since 2000. Early in my career, I worked as civil servant evaluating dossiers for Plant Protection Products within the environmental authority for 5 years in South America. Later, I developed myself in the area of ecotoxicological risk assessment, environmental fate modelling and regulatory aspects of conventional as well as biopesticide active substances for around 13 years in the consulting sector with a focus on Europe but covering USA, LATAM and Africa as well. Currently at FMC, in my role as regional regulatory manager with responsibility for Europe, Middle East and Africa for the biopesticides portfolio, allows me to stay in the regulatory arena supporting the registration of such Biological Plant Protection Products.

Jeremy Sweet

Environmental and

Research consultant, Sweet

Environmental Consultants

RNA interference for crop protection and production

The use of RNAi to improve food quality and provide crop protection will be discussed. Double stranded RNA can be produced in transformed plants to target specific plant, pathogen or pest genes through host induced gene silencing (HIGS) . In addition dsRNA produced in cultures and formulated can be applied externally to plants to silence genes in plants, pathogens or pests. Spray induced gene silencing (SIGS) can be enhanced by novel formulations to improve efficacy and reduce degradation of dsRNA in the environment.

Genetically modified plants expressing dsRNA are regulated under current EU GMO regulations. dsRNA can be very specifically targeted at genetic sequences in targets so that off-target effects are avoided or minimised. The recent increases in available genomic and transcriptomic sequence data allows the specific design of efficient and biosafe dsRNAs, minimizing the risk for off-target effects or silencing effects in non-target organisms. Regulatory systems for dsRNA applied as a pesticide have been developed in some countries (Australia, USA) and are under discussion in EU. Many of the biosafety considerations of HIGS and SIGS are similar but formulation also influences exposure and fate scenarios in SIGS.

Jeremy Sweet has spent the last 40 years conducting research on Crop improvement and plant diseases. Much of this work was conducted at NIAB Cambridge studying sustainable crop production, integrated disease management, environmental and agronomic impacts of GM crops, and gene flow to crops and wild relatives. He was Vice-Chair of the EU COST action iPLANTA studying RNAi in crop improvement and crop protection. He was a member of the EFSA GMO Panel for 12 years until 2018, providing scientific opinions on the risks associated with GMO applications in the EU. He has developed a number of the EFSA Guidance Documents on ERA and risk management and participated in an EFSA study of RNAi GM plants. He is an author in over 50 scientific papers on GMOs, numerous plant pathology papers and editor of 2 books. He developed his own consultancy in 2004 and is director of Sweet Environmental Consultants which provides research and advice on GMOs and Plant Health.

Regulation of low risk active substances under Regulation 1107/2009 - a need-to-know approach

Under Regulation (EC) No 1007/2009, low-risk active substances and plant protection products benefit from favourable conditions to be marketed. Annex II defines criteria to identify low-risk active substance, and 62 active substances (of which 24 are micro-organisms) meet the low-risk criteria.

With its Farm-to-Fork strategy, the EC supports the extension of the tools to protect plants for farmers with bio-control agents, and has amended the data requirements and approval rules for micro-organisms. The recent amendments bring the specific biological properties and ecology of micro-organisms to the centre of the assessment, making the rules more fit-for-purpose and flexible.

This need-to-know approach is what the Commission intends to explore for streamlining the regulation of other bio-control agents that are already on the market, or in the pipeline of companies in view of their placing on the market.

Erik Liegeois

Policy Officer, Pesticides

European Commision

DG Sante

Eric Liégeois is Chemical Engineer for Agricultural Industries from the University of Gembloux (Belgium). After a career as researcher, civil servant and manager in various positions in the Belgian Federal Administration, he joined the European Commission in 2002, first as seconded national expert at DG Environment where he was in charge of the Thematic Strategy on the Sustainable Use of Pesticides. Since 2007, he served as official in the Chemicals Unit at DG GROW, where he revised the Fertilising Products Regulation proposal and developed the concept of plant biostimulants.

He also worked on the Plastics Strategy and Circular Economy. He was leading a team in charge of Fertilisers, Detergents, Plant Protection Products, Biocides, Waste recycling and Plastics. Since April 2018 he joined DG SANTE in the team in charge of the authorisation of plant protection products where he is in charge of the biopesticides, low-risk products and risk reduction. He worked on several guidance documents related to micro-organisms and on the amendment of the regulations regarding data requirement and approval rules for micro-organisms used as plant protection products.

Session 2: Testing Biologicals

Development of methods for evaluation of plant biostimulants

Taking into account the issues of sustainability and climate change impact on food production has been, and will remain, crucial in order to achieve a sustainable agriculture. Research and development are ongoing in different areas, each of which plays an important part in contributing to a sustainable food production that would allow and promote biological diversity in a rapidly changing climate.

While striving for sustainable food production, additional fields of activity should be emphasized, notably that of biostimulants which despite its potential has yet to be given proper consideration. Biostimulants possess, among other properties, the ability to increase yields under unfavorable environmental conditions.

In order to be able to effectively utilize the potential of biostimulants in plant production and thereby contribute to increased yields of higher quality, adapted methods are needed to evaluate their effects. The goal of this project is to develop quick and cost-effective methods for evaluating biostimulants under Nordic conditions.

