

## 1<sup>st</sup> Annual Conference of the MiCropBiomes COST Action

**Exploiting Plant-Microbiomes Networks and  
Synthetic Communities to Improve Crops  
Fitness**



# Conference Overview

**Registration:** Wednesday 8:30 – 9:15 am

**Welcome:** Wednesday 9:15 – 9:45 am

Wednesday 11 <sup>th</sup> September	Thursday 12 <sup>th</sup> September	Friday 13 <sup>th</sup> September
<p><b>9:45 – 10:30</b></p> <p><b>Session 1</b></p> <p>Exploiting Plant-Microbiome molecular crosstalks: diversity, distribution and eco-evolutionary perspectives</p>	<p><b>8:45 – 10:30</b></p> <p><b>Session 4</b></p> <p>Crop-Microbiome assembly dynamics under specific environments</p>	<p><b>9:00 – 11:00</b></p> <p>Management Committee Meeting</p>
Break	Break	Break
<p><b>11:00 – 12:30</b></p> <p><b>Session 1</b></p> <p>Exploiting Plant-Microbiome molecular crosstalks: diversity, distribution and eco-evolutionary perspectives</p>	<p><b>11:00 – 12:00</b></p> <p><b>Session 5</b></p> <p>Communication, and engagement with society</p>	<p><b>11:00-13:00</b></p> <p><b>Social Programme - Natural History and Science Museum</b></p>
Lunch	<p><b>12:00 – 12:30</b></p> <p>Shared experiences for STSM/funded trainees</p>	
<p><b>14:30 – 16:15</b></p> <p><b>Session 2</b></p> <p>Crop microbiomes and plant diseases: from dysbiosis to increased defenses</p>	<p><b>14:00 – 16:30</b></p> <p>Working Group Meetings</p>	
Break	<p><b>16:30 – 17:00</b></p> <p>Plenary Conclusion WG1-WG5</p>	
<p><b>16:45 – 18:15</b></p> <p><b>Session 3</b></p> <p>From current microbial inoculants to synthetic communities</p>	<p><b>17:00 – 18:30</b></p> <p>Posters Session, Porto Wine Taste &amp; Group Picture</p>	
<p><b>19:00</b></p> <p><b>Congress Dinner</b></p>		





## Local Organisers

<b>Conceição Santos</b>	University of Porto, Portugal
<b>Maria Celeste Dias</b>	University of Coimbra, Portugal
<b>Joana Costa</b>	Instituto Pedro Nunes, Portugal
<b>Fernando Tavares</b>	University of Porto, Portugal
<b>João Prada</b>	University of Porto, Portugal
<b>Emil Gimranov</b>	University of Porto, Portugal
<b>Juliana Fernandes</b>	University of Porto, Portugal
<b>Marino Costa-Santos</b>	University of Porto, Portugal
<b>Sara Foubert-Mendes</b>	University of Porto, Portugal

## Scientific Committee

<b>Ahmad Abdelfattah</b>	ATB, Germany
<b>Anna Bonaterra</b>	University of Girona, Spain
<b>Caroline De Tender</b>	Ghent University, Belgium
<b>Claire Prigent-Combaret</b>	University Lyon 1, France
<b>Conceição Santos</b>	University of Porto, Portugal
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<b>Francesco Spinelli</b>	University of Bologna, Italy
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<b>Maria Celeste Dias</b>	University of Coimbra, Portugal
<b>Ralf Koebnik</b>	IRD, Montpellier, France
<b>Renata Artimová</b>	Institute of Forest Ecology, Slovakia





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# Scientific Program

## Wednesday 11<sup>th</sup> of September

08:30-09:15 **Registration**

09:15-09:45 **Opening & welcome by the organizers**  
**Conceição SANTOS** (Chair)

### **Session 1 Exploiting plant-microbiome molecular crosstalks: diversity, distribution and eco-evolutionary perspectives**

Chairs: Maria Celeste DIAS & Joana COSTA

#### **Invited Lecture**

09:45-10:15 – **Philippe VANDENKOORNHUYSE (Université de Rennes, FR)**: The holobiont and hologenome concepts and related emerging ideas for the next agriculture

#### **Oral Presentations**

10:15-10:30 – **Alberto SPADA (CREA, Research Centre for Viticulture and Enology, IT)**: Back to the holobiont: a journey through plant-microbe interactions for sustainable viticulture

*10:30-11:00 – Break*

11:00-11:15 – **Denis GUNIA (Leibniz Institute for Agricultural Engineering and Bioeconomy, DE)**: The edible antibiotic resistomes of apples ('Mandy' apples and 'Golden Reinders')



11:15-11:30 – **Esmeralda DUSHKU (Veterinary Research Institute of Hellenic Agricultural Organization-DIMITRA, GR)**: Integrated genomic and *in planta* assessment of novel plant growth-promoting rhizobacteria

11:30-11:45 – **Fabienne VAILLEAU (LIPME - INRAE , FR)**: Highlighting plant and bacterial key genetic factors of the molecular dialog between plant growth-promoting bacteria and *Arabidopsis thaliana*

11:45-12:00 – **Mogens NICOLAISEN (Aarhus University, DK)**: Microbe-mediated drought resilience in barley

12:00-12:30 – General Discussion

12:30-14:30 – *Lunch*

## **Session 2 Crop microbiomes and plant diseases: from dysbiosis to increased defenses**

Chairs: Joël F. POTHIER & Renata ARTIMOVÁ

### **Invited Lecture**

14:30-15:00 – **Vittorio VENTURI (African Genome Center, Mohammed VI Polytechnic University, MA)**: Cell-cell bacterial signalling in the plant pathobiome and microbial solutions for a more sustainable agriculture

### **Oral Presentations**

15:00-15:15 – **Claude BRAGARD (UCLouvain, BE)**: Unravelling the complex interaction between a fungicide, a candidate biocontrol agent and the bacterial wheat microbiome

15:15-15:30 – **Wafa ACHOUAK (CNRS-CEA-Aix Marseille University, FR)**: Phytopathogens-induced plant response, assemblage and network of the wheat rhizosphere microbiota



15:30-15:45 – **Tetiana KALACHOVA (Institute of Experimental Botany, CZ):** A look behind the curtains: exploring the dynamics of tripartite interactions between plants, bacteria and bacteriophages

15:45-16:00 – **Joana VICENTE (FERA, UK):** Detection of mushroom pathogens in complex microbial communities

16:00-16:15 – General Discussion

16:15-16:45 – *Break*

### **Session 3 From current microbial inoculants to synthetic communities**

Chairs: Anna BONATERRA & Claire PRIGENT-COMBARET

#### **Invited Lecture**

16:45-17:15 – **Emilio MONTESINOS (Laboratory of Plant Pathology-CIDSAV, ES):** Functional peptides in microorganisms and its potential in plant disease control

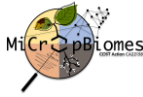
#### **Oral Presentations**

17:15-17:30 – **Núria DARANAS (University of Girona, ES):** Fate of *Bacillus velezensis* and *Lactiplantibacillus plantarum* strains applied to apricot, peach, and grapevine crops

17:30-17:45 – **Iakovos PANTELIDES (Cyprus University of Technology, CY):** From suppressive compost to targeted inoculants: synthetic microbial communities promote tomato growth and disease control

17:45-18:15 – General Discussion

19:00 – **Congress Dinner**



## Thursday 12th of September

### **Session 4 Crop-microbiome assembly dynamics under specific environments**

Chairs: Elodie VANDELLE & Gordana RACIĆ

#### **Invited Lecture**

08:45-09:15 – **Dejana PANKOVIC (Julius Kühn Institut Quedlinburg, DE):** Bacterial priming responses of resistance and growth parameters in spring barley

#### **Oral Presentations**

09:15-09:30 – **Catarina LEAL (Instituto de Ciencias de la Vid y del Vino - Logroño, ES):** Drought influences the fungal community structure, diversity, and functionality inhabiting the grapevine xylem and enhances the abundance of *Phaeomoniella chlamydospora*

09:30-09:45 – **Lyuben ZAGORCHEV (Sofia University, BG):** Interaction between soil microbiota, host plants, and stem parasitic plants of the genus *Cuscuta* under salinity

09:45-10:00 – **Marko PETEK (National Institute of Biology, SI):** Microbiome profiling of potato roots and leaves grown under organic vs integrated pest

10:00-10:15 – **Francesco SPINELLI (University of Bologna, IT):** The influence of fruit microbiota on ripening and quality and its dynamic during post-harvest

10:15-10:30 – General Discussion

10:30-11:00 – Break



## Session 5 Communication, and engagement with society

Chairs: Caroline DE TENDER & Ralf KOEBNIK

### Invited Lectures

11:00-11:30 – **Sonia GARCÍA MÉNDEZ (Ghent University & VIB, BE):** Fast track to environmentally adapted bacteria for soybean growth at northern latitudes

11:30-12:00 – **Céline DE PESSEMIER (Apha.bio, BE):** Unravelling mode-of-action of biocontrol agents to combat fungal diseases in wheat

12:00-12:30 – **Shared experiences for STSM/funded trainees**

12:30-14:00 – *Lunch*

14:00-16:30 – **Working Group Sessions**

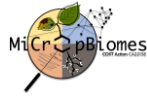
16:30-17:00 – **Plenary Conclusion WG1-WG5**

17:00-18:30 – **Poster Session, Porto Wine Taste & Group Picture**

## Friday 13th of September

09:00-11:00 – **Management Committee Meeting**

11:00-13:00 – **Social Programme - Natural History and Science Museum**



# Session 1

**Exploiting Plant-Microbiome molecular crosstalks: diversity,  
distribution and eco-evolutionary perspectives**

Chairs: Maria Celeste DIAS & Joana COSTA

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## **The holobiont and hologenome concepts and related emerging ideas for the next agriculture**

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Current agriculture has paid so far very little attention to plant microbiota. Worse, agriculture has likely jeopardized key microbiota components. By putting forward an efficient plant microbiota for host-holobiont protection and nutrient supply, an agriculture of holobionts has to emerge. The agriculture of holobionts will need paradigm shifts in breeding strategy and agricultural practices and is expected to restore damaged ecological functions, including soil fertility ecosystem service. In this presentation, I will provide an overview of the conceptual consequences of considering plants as holobionts and develop my vision of the agriculture of holobionts and related avenues for microbial ecology research.



## Back to the holobiont: a journey through plant-microbe interactions for sustainable viticulture

Alberto Spada<sup>1,2\*</sup>, Giuseppe Paradiso<sup>1</sup>, Marco Sandrini<sup>1</sup>, Chiara Biselli<sup>3</sup>, Teodora Basile<sup>1</sup>, Raffaella Balestrini<sup>4</sup>, Claudio Bonghi<sup>2</sup>, Agostino Fricano<sup>5</sup>, Vincent Arbona<sup>6</sup>, Luca Nerva<sup>1,7</sup>, and Walter Chitarra<sup>1,7</sup>

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**Keywords:** Grapevine rootstock; Domestication syndrome; Endophytes; Microbial recruiting; Multi-omics

Throughout centuries of anthropocentric breeding, plants have been selectively bred to enhance their quality traits and yield, often overlooking the importance of neglected attributes, like those involved in the interactions with beneficial microorganisms (1). For grapevine in particular this phenomenon led to an alteration in the distribution of photosynthetic products, shifting from defence mechanisms to growth, commonly described as the so called 'domestication syndrome' (2). Restoring the balance loss due to this condition is imperative to increase the resilience of plants to biotic and environmental stresses, which are becoming increasingly frequent due to climate change. Beneficial endophytes can be significant contributors to this task, so it is essential to unravel the concealed communication between grapevines and these microbes, as well as the mechanisms used by plants for their recruitment. To this aim, a Synthetic Community (SynCom) has been formulated, using selected grapevine endophytes and arbuscular mycorrhizal fungi (AMF), and applied to potted cuttings of diverse rootstock genotypes. Plant development and physiological parameters were monitored throughout an entire growing season. Root samples were collected for: i) DNA extraction and metabarcoding analysis to identify the root-associated microbiota



and ii) RNA isolation for transcriptomic analysis. Concurrently, leaf and root samples were collected for targeted metabolomics, focusing on compounds involved in microbial recruitment, including, among others, coumarins, strigolactones, salicylic acid, and jasmonic acid. AMF root colonization features and functional symbiosis markers have also been evaluated.

Employing a multi-omics approach, phenotypic, physiological, biochemical, and molecular data will be integrated (3), to improve our understanding of the complex interaction within grapevine and its associated microbiota, thus identifying genes involved in the recruiting of beneficial microorganisms. Beside basic research outcomes, these findings could help in the development of new breeding programs which will also consider these traits as selection criteria, thereby preserving the ability of grapevine to interact with soil microbiota. Furthermore, these results will provide useful information to augment holobiont resilience and reduce the chemical inputs needed nowadays in vineyards thus promoting more sustainable agricultural practices.

### References

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2. Sandrini M et al. (2022). Microbe-assisted crop improvement: a sustainable weapon to restore holobiont functionality and resilience. *Horticulture Research* 9, uhac160. doi.org/10.1093/hr/uhac160
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## The Edible Antibiotic Resistomes of Apples ('Mandy' apples and 'Golden Reinders')

Denis Gunia<sup>1,2\*</sup>, Ana-Maria Sanchez<sup>3</sup>, Prof. Dr. Neus Teixido<sup>3</sup>, Dr. Ahmed Abdelfattah<sup>1</sup>

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**Keywords:** Antibiotic Resistome; Apple microbiome; Whole Genome Sequencing; *Alphaproteobacteria*; *Actinomycetota*; *Firmicutes*; *Zymomonas*

Antibiotics are currently on verge of extinction worldwide due to antimicrobial resistance (AMR). In this regard, it is critical to have a comprehensive understanding of the antimicrobial resistance patterns across human-related environments. The field of food microbiomes remains understudied in terms of antibiotic resistance. This study presents the findings of antibiotic resistome in apple microbiome from two apple cultivars (Mandy apples and Golden Reinders) using culture-based technique and whole genome sequencing. The analysis of antibiotic resistance was carried out using different antibiotics including third generation of Cephalosporins (Ceftriaxone), Vancomycin, Penicillin G, Macrolides (Erythromycin), Tetracycline, Carbapenems (Imipenem) and 2nd generation Fluoroquinolones (Ciprofloxacin). From the 516 bacteria that were examined, 298 showed resistance to one or more antibiotics. Among the resistance isolates, 80 exhibited resistance to more than three different classes of antibiotics, which led to their classification as multi-drug resistant. The main taxonomic groups connected with these isolates were *Alphaproteobacteria*, *Actinomycetota* and *Firmicutes*. The bacteria exhibited a wide spectrum of antibiotic resistance profiles, with more than 50 % of them showing resistance to Ciprofloxacin, Ceftriaxone and Tetracycline. Interestingly, a single strain from *Zymomonas* genus exhibited high level of resistance to all the antibiotics examined, suggesting the potential for the emergence of superbugs in the future. The results from our study have demonstrated that apples possess a diverse array of microbiome with broad spectrum of resistance characteristics. Moreover, this work offers novel insights into the dynamics and incidence of antibiotic resistance in agricultural settings.



