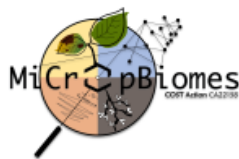


## **2<sup>nd</sup> Annual Conference of the MiCropBiomes COST Action**

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

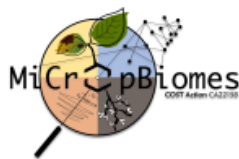


## Conference Overview

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

**Welcome:** Tuesday 9:15 am – 9:45 am

Tuesday 17 <sup>th</sup> June	Wednesday 18 <sup>th</sup> June	Thursday 19 <sup>th</sup> June
<b>9:45 – 10:30</b> <b>Session 1</b> Exploiting plant-microbiome molecular crosstalks: Diversity, distribution, and eco-evolutionary perspectives	<b>8:45 – 10:30</b> <b>Session 4</b> Crop-microbiome assembly dynamics under specific environments	<b>8:30 – 10:30</b> Management Committee meeting
Break	Break	Break
<b>11:00 – 12:15</b> <b>Session 1</b> Exploiting plant-microbiome molecular crosstalks: Diversity, distribution, and eco-evolutionary perspectives	<b>11:00 – 12:00</b> <b>Session 5</b> Communication and engagement with society	<b>11:00 – 14:00</b> Field trip
	<b>12:00 – 12:30</b> Shared experiences for STSM/funded trainees	
Lunch	Lunch	
<b>14:00 – 15:45</b> <b>Session 2</b> Crop microbiomes and plant diseases: From dysbiosis to increased defenses	<b>14:00 – 16:00</b> Working Group meetings	
	<b>16:00– 16:30</b> Glossary/Terminology session	
Break	Break	
<b>16:15 – 17:45</b> <b>Session 3</b> From current microbial inoculants to synthetic communities	<b>17:00 – 18:00</b> Posters session	
Group picture		
<b>20:00</b> Congress dinner		

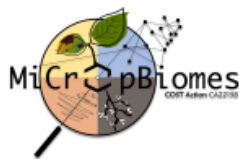


## Local Organisers

<b>Iakovos Pantelides</b>	Cyprus University of Technology, Cyprus
<b>Loukas Kanetis</b>	Cyprus University of Technology, Cyprus
<b>Maria-Dimitra Tsolakidou</b>	Cyprus University of Technology, Cyprus
<b>Stavroula Dimitriadi</b>	Cyprus University of Technology, Cyprus
<b>Styliana Efstathiou</b>	Cyprus University of Technology, Cyprus

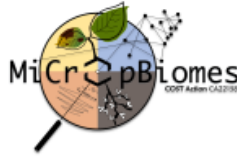
## Scientific Committee

<b>Conceição Santos</b>	University of Porto, Portugal
<b>Francesco Spinelli</b>	University of Bologna, Italy
<b>Ahmed Abdelfattah</b>	ATB, Germany
<b>Ellodie Vandelle</b>	University of Verona, Italy
<b>Joël Pothier</b>	Zurich University of Applied Sciences, Switzerland
<b>Anna Bonaterra</b>	University of Girona, Spain
<b>Caroline De Tender</b>	Ghent University, Belgium
<b>Gabriele Berg</b>	TU Graz, Austria
<b>Tanja Berić</b>	University of Belgrade, Serbia
<b>Gordana Racic</b>	Research and Development Institute Tamiš, Serbia
<b>Renata Artimová</b>	Institute of Forest Ecology, Slovakia
<b>Claire Prigent-Combaret</b>	University of Lyon, France
<b>Ralf Koebnik</b>	IRD, Montpellier, France
<b>Ivan Nikolic</b>	University of Belgrade, Serbia
<b>Iakovos Pantelides</b>	Cyprus University of Technology, Cyprus
<b>Loukas Kanetis</b>	Cyprus University of Technology, Cyprus



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# Scientific Program

## Tuesday 17<sup>th</sup> of June

08:30 – 09:15 Registration

09:15 – 09:45 Opening & Welcome by the organizers

### Session 1      Exploiting Plant-Microbiome molecular crosstalks: Diversity, distribution, and eco-evolutionary perspectives

**Chairs:** Ahmed ABDELFAH and Gabriele BERG

#### Invited Lecture

9:45 – 10:15      **Gabriele Berg (Institute of Environmental Biotechnology, Graz University of Technology, AT):** Plant-Microbiome interactions under the lens of the one health concept

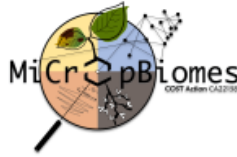
#### Oral Presentations

10:15 – 10:30      **Ahmed Abdelfattah (Leibniz Institute for Agricultural Engineering and Bioeconomy, DE):** Assembly and diversity of the fruit and seed microbiome sustainable Agriculture

10:30 – 11:00      *Break*

11:00 – 11:15      **Benjamin Dubois (Walloon Agricultural Research Centre, BE):** Improving plant microbiome profiling: host-DNA blocking strategies and high-accuracy long-read bioinformatics pipeline for Nanopore data

11:15 – 11:30      **Salme Timmusk (Swedish University of Agricultural Sciences, SE):** Plant-microbiome interactions under a changing world



- 11:30 – 11:45 **Ioannis Stringlis (Agricultural University of Athens, GR):** Lessons from model and crop plants on plant-microbiome communication
- 11:45 – 12:15 General Discussion
- 12:15 – 14:00 *Lunch*

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

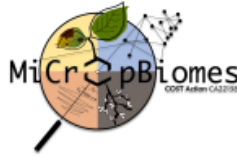
**Chairs:** Joël F. POTHIER and Renata ARTIMOVA

### Invited Lecture

- 14:00 – 14:30 **Lili Huang (Northwest A&F University, CN):** Bacterial Canker: Control vascular spread based seasonal dynamics of *Pseudomonas syringae* pv. *actinidiae* in Kiwi Plant

### Oral Presentations

- 14:30 – 14:45 **Matthias Noll (University of Coburg, DE):** Future climate and agricultural farming systems affect the fungal plastisphere of different biodegradable plastics at the early stage of field degradation
- 14:45 – 15:00 **Lena Pesenti (UCLouvain, Earth and Life Institute, Applied Microbiology, Plant Health Lab, BE):** Assessing the role of Salicaceae xylem endophytes on the establishment and progression of *Xylella fastidiosa* or *Brenneria salicis*
- 15:00 – 15:15 **Duška Delić (University of Banja Luka, Faculty of Agriculture, BA):** Seed microbiome diversity in balkan legumes: insights from *Lathyrus sativus* (Herzegovina) and *Phaseolus vulgaris* (Montenegro)
- 15:15 – 15:30 **Joanna Pulawska (The National Institute of Horticultural Research, Skierniewice, PL):** Universal properties of *Pantoea*



*agglomerans* T16/8, *Pantoea allii* T14/15 strains in protecting fruit and vegetable plants against diseases

15:30 – 15:45 General Discussion

15:45 – 16:15 Break

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

**Chairs:** Anna BONATERRA and Claire PRIGENT-COMBARET

#### Invited Lecture

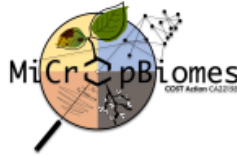
16:15 – 16:45 **Blanca Landa (Institute for Sustainable Agriculture, Spanish National Research Council, ES):** Unravelling and engineering the olive xylem microbiome to enhance its resilience against vascular pathogens

#### Oral Presentations

16:45 – 17:00 **Lucía Alonso Villar (Institute for Sustainable Agriculture, Spanish National Research Council, ES):** Development of synthetic bacterial communities (SynCom) with xylem-inhabiting bacteria for the biological control of vascular pathogens of olive crop

17:00 – 17:15 **Luca Nerva (Council for Agricultural Research and Economics - Research Centre for Viticulture and Enology, IT):** Microbiome-mediated adaptation: leveraging grape-associated microbiota to boost hologenome plasticity under climate change

17:15 – 17:30 **Fares Bellameche (Department of Life Sciences, University of Modena & Reggio Emilia, IT):** Ensuring the efficacy of the biopesticide Microfighter: survival of *Pseudomonas synxantha* DLS65 in diverse crops and its defense-stimulating role in grapevine



- 17:30 – 17:45 General Discussion  
18:00 Group Picture  
20:00 Congress Dinner

## Wednesday 18<sup>th</sup> of June

### Session 4 Crop-Microbiome assembly dynamics under specific environments

**Chairs:** Elodie VANDELLE and Gordana RACIC

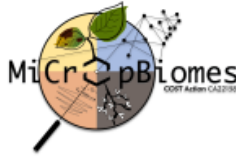
#### Invited Lecture

- 08:45 – 09:15 **Laura Weisskopf (Department of Biology, University of Fribourg, CH):** Chemical communication and its impact on the expression of biocontrol traits in plant-associated bacteria

#### Oral Presentations

- 09:15 – 09:30 **Wafa Achouak (Laboratory of Microbial Ecology of the Rhizosphere, Aix-Marseille Université-CEA-CNRS, Biosciences and biotechnology Institute of Aix-Marseille (BIAM), CEA Cadarache, FR):** Root exudates shape microbiota and rhizosheath formation in pearl millet: Implications for soil aggregation and carbon sequestration
- 09:30 – 09:45 **Bulbul Ahmed (African Genome Center, University Mohamed VI Polytechnic, MA):** Mapping the microbiome in extreme arid environments revealed microbial consortia with potential for saline stress tolerance
- 09:45 – 10:00 **Manikandan Ariyan (University of Tartu, EE):** Influence of tree mycorrhizal type and plant species identity on twig fungal communities





- 10:00 – 10:15 **Denise Khouri Chalouhi (International Centre for Genetic Engineering and Biotechnology (ICGEB) - Trieste Bacteriology Unit, IT):** An in-planta enrichment route to identify bacterial root endophytes
- 10:15 – 10:30 General Discussion
- 10:30 – 11:00 *Break*

## Session 5 Communication and engagement with society

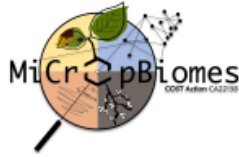
**Chairs:** Caroline DE TENDER and Ivan NIKOLIC

### Invited Lecture

- 11:00 – 11:30 **Eiko Kuramae (Netherlands Institute of Ecology NIOO-KNAW, NL):** Harnessing engineered microbial consortia and metabolites for sustainable grain yield and quality

### Oral Presentations

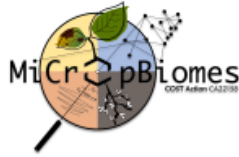
- 11:30 – 11:45 **Lara Amorim (Department of Biology, Faculty of Sciences, University of Porto, PT):** Engaging secondary school students in microbiome literacy: an international educational initiative by the WG5 Task Force
- 11:45 – 12:00 **Matthijs Van Hooste (Department of Biochemistry and Microbiology, Ghent University, BE):** Rooted in Resilience: Exploring the root & seed microbiome of sunflower for sustainable agriculture
- 12:00 – 12:15 **Eliza Loo (Heinrich Heine University, Düsseldorf, DE):** Rice phyllobiome dynamics during bacterial blight disease development
- 12:15– 12:30 **Sofia Montanari (University of Trento, IT):** Investigating the impacts of a new sustainable fungicide on plant-associated microbial communities



- 12:30 – 14:00 *Lunch*
- 14:00 – 16:00 Working Groups Meeting – Plenary Session
- 16:00 – 16:30 Glossary/Terminology Session
- 16:30 – 17:00 *Break*
- 17:00 – 18:00 Poster Session

### Thursday 19<sup>th</sup> of June

- 08:30 – 10:30 – Management Committee Meeting
- 11:00 – 13:00 – Field trip

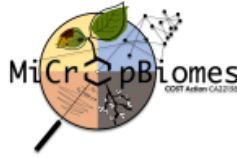


# Session 1

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

**Chairs:** Ahmed ABDELFAHAT & Gabriele BERG

\* \* \*



## Plant-Microbiome interactions under the lens of the one health concept

Gabriele Berg<sup>1,2,3\*</sup> and Ahmed Abdelfattah<sup>2,3</sup>

<sup>1</sup>Institute of Environmental Biotechnology, Graz University of Technology, Graz, Austria

<sup>2</sup>Leibniz-Institute for Agricultural Engineering, Potsdam, Germany

<sup>3</sup>Max-Planck-Institut für Kolloid- und Grenzflächenforschung, Potsdam, Germany

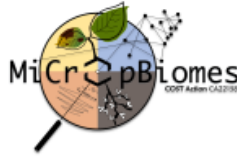
\*[gabriele.berg@tugraz.at](mailto:gabriele.berg@tugraz.at)

**Keywords:** plant microbiome, food-gut axis, microbiome management

Plant-microbiomes interactions are key processes for plant and ecosystem health as well as for one health. The latter is a concept integrating the health of people, animals, and plants into their environment (WHO). The plant microbiota, which consist of bacteria, archaea, protists, and fungi, is vertically transmitted by seeds and replenished horizontally from soil (1). All plants are holobionts and form a functional unit with their microbiome. Plant diversification and co-evolution shaped the plant microbiome and designed their specific composition and functional interplay, including natural biocontrol of pathogens. Plant microbial diversity is not only connected with microbial diversity in soil – recent studies showed their connection for the food-gut axis, confirming the farm-to-fork and one health concept (2). Human activities in the Anthropocene, and especially intense agriculture, are linked to a significant shift in diversity and evenness of the plant microbiota. This shift is characterized by a decrease in host specificity and symbionts, and an increase of r-strategic microbes, pathogens, and hypermutators. Findings from plant microbiome research over the past 20 years clearly call for management of the microbiome and joint attention to the crop holobiont. Plant microbiomes can be managed either directly by applying (i) microbiome transplants, (ii) microbes with beneficial properties, or (iii) microbiota-active metabolites, or indirectly by changing environmental conditions in a way that microbiomes also shift their structure and function from dysbiosis into a healthy state (3). Examples for the different strategies for plant protection and one health will be discussed.

### References

1. Abdelfattah A, et al. 2023. From seed to seed: the role of microbial inheritance in the assembly of the plant microbiome. *Trends Microbiol.* 31(4):346-355. doi: 10.1016/j.tim.2022.10.009.
2. Wicaksono WA, et al. 2023. The edible plant microbiome: evidence for the occurrence of fruit and vegetable bacteria in the human gut. *Gut Microbes.* 15(2):2258565. doi: 10.1080/19490976.2023.2258565.
3. Berg G, et al. 2020. Microbiome definition re-visited: old concepts and new challenges. *Microbiome.* 8(1):103. doi: 10.1186/s40168-020-00875-0.