Katja Ihrsen

Product manager

Lantmännens BioAgri

Following a couple of years of research studies and a PhD in agricultural management at the University of Bonn, Germany, Katja worked at DeLaval International for 3 years, first with advanced system trials, and later on with international customer relations. In 2008, she moved on to Lantmännen where she focused mainly on owner relations which gradually helped her develop a deeper understanding of the Swedish farming business(es), both on farm and at the various levels of the agricultural value chain. Since 2021, Katja is has been the product manager for biostimulants and biological seed treatment at Lantmännen BioAgri. She successfully introduced Lantmännen's first biostimulant in the Swedish market and leads the 3-year-project on biostimulants which started January 2022.

Carl Heiselberg

Chairman, Farmer

The Association for Danish Potato Farmers

Test of plant biologicals in farmers' fields

The EU "Farm to Fork" strategy outlines a 50% reduction of chemical pesticides by 2030. Plant biologicals is believed to be a part of the solution. However, the vision and goal are more based on wishful thinking than facts. For the last three decades, especially the universities have invested public resources in screening and developing of plant biologicals. The globally leading biotech and agrochemical companies have within the last decade changed their focus from chemical pesticides to naturally derived products for growth stimulation and prevention of pest and diseases. However, there is still very few documented products on the marked. These products will not be recommended and used before applied trials in farmers field can demonstrate the net benefit and when, where, and how to use these products.

Carl Heiselberg owns a farm near Vojens in Denmark, where he grows seed grass, spring barley, potatoes, and corn for biogas. Potatoes make up 230 hectares out of the farm's 450 hectares, and he grow potatoes for crisps, starch, granules as well as eating and baking potatoes. Carl has been a full-time farmer since 1999 and holds several board posts, among others in AKD (Danish share potato four factory). Since 2012 he has been chairman of the trade association for Danish Potato Farmers, with around 1000 members. Throughout the years Carl has tried many different biostimulants on his property, both on large plots and in strip trials.

Philipp Trénel
Senior Consultant
Danish Technological Institute

Svend Christensen
Head of department
Department of Plant and Environmental Science
University of Copenhagen

Development of novel methods for assessing the impact of plant biologicals on crop productivity, climate, environment, and biodiversity

As a part of the EU Green Deal Strategy the use of chemical pesticides and fertilizers must be reduced by 50 and 20%, respectively. Reducing the use of chemical products without alternatives runs the risk of a great indirect climate effect in terms of loss of yields to pests and diseases (Oerke, 2006). Plant biologicals (PB) can play a role as partly substitutes for these products. PB are a diverse group of microorganisms and products derived from natural organisms that can serve as plant biostimulants (i.e. products that increase the nutrient use efficiency, tolerance to abiotic stress, quality traits or availability of confined nutrients in the soil or rhizosphere) or biocontrol agents (BCA) in agriculture. impact of the applied PB on the climate, environment, and biodiversity.

Philipp Trénel holds a ph.d. in biology from Aarhus University and has been working as data scientist and R-specialist at DTI since 2008, where he is the product manager of the statistics and machine learning team. His scientific focus areas include optimal trial design and modern data analysis of sensor-intensive on-farm precision agriculture trials, including biostimulant trials. Philipp Trénel is part of the Innovation Foundation Innomission 3 project “Climate and Environmentally Friendly Plant Biologicals – Development of novel methods for assessing the impact of plant biologicals on crop productivity, climate, environment, and biodiversity”

Svend Christensen holds a Ph.D. (1993) from the Royal Veterinary and Agricultural University in Copenhagen. As a researcher, he has coordinated 10 larger research projects prior to becoming Research Director of the Department of Agricultural Engineering under the Danish Institute of Agricultural Sciences in 2001. In 2008 he was appointed Professor and Head of Department of the Department of Plant and Environmentsal Sciences at the University of Copenhagen.

Session 3: Biostimulants

David King
Head of Technical
UK, Eire and Nordics
Syngenta

‘Bio-stimulating’ our future

David King, Syngenta Head of Technical for the UK, Ireland and Nordics, describes the need for and the challenges involved with working with biological products, and how Syngenta has gone about developing practical solutions for farmers.

David has been involved in the Crop Protection Industry since 1989 working with both DuPont and currently with Syngenta as Head of Technical for the UK, Ireland and Nordics. He leads a Technical Team of 11 people who are responsible for bringing new technologies to help farmers and agronomists grow their crops in a world with increasing political, economic and environmental challenges.

The plant microbiome: from rhizosphere to seeds and applications to improve crop performance

The plant microbiome is considered as an accessory genome for plant providing complementary functions to their host such as nutrient mobilization and acquisition or functions to better cope with biotic and abiotic stresses. A number of microorganisms have been identified and selected for application as biofertilizer, plant strengthener or biopesticide and benefits seen in lab and greenhouse trials are highly promising. Nevertheless, field application remains a challenge as mechanistic understanding on plant-microbe interactions or on holobiont interactions is still missing.

Ecological understanding, science-driven product development and smart delivery approaches are likely to improve plant microbiome applications and will pave the way to the integration of microbiome functions in smart agricultural systems. In this talk different aspects on microbiome understanding and the use of new application approaches will be presented. Also, an overview of the EU-funded Coordination and Support Action MICROBIOMESUPPORT targeting food systems microbiomes will be given.