## Integrated Genomic and *in planta* assessment of novel Plant Growth-Promoting Rhizobacteria

Esmeralda Dushku<sup>1\*</sup>, Andigoni Malousi<sup>2</sup>, Athanasios Kargas<sup>3</sup>, Aikaterini Dourvanaki<sup>3</sup>, Evdokia Krystallidou<sup>3</sup> and Charalampos Kotzamanidis<sup>1</sup>

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**Keywords:** biostimulants; whole genome sequencing; *in planta* assays; kiwi; corn

Plant growth-promoting rhizobacteria (PGPR) are a heterogeneous group of beneficial bacteria that can support plant growth by promoting plant health, nutrition and stress tolerance. The incorporation of PGPR biostimulants into cropping systems is increasingly emerging as a promising strategy for sustainable agriculture and global food security. This presentation focuses on evaluating the PGP potential of novel bacterial strains isolated from the rhizosphere of kiwi (*Actinidia deliciosa*) and maize (*Zea mays*) by integrating *in vitro*, genomic and greenhouse-field trials. A total of 25 putative PGPBs were isolated from the genera *Bacillus*, *Cytobacillus*, *Peribacillus*, *Prieta*, *Pantoea*, *Paenarthrobacter*, *Arthrobacter*, *Pseudomonas*, *Microbacterium* and *Achromobacter*. The PGPB were analysed *in vitro* for their phyto-beneficial properties, including nitrogen fixation, phosphate solubilization, siderophore production, ammonium production, auxin production and ACC deaminase activity, in relation to resistance to abiotic stresses (pH and salt) and biofilm formation ability. Our results showed that 88% of the strains possessed at least four PGP traits, with most strains (90%) characterised as good auxin producers, while a few strains (20-30%) showed tolerance to abiotic stress and produced siderophores. The whole genome of the 25 strains was sequenced and analysed to uncover the molecular basis of their plant-promoting functions. Genome mining allowed us to confirm the PGP properties detected by *in vitro* analysis and to identify conserved and unique genes among the different PGPB species involved in plant-promoting functions. Four strains (*B. pumilus* CrA10, *P. dispersa* CrRR3, *P. megaterium* KWSR3, and *P. aurescens* CrR1) showing promising PGP properties were selected and encapsulated in calcium alginate microspheres (10<sup>9</sup> cfu/g). High viability (>90%) of the encapsulated bacteria was observed with minimal cell loss when stored for up to 8 months. The encapsulated PGPB were tested in lettuce, tomato, corn and kiwi plants under greenhouse or field conditions. Our results confirmed the PGP potential of the multi-species microbial inoculants and especially their high-value potential in accelerating seed germination,



promoting root growth and increasing plant biomass. Corn and kiwi plants grew better when they were given a multi-species PGP cocktail without fertilizer, compared to using fertilizer without bacterial inoculation. This work proposes a strategy for selecting potential PGP candidates and shows that alginate microbeads are effective carriers of biostimulants. This paves the way for developing an effective plant growth-promoting strategy.

**Funding:** This research was carried out as part of the project «Development and production of microbial biostimulants to improve the production and conservation of resources in the cultivation of *Actinidia deliciosa* and *Zea mays* (Hellenic Microbial Biostimulants-HeMiBio)» (Project code: KMP6-Q260559) under the framework of the Action «Investment Plans of Innovation» of the Operational Program «Central Macedonia 2014 2020», that is co-funded by the European Regional Development Fund and Greece.



## Highlighting plant and bacterial key genetic factors of the molecular dialog between Plant Growth-Promoting Bacteria and *Arabidopsis thaliana*

Rémi Duflos, Daniela Ramirez-Sanchez, Chrystel Gibelin-Viala, Corentin Chalas, Baptiste Mayjonade, Anaïs Botello, Sébastien Carrere, Alexandre Perrat, Coralie Fuertes, Fabrice Roux and Fabienne Vaillieu\*

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**Keywords:** microbiota, PGPB, *Arabidopsis thaliana*, natural genetic variation, GWAS

Plant microbiota includes, among others, Plant Growth-Promoting Bacteria (PGPB) that have the potential to mobilize and provision nutrients to the plants, alleviate abiotic stresses and provide protection against several pathogens. However, the effectiveness of microbiota on plant health is highly influenced by climatic and soil conditions, but it also depends on the genotype of the host plant, thereby deeply affecting their use in a wide range of agricultural conditions. In addition, while the combination of ‘omics’ tool and mutational studies has revealed key genes underlying the benefits conferred by a PGPB on a plant, studies reporting the genetic and molecular mechanisms associated with natural variation of a given PGPB remains scarce. There is therefore a growing interest in exploring natural genetic variation in plants interacting with PGPB, to exploit the beneficial effects of individual members of the microbiota, and improve their conditions of use. By studying native plant-microbiota interactions, we first identified strong ‘host genotype × bacterial genotype’ interactions for diverse vegetative growth-related traits. Then, we set up a Genome-Wide Association study (GWAS) to establish a genomic map of local adaptation in *Arabidopsis thaliana* in response to the main non-pathogenic bacterial members of its leaf microbiota. Finally, by genome sequencing with a long-read technology, we detected extensive genomic diversity for one of the most abundant and prevalent PGPB of the leaf compartment of *A. thaliana*. GWAS run on this specific PGPB revealed a complex genetic architecture that was dependent on the genotype of *A. thaliana* and the growth conditions. We therefore observed that on both sides of the interaction, on the plant side and the PGPB side, the genetic architecture was highly dependent on various parameters: the plant genotype, the developmental stage at which plants were inoculated, the growth conditions, and the identity of the bacterial strain. Strategies for functional validation of the key genetic factors identified on both sides will be discussed.

## References

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**Funding:** Support provided by COST CA22158 Action MiCropBiomes.



## Microbe-mediated drought resilience in barley

Menghui Dong<sup>1</sup>, Mette Vestergård Madsen<sup>1</sup>, Christopher James Barnes<sup>1,2</sup>, Miguel Sanchez Garcia<sup>3</sup>, Katarzyna Retzer<sup>4</sup>, Heinrich Grausgruber<sup>4</sup>, Louise Reinbach Rasmussen<sup>1</sup>, Mogens Nicolaisen<sup>1\*</sup>

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**Keywords:** microbial communities; barley; roots; drought; barley genomics

Understanding root microbiome - crop genome interactions can identify novel breeding strategies that harness the beneficial interactions between the host plant and microbes. I will give an introduction to the Horizon EU project BarleyMicroBreed, in which we are analyzing 16S/ITS amplicon sequencing data from 10,800 barley root samples. These root samples are taken from 600 barley lines including a number of introgression lines from crosses with *Hordeum spontaneum* and *H. bulbosum* grown under different environmental conditions in Lebanon, Morocco and Austria. Preliminary results show that barley variety explains most of the variation within both the bacterial and fungal communities (e.g. 26% for bacterial communities in the spring barley lines), with minimal influence from geospatial heterogeneity (ca. 2%). Surprisingly, over 70% of the ASVs are specific to fewer than 20% of barley varieties, contrasting with a core microbiome (i.e., shared by over 80% of varieties) accounting for only 8% of the ASVs, in the bacterial dataset.

**Funding:** Support was provided by the European Union, HORIZON CL6-2021-BIODIV, BarleyMicroBreed, Project No. 101060057.



## Detection of mushroom pathogens in complex microbial communities

Joana G. Vicente<sup>1\*</sup>, John Elphinstone<sup>1</sup>, Ian Adams<sup>1</sup>, Sam McGreig<sup>1</sup>, Marco Benucci<sup>1</sup>, Lynn Laurenson<sup>1</sup>, Hannah Marsay<sup>1</sup>, Adam Bryning<sup>1</sup>, Jennifer Cole<sup>1</sup>, Brian Carter<sup>1</sup>, Ann Barnes<sup>1</sup>, Christopher Field<sup>1</sup>, Ashleigh Elliott<sup>1</sup>, Andreja Dobrovin-Pennington<sup>2</sup> and Ralph Noble<sup>2\*</sup>

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**Keywords:** *Agaricus*; blotch; green mould; detection; sequencing

Bacterial blotch of mushrooms is a disease that has been shown to be caused mainly by the bacterial species *Pseudomonas tolaasii*, *P. costantinii* and several groups of '*P. gingeri*' in the UK and is considered to be the most important disease currently faced by the mushroom industry in the UK and elsewhere in Europe, causing losses that can frequently exceed 30% of production (1). Bacterial isolates obtained from mushrooms from several UK farms with symptoms of severe brown blotch, pitting and strong and mild ginger blotch were identified as *P. tolaasii*, *P. costantinii* and several groups of '*P. gingeri*' respectively. Selected representative isolates were whole genome sequenced. New TaqMan assays have been developed based on the recently obtained whole genome sequences, to detect groups of pathogenic *Pseudomonas* that were not detected by previously assays (2).

The number of outbreaks of compost green mould caused by *Trichoderma aggressivum* f. *europaeum* has generally been reduced through the implementation of sanitation measures, but the disease still occurs and early detection could be used to inform control strategies and to monitor general farm hygiene. Cultures obtained from mushroom substrates show that *T. aggressivum* f. *europaeum* was prevalent on two farms. PCR assays were selected for the detection of *Trichoderma* spp. at genus, species and subspecies level.

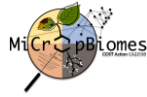
A study of microbial communities in cropping substrates obtained from four commercial mushroom farms, sampled at different cropping stages, was conducted to compare populations in healthy and diseased crops. Microbiome sequencing of bacterial and fungal communities showed differences between substrate and casing and some differences between farms. The methods used did not allow the identification of different *Pseudomonas* species, but successful detection of *Trichoderma* was achieved. A specific qPCR assay developed at Fera for *T. aggressivum* and MinION ITS sequencing detected these pathogens in mushroom casing at concentrations that did not produce visible green mould symptoms.



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**Funding:** *The project 'Improved understanding and control of bacterial blotch and green mould in mushroom production' (M065) was funded by The Agriculture and Horticulture Development Board (AHDB) of the UK.*



# Session 2

## **Crop microbiomes and plant diseases: from dysbiosis to increased defenses**

Chairs: Joël F. POTHIER & Renata ARTIMOVÁ

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## Cell-cell bacterial signaling in the plant pathobiome and microbial solutions for a more sustainable agriculture

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**Keywords:** pathobiome; quorum sensing; bacteria; signalling; SynComs

Plant health is thought to heavily depend also on its microbiome and signaling among microorganisms is crucial for the establishment of the microbial community. Understanding how bacteria undergo interspecies and interkingdom signaling in the microbiome is an important challenge for future studies. My laboratories study the plant microbiome at the site of infection as it could reveal potential commensal/resident bacteria that can communicate and cooperate with the pathogen: the concept of monostrain/monospecies infections is changing as different studies are beginning to indicate interactions between pathogens and the residential microbiota. In addition, understanding cell-cell communication systems among bacteria in the microbiomes will also shed light in their cross-talk and synchrony of these behaviors. Finally, these studies together with network analysis of plant microbiota, isolation enrichment strategies and the use of SynComs are helping us for the design of microbial solutions for a more sustainable agriculture.



## Unravelling the complex interaction between a fungicide, a candidate biocontrol agent and the bacterial wheat microbiome

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**Keywords:** Fungicide; Zymoseptoria; *Xanthomonas translucens*; biocontrol: wheat bacterial keystone species

Little is currently known about the influence of fungicide treatment on the wheat microbiome at the phyllosphere level. The perspective of emerging new bio-based biocontrol agents (BCA) is also stimulating the question of the impact of such treatment on the cereal bactobiome. Here we compared the effect of fungicide cereal treatment (REVYSTAR® GOLD) with a *Pseudomonas* candidate BCA on the wheat microbiome, at field level. A GFP-tagged BCA was used to highlight the distribution and presence of the strain in cereals.

We also highlight the complex interplay between a bacterial (*Xanthomonas translucens* pv. *undulosa*) or a fungal (*Zymoseptoria tritici*) plant pathogen and BCAs.

The impact of the cereal treatments on the cereal microbiome was analyzed via an innovative combination of the Flongle Oxford Nanopore sequencing technology and the newly developed PRONAME pipeline, providing crucial knowledge on keystone cereal bacteria and allowing for the identification of new or unusual cereal-associated species (1). This study also shows how metagenomic analysis of cereal microbiome is offering a strategic tool for the development of cereal integrated plant health management in the future.

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**Funding:** This research was funded by the Belgian Walloon Region ANTAGONIST project and the FRIA-FNRS.



