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Working Group Biological and Integrated Control of Plant Pathogens

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"From single microbes to microbiomes targeting one health"

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Monica Mezzalama, Tomislav Cernava and Davide Spadaro

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Preface

Welcome to the XVII IOBC-WPRS Working Group *Biological and Integrated Control of Plant Pathogens*!

The general goal of the Working Group is to promote cooperation among scientists, to exchange expertise on biological control against plant diseases caused by plant pathogens and to support the implementation of biological control strategies in agriculture in the West Palaearctic Region and beyond. The Working Group organizes workshops for scientists and extension people as well as producers and final users of biological control products.

The XVII workshop "From single microbes to microbiomes targeting one health" will focus on the role of microorganisms that are isolated, selected, characterized, and deployed to control plant pathogens going beyond plant health to protect organismal health in a holistic approach. A total of 146 contributions were submitted, five will be presented as invited talks, 48 as oral presentations, 44 as flash talks and 49 as posters. The contributions were divided in ten sessions. Application of biocontrol agents, as single strains or in consortia, will be presented and discussed in a context of climate change to control emerging and complex plant diseases. The partnership between industry and academia will be explored to improve and make more efficient the production and deployment of biocontrol products. Moreover, recent findings will be presented about how to explore new sources of biocontrol agents, the mechanisms that regulate the complex interactions among microbes, plants, and pathogens. Last, but not least a roundtable has been organized to discuss how to overcome current bottlenecks in the biocontrol product registration process to avoid unnecessary delays and costs. Sufficient time has been allocated to allow knowledge exchange and to deepen the discussion among participants about how biological control can be fostered under changing climate conditions that affect plant, animal, and human wellbeing.

Scientists representing 36 countries from the six continents will join and contribute to the quality and biodiversity of the meeting.

We look forward to meeting you in Torino,

Davide Spadaro and Monica Mezzalama, Chairs
Tomislav Cernava, Convenor

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Session I
Biocontrol for resilient cropping systems tackling climate change

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Biocontrol for resilient cropping systems tackling climate change

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Abstract: Climate change negatively impacts agricultural productivity, resulting in food insecurity, soil desiccation, soil degradation, weed infestation and diseases. Current management approaches do not suffice. To overcome climate change, we must integrate soil health management, water management, pest management, multi-omics technologies and machine learning to reinforce other control measures in the cropping systems. The objectives were to examine the influence of climate change, including drought stress, heat stress, and parasitic weed infestation, on yield components. The biocontrol agents for resilient cropping systems will undoubtedly be plant growth-promoting or pathogen-suppressive. Besides, being environmentally safe, reproducible, and adaptable. Some rhizobacteria fall in this category.

The rhizobacterial strains were evaluated for their traits that promote plant growth and stress tolerance. High-throughput sequencing of the complete microbial community's DNA was performed using the Illumina NovaSeq 6000 technology. The taxonomic analysis was performed using the Metagenomic Rapid Annotations using Subsystems Technology (MG-RAST). KBase online platform was used for functional profiling. The functional genes identified in the genome were linked to phosphate solubilization, iron transport, hormone regulation, nitrogen fixation, and oxidative and osmotic stress resistance. Also, secondary metabolites supporting bacterial biocontrol properties against phytopathogens, and abiotic stress, such as aerobactin-type non-ribosomal peptide siderophore, Stewartan-type ladderane, and Colicin-type NRPS, were discovered in the genome. Functional annotation of the metagenome-assembled genomes revealed their involvement in essential plant growth and development functions, such as sulfur and nitrogen metabolism. The intricate metabolic pathways mediated by the projected genes in the bacterial genome offer a genetic foundation for future understanding. This information is helpful for simulating crop yields in stress-prone areas in Sub-Saharan Africa. Metagenome datasets provide valuable information on rhizosphere microbial communities associated with crop and their functionality. In summary, the studies shed light on microbial taxonomic classification, functional categories, and the diversity and composition of the rhizosphere microbiome, which influences the microbial functions in managing and sustaining plant health against stressors. Metabolomics provides insights into stress-responsive small molecules of the biocontrol agents and how to optimise them for adequate control. It accelerates the hunt for metabolic traits for stress adaptation and early detection of and biocontrol of plant parasitic weeds. Multi-omics can be used for further studies on the selection and application of microbial resources for sustainable crop production. Biocontrol agents offer a sustainable approach by enhancing crop resilience while reducing dependence on chemical fertilisers or herbicides and alleviating the negative impacts of climate change. Researchers, policymakers, and all stakeholders are urged to champion multi-omics for biocontrol to tackle climate change.