Angela Sessitsch

Head of Bioresources Unit

Austrian Institute of Technology

Dr. Angela Sessitsch is Head of the Bioresources Unit at the AIT Austrian Institute of Technology. She studied biochemistry at the University of Technology in Graz, holds a PhD in Microbiology from the Wageningen University, the Netherlands, and is habilitated at the Vienna University of Natural Resources and Life Sciences. She has pioneered plant-associated microbiomes and is interested in understanding the interactions between plants, microbiomes and the environment as well as to develop applications. Her group explores the diversity and functioning of plant microbiota by applying a range of molecular approaches, interaction modes between plants and model bacteria, colonization behaviour of endophytes as well as various application technologies for biocontrol and crop enhancement applications. Together with her group A. Sessitsch published more than 200 peer-reviewed publications, she belongs to the Highly Cited Researchers and is co-inventor of several patents.

Andrea Genre

Associate professor

Department of Life Science and Systems Biology, University of Turin

Long-term effect of chito-oligosaccharide application on strigolactone biosynthesis and AM fungal accommodation

Arbuscular mycorrhiza (AM) is an ancient symbiosis between land plants and soil-borne fungi, supporting plant mineral nutrition. During AM establishment, the perception of root-exuded strigolactones boosts fungal release of short chain chitoligosaccharides (CO) that activate plant symbiotic responses. In this presentation I will demonstrate that exogenous CO application promotes AM development by changing the root gene expression pattern in Medicago truncatula roots over several weeks. CO treatment has a major impact on strigolactone biosynthesis and fungal accommodation related genes, leading to an increase in root strigolactone content and stimulating accommodation responses to AM fungi.

Andrea Genre obtained his PhD in Fungal Biology and Biotechnology in 2006 at the University of Turin (Italy), where he is currently carrying out his teaching and research as an Associate Professor. His main interest is in cell biology of arbuscular mycorrhizal (AM) intearctions and during his early studies he described a novel plant cell apparatus that anticipates the penetration of AM fungi in the lumen of root cells. Since 2012 he had the opportunity to start his own group and projects, which investigated the similarities between such pre-penetration responses and cell division, and described cell cycle reactivation in advance of arbuscule accommodation. He also contributed to the characterization of signalling molecules in AM fungi, eventually leading to their potential application as symbiosis promoters in agriculture. Andrea Genre is an Associate Editor for New Phytologist and Speciality Chief Editor for Frontiers in Plant Science.

Session 4: Biopesticides & Biological Control

Aofie Dillon

Principal scientist, Crop Protection, Fera

Notes from a Small Island – IPM in the UK

As policy makers around the world look to drive IPM uptake, what initiatives are the UK agencies pursuing? This talk will describe how, under the Sustainable Farming Incentive schemes, the UK plans to reward farmers for a more sustainable approach to land management, including better adoption of IPM.

Key considerations include: what are the current barriers, what solutions are being proposed and how is the industry responding to the challenge?

Aoife is the Principal Scientist in Crop Protection at Fera, specialising in biopesticides. As a translational science organization, Fera is focussed on science for impact – for public good, commercial advantage or both. Fera's key areas of expertise are Crop Health, Food Integrity, Next Generation Diagnostics and Sustainable Agri-Food Systems.

Aoife has 25 years' experience working in biocontrol, including microbial and low risk pesticides and, prior to joining Fera, Aoife worked for a variety of AgChem / biopesticide companies, including BASF, Exosect and EDEN Research, where she managed R+D and registration teams.

Over the course of her career Aoife has primarily focused on efficacy and non-target effect (invertebrates and gene flow) studies, as well as developing registration packages for microbials (entomopathogenic fungi), semiochemicals (pheromones) and natural substances (botanicals). Aoife was recently involved in the OECD Conference 'Innovating Microbial Pesticide Testing' (Sept 2022).

Insect Sex Pheromone Precursors from Engineered Oilseed Plants for Sustainable Pest Control

Pheromones are an environmentally friendly alternative to conventional insecticides for pest control. Camelina sativa seeds genetically modified to express sex pheromone precursor of several moth species, provided oil from which the precursor was isolated, purified, and transformed into the active pheromone.

Plant-derived pheromone proved equally effective as synthetic pheromone lures in monitoring diamondback moth, Plutella xylostella, in cabbage and disrupting mating of cotton bollworm, Heliothis armigera, in common bean fields.

Our study demonstrates the biological efficacy and economic feasibility of pheromone production in plant factories by metabolic engineering of an oilseed crop.

Christer Löfstedt

Professor of Ecology

Lund University

Christer Löfstedt, Professor in Ecology, Lund University, Sweden, combines fundamental research on chemical communication with applied aspects, including biological production of pheromones. He analyzed the evolution of sex pheromones in moths and demonstrated how yeast and plants can be turned into factories for pheromone production. He is a fellow of The Royal Swedish Academy of Sciences, codirector of the Max Planck Centre for Next Generation Insect Chemical Ecology, and a cofounder of SemioPlant AB, an R&D company with the aim of developing biologically produced pheromones for the market. He has published more than 200 original scientific papers.

Pam Marrone

PhD, CEO and Founder

Chestnut Bio Advisors

Long-term effect of chito-oligosaccharide application on strigolactone biosynthesis and AM fungal accommodation

All categories of ag biologicals are experiencing double digit annual growth and are more relevant today than ever as essential components of integrated pest management and crop production programs.

Innovation is exploding with many startups and investment capital in the space including a convergence of precision technology, data and biology. However, barriers remain to continued adoption by growers. This talk will summarize the state of the market, factors in market adoption, startup innovations and thoughts on why bioherbicides have lagged other biological categories.