## Phytopathogens-induced plant response, assemblage and network of the wheat rhizosphere microbiota

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**Keywords:** Fusariosis ; Rhizobacteria; Microbiota assembly; ISR; RNA-Seq analysis

*Fusarium* infection is a serious cereal disease that can cause damping-off, crown rot, and fusarium head blight. With the proposed EU-wide reduction in pesticide usage (already implemented in some member states), there is a pressing need for viable alternatives that offer comparable efficacy. Biological control stands out as a promising opportunity for developing environmentally friendly pest control mechanisms. To advance this, it is essential to evaluate the cost-benefit ratio of using biocontrol agents in agriculture. Additionally, it is crucial to understand how plants react to phytopathogens compared to phytobeneficial bacteria and how the latter enhance the plant's defense response to control the disease. Similarly, the role of the plant immune system in the assembly of a microbiome in the rhizosphere must be determined. In this study, we investigated the response of wheat seedlings to inoculation with *Pseudomonas brassicacearum* or *Paenibacillus polymyxa* strains, followed by a subsequent challenge with *Fusarium graminearum* or *F. culmorum* using an RNA-Seq approach. The structure of the rhizosphere-associated microbiota was analyzed through 16S, 18S, and ITS metabarcoding. The microbial assemblage and interaction network of the rhizosphere microbiota changed considerably following the *F. culmorum* challenge. A set of differentially expressed genes (DEGs) was identified for each treatment based on significant fold-change expression (adjusted  $p \leq 0.05$ ) compared to the respective mock treatment. Nearly 7,700 genes were modulated in the roots, and more than 12,000 in the shoots. While *F. culmorum* infection caused a massive reprogramming of gene transcription in wheat seedlings, the gene transcription in the roots of seedlings inoculated with each of the three bacterial strains remained nearly unchanged. Instead, these inoculated bacteria primarily modulated gene transcription in the shoots. Overall, the introduction of



phytobeneficial bacteria in the soil induces a shift in the assemblage and interaction network of the rhizosphere microbiota and triggers systemic resistance by modulating aboveground plant genes. Conversely, infection of aboveground plant tissue leads to the recruitment of a different root-associated microbiota, which may have the potential to protect the plant.



## A look behind the curtains: exploring the dynamics of tripartite interactions between plants, bacteria and bacteriophages

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**Keywords:** bacteriophages, plant-microbe interactions, *Pseudomonas*, plant immunity, microbiome

Bacterial diseases are responsible for major losses in vegetable crop production, and are particularly difficult to manage in close cultivation systems, aka greenhouses. While EU regulations still limit genome editing of plants, and in the context of increasing antibiotic resistance in the environment, bacteriophages (phages) appear as promising biocontrol agents. Indeed, bacteriophages are highly specific bacterial pathogens, self-amplifying and self-eliminating, with no detrimental impact on the plant host metabolism (Korniienko et al., 2022a). However, despite the promising laboratory trials, there are several limitations of phage field application, such as an unknown impact on plant-associated microbiome, fast evolution of the microbial community, but also - the lack of knowledge about the interactions between phages and plants.

We are dedicated to develop a working the strategy of protection of greenhouse tomatoes from bacterioses using bacteriophage-based approach. We have established a pipeline of bacteriophage isolation from the plant and environmental samples, and now investigate the tripartite interactions between phages, bacteria and plants using the model system consisting of *Arabidopsis thaliana*, hemibiotrophic bacteria *Pseudomonas syringae* pv *tomato* and our newly characterized phages Eir4 and Eisa9 (Korniienko et al., 2022b). We found that lytic bacteriophages are capable to persist in plant tissues, and pre-treatment with phage preparations has a priming effect on plant immune system. In particular, phage preparation application itself triggered a mild activation of defence responses (transcriptome remodeling, callose deposition, ROS production), and enhanced the physiological defences upon subsequent infection with virulent pathogen. At the same time, we have observed development of phage resistance in the pathogen population, emerging resistant bacterial strains having



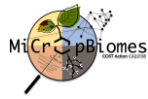
mutations in genes encoding for glycosyl transferases, likely involved in production of cell wall lypopolysaccharides. Notably, three out of four phage resistant isolates showed lower virulence *in planta*, suggesting it as another mechanism of decreasing disease severity, thus beneficial for practical application. We now investigate an impact on bacteriophages on leaf- and root- associated microbiome composition, which itself is a powerful indirect actor in plant immunity (Kalachova et al., 2023).

We aim to shed light on the mechanisms of interactions between plants, pathogenic bacteria and phages, opening the use of bacteriophages as efficient customizable biocontrol for sustainable agriculture.

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**Funding:** The work is supported by Technological Agency of Czech Republic (TAČR, grant no. TQ03000088). IEB Imaging Facility is supported by a project of the Ministry of Education, Youth and Sports “National Infrastructure for Biological and Medical Imaging (CzechBioImaging – LM2018129)”. NK and OB have received individual fellowships from the Visegrad Fund and FEMS.



# Session 3

**From current microbial inoculants to synthetic communities**

Chairs: Anna BONATERRA & Claire PRIGENT-COMBARET

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## Functional peptides in microorganisms and its potential in plant disease control

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**Keywords:** secondary metabolites; mechanisms of action; synthetic microbial communities.

Functional peptides (FPs) are considered one of the key components that influence or even regulate the interactions between the microbial components in the microbiome of plants and the interplay with the plant host. FPs are secondary metabolites produced by bacteria (cyclo-lipopeptides, cyclo-depsipeptides, bacteriocins, pseudopeptide antibiotics) and fungi (peptaibols, cyclic dipeptides, defensin-like peptides)(1, 2). Most of these peptides are antagonistic, by means of a lytic effect on competitor cells, but also through interaction with external structures necessary for pathogenesis (biofilm, LPS, motility) in plant pathogens, or affecting internal cell processes (DNA replication, protein synthesis, etc.). Other peptides have a priming effect on the plant by inducing defence responses against pathogens or by mitigation of abiotic stress. From the wide diversity of compounds and activities found, one can expect an enormous complexity of the interactions among the microorganisms within the plant microbiome. The discovery of new compounds produced by the non-culturable microorganisms in the plant microbiome through metagenomics is therefore of great importance. From the practical point of view, the capacity of certain strains of bacteria and fungi for the production of antimicrobial peptides (3), have been exploited to develop microbial biopesticides for plant disease or pest control. In the last years, a technology of biocontrol for developing commercial biopesticides, based on the use of synthetic microbial communities (SynComs) instead of using single strains, aims to create beneficial microbiomes associated to the plant crop. However, their development requires that the strain components have to be compatible and with different mechanisms of action to provide synergistic effects. I will present some examples and ongoing projects in this field, where a deep knowledge of the crop microbiome will help for the development of novel biological control strategies of pests and diseases of crops.



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## Fate of *Bacillus velezensis* and *Lactiplantibacillus plantarum* strains applied to apricot, peach, and grapevine crops

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**Keywords:** biological control agent; viability qPCR; population dynamics

*Bacillus velezensis* A17 and *Lactiplantibacillus plantarum* PM411 strains are two microbial biological control agents under development as plant protection products. These two strains were selected among a large collection of strains of *Bacillus* spp. and lactic acid bacteria (LAB) in previous studies (1, 2). The A17 strain was isolated from the flowers of *Lobularia maritima*, whereas the PM411 strain was isolated from the surface of a pear fruit. Both strains are well characterized in relation to antagonistic activity against plant pathogens, production of antimicrobial compounds, and plant defence response induction, and a strain-specific qPCR method has been developed for field monitoring. The ecological fitness of the strains was evaluated in different crops, geographical zones, and growing seasons (3). Both strains ( $2 \text{ g L}^{-1}$  of dried formulation) were spray-inoculated on apricot trees, peach trees, and grapevines. Depending on the crop, flowers, fruits, and leaves were picked at several sampling time points. The population dynamics of viable, viable but non-culturable, and dead cells were studied by comparing viability qPCR (v-qPCR), qPCR, and plate counting estimations. A17 showed high survival rates in apricot, peach, and grapevine organs. The A17 viability was confirmed since qPCR and v-qPCR estimations did not significantly differ and were rather constant after field applications. However, higher population levels were estimated by plate counting due to the non-selective characteristics of the growth medium used. The viability of PM411 was constrained by plant organ, crop, and climate conditions, being higher in apricot than in grapevine. PM411 survival declined after field application, indicating difficulties in its establishment. The PM411 field population was made up of dead, culturable, and viable but non-culturable cells, since significant differences between the three methods were observed. In conclusion, A17 and PM411 differ strongly in their survival in grapevine, peach, and apricot, suggesting the importance of survival and adaptation to stress in colonization of crops.



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**Funding:** Support was provided by the European Regional Development Fund (ERDF) through the Interreg V-A Spain France Andorra programme (POCTEFA 2014–2020) [grant number PALVIP EFA182/16].



## From suppressive compost to targeted inoculants: synthetic microbial communities promote tomato growth and disease control

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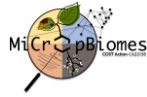
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**Keywords:** Suppressive compost; synthetic microbial communities (SynComs); tomato; *Fusarium oxysporum*; *Verticillium dahliae*

Soilborne pathogens such as *Fusarium oxysporum* and *Verticillium dahliae* represent a hard-to-control threat for many economically important crops. Suppressive composts represent an eco-friendly approach to control soilborne plant pathogens and enhance plant growth. This study aimed to design functional synthetic microbial communities (SynComs) promoting tomato health by harnessing insights from a previous exploration of suppressive compost microbiota. Two SynComs were constructed using representative bacteria isolated from the rhizosphere of tomato plants grown in a suppressive compost. SynCom1 consisted of a taxonomically diverse bacterial mix, while SynCom2 included only *Bacillus* strains. Pot experiments evaluated the effects of SynCom application via two methods: root drench in sterile substrate and seed biopriming. Root drench application revealed that SynCom1 benefitted tomato growth in pots, while SynCom2 promoted tomato growth but also suppressed disease symptoms caused by *Fusarium*. Seed biopriming with both SynComs promoted tomato growth and protected plants against both *F. oxysporum* f. sp. *lycopersici* and *V. dahliae*. These findings highlight the critical role of microbial community composition in influencing tomato health. This study also demonstrates the potential of using microbiota deriving from suppressive compost to design targeted SynComs as tomato inoculants. Characterizing key bacterial traits can facilitate the development of defined, controllable microbial communities with consistent plant growth promotion and disease suppression capabilities in tomatoes.



# Session 4

## Crop-Microbiome assembly dynamics under specific environments

Chairs: Elodie VANDELLE & Gordana RACIĆ

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## Bacterial priming responses of resistance and growth parameters in spring barley

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**Keywords:** AHL, *Ensifer*, *Bacillus*, barley accessions, genotype-strain dependence

The effects of priming with quorum sensing molecules and/or bacteria on plant disease resistance and plant growth are well documented under controlled conditions. Evidence for their efficacy under field conditions is accumulating but is mainly based on a limited number of genotypes. We investigated and compared the effects of bacterial priming: either based on N-acyl homoserine lactone (AHL) producing *Ensifer meliloti* strains (expr<sup>R3</sup>, atm, and expr<sup>R+ch</sup>) or *Bacillus* species (*B. pumilus* and *B. velezensis*). The priming efficiency of *E. meliloti* for leaf rust (1) and net blotch resistance (unpublished) was previously investigated in a diverse set of 198 spring barley accessions (IPK-SB224 panel) under greenhouse conditions. Responses were genotype dependent. Genotypes that positively responded to bacterial priming for resistance to both diseases ( $p < 0.001$ ) were selected for further experiments. Root and shoot growth of plants were promoted by C14 AHL in a hydroponic system. Results from a newly established greenhouse dual-pathogen response test (*P. hordei* and *P. teres f. teres*) indicate that the priming efficiency of *E. meliloti* expr<sup>R+ch</sup> is genotype dependent and may differ from the results obtained in single-pathogen system. The same genotypes were studied in field trials. Seeds were coated with *E. meliloti*, *B. pumilus* and *B. velezensis* (ABiTEP GmbH) before sowing, with additional bacterial drenching into the rhizosphere for *E. meliloti*. Plant growth parameters and symptoms of naturally occurring diseases were evaluated during vegetation. Priming inducers were detected in the rhizosphere of treated plots, but not in the control plots or in the bulk soil, indicating ongoing growth of the priming inducers applied. Reductions of naturally occurring leaf rust and powdery mildew symptoms, as well as changes on yield related parameters were genotype and strain dependent. Further field and greenhouse experiments are underway to confirm the observed plant genotype-strain dependence.



A plant genotype-dependent rhizosphere microbiome was also detected in the same set of spring barley accessions (2), requiring a microbiome-integrated approach to further investigate the genetic basis of plant genotype-strain dependence of priming responses.

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**Funding:** Presented data is part of the project “Priming for enhanced defense as a strategy to optimize crop resistance and as a possible breeding target - PrimedPlant3” financed by Bundesministerium für Bildung und Forschung (FKZ: 031B0196). This presentation is based upon work from COST Action CA22158 CropBiomes, supported by COST (European Cooperation in Science and Technology).

## Drought influences the fungal community structure, diversity, and functionality inhabiting the grapevine xylem and enhances the abundance of *Phaeomoniella chlamydospora*

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**Keywords:** abiotic stress; esca; global warming; microbiome dynamics; Petri disease; water deficit

Grapevine productivity worldwide is increasingly threatened by global warming, potentially exacerbating water scarcity. This study examines drought's effects on grapevine xylem's fungal communities, particularly regarding *Phaeomoniella chlamydospora*, linked to esca and Petri diseases. Investigating one-year-old grafted grapevines under varied water regimes, including severe, moderate, and no water deficit, the research assesses how drought influences fungal diversity, structure, and interactions within the xylem. Utilizing ITS high-throughput amplicon sequencing and droplet-digital PCR, the study tracks changes in fungal composition and *P. chlamydospora* prevalence over two growing seasons. The induced water stress not only altered the diversity and composition of the fungal microbiome in the xylem vessels but also affected co-occurrence networks, resulting in less complex networks with fewer correlations between taxa, potentially increasing grapevine vulnerability to various biotic and abiotic stresses. Severe water deficit significantly reduced microbial diversity, leading to a shift in the abundance of pathotrophs such as *P. chlamydospora* in the xylem. This underscores the interconnectedness between water stress, microbiome dynamics, and plant health. The combination of compromised plant defenses, altered physiological conditions, and shifts in the surrounding microbial community may create conditions conducive to increased *P. chlamydospora* abundance in the xylem vessels of young vines following water stress.