Key words: weed, diseases, integrated pest management, metabolomics, metagenomics, soil health, stress

Microbial inoculants affect the composition and function of the maize rhizosphere microbiome and mitigate the impact of abiotic stress factors on host plants

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Abstract: Recent agriculture relies on intensive farming practices to achieve high crop yields and is associated with potential environmental risks. The implementation of sustainable farming strategies such as the use of microbial inoculants has been proposed to address these concerns. Microbial inoculants are able to enhance plant performance and to mitigate plant stress responses to biotic and abiotic stress factors. However, their effectiveness can vary and their effects remain often inconsistent at field scale. Farming practice influences the soil and rhizosphere microbiota and can be a factor that affects the effect of applied microbial inoculants. Therefore, the impact of farming practice (tillage and fertilization intensity) and two growing seasons on the interaction between maize plants and a microbial consortium (BMc) of *Pseudomonas*, *Bacillus*, and *Trichoderma* were studied at field scale. Untreated and BMc inoculated maize plants were grown under intensive and extensive farming practice in a long-term field experiment. Severe periods of drought were experienced during one growing season of maize. A sufficient rhizosphere-competence of the BMc strains was observed in all treatments in both growing seasons. The BMc treated maize plants showed improved performance (growth, fitness) under the abiotic stress condition. Stimulation of shoot growth by BMc inoculation was associated with improved iron nutrition, crucial for drought adaptation. Furthermore, BMc inoculation modulated the plant hormonal balance and enhanced detoxification of reactive oxygen species (ROS). Amplicon sequencing revealed BMc-mediated alterations in rhizosphere bacterial and fungal communities, while metagenomic shotgun sequencing indicated enrichment of genes for antimicrobial lipopeptides and siderophores. ASVs positively associated with iron uptake were identified and these ASVs significantly increased due to BMc inoculation. Most iron-associated ASVs were classified as Comamonadaceae as the most important for iron uptake. In conclusion, the growing season affected the impact of the BMc on maize plants more than farming practice. Rhizosphere microbiome composition and function were shaped by BMc inoculation independent of farming practice and growing season.

Key words: microbiome, fungi, bacteria, amplicon and metagenome sequencing, rhizosphere competence, plant stress responses

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Selection of bacterial biocontrol agents to contrast the soilborne pathogen *Armillaria mellea* and support plant growth

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Abstract: The soilborne pathogen *Armillaria mellea* poses significant threat to a wide range of host plants, and its control is very difficult. In this study, we investigated the potential of bacteria isolated from soil as biocontrol agents against *A. mellea*, focusing on their antifungal efficacy and plant growth promotion. We were able to isolate 155 potential biocontrol agents, and after a series of screenings, we selected four *Pseudomonas* strains with *in vitro* biocontrol efficacy against *A. mellea*. *In vitro* dual culture assays revealed that three strains effectively inhibited fungal growth, while one strain prevented rhizomorph formation. Whole genome sequencing confirmed their biocontrol potential, identifying biosynthetic gene clusters related to antifungal compounds and siderophores. The impact of these biocontrol agents on the bacterial communities of olive plant roots and rhizosphere soil was assessed using amplicon metagenomics, revealing significant alterations in bacterial diversity and community structure. A Structural Equation Modeling approach suggested that the inoculation of biocontrol agents influenced plant biomass directly but also indirectly by modulating root and rhizosphere microbiomes. These findings highlight the potential of the selected bacterial isolates as sustainable solutions for managing soilborne fungal pathogens like *A. mellea*, promoting plant health, and aiding plant growth.

Key words: microbiome, microbial ecology, soilborne pathogen, metagenomics, amplicon sequencing

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Is it possible to manipulate the microbiome of growing medium for disease suppression during cucumber cultivation in soilless systems?

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Abstract: Vegetables are at the basis of a healthy human diet. Traditionally, vegetables were produced in soil-based cultivation, but due to problems with soil bound diseases soilless cultivation systems were developed. These systems can provide increased productivity per unit of area and reduce the input of water and fertilizers.

Relatively little is known about the micro-organisms present in soilless cultivation systems.

Therefore, we studied the bacterial microbiome of growing media, using a. o. metabarcoding and qPCR, in different experiments with cucumber cultivation in soilless cultivation systems. We also attempted to manipulate the microbial diversity and functionality present in the cultivation system by addition of different organic C sources, for example compost teas.