Pam Marrone spent her 30+ year career focused on biological products for pest management and plant health, having started and led three bioag companies. She is the board member and past treasurer of the Association for Women in Science, on the board of the Foundation for Food and Agricultural Research, is a Senior Fellow of the Arizona State University Swette Center for Sustainable Food Systems and is a past alumni-Trustee of Cornell University. She serves on the boards of Elicit-Plant (Chair), 180 Life Sciences (NASDAQ:ATNF), Stem Express and Pheronym and advises several other agtech/agbio startups.

She has a B.S. in entomology with Honors and Distinction from Cornell University and a Ph.D. in entomology from North Carolina State University. With co-founder Jim Boyd, the former CFO of Marrone Bio, Pam is currently in the process of launching the Invasive Species Control Corporation and Invasive Species Foundation, to bring effective, environmentally friendly biological solutions to control destructive invasive species.

Poster session

1. Integrating Biological control agents and Plant-resistant inducers into IPM strategies to control potato late blight

Isaac Kwesi Abuley, *Jens Grønbech Hansen*
Department of Agroecology, Aarhus University

In this study, we ascertained the efficacy of different biological control agents (BCA) and plant-resistant inducers (PRI) for controlling late blight in vitro and in vivo on resistant and susceptible potato cultivars. Subsequently, the most promising BCA and PRI (n = 2) were selected and tested as a stand-alone or combined treatment with fungicides. Two strategies for the combined treatment were evaluated. Strategy 1: BCA/PRI in low-risk periods and fungicides in high-risk periods. Strategy 2: BCA/PRI in the low-risk period and a mixture of BCA/PRI and fungicides in the high-risk period. The main result of these experiments showed that the BCA/PRIs were less effective against late blight as standalone treatments. However, the integrated strategy performed effectively as a routine fungicide application. This result was confirmed for both resistant and susceptible cultivars. The most prudent strategy was to alternate BCA/PRI with fungicides depending on the risk period.

2. Innovative IPM strategies for the management of diseases in wheat and onion (Innovate-IPM)

Isaac Kwesi Abuley, *Thies -Marten-Heick, Jens Grønbech Hansen, Peter Hartvig, Poul Lassen*
Department of Agroecology, Aarhus University

There is an increasing demand for reducing pesticides. Thus, control methods such as alternative products (e.g. biological control agents) that are environmentally benign are increasingly attractive. Several alternative products have emerged in the last decade. However, strategies including alternative products remain challenging to use due to their lower and unpredictable field performance. In this project, we claim that alternative products will fail as standalone treatments. However, there are clear opportunities to reduce fungicide usage by integrating alternative products, decision support systems (DSSs), and host resistance. Innovate-IPM will explore these IPM tools together with alternative products to manage Septoria tritici blotch (STB) (Zymoseptoria tritici) in wheat and Downy mildew (Peronospora destructor) in onions. Innovate-IPM will explore the key components of such an IPM strategy for downy mildew (onion) and STB (wheat). This will require development and testing to enable their successful implementation in the field. Overall, the project envisages a reduction of fungicides by 50% in both crops in the short term. Furthermore, a further reduction of up to 65% once confidence is built among the farming community.

3. Establishing a wheat-promoting microbial community

Adele Pioppi, Caja Dinesen, Xinming Xu, Ákos T. Kovács
Department of Biotechnology and Biomedicine, Technical University of Denmark

Important crops cultivated across the world are subject to yield losses due to pests and diseases, as well as climate change, resulting in high rates of undernourishment and food insecurity. Plant growth-promoting rhizobacteria represent a sustainable solution to boost crop resiliency. Understanding the interactions between plant roots and key bacterial groups, and especially bacterial communities, is crucial to harness their potential. In this work we focused on developing a wheat growth-promoting rhizosphere microbiome. This was pursued through successive wheat cultivation, with repeated re-inoculation of the root microbiome of faster and slower growing wheat plants. 16S amplicon sequencing revealed that the two selection lineages gave in fact rise to significantly different soil microbiome compositions, especially in the rhizosphere. Certain bacterial groups, including recognized wheat endophytes were particularly established across all plants independently of selection method, such as Pantoea and Paenibacillus; while Curtobacterium, Pseudomonas and Herbaspirillum seemed to most significantly differ in the rhizosphere microbiome of fast- and slow-selected plants. The investigation of larger microbial communities for biocontrol is a valuable complementary approach in addition to single strain studies, as a more complex system may better represent the natural field environment. In addition, the most important bacterial groups arising from such experiments are excellent starting points for further studies.

This project is part of INTERACT within the Collaborative Crop Resiliency Program (NNF19SA0059360) funded by the Novo Nordisk Foundation.