**Funding:** *This study has been funded by the GLOBALVITI project under the Strategic Program for Consortia of National Business Research (CIEN) (Spanish Ministry of Economy, Industry and Competitiveness, Center for Industrial Technological Development (CDTI)).*



## Interaction between soil microbiota, host plants, and stem parasitic plants of the genus *Cuscuta* under salinity

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**Keywords:** dodders, plant parasitism, soil enzymes, soil microbiota

Stem holoparasitic plants of the genus *Cuscuta* represent a major lineage of above-ground, economically important parasitic flowering plants. Their direct negative effect on host plants' growth and development is due to the exhaustion of photosynthates, minerals, and water. Besides this, they also serve as vectors of phytopathogens and were found to display a top-down effect on the soil microbiota. In the present study, we aimed to explore the combined effect of salinity and plant parasitism on the soil microbial diversity in the salt-sensitive model plants *Arabidopsis thaliana* and the salt-tolerant *Eutrema salsugineum*. Four soil enzymes, responsible for C, N, P, and S cycling and markers for soil health, along with 16S and 18S rDNA metagenomic analyses of prokaryotic and eukaryotic microbial diversity were used to characterize the occurring changes. All four soil enzymes tend to decrease in activity with increasing salinity, affecting negatively element cycling. *Cuscuta* parasitism leads to a less pronounced effect and trend toward preserved activity. Ascomycota and Chlorophyta were substantially affected by salinity and *Cuscuta* parasitism, but the relative abundance of individual bacterial phyla was not affected. While alpha diversity was affected by both salinity and *Cuscuta* parasitism, beta diversity was defined by the host plant, rather than abiotic or biotic stress.

**Funding:** This study is financed by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project No BG-RRP-2.004-0008 and grant KP-06-COST/09 of the National Science Fund, Ministry of Education and Science, Bulgaria, as a co-funding scheme for the COST Action CA22158 MiCropBiomes.



## Microbiome profiling of potato roots and leaves grown under organic vs integrated pest management

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**Keywords:** endophytes; 16 metagenomics; *Solanum tuberosum*; pest management practices

We sampled >500 leaves and roots of different potato cultivars grown in fields in Slovenia and isolated RNA from both surface-sterilized and non-sterilized material. We performed 16S rRNA metagenomic sequencing of amplified V4-V6 regions. We analysed the data using the QIIME2 pipeline utilising SILVA database, followed by community diversity analysis, statistical analysis of taxa abundance and dataset visualization in R and Python. We explored in more detail the impact of organic vs integrated pest management (IPM) practices on the microbial communities. We found that root community diversity was lower in IPM-managed fields compared to organic farming. We also identified bacterial clades with significantly different abundances between the farming practices. We plan to also further examine the bacterial taxa identified in the sterilised tissues as these might contain potential novel endophytes. Our findings contribute to understanding of the impact of pest management strategies on the interactions between plants and their microbiomes which is crucial for improving the sustainability and resilience of modern agriculture.

**Funding:** Support was provided by the Slovenian Research and Innovation Agency (ARIS) projects J4-3089, P4-0165 and P4-0431.



## The influence of fruit microbiota on ripening and quality and its dynamic during post-harvest

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**Keywords:** postharvest management, culturable microbiome, bacteria, fungi, PGPB, *Actinidia chinensis* var. *chinensis*, *Rubus idaeus*, chilling injuries, fruit firmness, VOCs, *Botrytis cinerea*

The carposphere microbiota of different fruit, namely raspberry and kiwifruit was dissected by using next generation sequencing (NGS) and culturomic methods for the fictional characterization of the different isolates. Fruit microbiota was correlated with the main quality parameter, such as soluble solid contents, aroma formation, fruit firmness and colour. In raspberry, the contribution of fruit-associated microbiota to volatile emissions was evaluated by performing an untargeted GC–MS analysis of VOCs occurring in control (C), sterile (S) and artificially reinoculated berries (R). The treatments significantly affected the fruit volatiles, thus confirming the role of bacteria in fruit aroma construction. In particular, aldehydes, monoterpenes, norisoprenoids, and other aroma-active compounds were significantly lower in S raspberries, and recolonisation could only partially restore the emission of terpenoid compounds. Several VOC-emitting bacterial taxa (including *Bacillus*, *Lactobacillus*, *Methylobacterium*, *Paenibacillus*, *Pseudomonas* spp.) are recurrently found in the raspberry-associated microbiome, suggesting that future applications aimed at the control of microbial colonisation may enhance fruit aroma. In kiwifruit, approximately 250 fungal and yeast and 500 bacterial strains were identified. Bacterial strains were also characterized for their potential beneficial functions and their ability to grow at low temperatures. Sampling was performed during first two months of storage with the aim to identify the fungal and bacterial species specifically selected by cold storage. Eight bacterial species were selected for post-harvest treatments on fruit to assess their potential ability to prolong storage, reduce losses or increase fruit quality. Different strains showed the potential to inhibit *Botrytis cinerea* growth. Furthermore, six strains were able to increase sugar content in fruit, however the effect was related to an increase in softening suggesting that these strains were inducing an earlier ripening. Interesting, the inoculation generally increases storage breakdown suggesting a possible microbial component of this physiological disorder.



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**Funding:** *Part of the experiments was performed in the facilities provided by the Agritech National Research Centre and received funding from the European Union Next-Generation EU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN0000022), spoke 2, Task 2.2.5. This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.*



# Session 5

## Communication, and engagement with society

Chairs: Caroline DE TENDER & Ralf KOEBNIK

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## Fast track to environmentally adapted bacteria for soybean growth at northern latitudes

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**Keywords:** Soybean, indigenous rhizobia, Flanders

Soybean (*Glycine max*), the world's most extensively farmed legume, serves as a crucial source of plant-based protein for both human diets and animal feed. However, due to its non-native nature, a significant yield gap must be crossed to produce soybean in northern latitudes. One strategy to overcome this is to develop rhizobial inoculants from strains isolated locally. In the 'Soy in 1000 gardens' project, we performed a large-scale trapping experiment for the identification of local soybean-nodulating rhizobia throughout the region of Flanders. With the help of 1200 citizen scientists, soybean was grown in their gardens, which allowed the identification of five *Bradyrhizobium* strains with great potential for their application in agriculture. These strains performed at least as well as the commercial inoculum, both in pot trials and in the field, showing the importance of identifying locally adapted rhizobia. The soil analysis provided information on parameters that influence nodulation, and the nodule microbiome analysis revealed the main taxonomic groups colonizing soybean nodules in Flanders. However, the high presence of some non-fixing genera such as *Tardiphaga* sp. as well as the high diversity found in most of the nodules pointed towards the rarity of efficient indigenous soybean-nodulating strains in this region. To summarize, we were not only able to raise public awareness about the benefits of legumes and microbes, but were also able to deliver indigenous strains, which performed well in the complex environment of the field, reflecting the original goal of our study to identify rhizobia



adapted to local conditions. Moreover, we present a fast and efficient pipeline that can be employed in other regions.

**Funding:** Support was provided by the Grand Challenge project GC03-C02: Soy in Flanders - Catching Rhizobia to Introduce High Protein Containing Soybean for a Sustainable Agriculture in Flanders.



## Unravelling mode-of-action of biocontrol agents to combat fungal diseases in wheat

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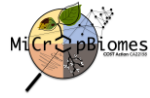
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Wheat is one of the most important crops worldwide with an annual production exceeding over 750 million tonnes. In Europe, wheat derived products are the main source of calories. With an ever-growing world population, wheat yields must remain stable, if not increasing. To protect crops against diseases and the subsequent yield losses, farmers rely heavily on the use of chemical pesticides. However, pesticides can be harmful to humans and other animals, as well as the environment. To mitigate those effects, the European Union has issued a set of initiatives in the Green Deal. A part of the Green Deal is the Farm to Fork strategy, which aims to reduce the use of pesticides by 50% by 2030. The increasing need for stable wheat yields driven by the increasing world population, along with the reduction of pesticide use encouraged by the Green Deal, create the demand for newer, environmentally friendly pesticides.

This project is in collaboration with Apeha.Bio, a company specialized in production of biological pesticides. The company houses a high-throughput screening platform that allows to discover the microbial dark matter and screen for microbial strains with potential biocontrol activity. Using this platform, three lead strains with very promising biocontrol activity against the fungal diseases Septoria Leaf Blotch (SLB), yellow rust and Fusarium Head Blight (FHB) have been isolated and screened. However, insights into the mode-of-action, i.e., how the strains work against the pathogens, remains unknown thus far.

To achieve this, we first want to study the interaction of the biocontrol agents (BCA) with the pathogen. Using (confocal) imaging, we will study the interaction both *in vitro* and *in planta* between the BCA and pathogen (*Fusarium graminearum* and *Zymoseptoria tritici*). To this end, we have generated a collection of tagged strains, including fluorescent proteins, luciferase and the state-of-the-art SNAP-tag. To monitor the behaviour of BCA and pathogen in the field, we have optimized a multiplex digital droplet PCR assay.

Together, these results will give a deeper understanding in the mode-of-action of the BCA against two of the most important plant pathogens in wheat.



# POSTERS



# Session 1

**Exploiting Plant-Microbiome molecular crosstalks: diversity,  
distribution and eco-evolutionary perspectives**

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## Genetic and microbiome attributes associated with berry development and aromatic potential in grapevine cultivars

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**Keywords:** grapevine microbiota; aromatic profile; berry microbiome

Climate change has severe consequences on grapevine productivity, yield and quality. Thus, studying gene networks, metabolic pathways and microbiota factors associated with proper growth, fruit development and plant resilience is important for exploring suitable systems towards addressing climate pressures without compromising berry quality. The quality characteristics of grapevine berries and the aromatic potential of wine involve molecular mechanisms regulating gene expression and biochemical pathways of secondary metabolite synthesis as well as the structure/function of microbiota communities. In the current work a comparative study of a set of genes involved in the quality characteristics and aromatic profile of grapes as well as analysis of microbiomes was performed in different grapevine varieties from Greece. In particular, the expression of genes encoding proteins such as terpene synthetases, monoterpene glycosyltransferases and transcription factors involved in the biosynthesis of secondary metabolites has been assessed across four berry developmental stages (pre-veraison, veraison, mid-veraison and maturity). Substantial differences in gene expression dynamics were witnessed among different varieties and locations. Microbiome analysis has been undertaken to unveil variable microbiota structures associated with grapevine tissue, genotype, and terroir attributes and how they might relate to the genotype- and terroir- specific mode of gene regulation. Findings will be discussed in the context of regulatory mechanisms and microbiota communities pertaining to berry secondary metabolites, aromatic profiles and varietal/terroir traceability.

## ***Thuricin17* signaling influence microbial dynamics in Canola**

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We studied the effect of three concentrations of Thuricin17 (Th17), a plant growth-promoting rhizobacterial signal molecule produced by *Bacillus thuringiensis*, on germination and vegetative growth of canola (*Brassica napus* L.) under stressful temperatures. We used Th17 in canola grown in sandy loam and clay soil in the experimental firm, to see the effect of Th17 on microbiota in root and rhizosphere soil. *Candidatus saccharimonas* and an unclassified Rubinisporaceae bacteria were identified as indicator species in root associated microbiota in clay loam field and sandy loam field, respectively. Four bacterial ASVs (*Rosemicrobium* sp., *Sphingomonas* sp and two unclassified Planctomycetes) were identified as indicator species in soil microbiota in both clay loam and sandy loam field. We found two planctomycetes bacteria as core, as-yet-unclassified phycisphaera-related group WD2101 bacteria and *Humisphaera borealis* in clay loam and sandy loam field, respectively. However, ASV8 (unclassified Rubinicporiaceae bacteria) was identified as both indicator species and core microbiota. Although thuricin17 treatment did not show significant effect on the microbial diversity, it showed independent effect on root and soil microbial communities. There was no significant difference in the alpha diversity of the community. The exploratory path model analysis showed that 31 ASVs were identified as endogenous variables, and five of the variables were exogenous, including bacterial ASV37, seed treatment, foliar treatment, clay and sandy loam soil type. For 19 bivariate relationships, the path analysis revealed statistically significant causal relationships where the calculation of Spearman's correlation coefficients would not have produced conclusions that these effects were statistically significant. Th17 tends to have a stimulatory impact at stressful temperatures, hence, supplementation with Th17 would have the potential as a plant growth promoter under stressed circumstances.

## Wild berries microbiomes and the importance of their compounds in biocontrol

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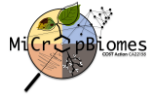
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**Keywords:** Microbiome; Biocontrol yeast; Volatilome; Wild berries

Lingonberries (*Vaccinium vitis-idaea* L.), rowanberries (*Sorbus aucuparia* L.), and rosehips (*Rosa canina* L.) are the most common wild berries in Northern Europe. These berries due to high content of bioactive compounds are attractive in the food industry and traditional medicine and may be involved in the spreading of microorganisms from the natural environment into agricultural ecosystems. Various microorganisms, especially yeasts, act as biological control agents by producing volatile organic compounds (VOCs), killer toxins, etc, which could modulate plant microbiome and may be important for developing natural tools for plant disease management and will have potential in safe food production. During our study, the Next Generation Sequencing-based metagenomic analysis of bacterial and fungal communities distributed on the surface of lingonberries, rowanberries, and rosehips was performed. According to alpha and beta diversity analyses, significant differences in the taxonomic composition of microbiota were observed. The bacterial community on rosehips was shown to be prevalent by Enterobacteriaceae, lingonberries - by Methylobacteriaceae, and rowanberries - by Sphingomonadaceae representatives. Among fungal microbiota, Dothioraceae dominated on rosehips, Exobasidiaceae on lingonberries, meanwhile, rowanberries were inhabited by a similar level of Aureobasidiaceae, Dothioraceae, Bulleribasidiaceae, and Filobasidiaceae fungi. Various yeasts were isolated through culture-dependent techniques, followed by molecular identification. The biocontrol ability of yeasts was tested based on the antagonistic activity against control strains and a broad spectrum of microorganisms. Subsequently, four isolated yeast species (*Hanseniaspora uvarum*, *Metschnikowia pulcherrima*, *Saccharomyces cerevisiae*, and *S. paradoxus*) were tested to assess their production of VOCs. The gas chromatography and mass spectrometry analysis revealed that the patterns of volatiles vary depending on tested yeast species but are dominated by alcohols and esters.



Our findings highlight the diverse plant-defined nature of microbial populations and demonstrate the high potential of counterparts for biocontrol.