Results show that bacteria can quickly colonize the growing media during cucumber cultivation, for example with numbers of 16S rRNA gene copies reaching 10^{10} copies/cm^3 of stonewool or coco coir within a first few weeks of greenhouse cultivation. As expected the taxonomic diversity of bacterial microbiomes in growing media differs significantly and is influenced by growing medium type and organic C added. Interestingly, addition of organic C in the form of biochar or spent mushroom substrate (SMS) had significantly greater effect on bacterial microbiome in inert growing medium than in organic growing medium.

This research provides evidence that steering diversity of microbiome in soilless growing media towards higher disease suppression is possible. Still, there remains a lot to be unraveled about the taxonomic diversity and metabolic functioning of microbiomes in soilless cultivation systems, before we will be fully able to harness the potential of microbes naturally present in these systems.

Key words: soilless cultivation, vegetables, microbiome, disease suppression

Introduction

Soilless cultivation systems have been standard for vegetable production in Dutch greenhouses since the early 1980's. The switch from soil to growing media in vegetables production, like stone wool or perlite, was dictated by ever increasing root diseases pressure in the greenhouse soil and a ban on the use of methyl bromide as soil disinfectant. The main advantages of the soilless systems are: 1) the possibility of producing vegetables where the soil is not suitable for horticultural production; 2) pathogen free start of the cultivation using the new growing medium for each cultivation cycle; 3) increased productivity and 4) increased water and nutrient use efficiency.

However, as it turned out, some of the root pathogens, such as *Pythium* or *Fusarium* species, were able to adapt quickly to the new cultivation systems. *Pythium* and *Fusarium* can easily spread via irrigation lines and still cause large scale problems in the greenhouses. On the other hand, currently the growers are discouraged from using a lot of chemical crop protection products, which were routinely used in the past in soilless cultivation systems. Therefore, growers urgently need alternative measures to prevent the spread of root pathogens and yield loss in the greenhouses with vegetables. In the past less attention was given to the microbes naturally inhabiting the soilless cultivation systems, looking at them mostly as more of a nuisance than a piece of the puzzle required for succesful vegetable production. Although the first results supporting the hypothesis, that *Pythium* disease can be suppressed by microorganisms naturally establishing in stonewool during cucumber cultivation, were published in 2000 (by Postma et al.), research into the natural microbiomes of soilless cultivation systems only recently gained momentum (Thomas et al., 2023).

In this article we present the results of manipulation of natural microbiomes of soilless growing media during the cultivation of cucumber in order to prevent the infection by root pathogen *Fusarium oxysporum* f. sp. *cucumerinum*.

Materials and methods

Greenhouse experiment 1

Cucumbers were cultivated in two greenhouses (144 m^2 each), one with stonewool mats and one with coco coir growbags. Cucumbers (cultivar Proloog) were propagated in stonewool blocks and transplated onto the slabs and growbags 3 weeks after germination. Treatment with biochar or spent mushroom substrate (SMS) were carried out at the propagation phase. Biochar and SMS were mixed with vermiculite and added to a plant hole) and at the start of greenhouse cultivation (biochar and SMS were spread on top stonewool slabs or coco coir growbags). Samples of the growing medium for the analysis of bacterial microbiome (metabarcoding) and qPCR of total bacteria were taken in week 2 and week 5 of the cultivation.

Greenhouse experiment 2

Cucumbers (cv Proloog) were cultivated in a greenhouse (144 m^2), in stonewool growing medium. Cucumbers were propagated in stonewool blocks and transplanted onto the slabs 3 weeks after germination. Four treatments were tested: 1) addition of SMS, 2) SMS tea (2 % volume of standard cucumber nutrient solution), 3) combination SMS and SMS tea and 4) non pathogenic *Fusarium oxysporum* Fo47.

Treatments with SMS, SMS tea or *Fusarium oxysporum* Fo47 were carried out in the propagation phase and during cultivation. During the cultivation, SMS treatment was applied once (at the start of cultivation), SMS tea was supplied to the plants continuously (2 % SMS tea in standard nutrient solution) and treatment with *Fusarium oxysporum* Fo47 was repeated biweekly. Pathogenic *Fusarium oxysporum* f. sp. *cucumerinum* was inoculated into the stonewool slabs during week 2 of the cultivation (to end density of 10^5 spores/ cm^3 growing media). Samples of growing medium for analysis of bacterial microbiome (metabarcoding) and qPCR of total bacteria were taken in week 2 of the cultivation before inoculation of pathogenic *Fusarium*.

qPCR and metabarcoding analysis of bacteria in growing media
DNA was extracted from the growing media with PowerSoil Extraction kit (Qiagen) using the manufacturer's protocol. For determination of total bacterial population with qPCR, protocol of Fierer et al. (2005) was used (primer set: Eub338/Eub518). Metabarcoding analysis was carried out by Eurofins Genomics (Illumina MiSeq) using V3/V4 variable region of 16S rRNA gene.