4. Coupling of secondary metabolite production in *Bacillus subtilis*

Caja Dinesen[1], *Carlos N Lozano-Andrade*[1], *Scott A Jarmusch*[2], *Ákos T Kovacs*[1]

[1] Bacterial Interactions and Evolution Group, [2]Natural Product Discovery Group,
DTU Bioengineering, Technical University of Denmark

The Gram-positive, soil-dwelling bacterium Bacillus subtilis has great potential as a biocontrol agent due to the production of a wide variety of secondary metabolites (SMs) with antimicrobial properties. Even though the interest in these secondary metabolites is increasing, there are still many unknown factors when it comes to the regulation of biosynthetic gene clusters including the interconnections between the production of different secondary metabolites. Our former study characterized a library of isolates for their ability to produce various lipopeptides and further dissected the natural product chemistry of mutant strains in respective gene clusters for these lipopeptides. This approach revealed a correlation between the production of the lipopeptide, surfactin and the bacteriocin, subtilosin A in B. subtilis. Interestingly, isolates and mutants lacking surfactin production contained increased level of subtilosin A. To further investigate this coupling among these SMs, the subtilosin A promoter was cloned before GFP and the construct was inserted in various knockout mutants related to lipopeptide production. The expression of subtilosin A is evaluated using a plate reader that detect the GFP signal in different strains to reveal the transcriptional response in the presence or absence of surfactin. Lastly, the production of subtilosin A is also evaluated using LC-MS to correlate with the gene expression determined in the plate reader assay.

5. Bacterial persistence and lipopeptide production are associated to effective postharvest control of mango anthracnose by *Bacillus subtilis/velezensis* strains

Carlos N. Lozano-Andrade[1,2], ***Camilo A Ramirez***[2]

[1] Bacterial Interactions and Evolution Group, DTU Bioengineering, Technical University of Denmark, [2]Grupo de Bacteriología Agrícola y Ambiental (BAA), Universidad de Antioquia

Bacillus-based biological control agents are promising alternatives to synthetic chemical fungicides for defeating plant diseases caused by fungi. In this work, we evaluated the ability of Colombian native aerobic endospore-forming bacteria (AEFB) to control mango anthracnose at postharvest stages. 120 strains were screened for in vitro antagonist activity against Colletotrichum gloeosporioides, a causal agent of mango anthracnose. Twenty-five of these strains showed the highest ability to inhibit the mycelial growth. Three of these isolates, identified as B. velezenis, were selected for experiments on fruits, along with 3 AEFB strains considered as models. Fruits treated with a spore solution of any of the isolates showed significant reductions in anthracnose severity, reaching up to 96% in comparison to non-bacterized control. Further studies showed that the remarkable biocontrol activity was associated with the germination of a minor portion of bacterial spores in the first day's post-inoculation and the consistent secretion of isoforms of iturin, fengycin, and surfactin, suggesting an antibiosis-like mechanism.

6. Rhizobacteria-mediated root architectural changes are correlating with systemic impact on antioxidant and carbohydrate metabolism

Chandana Pandey, *Victoria Naoumi, Daniel Buchvaldt Amby,*
Seyed Fazel Mirahmadi, Thomas Roitsch
Department of Plant and Environmental Sciences, Copenhagen Plant Science Centre, University of Copenhagen

Root system architecture is pivotal for plant anchorage and efficient uptake of water and nutrients resulting essential in plant fitness, crop performance and yield. Thus, the understanding and harnessing of root architecture for crop improvement are needed to address global challenges for the development of more resilient, productive, and climate-smart crops. Rhizosphere is a dynamic region of the soil surrounding plant roots and controlled by interactions between plants and plant growth promoting rhizobacteria (PGPR) that are in close association with the root. We check the effect of the inoculation with different PGPR (B. amyloliquefaciens-FZB42, Enterobacter-G20-XW and Pseudomonas putida-KT2440) on root growth in barley plants cv. Guld grown in germination pouches. Results reported a clear inhibitory effect on barley root growth in inoculated plants with G20-XW with an increase in root diameter and thickness at 7 days after inoculation and the results were further found to be consistent at 44, 47 and 66 hours after inoculation. There were also clear differences in root architecture in two-barley cv. Guld and Scarlet with contrasting responsiveness to G20-XW. This indicate that endogenous root developmental responses are affected. RNA sequencing revealed distinct qualitative and quantitative differences in gene regulation in response to G20-XW. Notably, significant increase in the activity of different antioxidant (GST, POX) and carbohydrate (PGI, PGM) enzymes correlated with elevated transcript levels of oxidative stress and sugar metabolic pathways. Genes from high-affinity nitrate transporters and CK biosynthetic pathways regulate the responses of the host plant and bacterial signals and ultimately affect root architecture.

7. Protist and bacterial community interactions in wheat rhizosphere

Christine Lorenzen Elberg, Rumakanta Sapkota, Anne Winding

Department of Environmental Science, Aarhus University

The predator-prey interactions between microorganisms like protist and bacteria play an important role for plant health by increasing nutrient availability and potentially suppressing pathogens. Despite of this vital role, understanding of the relationships between protists and bacteria is limited. We therefore aim to characterize protist communities associated with wheat rhizosphere, align with the bacterial communities, identify important interactions and the effect on wheat growth.

Microorganisms from bulk and rhizosphere soil samples from four wheat cultivars grown in a growth chamber were analyzed. By metabarcoding of protist using 18S rDNA, diversity was determined. By comparing with 16S rDNA metabarcoding of bacteria in the same samples, effects of habitat (bulk soil vs rhizosphere vs rhizoplane) and wheat cultivar were analyzed. Further, by network analyses of the microbial diversity, significant interactions were identified. Parallel to this, approximately 180 protists were isolated by growth on indigenous bacteria. Sanger sequencing identified the protists and the diversity of the indigenous bacteria in the cultures were analyzed by 16S rDNA metabarcoding.