**Funding:** *This research was funded by the Research Council of Lithuania (S-PD-22-058). This presentation is based upon work from COST Action CA22158 MiCropBiomes, supported by COST (European Cooperation in Science and Technology).*

## Volatile compound-mediated interactions between *Armillaria* and *Trichoderma*: implications for biocontrol strategies

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**Keywords:** Biocontrol; Volatile-based communication; GC-MS analysis; *Trichoderma atroviride*; *Armillaria ostoyae*

A complex network of biochemical signalling and ecological interactions between fungi is exemplified by the interaction between *Armillaria ostoyae* and *Trichoderma atroviride* (1), involving volatile organic compounds (VOCs). The aim of this study was to understand the basis of VOC production in each species and to explore the VOC-related background of the use *T. atroviride* as a biological control agent (BCA) against *A. ostoyae*. Using a dual culture assay (face-off between separately growing colonies), gene expression profiling, and GC-MS analysis, we aim to clarify the molecular communication between *A. ostoyae* and *T. atroviride* through VOC-mediated interactions. Our study aims to identify regulatory pathways that control VOC production based on sensing VOC from the other partner among both fungi through differential gene expression analyses. *T. atroviride* produces a range of VOCs including strong irritants, as 6-pentyl-2H-pyran-2-one (6PP). The compounds trigger the upregulation of gene expression connected to diverse chemical defence mechanisms and possibly necrosis in *Armillaria ostoyae*. This study aims to provide insight into the complex molecular dialogue between *A. ostoyae* and *T. atroviride* in order to identify a potential VOC target for fungal ecology and biocontrol strategies. The results obtained will provide a foundation for future research to understand fungal-fungal interactions at the level of VOCs for use in the development of agriculture, forestry, and environmental sustainability.

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## Interplay between the defense system of plants and the microbiome

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**Keywords:** plant defense; *Pseudomonas simiae* WCS417; microbiome; exudation; plant root

Plant roots interact with a diverse array of soil microbiota. Despite the presence of harmful microbiota in soil, plants usually remain healthy. We previously discovered that coumarins, a class of secondary metabolites, influence root microbiome assembly, favouring beneficial microbes over pathogens. Besides coumarins, structural defenses (cutin, suberin, callose, lignin) and chemical defenses (camalexin, glucosinolates) also act as gatekeepers in plant-microbiome interactions. To explore the roles of these components, we screened various *Arabidopsis* mutants *in vitro*, focusing on their interaction with the beneficial rhizobacterium *Pseudomonas simiae* WCS417. Some mutants exhibited reduced beneficial effects and impaired root colonization. Transcriptomic analysis revealed that camalexin affects bacterial chemotaxis, while other defense components mediate a growth-defense trade-off during colonization. We also assessed the effects of these defense components on microbiome assembly by growing mutants in soil and analysing unplanted soil, rhizosphere soil, and roots. This helped identify microbiota enriched in each defense mutant compared to wild-type plants and in specific compartments. Our findings highlight a compartment-specific influence of defense components on the microbiome and the enrichment or depletion of different microbial taxa when the function of a defense component is misregulated. Our goal is to understand how these defense components contribute to plant-microbiome homeostasis and apply this knowledge to develop microbiome-assisted agricultural strategies.

**Funding:** Support was provided by the Dutch Research Council (NWO/OCW), as part of the MiCRoP Consortium programme, Harnessing the second genome of plants (Grant no. 024.004.014).

## Bacterial community composition and antibiotic resistance in Lithuanian farmlands

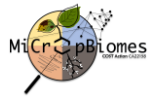
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**Keywords:** antibiotic resistant bacteria; 16s metagenomics; farmland microbiome; microbial diversity

Bacteria are ubiquitous in diverse environments, exerting significant influence as one of the most abundant microorganisms in nature. In farmland ecosystems, bacteria are crucial for soil fertility, plant growth, and the overall sustainability of agricultural practices. Understanding the genetic diversity of these microbiomes and their community associations is crucial for enhancing agricultural productivity and sustainability. It also helps address public health concerns related to antibiotic resistance. This study aims to investigate the distribution of bacteria in farmland ecosystems, focusing on soil, feed, and freshwater. Samples were collected from four different pastoral dairy farmland locations around Lithuania. The bacteria were identified using a combination of cultural and molecular methods, and 16S metagenomic analysis was used to understand the bacterial community composition in different environments within the farmland ecosystem. The results showed that in soil, the most abundant genera were *Pseudomonas*, *Nocardioides*, *Rhizobium*, and *Streptomyces*. Grass samples were dominated by *Pseudomonas*, *Sphingomonas*, *Rhizobium*, and *Massilia*. Water samples showed lower abundance of these genera, with *Acidobacteria*, *Nocardioides*, and *Pseudomonas* being the most prevalent. The study also found that 61 bacterial strains showed resistance against ampicillin, streptomycin, tetracycline, or chloramphenicol. A higher prevalence of antibiotic-resistant bacteria was found in soil samples, with 31 strains comprising more than half of all observed bacteria. High resistance was noted to both streptomycin and ampicillin, compared to other antibiotics. Additionally, several bacterial genera considered non-pathogenic or opportunistic to humans and animals were found in soil, water, and feed, including *Solibacillus*, *Enterobacter*, and *Acinetobacter* in soil, *Pantoea* in feed, and *Chryseobacterium* in water. However, potentially pathogenic genera, such as *Bacillus*, were also identified in soil samples. Exploring the genetic diversity of microbiomes and co-evolutionary relations is important for predicting community associations and identifying key molecular markers, which are essential for developing strategies to manage microbial communities effectively.



**Funding:** This project has received funding from the Research Council of Lithuania (LMTLT), agreement No S-MIP-23-7. 22-058). The performed studies are in frame with research networking of COST Action CA22158.

## An ERF transcription factor StPti5, a novel regulator of endophyte community maintenance in potato

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**Keywords:** Bacillus, potato, ERF transcription factor, knowledge networks, transcriptional regulation

As potato, the third most important crop in the world, is exceptionally sensitive to a wide range of stresses, studying its interaction with plant growth promoting bacteria is crucial to ensure an efficient environmentally friendly system of plant protection based on modulation of its microbiome. We investigated the mechanisms involved in potato - *Bacillus subtilis* interaction at the time of biofilm establishment on the root and later on when the bacteria are internalised. *B. subtilis* strains were isolated from tomato rhizosphere. RNA-seq analysis of roots and systemic tissue showed that immune responses in interacting roots were attenuated, while complex transcriptional network was triggered, including ERF, MYB, NAC and WRKY transcription factors, leading to induction of ISR in shoots 24h after biofilm production. At that time, *B. subtilis* was observed in systemic tissue, while its morphology was changed 24h later. The responses of plants are dependent on surfactin production by bacteria. The SrfA and ComQXPA mutants, that were not able to form biofilm on potato roots also triggered limited responses in the plant. We further studied plant responses to bacteria kin and non-kin interactions. We show that when kin strains were combined, there was no visible border between biofilm formed by each strain. On the other hand, when non-kin strains were combined, we observed biofilm growth separation and one of the strains dominated on the roots. Interestingly, the nonkin interaction of *B. subtilis* strains on the roots intensified the response of the plant to colonization. We focused our further research on the role of ERF transcription factor PTI5 in potato-*B. subtilis* interaction, as it was strongly regulated both in inoculated roots as well as in systemic tissue. While PTI5 is not involved in root biofilm formation, we show that bacteria abundance in systemic tissue is increased in PTI5 silenced plants indicating its involvement in balancing/limiting growth of internalized population of bacteria.

## Core microbiome of fungal and bacterial communities in different tissues of *Vaccinium* spp.

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**Keywords:** Agriculture, Biodiversity, Sustainable crop production, Holobiont, Metagenomic

Microorganisms colonise every part of the plant, from roots (rhizosphere) to leaf surfaces (phyllosphere), playing a crucial role in providing plants with nutrition, promoting stress tolerance/resistance, and modulating defence responses against pathogens. However, conventional agricultural practices, particularly the use of chemical products, can influence these associations by altering the plant's microbiome and causing a loss of biodiversity. Despite the growing interest in plant microbiomes, the role of microorganisms associated with perennial fruit plants and the factors influencing their composition has still not been studied in detail. To address this knowledge gap, in this study we evaluated the taxonomic composition of microbial communities colonising different plant compartments (leaves, fruits, and fruit endophytes) in ten blueberries (*Vaccinium* spp.) cultivars in two consecutive years. Our analysis included fungi and bacteria to determine the prevalence and relative abundance of various taxa. Fungal analysis revealed a dominance of Ascomycota and Basidiomycota, with a higher presence of Basidiomycota in fruit endophytes. Bacterial analysis showed a predominance of Proteobacteria, with Firmicutes more abundant in endophytes and Bacteroidetes on leaf surfaces. Additionally, we identified specific compositional patterns for each tissue analysed. Alpha diversity indicated higher fungal diversity in leaf epiphytes, while bacterial diversity was higher in fruit epiphytes. Beta diversity analysis confirmed significant differences between niches (epiphytic vs. endophytic) and across plant varieties and sampling years. Core microbiomes varied by niche, highlighting specific genera for the different compartments despite some similarities between the epiphytic compartments. Co-occurrence networks showed positive interactions within kingdoms and negative interactions between kingdoms, revealing complex microbial associations in plant ecosystems.

## Essential components of virulence of bacterial plant pathogen *Xcc* as revealed by dual RNA Seq analysis of the strains with epigenetically lowered virulence

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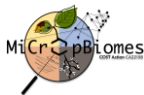
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**Keywords:** bacterial epigenetics; DNA methylation; virulence; *Xanthomonas campestris*; dual RNA-seq

A first step, selected strain of bacterial plant pathogen *Xanthomonas campestris* pv. *campestris* (*Xcc*) was treated by several epigenetic modulating chemicals working on different principles. Subsequently, genetic and phenotypic alterations were evaluated in differentially treated *Xcc* strains. As evident, some DNA demethylating chemicals unambiguously caused a durable decrease in *Xcc* bacterial virulence, even after its re-isolation from infected plants. The first-time use of chemicals to modify the activity of sirtuins also showed some noticeable results in terms of increasing bacterial virulence, but this effect was not typically stable. The molecular basis of the altered virulence was deciphered by using dualRNA-seq analysis of treated *Xcc* strains infecting *Brassica rapa* plants. Enriched KEGG analysis results identified the genes representing “bacterial secretion system” as the most different between variants with decreased virulence. Namely, the most different cluster belongs to the group *hrp* (hypersensitive reaction and pathogenicity) and *hrc* (hypersensitive response conserved) genes, representing genes involved in the type III secretion system (T3SS), which is crucial for the pathogenesis of *Xanthomonas* pathogens [1]. KEGG pathway analysis also highlighted the importance of the gum operon. The results of the present study should promote more intensive research in the generally understudied field of bacterial epigenetics, where artificially induced modification by epigenetic modulating chemicals can significantly increase the diversity of bacterial properties and potentially contribute to the further development of the topics, such as bacterial ecology and adaptation. For more details, see [2].



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**Funding:** This work was supported by the project LTC18009, program INTER-COST provided by the Ministry of Education, Youth and Sports of the Czech Republic and by the project EFRR “Multidisciplinary research to increase application potential of nanomaterials in agricultural practice” (No. CZ.02.1.01/0.0/0.0/16\_025/0007314).

## Soil, rhizosphere, and root bacterial community associated to six *Prunus* rootstocks

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**Keywords:** *Prunus* rootstocks, bacterial community, metabarcoding, soil, root, rhizosphere

Spain is considered the third sole largest world producer of almond (*Prunus dulcis* Mill.), accounting for 6% of total production. The production of almond usually requires the use of rootstocks, being GF-677 and Garnem the ones widely planted. However, other rootstocks are gaining importance in new almond orchards, including the ones of Rootpac series (RR, R<sup>®</sup>20, and R<sup>®</sup>40), that provide interesting agronomic characteristics including suitable development in replanted soils, low vigor and improved fruit size, respectively. Although the agronomic performance of all those rootstocks is already well known, there is currently no information on the specific root, rhizosphere, and bulk soil microbiome that may greatly influence other desirable traits, including nutrient use efficiency or drought stress tolerance. Thus, 16S rRNA metabarcoding was conducted to characterize the bacterial community present in bulk soil, rhizosphere, and roots of six *Prunus* rootstocks: GF677, Cadaman, Garnem, RR, R20, and R40. It was observed that both diversity and community composition varied significantly among the three compartments. Consequently, each compartment was evaluated individually for all rootstock. Both diversity and composition of the bacterial community were similar among the different rootstocks in the three compartments. Nevertheless, notable patterns were observed when differential abundance was calculated. In bulk soil, ASVs identified as *Vicinamibacteria* or *Vicinamibacteraceae* were enriched, while ASVs identified as *Gaiellales* and *Steroidobacter* were depleted through most of the rootstocks. In the rhizosphere, ASVs identified as *Vicinamibacteraceae*, *Gaiellales* and *Bacillus* were enriched across rootstocks while ASVs identified as *Oligoflexus*, *Solirubrobacter*, *Ilumatobacteraceae* and *Polyangiales* were depleted. In the roots, ASVs identified as *Ensifer* and *Steroidobacter* were enriched, while ASVs identified as *Myxococcaceae* and *Sorangium* were depleted in most rootstocks. The bacterial taxons identified in this study might be a target for isolation and testing for their potential as inoculants to ensure proper agronomic performance of the recently developed dwarfing rootstocks.

## Halophytic endophytes: A potential source of beneficial microbes for biotic and abiotic stress tolerance in crops

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**Keywords:** Biopesticides; Biofertilizers; Bioinoculants; Halophytic endophytes; Stress tolerance; Biocontrol

Alternative strategies of crop development are needed, due to climate change effects, as well as the significant reduction of agrochemicals' use according to the EU Green Deal alignment. Such an alternative strategy to improve tolerance to biotic and abiotic stress in crops can be the introduction of halophytic microbial endophytes. Halophytic endophytes potentially contribute to the host's adaptation to adverse environments, improving its tolerance against various biotic and abiotic stresses. Our group has created a Biobank of beneficial endophytic microbes from Cretan halophytic plants (plants growing in high salinity) that have been characterised and screened for plant-growth promoting abilities, salinity tolerance and pathogen antagonistic properties, with significant success in the lab and in the field. For the first time, the plant-associated microbiomes of halophytic plants in Greece have been used as an alternative to agrochemicals, according to EU Green Deal strategy. Furthermore, our molecular studies have provided unprecedented insights regarding the beneficial properties of endophytic microbes from halophytes, that will pave the way for their use in sustainable agriculture and plant protection, aiming to the future development of specialized microbial bioinoculants.