Results and discussion

Experiment 1: Microbiome of stonewool and coco coir during cucumber cultivation
Diversity of bacterial microbiome of the two studied growing media differed significantly during the cucumber cultivation (Figure 1). Similar differences between different growing media were reported by Grunert et al. (2016). The microbiome of each growing medium also changed during the cultivation of cucumbers. Biochar or SMS addition had significant effect on diversity of stonewool microbiome, while this effect is not visible in coco coir (Figure 1). Numbers of 16S rRNA gene copies/ cm^3 growing medium were comparable for the two growing media ($\approx 10^9$ copies of 16S rRNA gene/cm^3, data not shown).

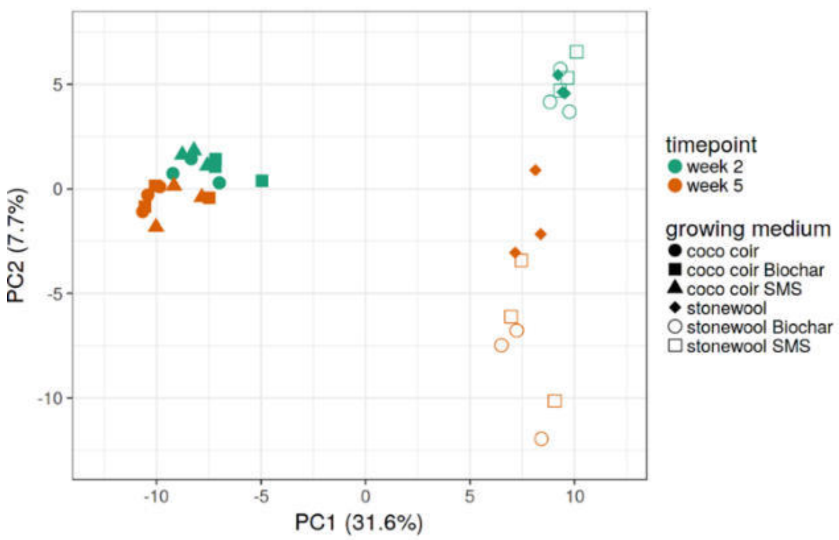

Figure 1. Principal components analysis (PCA) of bacterial community structure in stonewool and coco coir (with addition of biochar or SMS) during cucumber cultivation.

Experiment 2: Effect of manipulation of stonewool microbiome on infection of cucumber by Fusarium oxysporum f. sp. cucumerinum
Fusarium infection of cucumbers was slighthly influenced by addition of SMS or SMS tea alone, while combination of SMS+SMS tea or treatment with *Fusarium* oxysporum Fo47 slowed down the *Fusarium* infection significantly (Figure 2).

Bacterial microbiome of stonewool in the treatment with SMS + SMS tea was significantly different than that in untreated stonewool. Disease suppression in this treatment was positively correlated with higher relative abundance of *Bacillota* in treated stonewool compared to control.

These results show that there is potential for manipulation of stonewool microbiome in order to increase disease suppression in this growing medium.

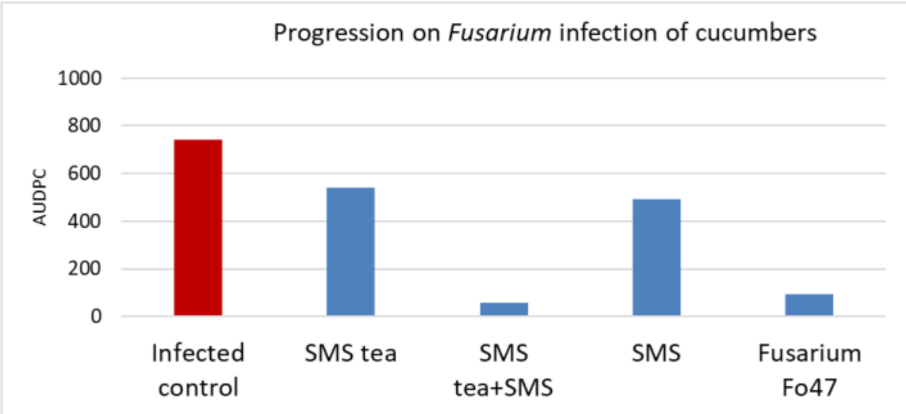

Figure 2. Progression of *Fusarium* infection of cucumbers grown in stonewool with different treatments (as AUDPC- area under disease progression curve).