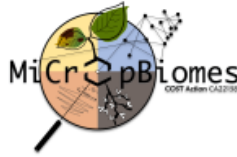
## **Assembly and diversity of the fruit and seed microbiome sustainable agriculture**

Ahmed Abdelfattah

Leibniz Institute for Agricultural Engineering and Bioeconomy, Potsdam, Germany

[\\*aabdefattah@atb-potsdam.de](mailto:aabdefattah@atb-potsdam.de)

The apple fruit microbiome is integral to fruit health, quality, and interactions with the environment, acting as a critical component of the plant's extended phenotype. This study investigates the diversity, assembly, and functional roles of apple-associated microbial communities, emphasizing the interplay between host, environment, and management practices. Leveraging high-throughput sequencing techniques, we explored the bacterial and fungal microbiomes of various apple cultivars, focusing on differences between external (epiphytic) and internal (endophytic) communities. Our findings reveal that microbial composition is influenced by cultivar genotype, rootstock selection, and environmental factors such as soil properties, climate, and orchard management practices. Storage conditions and post-harvest treatments further modulate microbial diversity, highlighting the dynamic nature of the fruit microbiome over time. Key microbial taxa associated with nutrient cycling, pathogen resistance, and fruit shelf life were identified, offering insights into potential applications for microbiome-based strategies to enhance fruit quality. Temporal analyses demonstrate shifts in community structure during ripening and storage, underscoring the role of fruit development in microbiome assembly. Additionally, vertical transmission pathways linking seed, flower, and fruit microbiomes suggest evolutionary strategies for microbial inheritance and seed dispersal. This research sheds light on the multifaceted factors shaping the apple microbiome and its potential to support sustainable agricultural practices. By integrating ecological, genetic, and functional approaches, our findings provide a foundation for the targeted manipulation of fruit microbiomes to improve crop resilience and productivity.



## Improving plant microbiome profiling: host-DNA blocking strategies and high-accuracy long-read bioinformatics pipeline for Nanopore data

Benjamin Dubois<sup>1\*</sup>, Mathieu Delitte<sup>2</sup>, Salomé Lengrand<sup>2</sup>, Claude Bragard<sup>2</sup>, Anne Legrève<sup>2</sup>, Anne Chandelier<sup>1</sup> and Frédéric Debode<sup>1</sup>

<sup>1</sup> Life Sciences Department, Walloon Agricultural Research Centre, Gembloux, Belgium

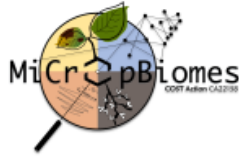
<sup>2</sup> Earth & Life Institute, UCLouvain, Louvain-la-Neuve, Belgium

[\\*b.dubois@cra.wallonie.be](mailto:b.dubois@cra.wallonie.be)

**Keywords:** Metabarcoding, Plant microbiome, PNA clamps, bioinformatics pipeline, long-read sequencing

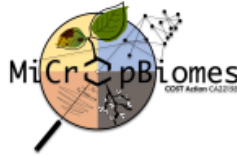
Understanding plant-associated microbiomes is critical for improving crop resilience, productivity, and sustainability. However, accurate profiling of microbial communities through amplicon sequencing remains challenging due to host DNA contamination and platform-specific limitations, especially when using long-read technologies like Oxford Nanopore. Here, we present two complementary developments aimed at improving both the experimental and bioinformatics steps of plant microbiome studies. First, we designed and validated a set of peptide nucleic acid (PNA) clamps that efficiently block the amplification of host plant DNA during PCR. Using wheat (*Triticum aestivum*) as a model, we developed PNA clamps targeting chloroplast and mitochondrial regions (V4 and V5-V7) as well as ITS regions (ITS1 and ITS2). These clamps enable targeted amplification of bacterial (16S) and fungal (ITS) DNA while minimizing host-derived reads. Our results show that the targeted regions share high sequence conservation across multiple cereal species, suggesting that these clamps can also be applied to a wide range of cereal crops. These systems are compatible with both Illumina and Nanopore metabarcoding protocols. Moreover, the associated publication provides a detailed roadmap to guide users wishing to develop custom PNA clamps for alternative host species. Second, we introduce PRONAME (1), a user-friendly and fully containerized bioinformatics pipeline for processing long-read metabarcoding data generated by Oxford Nanopore sequencing. The pipeline integrates a robust and innovative error-correction approach, combining consensus generation and polishing steps to achieve high accuracy. Validation tests on mock communities demonstrated that PRONAME can consistently reach a sequence accuracy of up to 99.7%. With the recent improvements in Nanopore basecalling models, it is expected that even higher accuracies are now attainable using this workflow. PRONAME is particularly well suited for researchers with limited bioinformatics expertise and supports end-to-end analysis from raw reads to final taxonomic assignments. Together, these advances contribute to overcoming two major limitations in plant microbiome studies: host DNA interference and sequencing errors. By combining targeted experimental approaches with high-fidelity data processing, our work enables more accurate and accessible characterization of plant-associated microbial communities, with direct implications for crop science, ecology, and biotechnology.

## References



1. Dubois B, Delitte M, Lengrand S, Bragard C, Legrève A, Debode F (2024). PRONAME: a user-friendly pipeline to process long-read nanopore metabarcoding data by generating high-quality consensus sequences. *Frontiers in bioinformatics*, 4, 1483255.

**Funding:** Support was provided by the Public Service of Wallonia (grant number D65-1417) and the European Food Safety Authority (grant number GP/EFSA/ENCO/2020/02). Trainings on these approaches is also provided through the COST Action MiCropBiomes (CA22158).



## Plant–microbiome interactions under a changing world

Salme Timmusk<sup>1\*</sup> and Taavi Pall<sup>1</sup>

Department of Forest Mycology and Plant Pathology, Uppsala BioCenter, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden

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**Keywords:** plant stress adaptation, plant-holobiont, host extended phenotype, land use intensification

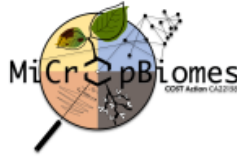
Climate change and land-use intensification pose a major threat to many agroecosystems, leading to losses in crop yield as well as species loss and alterations in community structure. Plants may adapt to stress situations, but this can lag rapid anthropogenic changes to the environment. Our data show that natural modulations of the microbiome using native, harsh, and wild habitats consortia directly affect plant phenotype and performance, thus increasing plant stress tolerance. The microbiome extends host evolutionary potential, and understanding how plant microbiome holobionts function in native, harsh, and wild habitats and how rhizobacteria mediate plant and ecosystem biodiversity in the systems enables us to identify key factors for plant fitness [1-3]. A systems approach to engineering microbial communities by connecting host phenotype adaptive traits would help us to understand the increased fitness of the crop holobionts supported by genetic diversity. Identification of genetic loci controlling the interaction of beneficial microbiomes will allow the integration of genomic design into crop breeding programs. It is important to understand the structure of microbial communities, functional and environmental patterns, enzyme pathways used, and how they are activated. DNA and RNA-based microbial community sequencing analysis methods are discussed.

### References

1. Pall T, Zagal-Venegas E, Pavlicek T, Nevo E, Timmusk S. Comparative analysis of microbial communities of soils under contrasting microclimates. doi.org/10.1101/20241127625671 2024.
2. Fetsiukh A, Pall T, Timmusk S. Decrease due to pollution in the rhizosphere microbial diversity can be amended by supplementation from adapted plants of another species. Scientific reports. 2024;14(1):18806. Epub 20240813. doi: 10.1038/s41598-024-68123-1. PubMed PMID: 39138231; PubMed Central PMCID: PMCPCMC11322436.
3. Timmusk S, Pall T, Raz S, Fetsiukh A, Nevo E. The potential for plant growth-promoting bacteria to impact crop productivity in future agricultural systems is linked to understanding the principles of microbial ecology. Frontiers in microbiology. 2023;14:1141862. Epub 2023/06/05. doi: 10.3389/fmicb.2023.1141862. PubMed PMID: 37275175; PubMed Central PMCID: PMCPCMC10235605.

**Funding:** Funding was provided by Swedish Research Council VR2021-05471 to ST





## Lessons from model and crop plants on plant-microbiome communication

Ioannis A. Stringlis<sup>1,2,\*</sup>, Jiayu Zhou<sup>1,3</sup>, Melissa Uribe-Acosta<sup>1</sup>, Dafni Paraskevopoulou<sup>2</sup>, Ronnie de Jonge<sup>1</sup>, Corné M.J. Pieterse<sup>1</sup>

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<sup>2</sup> Laboratory of Plant Pathology, Agricultural University of Athens, Athens, Greece

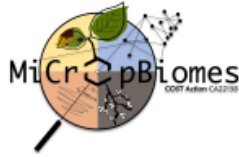
<sup>3</sup> Institute of Botany, Jiangsu Province and Chinese Academy of Sciences, Nanjing, Jiangsu, China

[\\*i.stringlis@aua.gr](mailto:i.stringlis@aua.gr)

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

Plant roots engage with a diverse soil microbiota, including both beneficial and harmful organisms, yet plants typically maintain health through sophisticated defense and communication mechanisms. To unravel how plants shape and respond to their microbiome, we employ three complementary approaches. First, we investigate in vitro the role of structural (cutin, suberin, callose, lignin) and chemical (coumarins, camalexin, glucosinolates) defense components in interactions with the beneficial rhizobacterium *Pseudomonas simiae* WCS417. Mutants lacking specific defenses show impaired root colonization and reduced plant benefits, with transcriptomic analyses revealing that camalexin modulates bacterial chemotaxis and other compounds mediate a growth-defense trade-off. Second, we assess the impact of these defenses on microbiome assembly by growing mutants in natural soil and analyzing microbial communities in bulk soil, rhizosphere, and roots. This approach reveals distinct microbial signatures associated with each mutant and compartment. Third, we compare plant traits and microbiome composition between wild tomato relatives resistant to soilborne pathogens and susceptible commercial cultivars, aiming to identify metabolites and microbes contributing to resistance. Together, these approaches aim to decipher the role of plant defenses in maintaining plant-microbiome homeostasis and inform the development of microbiome-assisted strategies for sustainable agriculture.

**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).

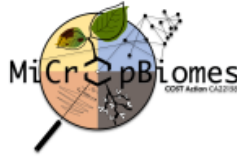


## Session 2

Crop microbiomes and plant diseases: From dysbiosis to increased defenses

**Chairs:** Joël F. POTHIER & Renata ARTIMOVA

\* \* \*



## **Bacterial Canker: Control Vascular Spread Based on Seasonal Dynamics of *Pseudomonas syringae* pv. *actinidiae* in Kiwi Plant**

Wei Liu, Mingming Yang, Nana Wang, [Lili Huang\\*](#)

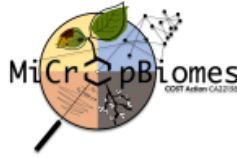
State Key Laboratory for Crop Stress Resistance and High-Efficiency Production and College of Plant Protection, Northwest A&F University, Yangling 712100, P.R. China.

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**Keywords:** Kiwifruit Bacterial Canker; *Pseudomonas syringae* pv. *actinidiae* (*Psa*); Temperature-dependent pathogenicity; Integrated disease management

Bacterial canker, caused by *Pseudomonas syringae* pv. *actinidiae* (*Psa*), is a most destructive disease affecting kiwi production worldwide. Traditional control methods have predominantly relied on chemical sprays, such as copper-based compounds, and the removal of infected plant materials during periods of high disease incidence. However, these measures have proven inadequate in controlling the disease, often resulting in trunk canker or even orchard devastation. Therefore, we shifted the strategy from a “treatment-focused” approach to a “prevention-oriented” one. Our research has revealed that *Psa* can invade multiple host organs and spread systemically within the vascular tissues. The type III secretion system (T3SS) and its effector proteins are key virulence factors of *Psa* biovar 3. Notably, a conserved *hrpR*-upstream locus and the two-component system RegAB have been identified as critical regulators of T3SS virulence, with phosphorylated RegA suppressing *hrpR/S* expression. Effector proteins HopAU1 and HopZ5 interact with plant receptors to activate immunity, while cyclic di-GMP (c-di-GMP) signaling and the type VI secretion system (T6SS) contribute to environmental adaptation and bacterial competition. Cool temperatures promote *Psa* infection and rapid spread, whereas temperatures above 24°C correlated with the decline in *Psa* populations and asymptomatics. Microbiome analysis showed *Psa* population of kiwi trees fell to the bottom in summer, then gradually rose to peak in early spring, further elucidating the influence of temperature on annual epidemic dynamics and pathogen population fluctuations. We uncovered that cool conditions exacerbate disease development by both suppressing ethylene-mediated host resistance pathways and enhancing pathogen virulence. Based on the novel insights, we developed a prevention technique that targets two crucial periods to inhibit pathogen invasion, colonization, and systemic movement within the host. Biological agents such as Zhongshengmycin, Kasumin, and Polymyxins, as well as developed compound products, are sprayed onto the trunks twice during the period “After fruit-picking and before leaf-fall” to protect trees from bacterial ooze-induced mortality the following spring. Additionally, trees are treated twice, “before and after flowering” to minimize *Psa* accumulation. This convenient approach, requiring only four treatments annually, dramatically reduces pathogen load in trees by 87.3% and lowers the tree mortality rate from 30% to less than 3%. The system has been implemented in nearly 70% of kiwi-growing regions in China, offering an effective solution for managing kiwi bacterial canker.

**Funding:** Support was provided by the Natural Key Research and Development Program (2022YFD1400200).



## Future climate and agricultural farming systems affect the fungal plastisphere of different biodegradable plastics at the early stage of field degradation

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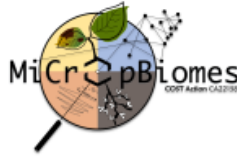
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**Keywords** Conventional farming, Organic farming, Climate change platform, Plastisphere microbiome, Polybutylene-succinate (PBS), Polybutylene adipate-co-terephthalate (PBAT), Soil

The use of biodegradable mulch films has an advantage over non-biodegradable ones, as it offers degradation by microbes under environmental conditions. Nevertheless, less is known about the microbial colonization of different biodegradable plastics under different agricultural systems and climate change conditions. In the current study, the plastic degradation experiment was conducted at the Global Change Experimental Facility platform, specifically in conventional and organic farming systems, both under ambient and future climatic conditions. In this study, we investigated the early fungal colonizers associated with polybutylene-succinate (PBS) and polybutylene adipate-co-terephthalate (PBAT) with polyethylene (PE) as a reference in comparison to the initial soil fungal community. We found a distinct pattern between soil and plastisphere fungi. Soil fungi were dominated by Sordariomycetes (mainly *Gibellulopsis*, *Fusarium*, and *Gibberella*), and fungi in plastics were dominated by Dothideomycetes (mainly *Mycosphaerella*, *Alternaria*, and *Cladosporium*). These microbes were previously reported as plastic colonizers and potential plastic degraders. We found that agricultural systems affect both fungal richness and community composition of the plastisphere. Plastic type significantly affected the fungal richness, but not the fungal community composition. The two different agricultural systems undergo different treatments, including crop rotation and fertilization, which in turn impact the fungal colonization of the biodegradable plastics. This study provides new insights into factors that affect early fungal colonization of different biodegradable plastics under real field conditions using high-throughput sequencing. These data are of high relevance to evaluate the plastic composition for adjusted rate of plastic biodegradation for upcoming mulch film products.