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**Funding:** The project was partially supported by the Emblematic Action of the Greek General Secretariat for Research and Technology, "Agro4Crete," Protocol Number: SAE013, Operational Program: SAE013. This project was partially supported the "BIOCONTROL" project that is implemented within the framework of the Single Action of State Aid for Research, Technological Development & Innovation "RESEARCH - CREATE - INNOVATE" of the EP. "Competitiveness, Entrepreneurship and Innovation" (EPANEK) 2014-2020 and is co-financed by the European Regional Development Fund (ERDF).

## Introducing the UK Crop Microbiome Cryobank with a case study in sugar beet

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**Keywords:** cryobank, data catalogue, soil type, amplicon sequencing, metagenomic sequencing

Sustainable agricultural practices are dependent upon soil microbiomes that play a key role in crop health. To develop sustainable agriculture practices for the future we need resources that target the microbiomes of crop plant systems. Here we introduce The United Kingdom Crop Microbiome Cryobank (UKCMCB)(1), a scalable genomic data resource linked to a cryobank collection of samples for six economically significant crops (fava bean, oil seed rape, spring barley, spring oats, spring wheat and sugar beet). The crops were grown in nine agricultural soils from across the UK under controlled conditions in a large-scale pot experiment. 16S-amplicon, ITS-amplicon and metagenomic short-read sequencing were conducted to analyse the microbiomes of rhizosphere and bulk soil for multiple crop-soil combinations. The UKCMCB resource AgMicroBiomeBase (<https://agmicrobiomebase.org/>) is the project data catalogue which links the genomic resources with soil metadata and with the cryopreserved samples. To show the utility of the resource we highlight a case study assessing the impact of soil type on the bacterial microbiome composition of sugar beet. The UKCMCB enables researchers to begin to understand the impact of agricultural practices on soil microbiomes and crop production. We aim to continue to develop the UKCMCB to enable data integration and data mining across additional data types, including microbial phenotypes and synthetic microbial communities.

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**Funding:** This work is funded under the BBSRC-UKRI Bioinformatics and Biological Resources Fund (BBR) under grant agreement BB/T019700/1.

## Phylogeny, functional traits and bacterial compatibility in wheat-associated *Pseudomonas*

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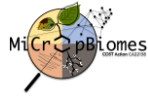
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**Keywords:** *Pseudomonas*, phylogenetics; functional traits; compatibility

Plant-associated *Pseudomonas* are widely reported in microbial communities of different plant tissues, types of soils and a variety of crops, presenting important plant growth-promoting properties. However, the understanding of how the diversity of its phylogeny and their functional traits are linked in this important bacterial group, remains unclear. We have studied 72 strains previously isolated from two wheat fields GH2 and Highfield (Harpenden-UK). A phylogenetic tree based in 76 concatenated gene sequences with 18 reference strains was generated. The phylogenetic tree showed nine clades. Interestingly, although with different isolation methods between the field trials, Highfield strains were associated with clades I-V and IX, while GH2 strains with clades VI-VIII. Ten functional activities were tested, and specific clades showed clear functional traits associations. Clades I-V were positive for zinc solubilization, while clades I and VIII were positive for ACC deaminase. Siderophores production, saline resistance, and aluminium phosphate solubilization were positive in all the strains. Additionally, carbon metabolism profiling was analysed by Biolog Ecoplates. Associations were also observed between different clades and carbon sources. Putrescine usage was associated with clades VII-IX while D-glucosaminic acid metabolism with clades I-V. Additionally, redundancy (RDA) analysis was performed for the bacterial clades and functional traits/carbon substrates. Compatibility tests between all the strains were performed. Seven strains from clade I inhibit clades II-V. Cophenetic distance analysis showed that non-compatible bacteria are phylogenetically closer. This study highlights that *Pseudomonas* derived from geographically similar sites can be phylogenetically distinct with a high level of functional redundancy between strains.

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# Session 2

**Crop-Microbiome assembly dynamics under specific environments**

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## Effects of long-term drought on soil vineyard microbiome diversity, structure and composition

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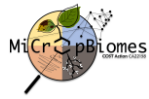
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**Keywords:** Soil Microbiome, Grapevine, Syrah, Drought, Crop Sustainability

Climate transition with frequent heat waves and long drought periods threatens grapevine productivity and wine quality in the Mediterranean regions. Microorganisms are known to contribute to plant fitness and to stimulate plant resilience against biotic and abiotic factors. In this work, it was assessed the impact of long-term drought on soil microbiome associated to grapevine in open field in Alentejo, renowned Portuguese wine region. Soil and plant tissues of drought tolerant Syrah cultivar and drought sensitive Touriga nacional cultivar exposed to three irrigation levels (100%- FI, 50%-DI ETC; rain-fed-NI) for 5 years were sampled both in 2023 and Syrah also in 2022. Metabarcoding analysis of soil bacteria (16S V4 rRNA) and fungi (ITS sub-region) were integrated with soil physiochemical properties and leaves' physiological data. Pre-dawn leaf water potential and stomatal conductance confirmed the imposed drought scenarios. Even though,  $\alpha$ - and  $\beta$ -diversity of prokaryotic and eukaryotic microbial communities differed more by season than water availability, samples clustered according to soil water content and pH ( $p < 0.05$ ). Fungal communities show higher differences in the structure across treatments than bacteria. In 2023, 16 bacterial against 61 fungal ASVs were significantly different in abundance between NI and FI. Beijerinckiaceae, Bradyrhizobiaceae (Alphaproteobacteria) and Nocardioidaceae, Streptomycetaceae (Actinobacteria) families resulted to be significantly more abundant in NI, while Ascomycota, Basidiomycota and Mortierellomycota are the most important fungal phyla in NI. With culturomics data, this study aims to gather insights into how soil microbiome is remodelled under drought and contribute to select bacterial and fungal taxa with potential to mitigate drought stress in vineyards.

**Funding:** This work is supported by the European program (*Partnership for Research and Innovation in the Mediterranean Area – PRIMA*) and the FCT with the project MiDiVine (H2020-



*PRIMA-S2-2020) and by the Faculty of Science of the University of Lisbon with the internal project Microdrygrape (FciênciasID).*

## Crafting synthetic bacterial communities for enhanced growth and abiotic stress tolerance in legumes

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**Keywords:** Comparative genomics; SynComs; Bacterial Community; Drought Stress; Rhizobacteria

Changing landscape, rapid urbanization, challenged fresh-water reservoirs, together with biotic and abiotic stresses, significantly impact global legume productivity. Yield losses because of widespread fungal and bacterial pathogens, and droughts are most common. The proposed research aims to identify organ distribution and specific assemblages of leguminous bacterial communities. Isolations were performed from rhizosphere and rhizoplane region of *Medicago sativa* L. plants collected from fields of the Public University of Navarra, Pamplona, Spain. Closely clustered bacterial isolates were picked from agar plates and treated as a community for subsequent analysis. Communities were assessed for mineral solubilization, production of phytohormones, extracellular hydrolytic enzymes, siderophores, and for 1-aminocyclopropane-1-carboxylate (ACC) deaminase synthesis. Several communities variably solubilized tri-calcium phosphate, and zinc sulphate on minimal agar medium. Extracellular enzymes including cellulase, protease, amylase, lipase, and chitinase were also produced by the bacterial communities. Qualitative indole-3-acetic acid (IAA) assay confirmed the synthesis of IAA by several of bacterial communities. Three of the bacterial communities also showed the characteristic ACC deaminase activity. This community-based culture collection will be used to design a synthetic community (SynCom) comprised of naturally occurring highly abundant bacterial groups from roots and shoots. Metagenomic analysis and plant experiments with selected bacterial consortia are in progress for the selection of resilient SynComs to be used as bioinocula for legumes and to characterize their potential in water-deficit conditions. The approach will help to recover silent and less active rhizobacteria to be used as biostimulants for enhanced resiliency in crops.

**Funding:** This project has received funding from the European Union's H2020 research and innovation programme under Maria Skłodowska-Curie grant agreement No 101034288.

## Transcriptional reprogramming of lettuce roots in response to chitin soil amendment, effect on plant growth, rhizobiome composition and disease resistance

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**Keywords:** Chitin; Lettuce; Soil amendment; Rhizobiome

Chitin soil amendment improves soil quality, plant growth and plant stress resilience, but the underlying mechanisms are not well understood. To gain a deeper understanding on the effects of chitin, several omics approaches were applied in a multidisciplinary project. We studied the growth promoting effects on lettuce upon treatment with chitin in two different soil types (potting and greenhouse). In both soils, lettuce grew bigger with chitin amendment. Lettuce grew generally better in the potting soil compared to the greenhouse soil. The rhizobiome composition was analyzed using metabarcoding. A decrease in  $\alpha$ -diversity was observed upon chitin treatment in both soils. Based on  $\beta$ -diversity, chitin amendment had a stronger effect on the fungal community compared to the bacterial one. Both soils contained different genera significantly altered upon chitin treatment. In potting soil, a known plant-growth promoting fungus was significantly more abundant and associated with other chitin degraders. Such association was not observed in the greenhouse soil. The transcriptional reprogramming of lettuce roots in response to chitin treatment was studied using RNA-Seq. Over 300 genes were significant differentially expressed with chitin amendment.

Our results suggest that chitin soil amendment promote plant growth indirectly by changing the rhizobiome, induce transcriptional and metabolomic changes in lettuce roots, and might activate induced resistance by priming lettuce plants (1).



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## Enhancing olive resilience to water deficit through *Pseudomonas reactans* treatment

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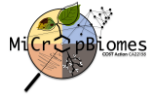
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**Keywords:** *Olea europaea* L., *Pseudomonas*, photosynthesis, oxidative stress

Agricultural ecosystems are increasingly facing extreme climate events due to climate change, with drought posing a significant threat to crop development and yield. This is true even for crops considered adapted to water-limited conditions, such as *Olea europaea* L. (olive tree). Employing eco-friendly solutions, such as plant-beneficial microorganisms, presents a sustainable agronomic tool to enhance olive tolerance to stress and boost agricultural production. In this work, it was exploited the benefits of pre-treating olive plants with the bacterium *Pseudomonas reactans* Ph3R3 in response to water deficit. Young potted olive plants were treated with a solution of *P. reactans* (via soil inoculation) or with distilled water, and then exposed to two watering conditions: well-watered or water-deficit. Plant water status, photosynthesis, pigments, carbohydrates, oxidative stress biomarkers, and total antioxidant capacity were evaluated 61 and 191 days after initiating the watering treatments. The benefits of the bacterial treatment were most evident under water-deficit conditions, with *P. reactans* increasing water stress tolerance. Particularly, *P. reactans* improved leaf dry biomass production and water availability, while also modulating photosynthesis and the antioxidant response. The treatment promoted the intercellular CO<sub>2</sub> availability, leading to improved net CO<sub>2</sub> assimilation rate and increased carbohydrates production (total soluble sugars and starch). Moreover, stress-protective strategies such as increased of total antioxidant capacity and carotenoid levels were upregulated, and oxidative stress levels were controlled. These findings highlight the potential of *P. reactans* to improve olive performance and to increase the capacity of olive plants to cope with drought stress. This approach seems a promising strategy for improving olive orchards sustainability under the climate change scenario. Future studies exploring the effects of *P. reactans* on other olive cultivars and (a)biotic stress, as well as on olive fruit yield and oil quality should be conducted.



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# Session 3

**Crop microbiomes and plant diseases: from dysbiosis to  
increased defenses**

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## Microbiome analysis unveils contrasting profiles between dutch elm disease tolerant and susceptible elm species

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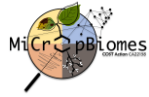
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**Keywords:** microbiome; stress resistance; *Ulmus glabra*; *Ulmus parvifolia*; *Ophiostoma novo-ulmi*

The forests are facing a series of urgent challenges due to climate change and the introduction of invasive pathogenic fungi and pests. Populations of wych elms (*Ulmus glabra*) and field elms (*Ulmus minor*) have been decimated due to the Dutch elm disease. This illness, caused by the Asiatic ascomycete (*Ophiostoma novo-ulmi*), appeared in Europe more than 100 years ago. Conventional breeding methods can be useful in such cases, but they often reach their limits due to missing resistant trees and their long generation times, as is the case with the Dutch elm disease. Therefore, the development of new approaches for resistance induction is essential to accelerate the development of resilient tree populations. The microbiome plays a crucial role in health and resilience in plant species. Microbiome modifications could be a faster alternative for resistance induction in tree species, as no crossings would be required. Approaches of microbiome modification could accelerate the development of resistant forest tree populations, ensure their long-term survival, and contribute to a resilient and sustainable forest ecosystem. As part of a new study, the microbiomes of the Dutch elm disease-tolerant Chinese elm (*Ulmus parvifolia*) and the non-tolerant wych elm (*Ulmus glabra*) are being compared using amplicon sequencing. The plants of both elm species, grown from seeds under the same environmental conditions, showed significant differences in both the bacterial and fungal microbiome. Microbial groups that were significantly increased in the tolerant species could be identified, which potentially could be associated with stress tolerance and colonisation resistance. Different methods will be currently tested to promote the microbiome enrichment of the susceptible Wyche elm with microorganisms from the resistant Chinese elm. This strategy could improve stress tolerance. Furthermore, a field trial was established to evaluate the potential improved stress susceptibility of treated Wyche elms.