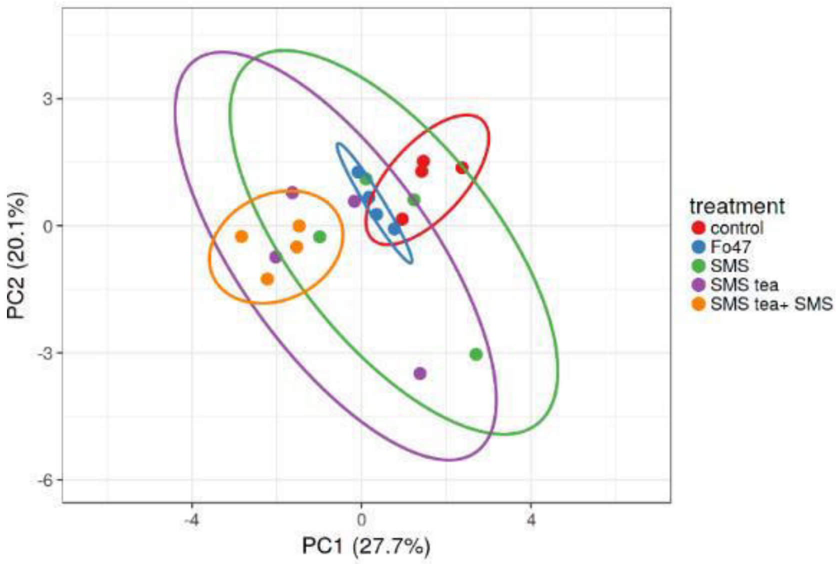

Figure 3. Principal components analysis (PCA) of bacterial community structure in stonewool with different organic additions during cucumber cultivation (week 2).

Acknowledgements

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References

Fierer, N., Jackson, J. A., Vilgalys, R., Jackson, R. B. 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Appl Environ. Microbiol. 71(7): 4117-4120. doi: 10.1128/AEM.71.7.4117-4120.2005.

Grunert, O., Hernandez-Sanabria, E., Vilchez-Vargas, R. et al. 2016. Mineral and organic growing media have distinct community structure, stability and functionality in soilless culture systems. Sci. Rep. 6: 18837. https://doi-org.ezproxy.library.wur.nl/10.1038/srep18837

Postma, J., Willemsen-de Klein, M. J., van Elsas, J. D. 2000. Effect of the Indigenous Microflora on the Development of Root and Crown Rot Caused by *Pythium aphanidermatum* in Cucumber Grown on Rockwool. Phytopathology 90(2): 125-133.

Thomas, P., Knox, O. G. G., Powell, J. R., Sindel, B., Winter, G. 2023. The Hydroponic Rockwool Root Microbiome: Under Control or Underutilised? Microorganisms 11(4): 835. doi: 10.3390/microorganisms11040835. PMID: 37110258; PMCID: PMC10141029.

Microbial consortia from the apple microbiome: Effective biocontrol agents adapted to diverse environmental conditions

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Abstract: The apple microbiome is a rich reservoir of microorganisms, offering a valuable source of potential biocontrol agents against major postharvest diseases. Recent advances have highlighted the importance of not only identifying effective individual isolates but also exploring their capacity to adapt to different environmental conditions.