**Funding:** Dr. Benjawan Tanunchai was supported for her postdoctoral position by Peter and Traudl Engelhorn Foundation. This work was partially funded by the internal research budget of Prof. Dr. Matthias Noll, Institute of Bioanalysis, Coburg University of Applied Sciences and Arts, Coburg, Germany.



## **Assessing the role of *Salicaceae* xylem endophytes on the establishment and progression of *Xylella fastidiosa* or *Brenneria salicis***

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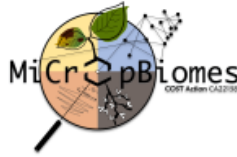
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**Keywords:** xylem microbiome, plant-microbe interaction

*Xylella fastidiosa* and *Brenneria salicis* are xylem-restricted bacterial pathogens responsible for devastating diseases in a wide range of host plants, including key members of the *Salicaceae* family. Yet, a puzzling phenomenon persists: some infected plants remain symptomless. The drivers behind this contrasting symptomatology remain largely unknown, but increasing evidence points to the resident xylem microbiota as a key player influencing pathogen establishment and disease progression. In this study, we explore the functional interplay between xylem-associated endophytes and vascular pathogens, aiming at identifying microbial interactions that may suppress infection and support plant health. From *Salicaceae* hosts, we isolated over 400 endophytic bacterial strains, complemented with targeted metagenomic amplification to better capture the xylem microbiome's composition and diversity. Initial confrontation assays revealed a subset of isolates capable of antagonizing either *X. fastidiosa* strain KLN59.3 or *B. salicis* LMG2698 *in vitro*. These strains were selected for whole-genome sequencing and further phenotypic characterization. Several are currently being engineered for fluorescent proteins expression, facilitating direct visualization of their spatial dynamics, colonization behaviour, and interactions with pathogens within plant xylem vessels. Co-inoculation experiments in *Populus*, *Salix*, and *Nicotiana tabacum* hosts are being used to validate in planta antagonistic effects and unravel potential protective mechanisms. By integrating classical microbiology, plant pathology, genomics, and imaging tools, our work contributes to a better understanding of plant-microbe-pathogen tripartite interactions within the xylem niche. These findings highlight promising candidates for the development of endophyte-based biocontrol strategies against xylem-inhabiting pathogens, and more broadly, emphasize the importance of microbiome-informed approaches for sustainable disease management.

**Funding:** Support was provided by the Fonds pour la Formation à la Recherche dans l'Industrie et l'Agriculture (FRIA grantee 2nd grant 40021505)



## Seed Microbiome Diversity in Balkan Legumes: Insights from *Lathyrus sativus* (Herzegovina) and *Phaseolus vulgaris* (Montenegro)

Mihajlo Voruna<sup>1</sup>, Jelena Zindović<sup>2</sup>, Duška Delić<sup>1\*</sup>

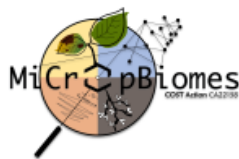
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The seed microbiome plays a vital role in early plant development, stress resilience, and ecological adaptation. Understanding the composition of seed-associated bacterial microbiome is critical for improving crop resilience, nutrient uptake, and sustainable agriculture. This study explores and compares the bacterial communities associated with seeds of *Lathyrus sativus* that is commonly grown for consumption in Trebinje locality of the Herzegovina region, Bosnia and Herzegovina (BiH) and a local *Phaseolus vulgaris* variety from Berane municipality in Montenegro (ME), commonly used in local cuisine. DNA extracted from seeds of *L. sativus* (BiH) and *P. vulgaris* (ME), were subjected to high-throughput 16S rRNA sequencing. Our results reveal distinct microbial signatures in both legumes. This research highlights the importance of seed-associated microbes in shaping plant-microbe interactions and supports the value of preserving local crop varieties and their native microbiomes for sustainable agricultural practices in the Balkans.

**Funding:** Support for the research was partially provided by the Funds of Ministry of Civil Affairs of Bosnia and Herzegovina supporting COST Actions (contract number 10-33-11-7188/24). 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).



## Universal properties of *Pantoea agglomerans* T16/8, *Pantoea allii* T14/15 strains in protecting fruit and vegetable plants against diseases

Joanna Puławska<sup>1</sup>, Artur Mikiciński<sup>1</sup>, Hubert Głos<sup>1</sup>, Monika Michalecka<sup>1</sup>, Anna Poniatowska<sup>1</sup>, Anna Jarecka-Boncela<sup>1</sup>, Magdalena Ptaszek<sup>1</sup>, Agnieszka Włodarek<sup>1</sup>, Katarzyna Góralska<sup>2</sup>, Krzysztof Ambroziak<sup>2</sup>

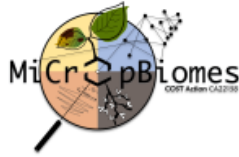
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**Keywords:** biocontrol, biostimulation

More than 2,500 bacterial isolates were obtained from the phyllosphere of various fruit plants and the soil under these crops. They were then assessed for their suitability for controlling the most important apple diseases. In the first step, their protective abilities against the causative agent of gray mold (*Botrytis cinerea*) were assessed in the screening test on apple fruits (Gala Must). Among the isolates with the strongest disease control properties (80-90% efficiency), 20 were selected, which were then assessed for the ability of the fire blight (*Erwinia amylovora*), brown rot (*Monilinia* spp.), bull's eye rot (*Phlyctema vagabunda*, *Neofabrea* spp.), blue mold (*Penicium*) and *Alternaria* rot control in the pear fruit test. Based on the results, two strains: *Pantoea agglomerans* T16/8, *Pantoea allii* T14/15 were found to possess the most universal activities. The selected strains also showed properties to control some diseases of vegetable plants and stimulate their growth. The genome sequence analysis of the selected strains revealed the presence of genes that can elucidate the mode of action of the selected strains. Additionally, they were tested for safety in a toxicological assay on rats. These two strains were commercialized as a product Bactim Pantoea Care.

**Funding:** The studies were performed in the frame of project BioSafeFood “Development of a technology for producing high-quality, consumer-safe fruit and vegetables using new biopreparations to protect crops against diseases”, co-financed by UE from the European Regional Development Fund under the Smart Growth Operational Programme 2014-2020, contract no. POIR.04.01.02-00-0100/17-00 NCBiR. (National Centre for Research and Development)



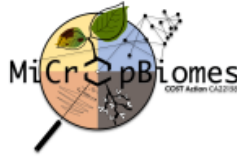
# Session 3

From current microbial inoculants to synthetic communities

**Chairs:** Anna BONATERRA & Claire PRIGENT-COMBARET

\* \* \*





## Unravelling and engineering the olive xylem microbiome to enhance its resilience against vascular pathogens

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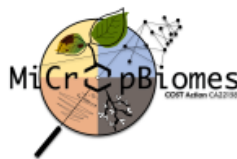
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**Keywords:** Plant-associated microbiome; *Olea europaea*; synthetic microbial communities; vascular plant diseases; *Verticillium dahliae*; *Xylella fastidiosa*

In natural environments, healthy plants coexist in continuous interaction with diverse communities of microorganisms—collectively known as the plant microbiome—which are increasingly recognized as key contributors to plant health and resilience. However, the potential role of these microbial communities in modulating resistance to vascular pathogens in olive trees (*Olea europaea* L.) has received limited attention and remains largely unexplored. In this study, we sought to address several foundational questions regarding the structure, function, and engineering of the olive xylem microbiome: Can the xylem microbiome be reliably extracted and analyzed? What is the nutrient composition of xylem sap? How does infection by vascular pathogens alter microbial communities within the xylem? What biotic and abiotic factors shape these microbial assemblages? Can specific microbial members be isolated and cultured? And finally, is it feasible to design synthetic microbial communities with traits beneficial for pathogen suppression (e.g., *Xylella fastidiosa* and *Verticillium dahliae*) and plant growth promotion? Our research has established the methodological framework required to explore these questions. We have identified key biotic and abiotic drivers influencing xylem microbial communities and successfully characterized several core microbiome members. Importantly, we have isolated and cultured specific bacterial strains from the xylem, some of which have been selected to form synthetic consortia. These microbial consortia can be introduced into the xylem of olive plantlets, enabling us to reshape their native microbiome toward enhanced resilience against vascular pathogens or improved physiological performance.

**Funding:** Support was provided by projects MCIN/AEI/10.13039/501100011033 (PID2020-114917RB-100), MICIU/AEI/10.13039/501100011033 “European Union Next Generation EU/PRTR” (TED2021-130110BC41), MCIN/AEI/10.13039/501100011033 Grant IJC2019-040423-I from the Spanish Ministry of Science and Innovation (AEI); project BeXyl (Grant ID 101060593, EU-Horizon Europe) and Qualifica Project QUAL21\_023 IAS. This abstract was elaborated within the COST Action CA22158 MiCropBiomes, supported by COST (European Cooperation in Science and Technology).



## Development of Synthetic Bacterial Communities (SynCom) with xylem-inhabiting bacteria for the biological control of vascular pathogens of olive crop

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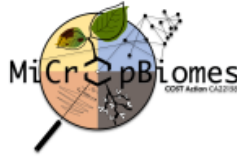
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**Keywords:** SynCom, biocontrol, *Xylella fastidiosa*, *Verticillium dahliae*, phenotypic characterization.

Infections caused by the fungus *Verticillium dahliae* (Vd) and the bacterium *Xylella fastidiosa* (Xf) are significant threats to economically important crops worldwide, such as olive (*Olea europaea* L.), almond (*Prunus dulcis* Mill.) and grapevine (*Vitis vinifera* L. As the European Union advances toward more sustainable agricultural practices under the framework of the European Green Deal, the use of endophytic microorganisms is emerging as a promising strategy for managing diseases caused by vascular pathogens like Xf and Vd. In particular, the development of synthetic microbial communities (SynCom) composed of olive xylem-inhabiting bacteria could provide a novel approach for biological control. A collection of over 100 xylem-inhabiting bacterial strains were isolated from olive trees and their phenotypic and genomic characterization was performed to evaluate their potential as biological control agents and as plant growth-promoting bacteria. Phenotypic characterization included the identification of biocontrol-related activities and traits, as well as the analysis of interactions with the indigenous xylem microbiome. The characterized activities were: i) the assimilation of carbon sources and tolerance to different stresses such as the presence of antibiotics, acid pH or sodium chloride, ii) the capacity to promote plant growth through the production of siderophores, indole-3-acetic acid, or nitrogen fixation, and iii) microbial antagonism through the production of antimicrobial enzymes (amylase, chitinase, protease, etc.), and iv) the inhibition of Xf and Vd growth. Based on the results of the previously mentioned activities, compatibility assays were performed with 12 of the most promising strains which allowed the development of 2 SynComs, each composed of 3 bacterial strains. These SynComs are currently being tested both *in vitro* and *in planta*. Specifically, the microbial antagonism and interactions between the SynCom and Vd and Xf are being analysed in microfluidic chambers. Moreover, SynComs are also being tested in different pathosystems to determine whether their *in vitro* efficacy translates to *in planta* conditions. These include Xf in tobacco (*Nicotiana benthamiana* Domin) as a model plant, Xf in olive, and Vd in olive. In addition, their ability to systemically colonize the xylem vessels when applied by endotherapy will be monitored over time.

**Funding:** Support was provided by projects MCIN/AEI/10.13039/501100011033 (PID2020-114917RB-I00), MICIU/AEI/10.13039/501100011033 “European Union Next Generation EU/PRTR” (TED2021-130110BC41), MCIN/AEI/10.13039/501100011033 Grant IJC2019-040423-I from the Spanish Ministry of Science and Innovation (AEI); project BeXyl (Grant ID 101060593, EU-Horizon Europe) and Qualifica Project QUAL21\_023 IAS. This abstract was elaborated within the COST Action CA22158 MiCropBiomes, supported by COST (European Cooperation in Science and Technology).



## Microbiome-mediated adaptation: leveraging grape-associated microbiota to boost hologenome plasticity under climate change

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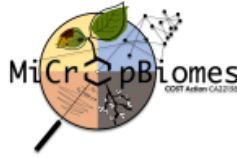
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**Keywords:** SynCom formulation; Drought stress; Arbuscular mycorrhizal fungi (AMF); Molecular cross-talk

Genetic improvement of plants has long been employed as a key strategy to enhance stress tolerance and optimize traits valuable for human needs. However, the continuous selection of specific genomic traits, along with the intensive use of external inputs to support elite genotypes, has led to unintended consequences, most notably a reduction in the biodiversity of plant-associated microbial communities. These microbes, collectively referred to as the plant hologenome (1), play a crucial role in plant adaptation to environmental stresses. In recent years, growing attention has been directed toward the isolation, characterization, and preservation of these plant-associated microbial communities, collectively termed the holobiont (2). In this context, a collection of endophytic bacterial isolates from grapevine woody tissues was assembled to create a tailored microbial inoculum. Selected strains were screened for plant growth-promoting (PGP) traits and tested in biocontrol assays against key grapevine pathogens. As a proof of concept, grapevine cuttings were inoculated with three distinct synthetic microbial communities (SynComs) prior to planting: i) SynCom1, composed of isolates with strong PGP activity, ii) SynCom2 enriched in biocontrol-active isolates, and iii) SynCom3 combining isolates from both groups. To explore potential synergistic effects, each SynCom was also co-inoculated with arbuscular mycorrhizal fungi (AMF), a well-studied group of beneficial symbiotic fungi. Plants inoculated with SynComs, SynComs+AMF, AMF alone, and uninoculated controls were monitored for growth and physiological performance. Ninety days after planting, a drought stress experiment was conducted by withholding irrigation until plants reached a defined threshold of severe water stress (stomatal conductance –  $g_s$  – below 0.01). Remarkably, plants inoculated with SynCom1, both alone and in combination with AMF demonstrated significantly higher resilience to drought compared to all other treatments. Indeed, the photosynthetic activity ( $P_n$ ) was still ongoing, and the stomatal conductance was significantly higher, when all the other conditions reached the severe stress ( $P_n \leq 0$  and  $g_s \leq 0.01$ ). Furthermore, the intrinsic water use efficiency (iWUE) was higher in the SynCom1-primed plants in comparison to the other treatments. To investigate the underlying mechanisms, leaf and root samples were collected at multiple time points for transcriptomic (RNAseq) analysis, while root samples were also analysed by metabarcoding to assess microbiome dynamics.

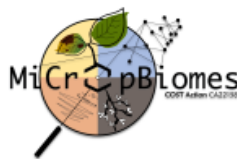


Encouraged by these promising results, SynCom1 was subsequently tested under field conditions on two grapevine cultivars (Glera and Nebbiolo) grown in two distinct regions of Northern Italy. Ecophysiological monitoring revealed improved plant performance in SynCom1-inoculated vines. Additionally, analyses of SynCom1-treated soil showed enhanced parameters, such as increased levels of available phosphorus, organic matter, and total nitrogen, as well as improved enzymatic activities (dehydrogenase, urease and  $\beta$ -glucosidase). Ongoing analyses aim to further elucidate the dynamics of crop–microbiome assembly and the molecular cross-talk driving the observed phenotypic outcomes.