**Funding:** Support is provided by the Agency for Renewable Resources (FNR).

## Rhizobacteria from Cypriot indigenous wine grape cultivars against grapevine trunk pathogens

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**Keywords:** *Bacillus*; Biological control; Esca; Grapevine Trunk Diseases; *Pseudomonas*

Grapevine trunk diseases (GTDs) comprise the most destructive biotic complex, limiting vineyard productivity and longevity globally. Based on recent data, the Cyprus vineyard is also seriously affected by GTDs. The scope of the present study is to decipher rhizobacterial communities from indigenous wine grape cultivars and select effective biological control agents (BCAs) towards GTDs management. The antagonistic activity of a collection of > 400 bacterial strains was screened *in vitro* against major GTD-related pathogens (*Phaeoaniella chlamydospora*, *Diplodia seriata*, *Neofusicoccum parvum*, *Eutypa lata*, *Ilyonectria liriodendri*, *Dactylonectria alcacerensis*, and *D. torresensis*). Strains exhibiting significant mycelial inhibition ( $\geq 50\%$ ) were selected for more detailed characterization. Based on 16S sequencing, strains were assigned to the genera *Acinetobacter*, *Bacillus*, *Bordetella*, *Chryseobacterium*, *Paenibacillus*, *Pantoea*, *Pseudomonas*, *Olivibacter*, *Rhizobium*, *Serratia*, *Streptomyces*, and *Variovorax*. Enzymatic traits related to antifungal action (lipase, esterase, protease, amylase, xylanase, pectinase, cellulase, chitinase), VOC activity, HCN production, antibiotics, and growth promotion traits were also evaluated. Results provided a pool of promising BCAs for successful GTD management. Solo and mixture applications of promising rhizobacterial BCAs, exhibiting optimum *in vitro* antifungal activity against the Esca pathogen *P. chlamydospora* (*Pchl*), were evaluated on potted grapevines under open field conditions. Selected BCAs were applied either preventative (4 days before the pathogen) or simultaneously on pruning wounds. Disease incidence and severity were evaluated in terms of the pathogen recovery from the grapevine wood, at 1 and 3-cm below the inoculation point. It was evident that the preventative BCA application significantly reduced disease incidence, compared to their simultaneous application with the pathogen. The *B. subtilis* strain M7 provided the best protection, where >88% of the plants not being infected at 3-cm below the point of inoculation. Interestingly, strain M6, “mix Bs”, and “mix all” treatments were also highly effective in reducing *Pchl* severity.

Furthermore, in a following 2-year-field-study that took place in 3 different established vineyards, the efficacy of commercial synthetic and biological plant protection



products, as well the strain M7 were evaluated against *Pchl* and *B. dothidea*. Applications were preventative on pruning woods that were inoculated 24 hours and 7 days post-treatment, respectively. Interestingly, the strain M7 exhibited auspicious results, given its non-formulated, “real-world”, field application. It was also evident that its efficacy was improved at 7d-pt; most likely due to better colonization & sealing of the pruning woods by the BCA.

## Establishing a *Fusarium oxysporum*-plant monitoring framework to decipher complex plant-microbe interactions

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**Keywords:** *F. oxysporum*; potato; tissue cultures; effectors; endophytes

Understanding complex interactions between plants and microbes is essential for effective agricultural practices and ecosystem management. Studies focusing on two-way systems can overlook intertwined nature of interactions seen in natural habitats. Hence it is crucial to establish systems for studying interactions between multiple associated organisms. We isolated a strain of *Fusarium oxysporum* (*F. oxysporum*) from our potato plants grown in sterile conditions of tissue culture. Through *in silico* analysis of the most abundant effectors from *F. oxysporum* species complex, the genome of our isolate was verified to lack Secreted in xylem (Six) effectors. A protocol for efficient dip inoculation of Desiree potato plants with adjusted concentration of *F. oxysporum* spores (microconidia) was established and two different inoculation methods were tested. Efficiency of inoculation and spread of the hyphae in the plant was evaluated using real-time polymerase chain reaction (qPCR), revealing higher concentration of *F. oxysporum* in lower plant tissues (roots) compared to upper ones. Additionally, *F. oxysporum* inoculation efficiency was verified by culturing surface-sterilized nodium and chopped plant material on MS-30 media. Moreover, exploration of both biotic as well as abiotic conditions was conducted since endophytic growth of *F. oxysporum* takes place within a specific framework. In context of biotic factors, different concentrations of *F. oxysporum* inoculum were tested and on the other hand concerning abiotic factors diverse incubation temperatures were examined. All tested conditions resulted in normal plant phenotype. Our study, together with employed tools, paves the way for more holistic approaches in terms of transition to sustainable agriculture.



## Impact of *Dactylonectria macrodidyma* inoculation on the rhizosphere and root-endosphere microbiomes of grapevine

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**Keywords:** Microbial diversity; plant-microbiome interaction; fungal microbiome; digital PCR; black foot disease; high-throughput sequencing; *Vitis vinifera*.

Grapevines establish intricate associations with a diverse array of microorganisms in the rhizosphere, which enhance growth, productivity, health, and wine quality. These microorganisms, influenced by plant species, genotype, and environmental factors, facilitate nutrient absorption, stress resistance, soil fertility, and disease management. However, grapevines are also vulnerable to various soilborne fungal diseases, such as black foot disease, which presents significant challenges to grape production worldwide. Recent research has leveraged high-throughput amplicon sequencing (HTAS) to gain deeper insights into microbial communities, their interactions within the grapevine rhizosphere and root-endosphere, and the impact of pathogenic microorganisms. This study aims to elucidate the composition and interactions within the fungal microbiome of the rhizosphere and root of grapevine grafted plants inoculated with *Dactylonectria macrodidyma* (black foot disease pathogen) using ITS HTAS. Additionally, we quantified *D. macrodidyma* in the root and rhizosphere over time using digital droplet PCR. Our results indicate that *D. macrodidyma* infection was more pronounced in clay soil plants. Initially, the pathogen concentration was higher in the rhizosphere, but it increased in the root over time, suggesting a progressive invasion of root tissue. Consequently, the root microbiome experienced more significant disruption due to pathogen inoculation compared to the rhizosphere. Over time, *D. macrodidyma*-inoculated plants exhibited reduced alpha diversity, particularly in sandy soil. Co-occurrence network analysis revealed that root microbiomes were more affected than those in the rhizosphere. In sandy soil, root network complexity decreased during the first nine months but returned to a stable state after a year,



whereas in clay soil, network complexity in roots progressively diminished. These findings underscore the complex relationships among microorganisms in the rhizosphere and root and highlight the profound impact of pathogens on plant microbiomes, stability, and protection. This research contributes to a better understanding of the microbial dynamics in grapevines and informs strategies for managing soilborne diseases.

## Exploring microbial communities in virus-infected raspberry plants

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**Keywords:** Beneficial bacteria; raspberry disease complexes; NGS

Prokaryotic plant growth-promoting rhizobacteria/endophytic bacteria, actinomycetes, and fungal organisms, as well as their possible mechanisms showed potential for suppressing viral infection in plants. So beneficial microbial organisms could play an important role in the integrated management of viral diseases via cross-protection, ISR, and the accumulation of defensive enzymes, phenolic compounds, lipopeptides, protease, and RNase activity against plant virus infection. Raspberry industry is one of the most profitable fruit productions in Bosnia and Herzegovina (BiH). However, in the previous research several important virus disease complexes were reported. Knowing that there is no direct way for controlling virus diseases, the main objective of this STSM was to investigate the impact of viral infections on the microbiome composition of raspberry plants. Therefore, raspberry plants collected from the main growing centres in BiH were tested for the presence of nine viruses (RLMV, RYNV, BRNV, RLBV, RBDV, TRSV, CLRV, ToRV, ArMV, ApMV). TNAs of 24 samples infected with single or multiple infection with RLMV, RYNV, BRNV, RLBV and RBDV were selected with 24 negative samples for all tested viruses for further investigation. Samples were made of plants from different locations (soil types), variety and growing type (commercial and nursery orchards). PCR amplification was then performed to check presence of plant growth-promoting bacteria in the virus positive and negative raspberry plants using universal primers for bacteria excluding detection of chloroplast. After agarose gel analyses the amplified samples were selected and product were cleaned and prepared for sequencing on Illumina Miseq following bioinformatical analysis. Overall, this research results represents an important step towards better understanding the microbial communities in virus-infected raspberry plants.

**Funding:** Support was provided by the Fonds of Ministry of Scientific and Technological Development and Higher Education supporting COST Actions. This presentation is based upon work from COST Action CA22158 Exploiting Plant-Microbiomes Networks and Synthetic Communities to improve Crops Fitness (European Cooperation in Science and Technology).

## Assessment of fungal community diversity and disease impact in stone fruit orchards

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**Keywords:** Fungal diversity; NGS sequencing; Disease; Stone fruits; Cova da Beira

In Portugal, the Cova da Beira region stands out as the main production area for stone fruits in Portugal, especially sweet cherries, peaches and nectarines. These fruits are mainly grown in the municipalities of Fundão, Covilhã and Belmonte, in the district of Castelo Branco. In recent years, market demand has increased, encouraging the use of improved varieties, which, together with their homogeneity, can increase the susceptibility of fruit orchards to certain biotic and abiotic agents, thus reducing their productivity. The most common diseases in these orchards are mainly caused by fungi and bacteria affecting several parts of the plant and in the spring of 2021 and 2022, signs of decline were observed in peach and nectarine orchards. This study aimed to assess the fungal diversity in symptomatic orchards using a metabarcoding approach, complemented by traditional culture-based methods. The combined results allowed a detailed characterization of the fungal communities in these production systems. Symptomatic plant material such as flowers, branches and exudates were collected from fourteen orchards and used for fungal isolation and molecular identification by ITS metabarcoding (Illumina). Culture-based methods have identified several harmful organisms that compromise production by affecting leaves, flowers and fruit (brown rot - *Monilinia* sp.; *Alternaria* sp., *Sordaria fimicola*, *Botrytis cinerea*, *Cladosporium* sp., *Stemphylium vesicarium*, *Ulocladium* sp.) and diseases that mainly affect wood (trunks and branches), including gummosis (*Cytospora leucostoma*), canker (*Diaporthe amygdali*, *Botryosphaeria dothidea*;) and rust (*Neofusicoccum parvum*). Organisms with antagonistic potential against some of these diseases were also isolated, namely *Epicoccum nigrum*. In the same samples, *Prunus* sp. dysbiosis was assessed by the structural diversity of the fungal community. Both beta and alpha diversity metrics were analyzed, revealing differences between sites, sample types, and species.



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## High-throughput sequencing approach for diagnosis and epidemiology of prunus crops in Beira Interior (Portugal)

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**Keywords:** High-Throughput Sequencing (HTS); Molecular Epidemiology; Plant diseases diagnosis; *Prunus* sp.

The emergence of plant diseases is a major threat to the sustainability of agriculture. Diagnostic challenges are increasing as globalization and climate change facilitate the introduction of new plant pests. In Portugal, the Beira Interior (BI) region is crucial for peach and cherry production. Recently, extensive agroforestry areas have been converted to intensive almond (*P. dulcis*) systems, which may have a significant impact on the agroecosystem dynamics, especially at the phytosanitary level. Worldwide, *Xylella fastidiosa*, *Xanthomonas arboricola*, *Pseudomonas syringae* and *Monilinia fructicola* are the main pests causing significant losses in *Prunus* crops. *Prunus* production in BI is severely affected by diseases of increasing intensity and severity, often without clear identification of the causal agents. The high density of genetically diverse *Prunus* species facilitates the coexistence of pests, promoting the development of highly adapted strains and the occurrence of coevolutionary phenomena, leading to the spread and emergence of new diseases. This coexistence of closely related strains with very different phenotypes poses a critical diagnostic challenge. High-throughput sequencing (HTS) methodologies are beginning to be used for the detection and identification of pathogenic microorganisms. Both short-read and long-read sequencing can be employed, but further studies are needed to validate the feasibility of these approaches. To address these issues, molecular epidemiological studies were conducted by characterizing the bacteriome of *Prunus* species using a long-read sequencing approach. This method aimed to assess the occurrence, distribution, and impact of pests. Preliminary results showed that important pathogen-related groups were detected that significantly influenced the microbiome structure. These results support this approach, and a comprehensive phytosanitary survey will be carried out



in the region. At the same time, the accuracy and sensitivity of this approach to pest detection will be assessed by comparison with official protocols (EPPO).

**Funding:** This work is supported by national funds through the FCT - Fundação para a Ciência e a Tecnologia, I.P., under the project XylOut (DOI: 10.54499/PTDC/ASP-PLA/3145/2021). This work is part of the activities of the core funding project of the Pedro Nunes Institute (IPN) Technology and Innovation Centre (CTI), under the terms of Call for Tenders (AAC) No. 03/C05-i02 /2022, within the scope of the Portuguese Recovery and Resilience Programme (PRR).

## Role of tomato and pepper plants as natural reservoirs of *Curtobacterium flaccumfaciens*

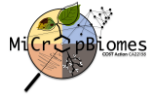
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**Keywords:** *Curtobacterium flaccumfaciens*, alternate hosts, coryneform bacteria, phytopathogenic bacteria

The role of alternative and alternate host plants as natural reservoirs of phytopathogenic bacteria is of great importance. Clarifying the ability of the phytopathogens to persist and survive in various habitats different than their primary hosts is a good bases for more in-depth studies on the mechanisms they possess to exert their pathogenic potential and is essential in order to enable the implementation of more efficient disease management strategies. This study aims at the identification and characterization of five actinobacterial strains (3t<sub>2</sub>, 12t<sub>1</sub>, 3p<sub>3</sub>, 4p<sub>1</sub> and 4p<sub>2</sub>) with presumed belonging to the species *Curtobacterium flaccumfaciens* accidentally isolated from tomato and pepper plants, which are not among the known primary host of this bacterium and establishing the potential role of both plants as natural reservoirs of this phytopathogen. All strains were identified as *C. flaccumfaciens* based on polyphase approach: MALDI-ToF MS, 16S rDNA sequencing and PCR. The strains possess phenotypic features which referred them to the group of phytopathogenic coryneform bacteria: Gram-positive cell wall, strictly aerobic growth, yellow/orange pigmented colonies; ability to hydrolyse esculin, starch and casein; catalase positive, oxidase-, urease-, indole- and nitrate-reduction-negative. All isolates produced antimicrobial substances against various phytopathogenic bacteria associated with tomato and pepper bacterioses – *Xanthomonas euvesicatoria*, *Xanthomonas vesicatoria*, *Xanthomonas gardneri* and *Clavibacter michiganensis* subsp. *michiganensis*. To confirm that the newly isolated *C. flaccumfaciens* strains can survive in tomato and pepper vascular system, both plants were artificially infected and grown in climatic chamber for a period of 28 days. Morphological alterations were observed only in the tomato plants, with defoliation of the first two to four leaves at the end of the test period. Then, viable coryneform bacterial isolates (n = 73) were successfully re-isolated only from the stems of the infected plants. The similarity between the re-isolates and the respective initial isolates, used for the artificial infection of the plants, was confirmed phenotypically and genotypically by RAPD-PCR. Our results showed that



solanaceous vegetables can act as reservoirs of *C. flaccumfaciens* and play a key role in the distribution of this bacterium.