Using a culturomics approach, 250 out of over 1000 initial microorganisms isolated from apples from different climatological conditions (mountain and valley) were screened to determine their potential to control *Penicillium expansum*, *Botrytis cinerea* and *Rhizopus stolonifer*, the main pathogens affecting postharvest pome fruits. While over 50 % of the individual microorganisms demonstrated promising results, we explored further through a novel strategy consisting of combining isolates from different environments into microbial consortia to enhance the final product's biocontrol resilience and stability under variable environmental conditions, that could take place in the field and different geographical production areas. In this study, we evaluated the efficacy and population dynamics on the apple surface of four initial candidates isolated from mountain and valley orchards. Their control efficacy against *P. expansum* was tested using fresh cells at different doses, and their population dynamics on fruit surfaces were assessed under 85 % and 40 % relative humidity conditions, emulating mountain and valley environmental conditions, respectively. A bacterial isolate from valley orchards (BV) and a yeast isolate from mountain orchards (LM) were selected for their superior disease control at lower doses and their stable population dynamics over 12 days. The two selected candidates were produced in 2-liter bioreactors and formulated individually (BV and LM) and as a consortium (BV + LM). Their efficacy was analyzed using fresh and formulated cells against *P. expansum* and *B. cinerea* at 0 °C and 20 °C. The results showed that the consortium of candidates, both fresh and formulated, could better control the incidence and severity of tested diseases at both temperature conditions in comparison with those applied individually. Our results underscore the potential of microbial consortia as an innovative and sustainable biocontrol strategy, not only for the efficacy but also for the environmental conditions broadening action. This approach advances postharvest disease management by enhancing both the efficacy and resilience of biocontrol formulations, offering a promising alternative to chemical fungicides. This work has been financed by 'Generalitat de Catalunya' (CERCA Programme and 2021 SGR 01477), the Government of Spain (project PID2020-117607RR-I00) and UdL-IRTA doctoral grant.

Key words: apple microbiome, consortia, biocontrol agents, postharvest, formulation

Identification of treatments that prolong chronological lifespan of the biocontrol yeast *Metschnikowia pulcherrima* in a temperature-dependent manner *in vitro* and on tomato seeds

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Abstract: *Metschnikowia pulcherrima* is an ascomycete, oleaginous yeast that is, for example, studied in biotechnology and as a biocontrol solution against fungal plant pathogens. This interesting yeast antagonizes other fungi by producing the iron-chelating compound called pulcherriminic acid, by being strongly competitive, and by other, yet unidentified mechanisms. A successful biocontrol product must be effective in protecting crops, but cost-effective production methods and stable formulations that remain active during storage are equally important. A collaborative project within Agroscope, has the goal to bridge the gap from laboratory research to application by optimizing the production and the formulation of *M. pulcherrima*. The first part of the project aims to study and characterize the chronological lifespan (CLS) of *M. pulcherrima*. CLS measures how long nondividing cells survive, which is an important criterion of optimization for the storage/stability of the product. Currently, there is no description of compounds or treatments that prolong CLS of *M. pulcherrima*. We have chosen 13 treatments or compounds that are known to improve CLS in *Saccharomyces* cerevisiae. Among these 13 different treatments, osmotic stress, metformin, spermidine, and quercetin improved CLS in *M. pulcherrima* the most (at least 3 months of storage). Surprisingly, the effect of some factors depended on the storage temperature. For example, metformin and spermidine only had a CLS prolongation effect at 22 °C and 30 °C, respectively. The treatments tested so far, and after 3 months of storage, did not have a negative impact on the antagonistic activity against *Botrytis cinerea*.

Key words: biocontrol, yeast, lifespan, antagonism

Deciphering the mode of action and the environmental impact of a fungal biocontrol agent against *Botrytis cinerea*

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Abstract: *Botrytis cinerea* is an airborne phytopathogenic fungus damaging a wide variety of crops and capable of developing resistance to chemical fungicides. The need to find new protection tools, including biocontrol, is thus crucial. However, encouraging farmers to use biocontrol solutions requires providing them with recommendations for improving their efficacy in the field and demonstrating their safety for the environment. It is therefore important to understand their mode of action and the optimal conditions for their use, as well as their non-intentional effects. A fungal strain was isolated from healthy tomato plants in a greenhouse infected by *B. cinerea*. This strain has the ability to protect tomato plants against *B. cinerea* when applied on pruning wounds as a preventive treatment. It has been identified as belonging to the *Fusarium* species complex but its precise taxonomic identity is still unknown. Its modes of action are supposed to be based on competition for nutrients and pH acidification and its unintended effect on the indigenous microbial community has not been evaluated. Therefore, our objectives were (i) to specify the phylogenetic position of this fungus using several molecular markers, (ii) to decipher its functional potential as a biocontrol agent using whole genome sequencing and (iii) to evaluate its effect on the microbiota of tomato plants under controlled conditions. The fungal biocontrol agent was confirmed to belong to the *Bisifusarium* genera. Genome sequencing revealed a genome size of 33 Mbp with 7,507 genes representing 32.4 % of the total genome. Interspersed repetitive sequences accounted for 0.8 % of the genome while tandem repeats accounted for 2.5 %. Genome annotation highlighted that 38 % and 37 % of the coding genes are dedicated to cellular process and metabolic process, respectively. The cell killing process and the immune system process represented only 2 % and 5 % of the genes, respectively. The effect of this biocontrol agent on both epiphytic and endophytic plant microbiota was evidenced by analyzing microbial abundance and taxonomic composition with bacterial and fungal qPCR and metabarcoding methods, respectively. Molecular biology tools make it possible to clarify and better understand the modes of action of microbial biological agents. However, more extensive and in-depth experiments in field conditions are necessary to achieve a comprehensive understanding of their optimal conditions of use in the field.