8. Combining above- and belowground biological control agents for improved pest control in strawberry tunnel production

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Strawberry production in tunnels can extend the growing season, and increase yield and fruit quality, but also the risk of pest outbreaks. Arthropod predators are used for biological control in tunnels, but does not always ensure sufficient pest control. Microbial control agents, such as entomopathogenic fungi (EPF), can provide direct control of belowground insect stages, but EPF can also affect population growth of arthropod pests aboveground, likely due to induced plant defences. We investigated the potential of combining macro- and microbiological control agents, above- and belowground, by release of the predatory mite Neoseiulus cucumeris and soil drenching of the EPF Metarhizium brunneum in laboratory and field trials.

Laboratory experiments were conducted with soil application of three M. brunneum isolates to investigate their individual effect on the population growth of the two spotted spider mite, Tetranychus urticae, in clip-cages. The M. brunneum isolate KVL16-36 resulted in least eggs and nymphs of T. urticae. Next experiment tested the combination of M. brunneum and N. cucumeris on T. urticae populations using whole plant assays. Here, combined treatment of KVL16-36 and predatory mites reduced populations of T. urticae more than single treatments. Finally, this combination was tested in two commercial strawberry tunnels during the growing season, where the abundance of pests (T. urticae, aphids, thrips) were recorded. Delayed pest population development were observed in the treatments applied EPF, with variation between the two sites. The results indicate potential for improved pest control in strawberry tunnels when biocontrol strategies targeting more pests are used, combining direct effects with indirect effects through induction of plant defence mechanisms.

9. Identification of plant growth-promoting microbial consortia and individual strains from a Danish soil

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Environmental soils are natural reservoirs of untapped microbes with the potential to improve plant performance. How plants enrich a beneficial community from a complex and highly diverse soil-borne microbiota to promote growth is poorly understood. Here, we explored the mechanism of the establishment of a plant growth-promoting microbial consortia from soil samples collected from an alder swamp in Denmark. By applying a series of dilutions of a soil suspension (a soil slush) followed by inoculation of them into sterilized soil, we found a dilution of the slush that can increase Arabidopsis rosette size two-fold as compared to plants grown in the original sterile soil. Further dilutions of the slush did not show a growth-promoting effect. Through 16S analysis and metagenomics analysis of plant-associated microbiome growing in the diluted soil slush, we will identify the composition and formation of the beneficial community as well as the functionality that could explain the beneficial effect of the consortium. Meanwhile, culture-dependent isolation of individual strains followed by mono-association based screening has identified two bacterial endophytes showing synergistic positive effect on plant organ size, possibly being responsible for the plant growth promoting phenotype of the diluted soil slush. In controlled agar plate experiments, we demonstrated that the two strains can promote a significant increase in leaf size, lateral root initiation and total root length. Ongoing experiments focus on elucidation of the plant growth-promoting mechanisms utilized by the two beneficial strains and to evaluate their potential as biostimulants in different crops.

10. Ménage-à-quatre

Combined effects of rhizobia, pea enation mosaic virus and its pea aphid vectors on leguminous biomass and hormones

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Assessing multitrophic interactions effect is fundamental to understand crop ecology, therefore to improve management. Plant mutualists can contribute to defences against plant antagonists whereas plant antagonists can help each other by interfering with plant defences. While most studies focused on dual interactions, only a few addressed multitrophic interactions. Such knowledge cannot be inferred.

Thus, we investigated biomass and hormones of fava bean while interacting with the mutualistic rhizobacteria, and the antagonistic pea enation mosaic virus (PEMV) and its pea aphid vector. Rhizobia promotes Induced Systemic Resistance (ISR), mediated by Jasmonic Acid (JA), whereas aphid and virus induce salicylic acid (SA) to repress ISR. Previous studies showed rhizobia antagonising both aphids and virus. Conversely, virus and aphids are supposed to antagonise rhizobia because SA interferes with nodulation. Moreover, it was proposed that attack on the shoot causes resources re-allocation to the root. Our results confutate reallocation of biomass.

Rhizobia increased plant biomass while aphid and virus decreased roots biomass more than shoot's and top stem's more than leaves'. These effects cancelled each others in shoot, but not in root, where rhizobia affects more than the antagonists combined. Additionally, the virus reduced nodules number and the effect is stronger in combination with aphids. However, the nodule average weight was unaffected. Hence, only initiation is affected. Surprisingly, virus increased both SA and JA. Even more surprisingly, combining virus with aphid decreased SA content compared to virus alone. This suggest a damage threshold for SA induction. Despite the lower SA, we observed a significant decrease in JA. Consequently, SA alone cannot explain the interference with JA. This suggests another interference target in the oxylipin pathway. Similarly, rhizobia did not increase JA, suggesting that ISR promotion works on increased sensitivity to JA rather than concentration.

11. High-throughput exploration of microbial diversity for novel bio-fungicides

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Chemical pesticides have been shown to reduce biodiversity and contaminate ecosystems. Furthermore, the continuous application of chemical pesticides results in resistance development among plant pathogens, rendering them both ineffective and detrimental to the environment. While microbes represent a sustainable and ecologically friendly alternative, microbial products thus far show varying efficiency in the field. With an estimated 10^11 -10^12 microbial species on earth, diversity within microorganisms represents an underexploited reservoir for microbial solutions. To address the enormous challenge of testing and evaluating diverse microbes across lab and field conditions, SABS (Smarter AgroBiological Screening) has developed an automated high-throughput (HT) pipeline for screening our DTU collection of 45,000 strains against two major fungal pathogens of cereals; Fusarium graminearum and Zymoseptoria tritici. In collaboration with FMC we transfer promising strains from lab to field in a step-wise in-vitro to in-vivo pipeline, and by analyzing the combined data we are able to mine for any correlations from in-lab assays to success in-field.