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## From benign inhabitant of yeast to microbial biocontrol agent

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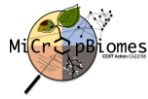
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**Keywords:** Delivery system; Biocontrol agent; Nisin; Virus-like particles

Viruses are inherently organized supramolecular structures, designed by nature for own genome packaging and delivery. Virus-like particles (VLPs) are envisioned as a tool to enhance the efficacy of active compounds by taking advantage of viruses' innate capacity to protect relevant cargo. These nanoparticles resemble natural viruses in all but the ability to replicate due to the lack of genetic material. The void internal space can be utilized to encapsulate small molecules, peptides, proteins, and nucleic acids, while the surface of VLPs can be modified to achieve targeted delivery.

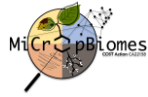
*Totiviridae* family dsRNA viruses inhabit range of yeast *Saccharomyces cerevisiae* strains, from wild type to those involved in food production. These viruses lack extracellular stage, spreading by passing on to the progeny cells. The members of *Totiviridae* family encode the sole structural capsid protein Gag, self-assembling into nanoparticles. The VLPs were obtained by synthesis of the recombinant major capsid proteins in *S. cerevisiae* and *E. coli*, and purified by the tandem ultracentrifugation to nearly homogeneous state. The different VLPs were found closely resemble the size of the original L-A and L-BC viruses, as demonstrated by transmission electron microscopy and dynamic light scattering technique. The particles were found exceptionally stable in different conditions over half a year period, as confirmed further by fluorescent thermal shift assay. The development of nano-delivery system was advanced by performing passive encapsulation. The particles were incubated with the broad-spectrum antimicrobial peptide nisin, a highly promising biocontrol agent in food industry and plant protection. After separation of unbound content, the particles were exposed to range of Gram-positive and Gram-negative bacteria. Encapsulation and cargo delivery to the target cells were found successful, as evidenced by the inhibition zones resulting from the release of encapsulated nisin.

The goal of this study was the evaluation of the properties of VLPs purified from *S. cerevisiae* and *E. coli* to establish their potential use as a system, alternative to available microbial biocontrol agents. In addition to high stability, the efforts to reversibly entrap cargo load by passive diffusion succeeded. We are in process of the development of biologic particles into the universal platform for delivery of range of



bioactive materials. The bioengineering of capsids of yeast viruses is performed to prepare nanoparticles with universal cargo loading and facilitated delivery properties.

**Funding:** *This research was funded by the Research Council of Lithuania (S-MIP-23-28).*



# Session 4

**From current microbial inoculants to synthetic communities**

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## Love my grape: unraveling the potential of local bacterial isolates obtained from grapevine rhizosphere

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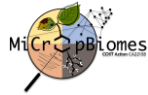
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**Keywords:** Grapevine, rhizosphere, bacteria, BCA, PGPR

Grapevine is one of the most important crops in Italy, especially in the Veneto region. However, viticulture still depends heavily on the use of many fungicides and chemical fertilizers to fight against different fungal diseases as well as to increase yields. Therefore, alternative sustainable, and eco-friendly methods should be explored. This research aims at identifying and characterizing microbial strains isolated from local vineyards to be applied as alternatives or integrations to chemical inputs to enhance vineyard resilience against biotic and abiotic stresses. A total of 105 bacterial isolates were obtained from the rhizosphere of three different vineyards located in the Verona province, among which several were selected due to their noticeable activities in terms of biological control of grapevine fungal pathogens (3 strains) and/or plant growth promotion (4 strains). Selected isolates were further subjected to whole-genome sequencing to unravel the genomic features related to their activities. This revealed that all bacterial biological control agents (BCAs) belong to the *Bacillus velezensis* species, while 3 isolates chosen for their plant growth-promoting (PGP) behavior represent new species of the *Pseudomonas* genus. Among them, *Pseudomonas* sp. 525B isolate emerged as a promising PGPR and displayed a significant biological control capacity towards *Botrytis cinerea*, especially through the secretion of volatile organic compounds. These results indicate that vineyard microbial biodiversity represents an important reservoir of endogenous microbe-based potential alternatives to chemicals to improve viticulture sustainability.



**Funding:** This work is supported by the European program (*Partnership for Research and Innovation in the Mediterranean Area – PRIMA*) and the Italian Ministry of University and Research with the project VINEPROTECT (*PRIMA-S2-2021*).

## Impact of bio-based and biodegradable plastic (PBSA) and nitrogen fertilizer on plant biomass and health: Interactions with *Fusarium solani* in mung bean (*Vigna radiata* L.)

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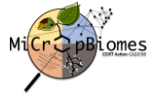
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**Keywords:** PBSA, Plant health, Ammonium sulfate fertilization, Mulching film, Plastic accumulation

Bio-based and biodegradable plastics such as mulching films are widely used in agricultural field sites. However, there are limited studies of their impact on plant development and health even though an important soil-borne plant pathogen *F. solani* has been reported to associate with various types of bio-based and biodegradable plastics, especially polybutylene succinate-co-adipate (PBSA). To evaluate the influence of PBSA amendment in soils on plant development and health, *F. solani* and mung bean (*V. radiata*) were used as models in a modified petri-dish test using soil suspensions. Mung bean seeds were incubated in suspensions with two dilutions (high vs. low dilution with low vs. high PBSA amendment) of soils preincubated 1 year with PBSA under different treatments (combinations of N fertilizer (ammonium sulfate) and PBSA load) in the modified petri dish test. Plant development and disease incidence were recorded with both microscopic and molecular techniques (specific PCR and Illumina amplicon sequencing). Treatment with PBSA and N fertilizer in non-sterile soil suspensions strongly increased the disease caused by *F. solani* on *V. radiata* at both low and high soil dilution. At high soil dilution, the *F. solani* disease incident was 67.5% while at the low dilution the disease incidence reached 92.5%. In contrast, in treatments PBSA but without N fertilizer, non *F. solani* disease was observed. Apart from *F. solani* infection, other soil fungi can also infect the mung bean seedlings,



especially at low soil dilution levels. Nevertheless, based on this short-term study, we found no evidence that PBSA alone can significantly increase the overall disease incidence.

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## ***Pseudomonas synxantha* DLS65 as the active ingredient of Microfighter, an innovative bio-pesticide against oomycetes and bacteria**

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**Keywords:** Biocontrol; downy mildew; olive knot disease; tomato bacterial speck; soil microbiota

Currently, copper-based pesticides are very commonly used in agriculture, despite the environmental concerns posed by copper accumulation in the agro-environment, thus posing a threat to its safety, human health and biodiversity (EFSA, 2018). As an effective alternative, the use of beneficial microbes is a promising approach to reduce the use of copper. The EU-financed project “LIFE Microfighter” (<https://webgate.ec.europa.eu/life/publicWebsite/project/LIFE21-ENV-IT-LIFE-MICROFIGHTER-101074218/innovative-zeo-biopesticides-based-on-useful-microorganisms-for-eliminating-the-use-of-copper-based-pesticides>) has the ambition to reduce the input of copper by at least 50% in specific cases. Microfighter (tentative product name) is an innovative biopesticides, where the antagonistic bacterium *Pseudomonas synxantha* DLS65 is coupled with a natural zeolite (chabasite) that may support its viability and availability in orchards, vineyards and other cropping systems. Experiments are currently done (Technology Readiness Level, TRL 7 and TRL 8) in three EU countries, Spain, Croatia and Italy, to lower the impact of fungal (downy mildew of grape) and bacterial (olive knot and tomato speck) diseases, together with the reduction of copper inputs in vineyards, olive groves and tomato fields. Contemporarily, soil metagenomics analyses are done, to check and monitor the abundance and biodiversity of microbial communities along 3-4 seasons and to confirm the positive effects of decreasing copper inputs on soil microbiota richness. From the technical point of view, DLS65 showed a tolerance to copper (500 ppm) in liquid and solid medium, therefore confirming its viability in IPM strategies, together with a reduced copper use: such tolerance was molecularly confirmed through the detection of the *cusABL* genes cluster. Traits linked to plant growth promotion by DLS65 were confirmed as well, demonstrating its ability to produce siderophores, ammonia and to solubilize phosphates. The antimicrobial activity of DLS65 appears to be linked to the production of volatile organic compounds (VOCs) as well, since the growth and virulence traits of *Pseudomonas syringae* pv. *tomato* and *Pseudomonas savastanoi* pv. *savastanoi* were severely affected. Results confirmed the excellent performance of

DLS65 associated to a zeolite to control the olive knot disease in commercial olive groves; additionally, the same product significantly reduced the incidence of downy mildew of grape in semi-field experiments (Italy) and in commercial vineyards (Croatia), although its control mechanism(s) remain largely unknown. To unravel the molecular interaction between *P. synxantha* DLS65 and *Vitis vinifera*, making grapevine tolerant to the downy mildew, a transcriptomic approach was attempted, to seek specific gene expressions that may explain the reduced disease severity by *Plasmopara viticola*. Our findings confirmed that *P. synxantha* strain DLS65 is a promising biocontrol agent against both bacteria and oomycetes, and a natural zeolite (chabasite) is an optimal supporting material to prepare a commercial biopesticides that maintains the viability and the bioactivity of the pseudomonad, together with easiness of field application.

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## Impact of bacterial interactions in a community: an experimental evolution approach in planta

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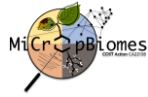
**Keywords:** Synthetic community; experimental evolution; plant growth-promoting rhizobacteria; *Pseudomonas*

Plants, via root exudates, shape and select their rhizospheric microbiota from the soil's microorganism reservoir. Thus, the rhizosphere hosts a huge diversified microbial community, including bacteria able to interact with plants, by means of direct or indirect plant beneficial properties; these bacteria are known as plant growth-promoting rhizobacteria (PGPR). The interactions between these partners influence plant development and physiology. Our work aims to prove that plants will select a community of PGPR that best interact between them and with the plant. To support this hypothesis, we realized an in vitro experimental evolution on maize plants (PR37Y15) with a PGPR assembly, composed of 10 fluorescent *Pseudomonas* ("ancestral" *Pseudomonas* SynCom), half of them with a high number of plant beneficial properties (8 or more) and the other half with a lower number (6 or less). Our experimental evolution (1) consists on the inoculation of the "ancestral" assembly on the plants and after 7 days of growth, bacterial assemblies retrieved from the roots are used as new inoculums for a fresh batch of plants. Our experiment was carried out over 6 months and community composition has been analysed for each plant. We have evidenced (i) that plants select PGPR with few plant beneficial properties and (ii) that there is coevolution between the PGPR within the assembly, leading to "evolved" assemblies with fewer PGPR and with changes in their genomes. Meanwhile, competition assays (ratio 1:1) have been done to evaluate the fitness of "evolved" *Pseudomonas* strains compared to "ancestral" *Pseudomonas* strains. We are now analysing the genomes of evolved clones in order to understand the adaptive mechanisms selected during the interaction of this *Pseudomonas* SynCom with plant.

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# Session 5

## Communication, and engagement with society

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## Microbes - the invisible heroes of earth: educating the next generation on microbiomes

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**Keywords:** microbiomes case studies, microbiology literacy, secondary school biology education, hands-on learning, inquiry-based learning

A new and pioneering educational program, "Microbes - the Invisible Heroes of Earth," is making its way into Portuguese secondary schools, aiming to enrich science education and enlighten students about the critical roles of microbiomes and the innovative concept of holobiome. The program seeks to cultivate a generation of informed and inquisitive individuals capable of addressing future scientific challenges by exploring case studies of both human microbiomes and plant microbiomes, giving students an opportunity to gain practical insights into real-world applications and implications of microbiome research. The program employs educational strategies designed to seamlessly integrate into existing science curricula and meet the demands of the Ministerium of Education regarding essential learnings. It emphasizes active learning through challenges and practical laboratory work, allowing students to engage in hands-on experiments that encourage inquiry-based learning (Pedaste et al., 2015). Through an interdisciplinary approach, the program not only covers biological aspects but also integrates ecological, medical, and agricultural perspectives. This holistic approach aims to provide students with a broad perspective of the ubiquitous presence and importance of microbes in various ecosystems and human health. Furthermore, the program promotes the development of critical thinking and scientific literacy. By engaging with contemporary research and ethical debates surrounding microbiome studies, students will be encouraged to form evidence-based opinions and consider the broader societal implications of microbiomes. In addition to student-centred learning, the program places significant emphasis on professional development for educators. Workshops and continuous professional development (CPD) sessions are being designed to equip teachers with the necessary skills and knowledge to effectively deliver the program. This includes training in the latest pedagogical strategies and hands-on laboratory techniques, contributing to more confident and proficient educators, guiding students through the program's content.



Key themes explored within the program include (1) the cultivation and study of microbiota, where students learn how to grow microbiota in laboratory settings and investigate their characteristics; (2) microorganism-host interactions, which examine how microorganisms interact and communicate with their hosts; (3) the impact of antimicrobial substances, helping students understand how these substances affect the balance of holobiome (Penumutchu et al., 2023); (4) the costs and risks associated with holobiome imbalances, emphasizing the consequences and dangers of disruptions in these microbial communities (Cavicchioli et al., 2019). Evaluation and feedback mechanisms are integral to the program's success. Regular assessments, student feedback, and teacher evaluations will be used to refine and adapt the program strategies and resources, ensuring it remains relevant and impactful.

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