Key words: biological control agent, mode of action, plant microbiota

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Prospective for biocontrol implementation of the chestnut blight fungus *Cryphonectria parasitica* in Friuli Venezia Giulia

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Abstract: The fungus *Cryphonectria parasitica*, the causal agent of chestnut blight, gives rise to important economic losses worldwide. The disease can be efficiently managed through the *Cryphonectria*-Hypovirus pathosystem. Indeed, hypoviruses, specifically *Cryphonectria hypovirus* 1 (CHV-1), which is mainly transmitted through hyphal anastomosis among vegetative compatible (VC) *C. parasitica* types, can reduce the virulence and pathogenicity of the fungus. However, the recrudescence of the disease in Friuli Venezia Giulia required a new study of the *C. parasitica* population to evaluate the implementation of biocontrol strategy in the region. Therefore, isolates of *C. parasitica* were characterized both by the analysis VC-types and mating-type idiomorphs. In addition, the presence and occurrence of CHV-1 was studied and by the susceptibility of commercial and local chestnut cultivars to the disease was evaluated in an in vitro experiment. The data obtained pointed out a high genetic variability of the fungus and a low occurrence of hypovirulent strains, revealing a challenging prospective to find a successful biocontrol solution.

Key words: chestnut blight, *Cryphonectria parasitica*, hypovirulence

Introduction

Chestnut blight, caused by the fungus *Cryphonectria parasitica*, poses a threat to chestnut trees, endangering the sustainability of its cultivation in many countries. The fungus produces perennial necrotic lesions, commonly known as cankers, on the bark of susceptible hosts, particularly in young and grafted trees, which can ultimately lead to their death (Rigling and Prospero, 2018). Because of a viral infection of *C. parasitica* by hypoviruses, which can reduce the virulence and pathogenicity of the fungus, the disease on European chestnut (*Castanea sativa*) is not as severe as in North America, resulting in superficial cankers that do not cause dieback of infected trees (Anagnostakis, 1987).

The *Cryphonectria*-Hypovirus pathosystem is an effective example of biocontrol, which has been succesfully applied in Europe (Cortesi and Milgroom 2004). *Cryphonectria hypovirus* 1 (CHV-1) is the most important virus that infects *C. parasitica*, which is transmitted horizontally through hyphal anastomosis among vegetative compatible (VC) strains of *C. parasitica* and through asexual spores (Rigling and Prospero, 2018). Even though virus-containing *C. parasitica* strains, referred to as hypovirulent, are currently present in many chestnut-growing areas of Europe, virus-free (virulent) strains of *C. parasitica* continue to cause economic losses and dieback of chestnut trees, probably due to a combined effect of the pathogen and changing climate (Waldboth and Oberhuber, 2009). Therefore, the knowledge about the VC type composition of *C. parasitica* strains in a specific cultivation area combined with data on the presence and frequency of CHV-1 are essential parameters to monitor a mycovirus-mediated biocontrol program and to take plant protection measures accordingly.

In the Friuli Venezia-Giulia region (northern Italy), in the area named 'Valli del Natisone', local European chestnut cultivars have been cultivated and valued for various purposes, including fresh consumption, animal feed, and timber production (Donno et al., 2002). These local *C. sativa* cultivars, due to their excellent nutraceutical and sensory properties, can be commercially competitive similar as the international varieties, which nowadays cover the complete area of 2.200 ha of chestnut stands (Donno et al., 2002). Therefore, initiatives to protect and study the local cultivar biodiversity and to adopt effective crop management practices could contribute to renewed attention to the regional chestnut sector.

The observed recrudescence of chestnut blight in the Friuli Venezia Giulia region in recent years requires effective management strategies. Two main strategies to counter chestnut blight are cultivating tolerant genotypes and using biocontrol via virus-induced fungal hypovirulence.