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## Ensuring the efficacy of the biopesticide Microfighter: survival of *Pseudomonas synxantha* DLS65 in diverse crops and its defense-stimulating role in grapevine.

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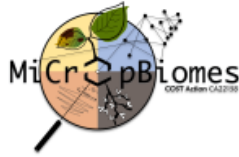
**Keywords :** biocontrol; colonization; defense-related genes

The search for sustainable alternatives to chemical pesticides in agriculture has driven the development of several commercial microbial-based products. In this context, the EU-funded project “LIFE Microfighter” is evaluating the efficacy of *Pseudomonas synxantha* strain DLS65, formulated with natural zeolites (chabasite) into a biopesticide named 'Microfighter', to control major fungal and bacterial diseases with the reduction of copper-based pesticides in vineyards, olive groves, and tomato fields. In this study, we investigated the persistence and survival of strain DLS65, the microbial active ingredient of Microfighter, on three economically important crops: olive, tomato, and grapevine. Our findings demonstrate that DLS65 exhibited crop-dependent colonization and persistence behaviour within the phyllosphere of olive and grapevine in field conditions. Under greenhouse conditions, the eGFP-tagged DLS65 strain successfully colonized both the rhizosphere and phyllosphere of tomato plants at least two weeks post-inoculation. Moreover, transcriptomic analyses (1) revealed that Microfighter treatment consistently triggered the upregulation of key defense-related genes in grapevine, including those associated with salicylic acid and jasmonic acid signalling pathways (2). These results indicate that DLS65 can adapt to different plant hosts and effectively activate plant immune responses, suggesting a broad-spectrum potential for disease management. This research supports the integration of Microfighter into an eco-friendly strategy to promote crop protection and reduce dependency on chemical pesticides.

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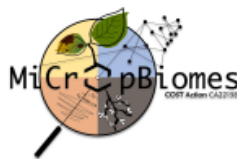


# Session 4

Crop-Microbiome assembly dynamics under specific environments

**Chairs:** Elodie VANDELLE & Gordana RACIC

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## Chemical communication and its impact on the expression of biocontrol traits in plant-associated bacteria

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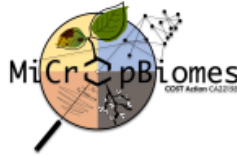
**Keywords:** *Pseudomonas*; *Streptomyces*; *Phytophthora infestans*; volatiles; siderophores; biocontrol

Plants are colonized by a diverse microflora both at the root and at the shoot level. This contributes to plant health, e.g. by secreting antimicrobial compounds, by inducing the plant immune defences or by depriving pathogens of important resources such as iron. We are interested in understanding how the microbiome contributes to plant health and use crops of agronomical relevance such as potato and grapevine to answer this question. We have recently discovered that plant-associated bacteria and fungi can detect the presence of competing microbes via their volatile emission and react by upregulating antimicrobial volatile emission and/or siderophore production. In turn, some of the volatile chemical signals emitted by beneficial root-associated *Pseudomonas* can remotely influence the behaviour of other rhizosphere inhabitants, leading to diminished siderophore production, reduced motility and increased biofilm formation. These modulated traits are of relevance for both the ability of the microbes to successfully colonize their host plant and to competitively inhibit plant pathogens. These new findings lift the veil on the complex chemical communication taking place within the plant microbiota, which can be mediated by both volatile and non-volatile signalling compounds. Understanding the basis of this communication and identifying the chemical signals leading to up- or downregulation of biocontrol-relevant traits such as siderophore production or emission of antimicrobial volatiles in plant-associated bacteria will open significant avenues for improved microbe-mediated crop protection in the future.

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**Funding:** Support was provided by the Swiss National Science Foundation (grant nr. 207917 to L.W.).



## Root exudates shape microbiota and rhizosheath formation in pearl millet: implications for soil aggregation and carbon sequestration

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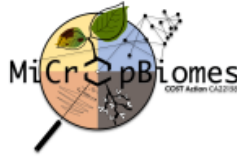
**Keywords:** Pearl millet, exudates, soil aggregation, microbiota, carbon storage

The rhizosheath—a dense layer of soil adhering to plant roots—plays a vital role in water and nutrient uptake. In pearl millet, genome-wide association studies (GWAS), bulk segregant analysis, and gene expression profiling revealed that rhizosheath formation is under complex genetic control, primarily influenced by root exudation. To investigate how root exudates affect microbiota composition, interactions, and rhizosheath structure, we studied four pearl millet lines with contrasting soil aggregation capacities. We combined 16S rRNA and ITS metabarcoding with FTICR-MS metabolomics to profile microbial communities and metabolic signatures across plant compartments and root exudates. Our results revealed significant differences in microbial diversity and network structure among the lines.  $\beta$ -NTI analysis showed that bacterial communities in the rhizosphere are mainly shaped by deterministic processes, particularly homogeneous selection in root tissue (RT) and root-adhering soil (RAS), while fungal communities were more influenced by stochastic processes. In bulk soil, both deterministic and stochastic forces were at play, with deterministic processes dominating. Metabolite profiles in shoots, RT, and RAS correlated with soil aggregation levels, highlighting the role of plant genotype in shaping both microbiota and metabolic outputs. Notably, antimicrobial compounds like DIMBOA and H-DIMBOA were exclusively found in low-aggregating lines. Using a conceptual model and dual carbon isotope labelling, we confirmed a priming effect across all lines. Interestingly, the priming amplitude (Clost:Cnew ratio) was higher in low-aggregating lines, suggesting greater carbon stabilization potential in high-aggregating genotypes.

These findings highlight the central role of root exudates in modulating plant-microbe-soil interactions, offering new insights into mechanisms underlying rhizosheath formation and their potential for enhancing soil health and carbon sequestration.

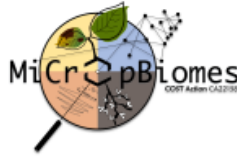
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**Funding:** This work was supported by the French Agence Nationale de la Recherche (ANR grant RootAdapt ANR17-CE20-0022–01).



## Mapping the microbiome in extreme arid environments revealed microbial consortia with potential for saline stress tolerance

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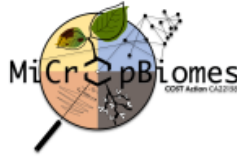
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**Keywords:** microbiome, mapping, saline, arid

Our study focused on mapping microbes associated with different plants – tree, shrub and small vegetation and soil and development of consortia for sustainable agriculture. We sampled in five different ecosystems in the Southern Morocco, which is featured by drought and saline - sabkha (salt flats), dunes, grara (seasonal salt flats characterized by periodic water retention), riverbeds, and lagoons. Over the last two years, we sampled plant shoot, root, rhizosphere soil, and bulk soil for culture dependent and culture independent (sequencing) methods to reveal the complete genetic composition and the dynamics of microbial communities. Metabarcoding approach identified more than 10000 ASVs across more ten plus phylum of bacteria and fungi. Alpha and beta diversity analysis revealed that there is significant difference among plant and sample types. Soil physio-chemical analysis and Pearson's correlation and Mantel test showed that some key soil properties (e.g. electro conductivity, soil organic matter) plays as key variables for the microbial abundance across ecosystems. Our initial findings of culture dependent approach identified 65 halotolerant microbial strains, for example nine different *Bacillus* sp. Including *B. velezensis* as well as other bacteria of *Kushneria*, *Halomonas*, *Thalassospira*, *Maritimibacter* and *Marinococcus*. By considering the ecosystems origin and features and different PGP traits of those isolates, we have formed nine consortia and now they are under evaluation in the greenhouse on the growth performance of salt stress on wheat. These findings underline the significant impact of salinity on the composition and diversity of microbial communities in sabkha ecosystems.

**Funding:** The Authors would like to acknowledge the support through the PHOSBOUCRAA—sponsored by OCP (OCP Foundation, PHOSBOUCRAA foundation, African Genome Center, Mohammed VI Poly-technic University, under the project entitled “Cartographie du microbiome dans le Sud du Maroc et son impact sur la solubilité du phosphore et les performances agronomiques grâce aux approches de séquençage à haut débit et grâce aux approches de Machine Learning.”



## Influence of tree mycorrhizal type and plant species identity on twig fungal communities

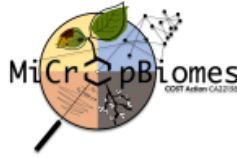
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**Keywords:** Plant Mycorrhizal type; Plant species identity; twig microbiome

Plant species and their associated mycorrhiza types exhibit differences in microbiome composition and carbon and nutrient cycle abilities. Also, various studies show that different types of mycorrhizae are crucial for facilitating plant interactions and influencing biodiversity - productivity in various forests. We established the MycoPhylo experimental plot to test hypotheses about the impact of plant species, species diversity, mycorrhiza type, and phylogenetic context on soil microbial diversity, functioning, and processes. MycoPhylo plot consists of 116 plant species from three mycorrhiza types: Ecto (EcM), Ericoid (ErM), and AM in 237 plots, with plant diversity ranging from one to four and mycorrhiza type diversity from one to three per plot. Initially, we examined the young tree's twig fungal communities. We have collected soil from the 237 plots and extracted DNA from them. Then, we performed PCR using ITS primers and sequenced the samples using the PacBio sequencing platform. The initial results show the mycorrhizal type greatly influenced the fungal communities in the twig of EcM plant species, which had higher alpha diversity indices than AM and ErM. In the Beta diversity analysis, AM and ErM were grouped in the same cluster, while ErM formed a distinct cluster. In conclusion, our study demonstrates the significant impact of host specificity and phylogenetic factors on young tree's twig fungal communities, highlighting the mycorrhizal type's crucial role in shaping microbial diversity.



## **An *in planta* enrichment route to identify bacterial root endophytes**

Denise Khouri Chalouhi<sup>1</sup>, Iris Bertani<sup>1</sup>, Alfonso Esposito<sup>1,2</sup>, Silvano Piazza<sup>1</sup>, Cristina Bez<sup>1\*</sup>, Vittorio Venturi<sup>1,3\*</sup>

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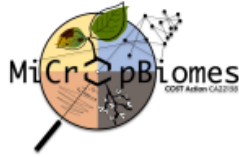
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**Keywords:** super endophytes; plant microbiome; rice; nitrogen depletion; enrichment

Microorganisms live in close association with plants forming ecological interaction webs and providing beneficial traits such as nutrition, growth and tolerance to biotic and abiotic stresses. Via the rhizosphere, plants recruit bacteria which colonize internal plant tissues creating a spatial gradient between the rhizosphere and the endosphere. This study presents a high throughput *in planta* endophyte enrichment scheme designed for the identification of ‘super’-endophytic bacteria which can serially colonize the rice root endosphere. *Oryza sativa* (rice) plants were grown in bulk soil and endophytes were then recovered from roots. The recovered endophytes mixture was used as inoculum for the first generation of rice plantlets which were then grown under no stress or nitrogen (N) depletion. The total endophytic community was then purified and used as a second inoculum for a new set of plants; this procedure was repeated for four generations. Enrichment patterns of root bacterial endophytes were observed, such as *Kosakonia* in the non-stressed plants and *Ferrovibrio* in plants grown under nitrogen starvation. This enrichment method proved to be suitable for the identification of endophytes which can efficiently colonize the root endosphere.

**Funding:** This work was supported by an ICGEB pre-doctoral fellowship and by the grant PGR10061 from the Italian Ministry of Foreign Affairs and International Cooperation (MAECI, Italy).

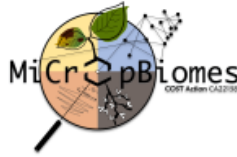


# Session 5

Communication and engagement with society

**Chairs:** Caroline DE TENDER & Ivan NIKOLIC

\* \* \*



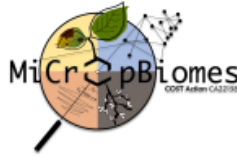
## **Harnessing engineered microbial consortia and metabolites for sustainable grain yield and quality**

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Addressing the critical challenge of enhancing soybean productivity while reducing chemical inputs, in this talk I will present cutting-edge field research investigating the transformative role of beneficial microbial consortia. Our multi-season studies demonstrate that inoculating soybean crops with nitrogen-fixing *Bradyrhizobium* species, *Azospirillum brasilense*, *Bacillus subtilis*, and microbial secondary metabolites (MSMs) significantly improves rhizosoil fertility, nutrient uptake, nodulation, photosynthetic efficiency, and grain yield (up to 12.2%) and protein concentration. Notably, consortia enriched with lipochitooligosaccharides enhanced nitrogen and phosphorus assimilation, while also reducing oxidative stress and improving drought resilience. Metagenomic analyses confirmed functional shifts in rhizosphere microbiomes associated with enhanced plant metabolism. This effective microbial approach offers a biologically based alternative to inorganic nitrogen fertilizer, reducing environmental impact. Increasingly adopted as a sustainable agricultural practice in tropical regions, it represents a significant advancement towards resilient and eco-efficient soybean production systems.



## Engaging secondary school students in microbiome literacy: an international educational initiative by the WG5 Task Force

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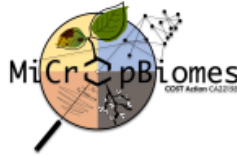
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**Keywords:** education on microbiomes, secondary schools, scientific literacy, citizenship awareness

Public perception of microorganisms is often negative due to their association with diseases and food spoilage. However, most are harmless or beneficial. Microbiomes are vital to sustain life, influencing host health (humans, animals, plants), and are affected by human activity. Raising awareness and improving education about microbiomes can enhance understanding of human health, agroecosystem resilience, and scientific literacy - especially among youth - encouraging interest in biology, health and agrifood careers. Within the MiCropBiomes COST Action, we formed an international task force (WG5.Edu) to design an educational program. The first step involves surveying microbiome literacy among secondary school students in several European countries (e.g., Belgium, Portugal, Greece, Tunisia). This baseline will inform strategies to increase literacy through the development of digital resources of learning plans and educational activities. These efforts align with the One Health concept and the United Nations Sustainable Development Goals, highlighting the interconnection between environmental, plant, animal and human health. Our mission is to raise microbiology literacy in schools, with a special focus on plant microbiomes. We will begin by introducing the MiCropBiomes project and administering a diagnostic questionnaire to assess student knowledge across five key areas: 1) general understanding of plant microbiota; 2) microbiota in agriculture and food security; 3) impact on food quality and nutrition; 4) environmental functions of plant microbiota; and 5) human influence on plant microbiota. The questionnaire, developed in English, contains mainly close-ended items such as multiple-choice and Likert-type questions. These formats facilitate quick responses, enable standardized data collection, and support efficient, objective analysis. It will be translated to suit each country's language and educational context. Task force members will coordinate local implementation and provide feedback on the process and results. Recognizing the diversity of educational systems and student backgrounds will enable us to: (1) conduct a SWOT analysis of microbiome literacy in European schools; and (2) adaption teaching strategies to local needs. These may include project-based learning, real-world problem-solving related to microbiomes (soil health, food systems, human/plant health), and international virtual exchanges between classrooms. This initiative seeks to expand MiCropBiomes program across multiple countries, strengthen international collaboration, and ensure relevance and impact by tailoring the approach to local realities.