In our first campaign, 2,000 strains were screened in HT assays, and 150 strains went on to plant-based assays. Thus far, we have studied the correlation between lab, detached-plant tissue assays, and whole-plant assays. As field studies represent the final, ecologically relevant assay in the pipeline, successful strains are now being scaled up for 2023 field application. Based on these learnings, we aim to adjust and re-design our pipeline, and introduce machine learning for AI-assisted selection in future campaigns. Therefore, by tapping into high diversity strain collections and identifying assays that predict success in the field we aim to accelerate the discovery of novel biologicals. growth-promoting mechanisms utilized by the two beneficial strains and to evaluate their potential as biostimulants in different crops.

12. The host-soil-microbiome nexus suppresses bacterial pathogens

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Reductionist approaches to science are largely responsible for our inability to harness the full potential of phytobiomes for sustainable agriculture. Crop health is often attributed to either a resistant genotype, or a disease suppressive soil, or a microbe with anti-pathogen activity. However, it is more likely to be a mixed and somewhat inseparable result of them all. Here we attempt to embrace this complexity and understand how microbiomes from the host-soil interface suppress bacterial pathogens. In cultivation bioassays, we measured the disease development and microbiome dynamics due to pathogen inoculation (i) in the soil and (ii) on the host, for 6 cultivars of A. bisprous and in ten soil types.

We were able to associate specific co-occurring bacteria in the soil microbiome with pathogen invasion and disease suppression, across ten different soil types. Disease suppression was transferrable via a microbiome extract from suppressive to conducive soils. The response of the host cultivars to the pathogen differed widely with the inoculation location, highlighting the role of the microbiome in disease development. Resistant cultivars actively recruited a diverse host microbiome from the soil which comprised many of disease suppressive genera. Strong trade-offs between productivity and disease suppression in the cropping system were also regulated by host-soil-microbiome nexus.

We concluded that the resilience of the host to bacterial diseases is determined not only by genotype, but also by the assembly of- and interactions within- the host-soilmicrobiome nexus. This knowledge on microbiome assembly and its dynamics presents new prospects for the design of ecological disease control strategies.

13. Interkingdom priming of tomato for improved drought stress and herbivore attack responses by the cytokinin-producing plant growth promoting bacterium *Pseudomonas fluorescens* G20-18

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The application of beneficial bacteria is receiving increasing attention as environment friendly alternative to agrochemicals for sustainable crop production within climate change scenarios. However, the mechanisms involved in this interaction are still poorly understood, notably in relation to the widespread production of phytohormones by rhizobacteria of the genus Pseudomonas [1]. Thus we characterised the impact of inoculation of tomato seedling roots by the cytokinin-producing Pseudomonas fluorescens G20-18 on development and abiotic and biotic stress responses. G20-18 stimulated root and shoot growth [2], which correlated with induction of distinct activity signatures of key metabolic enzymes [3]. G20-18 also improved cell- and eco-physiological drought stress responses [4]. Likewise, G20-18 boosted the protection against the herbivore Spodoptera exigua. RNA sequencing revealed distinct qualitative and quantitative differences in gene regulation in response to G20-18. Notably, the number of genes differentially regulated in response to G20-18 was several fold higher during drought stress [4] and insect attack, indicating the priming for a much stronger transcriptionally regulated systemic drought stress and insect pest response. The relevance of the ability of G20-18 to produce cytokinins for the interorganismic signalling to mediate the observed improvement of stress responses of the host plants was evident from functional approaches with cytokinin deficient G20-18 mutants.

14. Novel amplicon-based approach to track plant growth-promoting species in natural soil

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Species of the genus Bacillus are used widely as plant growth-promoting rhizobacteria. To exploit the Bacillus genus in agricultural or industrial applications, the organism must be profiled at the species level as its bioactivities differ markedly between species. Standard 16S rRNA gene amplicon profiling does not allow for accurate species differentiation. Recently, we developed an amplicon sequencing-based high-resolution method targeting a conserved gene enabling taxonomic differentiation of Bacillus species in soil samples without either laborious cultivation or expensive metagenome sequencing. Several studies demonstrated the application of the Bacillus genus as an eco-friendly approach to alleviate salt-stress impact and to improve wheat growth significantly.

Therefore, in this work, we developed a Bacillus Amplicon Sequence Tool (BAST) to investigate which specific root-associated Bacillus species are recruited by plants under salinity stress. By coupling our high-throughput sequencing method with plant assay we claim to identify effective strategies applied by plants to adapt to salt stress and further contribute to improving plant performance under stressful environments. Further, our approach using BAST facilitates the quantitative description of complex microbial interactions within the plant rhizosphere and specific contribution of the Bacillus genus.

This study innovatively integrates Bacillus amplicon sequencing methodology with plant-microbe interactions studies which proposes novel guidance to large-scale exploring bioactive Bacillus species in the field.

15. Above- and below-ground applications of fungal biocontrol agents have variable effects on spider mites in three strawberry cultivars

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The two-spotted spider mites, Tetranychus urticae (Koch) are among the most notorious pests of strawberries, affecting yield and quality. Entomopathogenic fungi (EPF) can be used as biopesticide, but the EPF also display a plant-associated lifestyle as endophytes. Root inoculations with EPF can affect aboveground herbivores, but the underlying mechanisms remain unclear.

Here, we applied two EPF strains (Metarhizium brunneum KVL 16-36 and KVL 19-39) and another biocontrol fungus (Clonostachys rosea KVL19-38) either as foliar sprays or by root inoculation in three strawberry cultivars (Clery, Faith and Rumba), and we evaluated the effects on T. urticae individual development in bioassays and T. urticae population growth on whole plants, respectively. In the foliar spray experiment, M. brunneum KVL 19-39 significantly decreased egg survival and hatch rates, and increased mite development time compared to the other treatments.

However, in the root inoculation experiment, M. brunneum KVL 19-39 unexpectedly promoted T. urticae population growth while M. brunneum KVL 16-36 decreased the population growth, though only in the cultivar Rumba. In addition, C. rosea KVL19-38 also inhibited T. urticae population growth in both cultivars Rumba and Clery, but not in Faith. The absence of EPF colonization in leaves led us to hypothesize that the EPF may have affected the plant secondary metabolites and/or rhizosphere microorganisms, leading to changes in the population of T. urticae. Fungal biocontrol agents may therefore have different effects on the same pest organisms depending on application method and plant cultivar.

16. High-throughput sequencing reveals novel RNA mycoviruses in *F. culmorum* isolates

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Fusarium head blight (FHB) continues to be one of the major problems infecting both wheat seedlings and heads and is considered a disease complex caused by several highly specialized pathogens including Fusarium graminearum and F. culmorum. Fusarium spp. and its disease route have been studied extensively; however, mycoviruses infecting them remain an elusive factor potentially influencing the fungal phenotype.

While the majority of known mycoviruses do not appear to cause detrimental effects to their hosts, few viruses have been reported to affect fungal phenotype, including growth and induce hypovirulence. Thus, these viruses have a biocontrol potential. With the objective to investigate hypovirulent mycoviruses, we isolated F. culmorum from several wheat (Triticum aestivum) cultivars and screened them for mycoviruses by extracting and sequencing double stranded RNA (dsRNA).

Based on the RNA-dependent RNA polymerase domain analysis, we found two dsRNA and three ssRNA mycoviruses belonging to Hypoviridae, Unirnaviridae, Partitiviridae, Mymonaviridae and Mycoaspiviridae families. Further efforts to characterize biological effects of these viruses are still ongoing.

17. Smart selection of rhizospheric microbiome by multi-passaging approach to mediate salinity tolerance in *Vigna radiata*

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The increasing global mean temperature coupled with cumulative stress due to the indiscriminate use of chemical fertilisers and pesticides causes salt deposits to accumulate in the soil. Excessive accumulation of salt in soil leads to salinity stress and negatively impacts plant physiology and yield. Due to reported beneficial effects of microorganisms on crops, the application of plant growth promoting bacteria residing in rhizosphere is gaining popularity.

The introduction of '-omics' technology has enabled scientists to gain a deeper understanding of the overall microbial community structure and functions of diverse root-associated microbial communities present in the rhizosphere. Single strains or a consortium of bacterial strains have limited efficiency due to challenges in colonisation and competition from the indigenous microbiome. Additionally, the applied bacteria may disturb the existing networks of beneficial microorganisms. In this study, a plant-assisted microbiome selection strategy was employed, in which the host plant was made to select a microbiome that aids in plant growth and development under stressed conditions. A multi-passaging approach of microbiome selection was utilized for the model host plant Vigna radiata, owing to the latter's short life cycle and economic importance in India. The domesticated microbiome was introduced to the plant via a series of passages, along with successive increase in salt stress after every alternate passages.

The results of this study demonstrated that rhizospheric microbiome acclimatised across successive passages, and enhanced plant's tolerance to saline stress. In salinity-stressed plants treated with a acclimatised soil microbiome, there was an increase in root and shoot lengths, as well as gradual decrease in the levels of stress indicators like proline, malondialdehyde, and membrane stability index, relative to control plants. The combination of next-generation sequencing (NGS) and quantitative polymerase chain reaction (qPCR) tools demonstrated an increase in the abundance and diversity of Proteobacteria and Actinobacteria in microbiometreated plants during consecutive passaging stages. This work highlights the efficacy of multipassaging method of acclimatizing the microbiome as a promising sustainable approach for alleviating salinity stress in higher plants.

18. From endophyte discovery to biological control

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The endobiome of plants includes a diverse fungal community ranging from true endophytes (who never cause disease in that specific host) to latent pathogens. We have studied endophytic fungal communities using amplicon sequencing and isolated endophytes – and latent pathogens - from healthy plant materials including cereals and dicots (vegetables).

The amplicon sequencing studies have revealed effects of plant development stage, source of inoculum, pathogen pressure and plant genotype as factors influencing their composition. We have isolated endophytic fungi from healthy plant tissues subjected to natural pressure and studied - with some success – their potential as biological control agents to combat serious cereal diseases, namely Fusarium Head Blight and Septoria blotch in wheat. Studies of mechanisms involve using RNAseq implicate induced resistance as the primary mechanism.

Meet the exhibitors

You can meet the following exhibitors in the foyer right outside the auditorium during the breaks in the symposium programme:

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