## Rooted in Resilience: Exploring the root & seed microbiome of sunflower for sustainable agriculture

Matthijs Van Hooste<sup>1</sup>, Sylvie Weckx<sup>3</sup>, Anneleen Wieme<sup>1,3</sup>, Lisa Joos<sup>1,2</sup>, Shira Houwenhuyse<sup>1</sup>, Sofie Goormachtig<sup>4,5</sup> and Caroline De Tender<sup>1,2\*</sup>

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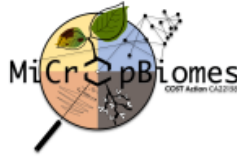
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**Keywords:** Microbiome, drought-resistance, germination, plant growth-promoting microorganisms

The seed microbiome holds the potential to support germination, early plant development, and resilience to environmental stress, such as drought. While root and soil microbiomes are well-studied, these early-stage communities remain underexplored. This study characterises the bacterial and fungal communities associated with sunflower (*Helianthus annuus*) seeds, seedlings, and soil, aiming to: (1) identify plant growth-promoting and drought-resistant microorganisms for isolation studies and application, and (2) compare them with the root and soil microbiome to explore microbial selection and potential horizontal transfer. Using samples collected from four Flemish sunflower fields, we performed metabarcoding analyses (16S rRNA and ITS2) on multiple plant compartments including inner seeds, outer seed shells (seed coats), roots (rhizosphere and endosphere), and surrounding soil. Preliminary results show a clear gradient in microbial diversity from soil to seed, with inner seeds harbouring higher bacterial diversity than outer seed shells. Key drought-associated genera such as *Pseudomonas*, *Sphingomonas*, and *Streptomyces* were found in seed and root compartments, indicating their promise for sustainable microbial applications. This work lays the groundwork for the collaboration between Ghent University and Eurochem ISP, to combine academic microbiome research with industrial expertise in seed treatment. Metabarcoding-based cultivation and identification of promising strains will be used to enrich the industrial knowledge base for microbial seed coatings. Ultimately, this collaboration aims to translate microbiome insights into practical, sustainable solutions that support farmers in coping with climate-related challenges.

**Funding:** This research was funded through a Baekeland mandate from VLAIO (Flemish Agency for Innovation & Entrepreneurship).





## Rice phyllobiome dynamics during bacterial blight disease development

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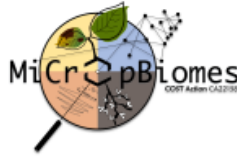
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**Keywords:** Resilient microbiota, metabolic balance, disease resistance, *Xanthomonas oryzae*

*Xanthomonas oryzae* pv. *oryzae* (*Xoo*) hijacks the SWEET sugar transporter to cause bacterial blight disease in rice. While the role of *SWEETs* as susceptibility genes is well-defined in the pathogenesis of *Xoo*, the effects of *SWEETs* on the microbiota as a consequence of nutritional and metabolic alteration during bacterial blight development are not known. In this talk, I draw inferences from my recent study on the contribution of *SWEETs* in the spatial distribution of microbes along the root of *Arabidopsis thaliana* as a basis to investigate the alteration of rice phyllospheric microbiota during *Xoo* infection. Plant roots are heterogeneous in cellular architecture, transcriptome, metabolic states, and immune responses. We hypothesized that physicochemical differentiation along the root axis may also affect spatial colonization by root microbiota. To test our hypothesis, we developed two growth systems, ArtSoil and CD-Rhizotron, to grow and dissect *Arabidopsis* roots into three segments. We identified distinct endospheric and rhizosphere bacterial communities in the segments, supporting the hypothesis of microbiota differentiation along the axis. Root metabolite profiling of each segment revealed differential metabolite enrichment and specificity. Bioinformatic analyses and GUS histochemistry indicated microbe-induced accumulation of SWEET2, 4, and 12 sugar uniporters. Profiling of root segments from *sweet* mutants showed altered spatial metabolic profiles and reorganization of endospheric root microbiota. This work reveals the interdependency between root metabolites and microbial colonization, and the contribution of *SWEETs* to the root microbial ecosystem.



## Investigating the impacts of a new sustainable fungicide on plant-associated microbial communities

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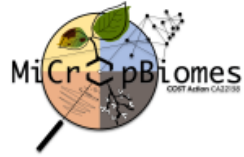
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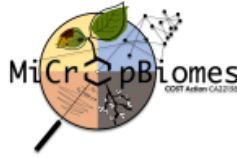
**Keywords:** sustainable fungicide, amplicon sequencing, grapevine, phyllosphere, microbiome

Downy mildew (caused by *Plasmopara viticola*) and powdery mildew (caused by *Erysiphe necator*) negatively impact grapevine health and production. Disease management typically relies on the use of conventional fungicides, which raises concerns about environmental sustainability and potential impacts on non-target microorganisms. This study aimed to evaluate the efficacy of a new sustainable fungicide, based on choline pelargonate (CP), against *P. viticola* and *E. necator* and to assess potential side effects on phyllosphere microorganisms under field conditions. Field trials demonstrated the efficacy of CP against grapevine downy mildew and powdery mildew in two vineyards and showed high efficacy against powdery mildew. Phyllosphere microbial communities were isolated from grapevine leaves and bunches of untreated plants (control) and plants treated with CP or with a combination of copper and sulfur as reference fungicides. Results showed that the composition of fungal and bacterial communities associated with grapevine leaves and bunches differed according to vineyard location and seasonal time point. Therefore, the effect of CP on the microbial structure was influenced by vineyard- and season-specific differences in the microbiome composition of the grapevine phyllosphere. This study demonstrated that CP is an effective alternative to manage grapevine diseases, for further development as an alternative solution to conventional fungicides.

**Funding:** Bioinformatic analyses were partially supported by the COST Action MiCropBiomes, CA22158, supported by COST (European Cooperation in Science and Technology).



# Posters



## Implementation of PCR-based molecular techniques for studying PGPR properties in soil bacteria

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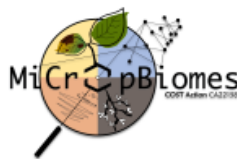
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**Keywords:** Nitrogen fixation, Phosphate solubilization, IAA production PGPR

In the present study, rhizospheric soil samples were collected from agricultural regions in Erzurum and nearby locations and aseptically transferred to the laboratory. Pure bacterial isolates were obtained, and active ones were selected by using conventional methods according to the PGPR criteria that include nitrogen fixation, phosphate solubilization, and IAA production. Conventional microscopic examinations and molecular techniques were used for the identification of the active isolates. Molecular identification of the isolates was done by using PCR with universal primers for 16S rRNA gene region, sequencing of amplicons, and the BLAST analysis of the NCBI database. These active isolates were then used to develop PCR-based molecular techniques that could be used to investigate these properties. According to the results, 34 active isolates with PGPR properties were determined, and their molecular characterization was done. In addition, using these isolates, new pairs of specific primers were designed for the nitrogen fixation-related *nif*, phosphate dissolution-related *pqq*, and IAA production-related *ipdc* gene regions (1-3).

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## Harnessing microbial networks for sustainable peach (*Prunus persica*) production: insights into microbiome diversity and structure

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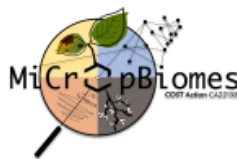
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**Keywords:** peach, *Prunus persica*, microbial diversity, 16S rRNA sequencing

This study focuses on the analysis of the peach (*Prunus persica*) microbiome in different plant compartments – soil, fruit, and leaves – with the aim of identifying the impact of environmental factors such as cultivation location, cultivar, and season on the composition and diversity of microbial communities. Samples were collected over three consecutive years (2021–2023) from three Slovak orchards located in Čachtice, Veselý, and Nové Zámky and analyzed using 16S rRNA gene sequencing. The results revealed the highest alpha-diversity in soil samples, particularly in Nové Zámky for the cultivar Favorita (Shannon index above 5.5). Fruit and leaf samples showed lower diversity, with the lowest values recorded in Čachtice for the cultivar Crethaven (average around 2.6). Beta-diversity analysis using PERMANOVA highlighted compartment type as the primary factor influencing microbial community structure ( $R^2=0.26$ ;  $p=0.001$ ), followed by cultivar ( $R^2=0.06$ ;  $p=0.001$ ), and sampling year ( $R^2=0.01$ ;  $p=0.01$ ). Surprisingly, location showed the least effect ( $R^2=0.01$ ;  $p=0.01$ ). The most common microorganisms identified in the soil included bacteria from the phyla Proteobacteria, Actinobacteria, Bacteroidetes, and Firmicutes. In fruit and leaf samples, dominant genera included *Pseudomonas*, *Bacillus*, *Sphingomonas*, *Microbacterium*, *Massilia*, and *Methylobacterium*, known for their plant-beneficial traits and potential to suppress pathogens. Additionally, opportunistic microorganisms such as *Erwinia* and *Xanthomonas*, which may affect fruit quality, were also detected. This study highlights the complexity and variability of microbial composition influenced by ecological and agronomic conditions. The findings contribute to the development of sustainable orchard management strategies that consider the natural microbial balance as a key factor in plant health and productivity.

**Funding:** This research was financially supported by a project of the Ministry of Education, Science, Research and Sport of the Slovak Republic, grant no. VEGA 1/0661/19 “Plant microbiome and safe food”.



## Effects of *Trichoderma* spp. on gene expression in tomato plants under drought conditions

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**Keywords:** Signalling pathways; defense related genes; Water deficit; qRT-PCR

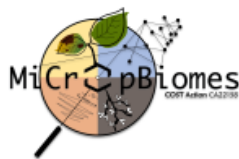
*Trichoderma* spp. are cosmopolitan filamentous fungi that inhabit soil and the rhizosphere. These opportunistic and avirulent plant symbionts are characterized by rapid multiplication and efficient substrate colonization (1). During the *Trichoderma*-plant interaction, fungi can significantly enhance nutrient absorption, promote growth, and improve tolerance to abiotic and biotic stresses (2, 3).

In this research, the adaptive responses of tomato plants (cv. Gružanski zlatni) to progressive drought with addition of *T. harzianum* and *T. brevicompactum* to the rhizosphere were studied. Plants were grown in pots under controlled conditions of the plant phenotyping platform at SUA in Nitra, Slovakia. The expressions of PR1b1 (salicylic acid related), PINII (jasmonic acid related), TPX1 (lignin and suberin related), FLS (flavonoids related), SIJAF13 (anthocyanins related) and C3H (caffeic and chlorogenic acid related) as well as NCED1 (ABA related) were examined by quantitative reverse transcription real-time polymerase reaction (qRT-PCR).

All treatments in which *Trichoderma* was applied demonstrated the activation of the jasmonic acid signalling pathway, as indicated by the expression of the PinII gene. The salicylic acid signalling pathway was activated only in plants exposed to drought in the treatment with *T. harzianum*, as indicated by the expression of the PR1b1 gene. These changes indicate that *Trichoderma* treatment promotes a better potential of plants for resistance to biotic stresses. The increased expression of the NCED1 and SIJAF13 genes indicate that both *Trichoderma* strains might influence the accumulation of ABA and anthocyanin content in all treatments, indicating enhanced potential for plant resistance to abiotic stresses. The increased expression of the C3H gene indicates an increased content of caffeic and chlorogenic acid, which was detected in tomato leaves in optimally watered plants in the presence of *T. harzianum* as well as in the drought treatment in the presence of *T. brevicompactum*. These observed changes in the plants indicate they are more resistant to biotic stress and transport nutrients and water more efficiently.

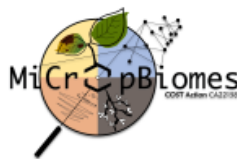
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**Funding:** Support was provided by the Provincial Secretary for Higher Education and Scientific Research, Autonomous Province of Vojvodina project, *Use of Trichoderma spp. in sustainable agriculture*, Grant No. 142-451-3172/2022-01/01. This presentation is based upon work from COST Action MiCropBiomes CA22158, supported by COST (European Cooperation in Science and Technology).



## Yeast as biocontrol agents: insights from killer phenotypes and volatile organic compounds production in rowanberry and rosehip ecosystems

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**Keywords:** Biocontrol; Volatile organic compounds; Killer systems; Yeasts; Food

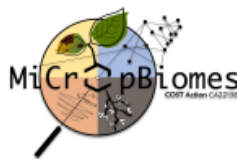
Yeasts produce toxins, volatile organic compounds (VOCs), and other secondary metabolites, which could influence biocontrol strategies for agriculture, food preservation, environmental protection, and industrial biotechnology. This study comprehensively profiled killer systems and VOCs from yeasts inhabiting rowanberry (*Sorbus aucuparia* L.) and rosehip (*Rosa canina* L.) to uncover their biocontrol potential and application in a food product. Yeast cultures were isolated by culture-dependent approaches and identified by molecular methods. DsRNA was extracted to confirm the viral origin of the killing phenotype. The biocontrol properties of killer yeasts were assessed in a real food system – juice. VOCs of selected *H. uvarum*, *M. pulcherrima*, *S. cerevisiae*, and *S. paradoxus* yeast strains were tested using GC-MS. About 70% of isolates from spontaneous fermentations of rowanberries and rosehips were *H. uvarum* and *M. pulcherrima*. The remaining 30% consisted of *Pichia*, *Saccharomyces*, *Torulaspora*, and *Candida* yeasts. Killing activity was revealed in *S. cerevisiae*, *S. paradoxus*, and *T. delbrueckii* yeasts. This study shows that *S. cerevisiae* K2-type killer toxin reduced fungal contaminants in the grape juice. VOC analysis revealed species-specific profiles: *H. uvarum* and *M. pulcherrima* predominantly produced ethyl acetate, while *S. cerevisiae* and *S. paradoxus* emitted 2-methyl-1-butanol. *S. paradoxus* killer properties-possessing strains produced more ethyl acetate, ethanol, ethyl propionate, propyl acetate, 2-methyl-1-propanol, 3-methyl-1-butanol, and 3-methylbutanoic acid compared to non-killer strains. These findings highlight the potential of killer yeasts and their distinctive VOCs as effective biocontrol agents in food and agricultural systems.

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## Bacterial contamination in plants cultivated under in vitro conditions and its elimination using nanoparticles

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**Keywords:** in vitro environment; plant micropropagation; bacterial contamination; *Curtobacterium*; nanomaterials

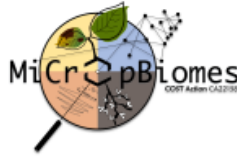
The controlled and aseptic environment of plant tissue culture laboratories represents a unique ecological niche, inhabited by specialized microorganisms capable of surviving standard decontamination protocols. In this study, bacterial contaminants were collected from in vitro cultures originating from three laboratories—two academic and one commercial—focusing on the micropropagation of various plant species. Our findings suggest that the composition of bacterial contaminants is influenced by the plant species used, with *Prunus* genotypes showing a high prevalence of *Curtobacterium* spp. Among the isolated bacteria, *Curtobacterium* sp. A7\_M15 strain was selected as a model for testing decontamination strategies.

To eliminate this contaminant, we applied six types of nanomaterials, including silver, copper, selenium, and reduced graphene oxide composites. Two nanocomposites—rGO-Cu-Ag and AgSe-NPs—showed complete inhibition of bacterial growth on the culture medium surface at concentrations  $\geq 100 \text{ mg} \cdot \text{L}^{-1}$  Ag. However, their ability to eliminate internal bacterial presence within explants was limited, likely due to the endophytic nature of *Curtobacterium* sp. Notably, treatments did not impair plant biomass or vitality at effective concentrations. These results highlight the complexity of managing microbial communities leaving in plants cultivated in artificial environments and support the use of nanomaterials as surface-level antimicrobial agents in plant micropropagation systems. More information about the conducted research can be found in the publication by Tekielska et al., 2024.

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**Funding:** This work was supported by the project QK22010031 “Use of innovative potential of nanotechnology to enhance the rentability of selected areas of agricultural production” (funded by The Ministry of Agriculture of the Czech Republic).



## Comparative metagenomic profiling of rhizosphere microbiome in lentil landraces collection

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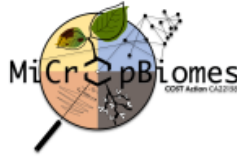
**Keywords:** *Lens culinaris*; germplasm; microbial diversity; N<sub>2</sub>-fixing bacteria; archaea

Lentil is a key legume crop in Mediterranean agriculture and diet, valued for its nutritional and health benefits. As a legume, it contributes to soil fertility and structure through associations with rhizobia. However, beyond these symbiotic relationships, little is known about the broader rhizosphere microbiota and its role in plant health. A collection of lentil landraces, including commercial cultivars, was used to assess the rhizosphere microbial diversity of lentil plants grown under uniform cropping practices in the same field. Targeted amplicon sequencing was employed to analyze the rhizosphere microbiota of the lentil landrace collection. The identified core microbiome reveals common lentil-microbe interactions, with a prominent presence of diazotrophs and other microbial taxa, suggesting that lentil genotype plays a significant role in shaping these communities. The genetic diversity of the lentil landraces collection was characterized using SSR molecular markers, underscoring the impact of genotype on distinct rhizosphere microbiome signatures and plant-soil interactions. Furthermore, the genotype-specific presence of certain soil fungal pathogens indicates a potential role of the lentil holobiome in mediating these associations under uniform field conditions. The insights gained from this study highlight the importance of plant genotype-rhizosphere microbiota interactions in addressing challenges related to climate change and food security.

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## Auxin secretion by soil bacteria accelerated immune system maturation in young seedlings

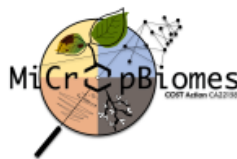
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The plant immune system is essential for controlling bacterial colonization. Protecting the plant from pathogens and managing proper interactions with the root microbiota. The immune system is routinely explored in mature roots and leaves. However, how the immune system is developing after seed germination during the first days of root growth, and whether the soil microbiota affects the immune system development, is largely unknown. We have found that the root immune system is not fully active from the moment of seed germination but gradually matures during the first days of root outgrowth. Accordingly, most of the soil bacteria that activate an immune response in mature seedlings did not induce a response in young roots, and attenuation of pathogen colonization is impaired. However, we have found that interaction of seedlings with specific soil bacteria accelerates the ontogeny of the plant immune system, we termed these bacteria 'Immunomodulators'. Looking for the mechanism of action of the immunomodulators revealed that auxin secretion by the bacteria served as a developmental signal to trigger immune system ontogeny. Finally, we demonstrated that Immunomodulators are 'keystone' bacteria able to affect the colonization of other bacteria and shape the microbiome composition irrespective of their abundance in the community.



## **Cultivating resilience in wheat: mitigating arsenic toxicity with seaweed extract and *Azospirillum brasilense***

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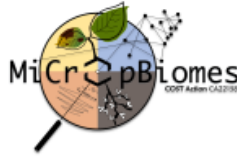
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**Keywords:** Arsenic (As) toxicity; *Azospirillum brasilense*; seaweed extract; crop growth; Wheat

Arsenic (As) toxicity is a serious hazard to agricultural land due to growing industrialization, which has a negative effect on wheat crop yields. To address this issue, using seaweed extract and *Azospirillum brasilense* has emerged as an effective strategy for improving yield under stress conditions. However, the combined application of *A. brasilense* and seaweed extract in wheat crops under As toxicity has not been fully explored. The effectiveness of combining *A. brasilense* and seaweed extract in reducing As toxicity in wheat production was examined in this study through a 2-year pot experiment with nine treatments. These treatments included a control with no additives and two As concentrations (50 and 70  $\mu\text{M}$ ). At 50 and 70  $\mu\text{M}$ , As was tested alone, with seaweed extract, with *A. brasilense*, and both. Significant results were achieved in reducing As toxicity in wheat crops. Arsenic at 70  $\mu\text{M}$  proved more harmful than at 50  $\mu\text{M}$ . The application of *A. brasilense* and seaweed extract was more effective in improving crop growth rates, chlorophyll levels, and stomatal conductance. The combined application notably decreased As concentration in wheat plants. It was concluded that applying *A. brasilense* and seaweed extract not only improves wheat growth but can also improve soil parameters under As toxicity conditions by increasing organic matter contents, boosting nutrient availability, and increasing the production of antioxidant enzymes.

**Funding;** The author(s) declare that no financial support was received for the research.



## Recruitment of root associated microbial communities in two citrus rootstocks exposed to water and salinity stresses

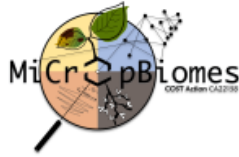
Alexandros Mosca\*, Giulia Modica, Giulio Dimaria, Gabriella Cirvilleri, Alberto Continella and Vittoria Catara

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**Keywords:** amplicon-based metagenomics; bacterial communities; fungal communities; abiotic stress; citriculture

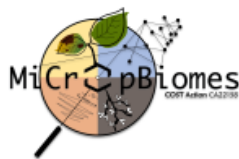
Rootstocks are a fundamental agronomic tool in citrus cultivation, as they influence not only yield and fruit quality but also tree resilience to biotic and abiotic stresses. In Mediterranean regions, the presence of Citrus tristeza virus (CTV) has led to the replacement of sour orange rootstocks with CTV-resistant alternatives. However, water scarcity and soil salinity remain major limitations to citrus production. Recent studies suggest that root-associated microbial communities may play a crucial role in citrus plant stress tolerance<sup>1</sup>. To explore this, we investigated the rhizosphere and endorhizosphere microbial communities of two citrus rootstocks, Bitters (*Citrus sunki* × *Poncirus trifoliata*), a recently introduced rootstock, and Carrizo Citrange (*C. sinensis* × *P. trifoliata*). These genotypes differ in tolerance to abiotic stresses, with Bitters showing higher tolerance than Carrizo under water and salinity stress<sup>2,3</sup>. Using amplicon-based high-throughput sequencing, we analysed bacterial and fungal communities to assess how water and salinity stress influence the root-associated microbiota in each rootstock. Phenotypic data showed that Bitters plants under salinity stress differentiated due to increased proline content and root volume, while under water stress Carrizo plants were more related to chlorophyll and catalase. The composition of microbial communities differed between rhizosphere and endorhizosphere in both rootstocks. Six bacterial phyla (Proteobacteria, Planctomycetota, Actinobacteriota, Verrucomicrobiota, Acidobacteriota, Firmicutes) accounted for >80% of relative abundance. Ascomycota and Basidiomycota dominated the fungal communities, along with Rozellomycota in the rhizosphere and Glomeromycota, particularly in non-stressed plants, in the endorhizosphere.  $\beta$ -diversity analysis revealed that microbial communities in both rootstocks underwent significant differentiation in response to both stresses as compared to control plants. In the rhizosphere, the two rootstocks maintained distinct bacterial communities under water stress, while fungal communities did not differ. Within the endorhizosphere, bacterial communities were shaped by genotype and treatments. However, fungal communities responded solely to stress. Genotype-specific differences in the structure of microbial networks in both rootstocks were also observed. In bacterial networks, central nodes were ASVs represented by specific core genera, whereas no core taxa were found as central nodes in fungal networks. These results highlight the role of rootstock genotype and stress in shaping the root microbiota, promoting potentially beneficial microorganisms for stress resilience.



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**Funding:** PON “RICERCA E INNOVAZIONE” 2014–2020, Azione II—Obiettivo Specifico 1b.- WATER4AGRI FOOD”, n. ARS01\_00825, Cod. CUP: B64I20000160005.



## Establishing the role of peptaibols in biological plant protection through their comprehensive investigation

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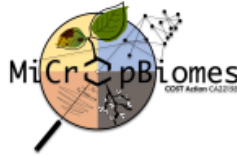
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**Keywords:** Trichoderma, peptaibol, computational modelling, biocontrol

Extreme weather conditions, emerging plant pathogens, and problems caused by chemical pesticides affecting agriculture are driving the search for new and sustainable solutions. The filamentous fungal *Trichoderma* strains have already proven to be successfully applied biocontrol organisms in practice, but their produced secondary metabolites, like peptaibols, may open alternative ways in biological plant protection. Due to their characteristic properties, peptaibols are extremely stable compounds, which can effectively inhibit bacterial and fungal strains, and they are able to enhance the systemic resistance in plants against pathogenic microorganisms. During our work we approach the study of peptaibols from several perspectives: the total peptaibol production (peptaibiome) of *Trichoderma* strains are determined using analytical methods; through laboratory studies, we gain information about the effect of peptaibols against microorganisms, while with the application of computational molecular modelling techniques, we can gain a deeper understanding of the background mechanisms of their action and structure. Based on our results, peptaibols with the most suitable effect are inspected on plants to determine the extracts that may have a positive effect and provide protection against pathogens. Our results may facilitate the selection of practically applicable peptaibol extracts, which may open new possibilities in agriculture for biological plant protection.

**Funding:** This work was supported by the Hungary–Serbia IPA Cross-border Co-operation Programme (project FERTILEAVES, HUSRB/23S/11/027) and by the Scholarship Program of the Ministry of Culture and Innovation, financed from the National Research, Development and Innovation Fund (EKÖP-24-4SZTE-629 and EKÖP-24-4-SZTE-605).



## Interplay of microbiome and epigenetics in modulating stress responses in trees

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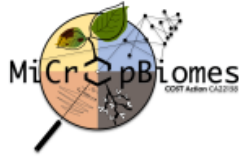
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**Keywords:** microbiome; epigenome; forest trees, stress resistance

Forests are currently facing significant challenges due to climate change and the introduction of invasive pests and pathogenic fungi. The microbiome and epigenetic factors play vital roles in plant health and stress resilience, and their modulation could be practically applied to improve stress resistance in endangered forest tree species. We hypothesized that stress resistance in trees might depend not only on genetic differences but also on microbiome composition and epigenetic factors. To evaluate this hypothesis, we compared two elm species—the wych elm (*Ulmus glabra*) and the stress-tolerant Chinese elm (*Ulmus parvifolia*)—as well as two Douglas fir varieties: The Coastal Douglas fir (*Pseudotsuga menziesii* var. *menziesii*) and the Rocky Mountain Douglas fir (*P. menziesii* var. *glauca*). In the first study, we analyzed the endogenous fungal and bacterial microbiomes and epigenetic variation (at DNA and RNA levels) in seedlings of both elm species. Seedlings of the same age were grown under identical environmental conditions to minimize confounding variables. Results revealed significant differences in microbiome composition and epigenetic profiles between the two elm species. Using metabarcoding analysis, we observed distinct bacterial and fungal communities in each species. Microbial taxa enriched in *U. parvifolia* were identified, potentially linked to stress tolerance and colonization resistance. Differential abundance analysis at the genus level highlighted 14 taxa (defined as taxa with >0.2% abundance in *U. parvifolia*) that were significantly more abundant in *U. parvifolia* than in *U. glabra*. Epigenetic changes were assessed using reversed-phase ultraperformance liquid chromatography (UPLC) coupled with sensitive mass spectrometry. Lower levels of N6-methyladenine (N6-mdA) were detected in *U. parvifolia* compared to *U. glabra*. N6-mdA is recognized as a predominant epigenetic marker in bacterial species, though its presence has also been documented in plants, including various tree species. These results once again raise questions about the microbiome's potential influence on the observed differences, as noted in prior findings (Hoenicka et al., 2024). A second study is currently underway to compare the microbiomes of the two Douglas fir varieties, which exhibit differing stress resistance. Preliminary results confirm microbiome differences between the varieties. For example, bacterial communities associated with Douglas fir seedlings showed significant compositional differences.

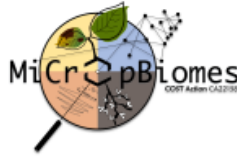
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**Funding:** Support is provided by the Agency for Renewable Resources (FNR).



## Impact of ozonated irrigation on rhizosphere microbial communities in young grapevines: a DNA- and RNA-based comparison

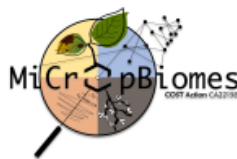
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**Keywords:** Ozonated irrigation, Rhizosphere microbiome, DNA/RNA amplicon sequencing, Biological control agents, Grapevine trunk disease pathogens.

The implementation of ozonated water as a sustainable strategy in viticulture raises new questions about its effects on the rhizosphere microbiome, particularly with respect to microbial diversity, disease risk, and biocontrol potential. In this study, we evaluated the impact of ozonated drip irrigation on fungal and bacterial communities associated with the rhizosphere of one-year-old *Vitis vinifera* plants. The experiment was conducted in two field plots in Valdegón (La Rioja, Spain), where rhizosphere samples were collected at 0, 2, 4, and 6 months post-planting. A total of 64 samples were analyzed using amplicon sequencing of the fungal ITS2 and bacterial 16S rRNA V4 regions, from both DNA (total community) and RNA (active community) extracts. Alpha diversity analysis revealed significant temporal and treatment-related effects. For fungi, DNA-based richness (Chao1) was significantly higher in ozonated samples at T2, while RNA-based diversity remained stable across treatments and time, suggesting that the active fungal community was more resilient. For bacteria, DNA-based evenness (Shannon, Simpson) showed statistically significant differences between treatments at T1, indicating that ozonated irrigation affected the structure of the total bacterial community early in the growing season. RNA-based bacterial diversity was less impacted overall but showed a significant increase in richness (Chao1) at T1, suggesting a short-term stimulation of transcriptionally active bacterial taxa. Focusing on grapevine trunk disease (GTD) pathogens, DNA analyses identified genera such as *Cadophora*, *Diplodia*, and *Ilyonectria*, with increased abundance in ozonated treatments. Notably, *Dactylonectria*, a black foot pathogen, showed high RNA activity despite undetectable DNA levels, highlighting its metabolic activity even at low abundance. Conversely, RNA signals for *Phaeomoniella* and *Eutypa* were negligible. Biological control agents (BCAs) responded differently to ozonated irrigation. The fungal BCA *Aureobasidium* increased in DNA-based abundance under ozonated conditions, while *Trichoderma* was more abundant in control samples, suggesting that ozone may not favor its establishment. RNA activity for both was low. Among bacterial BCAs, *Bacillus* was highly abundant across all treatments with moderate RNA activity, while *Pseudomonas*, *Rhizobium*, and *Azospirillum* were present at lower levels, with no consistent enrichment under ozonated irrigation. Together, these results demonstrate that ozonated irrigation can induce short-term, taxon-specific effects on both the structure and function of rhizosphere microbiota. The integration of DNA and RNA profiling was essential to distinguish between microbial presence and activity, providing deeper insight into the ecological impact of ozonated water and its potential application in integrated disease management for viticulture.



## Endophytes of CWRs grow in extreme environments: a potential source of beneficial microbes for sustainable agriculture

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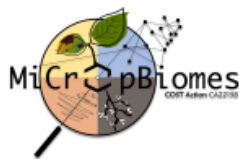
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**Keywords:** Biopesticides; Biofertilizers; Halophytic endophytes; Stress tolerance; Biocontrol

Crop wild relatives (CWRs) thriving in extreme environments could be valuable sources of novel beneficial endophytic microbes with potential applications in enhancing crops resilience against biotic and abiotic stresses. These microorganisms have evolved alongside their host plants, helping them survive in harsh conditions such as drought, salinity, high temperatures, nutrient-poor soils, as well as against pathogens attack. The isolation and characterization of beneficial endophytic microbes from CWRs that enhance stress tolerance, improve nutrient uptake, and promote overall plant health turns to be of great importance for sustainable crop production. Since these microbes can be applied to crops, they may reduce dependence on chemical fertilizers and chemical pesticides, leading to more environmentally friendly and sustainable agricultural practices, while maintaining productivity in challenging agricultural conditions. The concept of this project is the isolation, identification and characterization of endophytes from CWRs, collected from extreme environments, to generate a Biobank of beneficial microorganisms for use in crops' production. Many of our isolated microbes produce bioactive compounds that stimulate plant growth, modulate stress-responsive genes and improve water and nutrient use efficiency. Additionally, several function as bio-stimulants activating the plant's innate immune system to defend against pathogens. These traits can be harnessed to develop bio-inoculants that improve crop performance under adverse conditions, making agriculture more adaptable to climate change. Moreover, using native microbes from CWRs adapted to extreme conditions ensures that these beneficial interactions are naturally suited to the stress factors encountered in modern agricultural systems. Incorporating endophytic microbes from such wild relatives into crop management strategies aligns with the principles of sustainable agriculture by prioritizing biological solutions over synthetic inputs. Our Biobank contains more than 1500 microbial isolates (Bacteria, Fungi and Yeasts), from various CWRs originating from different extreme environments, and their experimental use in crops have revealed highly promising results so far.

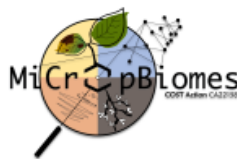
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**Funding:** The project was partially supported by the Emblematic Action of the Greek General Secretariat for Research and Technology, “Agro4Crete,” Protocol Number: SAE013, Operational Program: SAE013.



## Exploiting brassica-derived microbiomes for disease management: protective effects of *Paraburkholderia* strains against *Verticillium dahliae* and *Botrytis cinerea*

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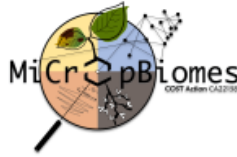
**Keywords:** Biological control, *Eruca sativa*, *Paraburkholderia eburnea*, *Paraburkholderia oxyphila*, plant diseases

Brassica plants are well-recognized for their ability to influence soil microbiota, a trait that has been harnessed for effective disease management. In this study, we isolated the rhizospheric microbiome of plants treated with rocket extract (*Eruca sativa*) (1). Our findings revealed the presence of strains from the genera *Pseudomonas* and *Paraburkholderia*, which exhibited significant in vitro activity against the soil-borne pathogen *Verticillium dahliae*. Further investigation showed that these isolated strains notably reduced the percentage of diseased leaves in eggplants infected by *V. dahliae*. The plant-protective action of two strains, *Paraburkholderia eburnea* EP3 and *Paraburkholderia oxyphila* EP4, was linked to the activation of plant defense mechanisms. Genetic analysis of the strains revealed common biosynthetic gene clusters, including those involved in siderophore synthesis. The plant-protective efficacy of *Paraburkholderia oxyphila* EP4 was also tested in tomato and cucumber plants against *Botrytis cinerea*, demonstrating that root application of EP4 was as effective as both leaf application and a combined leaf/root treatment. Additionally, we observed that EP4 could effectively colonize both the root and leaf systems of tomato and cucumber plants. Furthermore, EP4 triggered the expression of the defense-related gene *PR1*, a marker of the salicylic acid-dependent defense pathway.

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**Funding:** This research was financially supported by the European Union- Next Generation EU, Greece 2.0 National Recovery and Resilience plan.



## Effect of different soil management interventions on soil biodiversity and relationship of soil-borne pathogen levels and plant disease incidence

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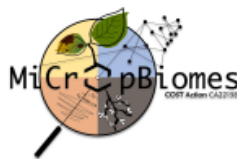
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**Keywords:** Soil microbiology, biodiversity, metabarcoding, soil-borne diseases, sustainable management, biocontrol

The management of soil biodiversity has an impact on sustainable crop production. Using nine field trials in the UK, we have compared the effects of soil management practices on soil biodiversity using DNA barcode sequence data. A post-sequencing DNA-barcode biodiversity standard analysis method (DNA-BSAM) was developed and employed to compare standardised data. Use of the DNA-BSAM revealed that there was a large variance in the effects of various management interventions at different sites, and location had an equivalent or greater effect than most management interventions for most metrics. Some sites in diverse locations that had the same organic amendments' interventions, showed different responses. We also observed strong differences in soil microbiomes by location, suggesting that any effect of management may be dependent on location. Field trial sites with raspberry, daffodil, and onion crops were assessed for soil-borne diseases, and assays were used to detect the level of disease in the soils. In each trial, no clear relationship was found between plant disease levels and pathogens in soils as, in the years of sampling, some sites had high level of pathogens whilst other sites had very low levels. A glasshouse experiment with strawberries growing in pots inoculated with the pathogen *Verticillium dahliae* and with treatments of biocontrol agent *Gliocladium catenulatum* revealed that colonisation of these organisms was highly variable; the inoculation of *V. dahliae* did elevate the disease levels, but no suppressive action of *G. catenulatum* was observed. The qPCR tools used to quantify the organisms showed potential to monitor target populations of these organisms in soils.

**Funding:** these studies were conducted within the Soil Biology and Soil Health (SBSH) Partnership, funded by the Agriculture and Horticulture Development Board (AHDB) and the British Beet Research Organisation (BBRO) of the United Kingdom and were part of PhD projects at the University of Lincoln, Newcastle University and Fera Science.



## **Rhizospheric microbial dynamics of gladiolus corms: insights into quorum sensing and quorum quenching for disease management and growth enhancement**

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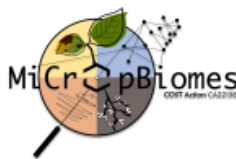
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**Keywords:** Gladiolus, Rhizospheric bacteria, Characterization, Disease management, Growth enhancement

Gladiolus, a widely cultivated cut flower known for its aesthetically pleasing multi-coloured spikes, has earned significant commercial popularity. Comprehensive understanding of the rhizosphere bacterial community associated with gladiolus is imperative for unlocking their potential benefits. Molecular characterization is considered to be an effective method to gain insights in structural and functional aspects of microbial populations. The soil characteristics and bacterial communities in the rhizosphere are typically influenced by quorum sensing (QS) and quorum quenching (QQ) mechanisms. This study aims to explore the niceties and diversity of rhizospheric bacterial populations linked with gladiolus corms, with a specific focus on understanding the dynamics of QS and QQ mechanisms in their complex interactions. Isolation of bacterial strains was achieved through the serial dilution method on nutrient agar (NA) media. Identification of the isolates was accomplished by amplifying 16S rRNA gene sequences through polymerase chain reaction (PCR) using universal primers. Sequence analysis was conducted using BLAST on the National Center for Biotechnology Information (NCBI) database. The characteristics of the isolated bacteria were elucidated using biosensors. The study identified three QS strains and five QQ strains. A consortium of quenchers was formulated utilizing five strains that demonstrated efficacy in mitigating disease impact on gladiolus and fostering growth. Among the three treatments—Scale, Descale, and Descale and cut half (DSC) - the DSC treatment emerged as the most effective. This treatment exhibited a broader range of variation in biological parameters over time, aligning with prevailing trends in the local market.

**Funding;** *No financial support was received for the research.*



## Microbial communities associated with the infections of the common walnut

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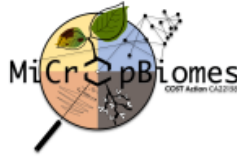
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**Keywords:** Common walnut, Pathogenic microbiome, Amplicon sequencing; *Pseudomonadota*, *Raoultella*, *Brucella*

Ensuring the production of walnuts is an essential economic task in Hungary, as the area under production exceeds 9,200 hectares. One of the key pillars is to identify the bacteria involved in the walnut husk fly (*Rhagoletis completa*)-born infection processes. The aim of the study was to reveal the composition of the bacterial communities of infected walnuts and leaves collected from two and one walnut plantations with different severity of infestation in Zala and Győr-Moson-Sopron County in Hungary, respectively. Pupae and imagos emerging from the infected walnuts were grown in sterile conditions to analyse the bacterial communities adapted to the tissues of the flies. Amplicon sequencing of the 16S rRNA gene was performed on Oxford Nanopore MinION platform. The bacterial community of the imagos proved to be the most diverse and the healthy leaf was the least diverse sample. The taxonomical composition showed the dominance of Pseudomonadota (up to 50%) in bacterial communities of different developmental stages of the walnut husk fly. *Pseudomonas* and *Stenotrophomonas* spp. were abundant in the pupae, while *Brucella* and *Raoultella* were more frequent in the imagos. Healthy walnuts and leaves were inhabited exclusively by the members of Cyanobacteriota. The microbiota of the more severely symptomatic plants was dominated by Pseudomonadota: *Stenotrophomonas* and *Pseudoxanthomonas* were present with higher sequence numbers in walnuts; *Pseudomonas*, *Raoultella*, *Daeguia* and *Enterobacter* were found more frequent in leaf samples. The results show that the microbiota of infected walnut trees contains a number of potential plant pathogenic bacteria that are spread by the walnut husk fly and play a role in the overall decline of walnut trees.

**Funding:** This study was supported by the National Research, Development and Innovation Office (grant 2022-1.2.6-TÉT-IPARI-TR-2022-00009).





## Role of *Musa* spp. phytomicrobiome in the genotype specific responses to infection by *Fusarium oxysporum* f. sp. *cubense*

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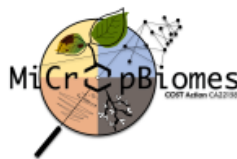
**Keywords:** Panama disease; soil-borne pathogen; plant endophytes; metabarcoding; dysbiosis

Banana (*Musa* spp.), a major crop providing food security to millions of people, is highly threatened by *Fusarium oxysporum* f. sp. *cubense* (Foc), the causal agent of the Fusarium wilt. The importance of plant associated microbiomes is increasingly acknowledged for developing disease protection strategies and recent studies have focused on the potential of banana endophytes<sup>1</sup>. Understanding of the *Musa* spp. phytobiome is crucial for effective microbiome engineering. In particular, identification of keystone species capable of mitigating disease and elucidating their interactions with the pathogen are essential. In this work, the endophytic bacterial communities associated with both resistant and sensitive *Musa* spp. cultivars were characterized using advanced sequencing technologies. The dysbiosis caused by Foc infection was assessed to identify keystone species that could play a role in the resistance against the pathogen. Following identification, isolation methods were implemented to target these species, aiming to develop a synthetic community representative of the core endophytes diversity. Our study revealed differences in the bacterial endophytic microbiomes of resistant and sensitive banana cultivars, with a high diversity in the resistant cultivar. A core microbiome, associated to asymptomatic plants, was identified and some taxa were linked to interesting biocontrol traits. Based on these results, root endophytic bacteria were isolated and screened for *in vitro* antagonism against Foc. A diverse array of candidates was selected to be further evaluated *in planta* alone or as part of synthetic communities, aiming to study the interactions with the pathogen in banana vitroplants. This research highlights the potential of microbiome engineering as a novel strategy for disease management in banana crops. By focusing on the ecological interactions within the *Musa* spp. phytobiome, the study contributes to the understanding of plant-microbe interactions and offers a pathway for developing sustainable control measures against soil-borne pathogens like Foc.

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**Funding:** Support was provided by the Fonds pour la Formation à la Recherche dans l'Industrie et l'Agriculture (FRIA grantee 1st grant 40030076) and the Fonds de Support à la Recherche (FSR, UCLouvain).



## Developing a monitoring framework for *Fusarium oxysporum*–plant interactions and characterizing an endophytic *F. oxysporum* isolate to understand complex plant-microbe relationships

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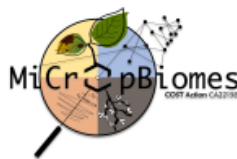
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**Keywords:** *F. oxysporum*; tissue cultures; effectors; endophytes; sustainable agriculture

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, to further characterize our isolate, we conducted inoculation assays on both bean and tomato plants and performed radial growth experiments to compare growth rate of our isolate with other endophytic and pathogenic *Fusarium oxysporum* strains. Our study, together with employed tools, paves the way for more holistic approaches in terms of transition to sustainable agriculture.

**Funding:** Financial support was provided by Slovenian research agency (ARIS) project J4\_3089 and STSM grant obtained in the frame of COST MiCropBiomes action (CA22158).



## Phytoplasma-driven shifts in the endophytic bacterial communities of European blueberry plants

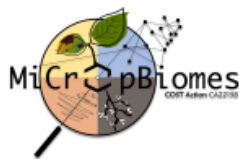
Juliana Lukša-Žebelovič, Martynas Dėlkus, Marija Žižytė-Eidetienė, Algirdas Ivanauskas, Deividas Valiūnas and Elena Servienė

State Scientific Research Institute Nature Research Centre, Vilnius, Lithuania

**Keywords:** *Vaccinium myrtillus* L.; endophytic microbiome; microbial diversity; 16S rRNA sequencing  
Phytoplasma

Phytoplasma infections threaten the ecological and economic value of *Vaccinium myrtillus* L. (European blueberry), yet their impact on the plant's endophytic microbiome remains poorly understood. This study employed next-generation sequencing of the 16S rRNA V3–V4 region to compare bacterial communities in healthy and phytoplasma-infected plants (strains related to '*Candidatus Phytoplasma pruni*' and '*Ca. P. trifolii*'). Analysis of 1.286 million raw reads revealed higher ASV richness in infected plants compared to healthy plants, though alpha (Shannon/Faith's PD) and beta diversity metrics showed no significant differences. Pseudomonadota dominated both groups (about 48%), but infected plants exhibited increased Acidobacteriota (13.8% vs. 9.5%) and Myxococcota (10.6% vs. 3.3%), while Actinomycetota was more prevalent in healthy plants (17.8% vs. 9.0%). ANCOM-BC identified Azospirillales ( $LFC = 4.19$ ) and *Caulobacteraceae* ( $LFC = 5.04$ ) as strongly associated with infection, whereas Gammaproteobacteria and *Rhizobium* were linked to healthy plants. These findings suggest that phytoplasma infection subtly alters microbiome composition, favoring taxa that may thrive under stress or contribute to pathogen resilience. This study provides foundational insights into microbiome-phytoplasma dynamics, highlighting potential diagnostic biomarkers and ecological implications for disease management.

**Funding:** This research received no external funding.



## Exploring *Rhodiola rosea* bacteria for plant growth enhancement

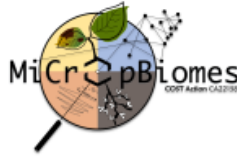
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**Keywords:** Endophytes; Plant growth-promoting bacteria; Adaptogenic plants; Microbial inoculum

Endophytes promote plant growth, enhance stress tolerance, and provide disease resistance. Plants adapted to survive under suboptimal growth conditions have an improved capacity to enrich beneficial microbiota in the rhizosphere and could serve as a source of plant growth-promoting bacteria. *Rhodiola rosea* L. is a succulent plant from the *Crassulaceae* family. It is adapted to survive in sandy or rocky soils and dry tundra and is known for its adaptogenic properties. In this study, we isolated and identified endophytic bacteria from the rhizobium of *Rhodiola rosea* plants using both metataxonomic and culture-dependent approaches. The isolates were screened for plant growth-promoting traits, including siderophore production, indole acetic acid synthesis, antagonistic activity, and reactive oxygen species production. The co-cultivation of selected isolates with germinating tobacco seedlings revealed a radicle- and hypocotyl-growth-modulating effect. A bacterial inoculum consisting of three *Bacillus toyonensis* (two from *Rhodiola* and one from tobacco plants) and one *Peribacillus frigoritolerans* species (from *Rhodiola* plants) was selected and used to inoculate apple shoots. The results showed that this microbial inoculum significantly enhanced the growth of ex vitro acclimatised apple plantlets. Our findings highlight opportunities for endophytes from *Rhodiola rosea* to serve as effective microbial inoculants, enhancing non-host plants such as apple growth and health. This work emphasises the potential of engineered microbial communities to enhance agricultural practices and improve plant performance across diverse species.

**Funding:** This presentation is based upon work from COST Action MiCropBiomes, [CA22158](#), supported by [COST](#) (European Cooperation in Science and Technology).



## Isolation and characterization of culturable bacteria from plant environments to build-up microbial consortia with biostimulant/biofertilizer activity

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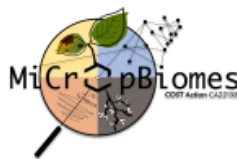
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**Keywords:** Microbial consortia, Bottom-up construction, Compatibility

Agriculture today faces significant challenges that need the development of new approaches to increase productivity and quality of crops while managing sustainably. Microbial consortia that function as biostimulants or biofertilizers are promising biological products that can enhance water absorption, nutrient acquisition, tolerance to abiotic stress, plant vigour, quality, and crop yield. The aim of this study was to isolate strains from different plant environments and composed of various genera that are known to be beneficial to plants, particularly focusing on diazotrophic bacteria and considering additional characteristics such as phosphate solubilization, hormone production, ACC deaminase activity, and siderophore production. We processed 115 soil and rhizosphere samples from cultivated and wild plants of different species collected from the area of Catalonia (Spain), resulting in 435 isolates. 235 isolates were selected and identified through 16S rDNA sequencing, allowing us to assess microbial diversity. The most abundant genera identified were *Stenotrophomonas* (19%), *Pseudomonas* (10%), *Agrobacterium* (5%), *Rhizobium* (5%), *Sphingomonas* (3%), *Variovorax* (3%), *Achromobacter* (3%), and *Flavobacterium* (3%). Four isolates from different species (S1, S2, S3, and S4) were selected to construct a bottom-up synthetic microbial consortium. Assessing the compatibility of these strains is crucial for predicting their performance as biostimulant/biofertilizer microbial consortia, and our results indicated that these strains were highly compatible. Then, we are currently conducting plant experiments using tomatoes and wheat plants to study beneficial effects.

**Funding:** Support was provided by a student grant (2024-FI-1 00773). This presentation is based upon work Action MiCropBiomes, CA22158, supported by COST (European Cooperation in Science and Technology).



## ***Stemphylium vesicarium* affecting pear: exploiting bacterial isolates from carposphere as potential microbial biocontrol agents**

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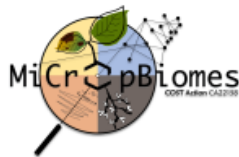
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**Keywords:** Brown spot of pear; Bacterial antagonists; Biological control

The Farm to Fork Strategy, part of the European Union Green Deal (1), foster the importance for innovative and sustainable solutions for pathogen management by limiting the use of synthetic pesticides in agriculture. This study explored the potential of bacteria inhabiting the carposphere of pear cv. Abbé Fétel as candidate microbial biocontrol agents (*mBCAs*) against *Stemphylium vesicarium*, the causal agent of brown spot of pear (BSP). In 2023 and 2024, pear fruits were collected from orchards located in Regione Emilia Romagna conducted with organic (BIO) and integrated pest management (IPM), respectively. Bacteria were isolated, morphologically characterized and screened *in vitro* by means of dual assays to check their antagonistic potential against a set of *S. vesicarium* strains. A total of 639 bacterial isolates were collected, displaying an *in vitro* mycelial growth inhibition up to 87.44%. The most prospective isolates were molecularly identified at genus level by sequencing the 16S rRNA region and were further tested for their ability to inhibit six additional *S. vesicarium* strains *in vitro*, and *in vivo* on detached fruit (2). The selected strains were assigned to the genus *Bacillus*, *Pseudomonas*, *Erwinia*, *Pantoea*, *Micrococcus*, *Frigobacterium* and *Variovorax*. The *in vitro* antagonistic performance of the selected isolates was slightly variable, depending on the *S. vesicarium* strain tested. Concerning the biocontrol potential on detached fruits, *Bacillus* (DLS321 and DLS323), *Pantoea* (DLS311) and *Pseudomonas* (DLS329) resulted the most effective, with BSP severity reduction up to 80%. Intriguingly, the *in vitro* and pear fruit bioassay results were not always correlated. Therefore, further studies are needed to investigate the mechanisms by which these *mBCAs* might exert their protective effects against *S. vesicarium* (3). Here, high-throughput genomic approaches may shed light on those specific secondary metabolites and their biosynthetic gene clusters (BGCs) for the development of efficient and sustainable *mBCAs* for engineering pear microbial communities and controlling BSP disease.

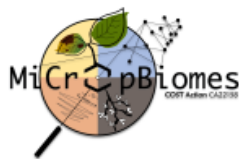
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## Effect of *Bacillus* species on the germination and establishment of *Pinus sylvestris*

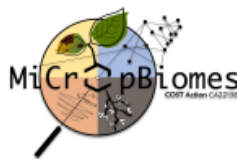
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**Keywords:** Climate change; Woodland expansion; Bioinoculants; Seed germination; Seedling development

In line with the recommendations from the Committee on Climate Change (CCC), the United Kingdom is committed to creating 30,000 hectares of new woodland annually to achieve net-zero emissions by 2050. To achieve that, efficient and reliable propagation of native woodland species is essential. Scots pine (*Pinus sylvestris*), a significant woodland species in the UK and northern Europe, faces challenges such as low seed quality and availability and poor germination and establishment. Biological seed treatments, although commonly used in high-value vegetable crops, have not been tested on tree species and might provide a route to improving tree seed quality. This study investigates the role of the bacterial genus *Bacillus*, known for its plant growth-promoting properties, on *P. sylvestris* seed performance. *P. sylvestris* seeds were surface-sterilised using 15% v/v hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and inoculated with individual *Bacillus* species at a concentration of 10<sup>4</sup> cfu/mL. The treated seeds were then kept in a growth cabinet at 20 °C and 55% ± 5% relative humidity (RH) for 12 days to evaluate their potential as bioinoculants under controlled conditions. The influence of seven *Bacillus* species on *P. sylvestris* seed germination and seedling development was assessed by evaluating key parameters such as maximum germination percentage, speed of germination, root and stem length, stem diameter, and wet and dry biomass. Seeds inoculated with *Bacillus megaterium* exhibited the highest germination rate, while *B. atrophaeus* and *B. amyloliquefaciens* promoted the fastest germination. Additionally, *B. amyloliquefaciens*, *B. licheniformis*, and *B. megaterium* enhanced seedling biomass by promoting root elongation. These findings highlight the potential of *Bacillus* species as effective seed treatments to improve *P. sylvestris* establishment. Further research is needed to elucidate the underlying mechanisms of seed–bacteria interactions and to optimise their application in commercial tree seed improvement.





## Screening for potential biocontrol agents in the microbiome of walnut rhizosphere and phyllosphere in Hungary and Turkey

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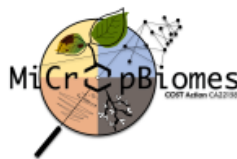
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**Keywords:** Biocontrol; Phyllosphere; Rhizosphere; Walnut

The English walnut (*Juglans regia*) is a commercially significant hardwood species. Both Hungarian and Turkish walnut products are highly sought in global markets. However, as a result of climate change, plant health issues and cultivation difficulties have continuously declined both walnut quality and yield. Various pests as well as bacterial and fungal pathogens cause the decline of walnut trees, which increasingly affect trees due to the weakening of their natural resistance. Chemical pesticides are presently the primary means of control. However, the chemical residues accumulate in the food chain, which rises both health and environmental concerns, intensifying the demand for eco-friendly, sustainable alternatives like biocontrol methods. In Hungary, our research focused on the microbial diversity associated with walnut trees. Soil samples were collected from orchards with varying degrees of disease severity. Alongside walnut pathogens (*Agrobacterium tumefaciens*, *Alternaria angustifolia*, *Aspergillus* sp., *Neofusicoccum parvum*, *Nothophoma spiraeae*, *Pantoea agglomerans*), potential biocontrol agents, including *Bacillus*, *Pseudomonas*, *Simplicillium*, *Streptomyces* and *Trichoderma* species were identified and are under detailed characterization. In Turkey, Anatolia, particularly the basin of Lake Van, is a key genetic center of *Juglans regia* and an important region for its cultivation. We aimed to detect *Xanthomonas arboricola* pv. *juglandis* (*Xaj*), the causal agent of walnut bacterial blight, and to identify potential biocontrol agents. Surveys conducted in 2024 across Bitlis, Hakkari and Van provinces found no evidence of *Xaj* or disease symptoms. Bacterial isolates obtained from walnut leaves in surveyed orchards yielded 47 potential biocontrol agents. Screening for plant growth-promoting traits revealed that 87% of the isolates produced indole-3-acetic acid, 76.5% exhibited siderophore production, 27.6% demonstrated nitrogen fixing ability, and 19% solubilized phosphate. Our findings provide the basis of future research on biocontrol strategies in walnut cultivation both in Hungary and Turkey.

**Funding:** This study was supported by the National Research, Development and Innovation Office (Hungary, grant 2022-1.2.6-TÉT-IPARI-TR-2022-00009) and TUBITAK Project No. 222N041 (Turkey).



## Functional analysis of alfalfa rhizobacteria for developing plant growth-promoting and stress-tolerant SynComs

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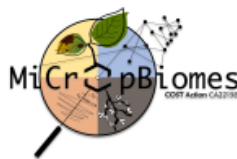
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**Keywords:** Bacterial genomics; SynComs; Bacterial Community; Drought Stress; Rhizobacteria

The dynamic environmental conditions, rapid urbanization, and strained freshwater resources, coupled with both biotic and abiotic stressors, significantly impact global legume production. The most common factors contributing to yield reduction are the incidence of fungal and bacterial diseases, as well as drought conditions. The proposed research aims to identify organ distribution and specific assemblages of leguminous bacterial communities. Microbial isolations were conducted from the rhizosphere and rhizoplane regions of *Medicago sativa* L. plants using three different culture media: tryptic soy agar, LB agar, and King's B agar. 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 the 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.

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## Supporting the crop microbiome as key in sustainable agriculture

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**Keywords:** Microorganisms, Biostimulants, Agriculture

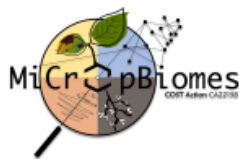
Increasing crop productivity by mitigating climate change and preserving agroecosystems is one of the significant goals of sustainable agriculture. INTERMAG's activity is focused on the development and production of modern preparations for agriculture. Currently the company is one of the biggest manufacturers of fertilizers and biostimulants. As a company, we are aware of that it is only through the combined efforts of world-class specialists in technology, chemistry, biology and microbiology that we collectively offer a broad field of research for the improvement of crop quality, crop productivity, and sustainability of existing systems to produce more and better-quality agricultural products. In sustainable agriculture, successful application of microbes helps in maintaining soil health, improving water holding capacity, carbon storage, root growth, availability and cycling of essential nutrients, filtering pollutants, and also in protection of biodiversity. The diversity and abundance of soil and rhizosphere microorganisms influence plant composition, productivity, and sustainability. Deploying microbes to improve agriculture productivity is an extremely attractive approach. Although both soil and plant microbiomes have important roles in crop performance and yield, the greater chance of success in using plant microbiomes is driven by eco-evolutionary interactions between plant species and their microbiomes (1). However, to realize the full potential of the crop microbiome to support productive and sustainable croplands would require a better understanding of the structure and function of the crop microbiomes, and their interactions with host species across the globe soil types.

The world population is expected to reach 9.7 billion in 2050, with a subsequent increase in demand for food and water. In this scenario, food production needs to increase by 70% by 2050 (2). Meeting the projected demand for healthy and sustainable food production is a crucial challenge for current agriculture.

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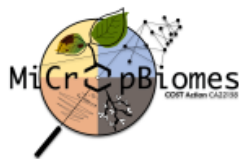
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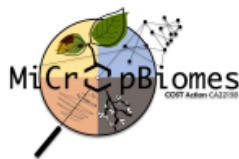


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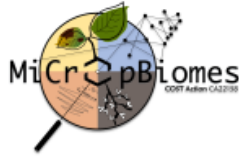
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