The objectives of the present study were: i) to characterize the genetic diversity of *C. parasitica* on the base of VC- and maiting-types analyses; ii) to assess the susceptibility to the fungus of three local chestnut varieties from Friuli Venezia Giulia; iii) to detect the presence and occurrence of CHV-1 infecting *C. parasitica* in Friuli venezia Giulia.

Materials and methods

Sampling and isolation of **C. parasitica**

Samples of bark cankers of 58 chestnut trees showing characteristic symptoms of chestnut blight were collected in the area 'Valli del Natisone' in 2023. The area can be divided in two subzones, which were named 'Valli del Natisone 1' and 'Valli del Natisone 2'. Canker apperance was distinguished as ‘virulent lesion’ or ‘healing lesion’. The samples were incubated in moist chambers at room temperature for 7-20 days. Pycnidia grown on the bark surface of the samples were removed and inoculated on Potato Dextrose Agar (PDA) medium (VWR, Leuven, Belgium) at room temperature in the dark. A total of 49 pure isolates were obtained. Cultures were mantained on fresh PDA at 20 °C.

C. parasitica *vegetative compatibility and mating type analysis*

Fungal genomic DNA was isolated from pure cultures following the protocol described by Ahmad and Baric (2022). Each *C. parasitica* strain was characterized by PCR amplification of six VC loci (Mlinarec et al., 2018; Ahmad and Baric, 2022) as well as the mating type locus (MAT locus) (McGuire et al., 2001). Isolates were assigned to EU types (VC types) according to the vic allelic data defined by Cortesi and Milgroom (1998).

Suceptibity test of chestnut cultivars

Three local cultivars of chestnut named 'Obiacco', 'Marujac', and 'Muron', and the commercial hybrid cultivars 'Bouche de Bétizac' (*C. sativa* × *C. crenata*), were assessed in an in vitro assay for their susceptibility to *C. parasitica*. Mycelium plugs of five virulent *C. parasitica* colonies were, inoculated on wounded one-year-old detached stems (8 cm in length; 5 replicates). The inoculated plant material was incubated in humid boxes at 25 °C. Canker size on detached stems was measured at 28 days post inoculation (dpi).

Detection of the presence of CHV-1

Total RNA was extracted and processed to prepare complementary DNA (cDNA) and an adapted PCR protocol was used to dected the nucleic acid of the hypovirus (Ahmad et al., 2024).

Results and discussion

C. parasitica *genetic variability*

The VC type analysis of the *C. parasitica* isolates reveled a high genetic variability of the fungus in the investigation area. The two most common of the 16 VC types observed, EU-17 and EU-14, occurred at a frequency of 16.3 % and 12.2 %, respectively. The VC types showed different distribution patterns between the two subzones. Seven VC types were found in both areas, but at different frequencies, while four VC types were found in Valli del Natisone 1 and five VC types only in Valli del Natisone 2 (Figure 1). The two mating type alleles occur at a ratio of approximately 1:1 in the two sampling subzones (Table 1), thus an active sexual reproduction of the pathogenic fungus can be assumed.

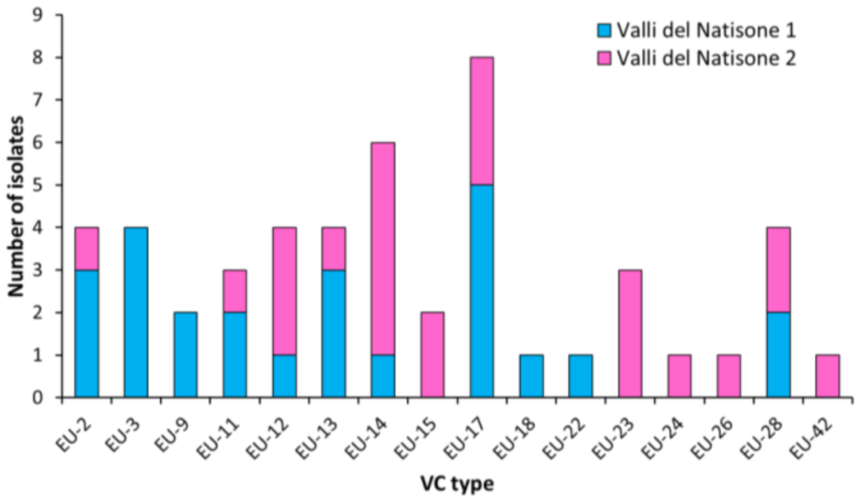

Figure 1. Absolute distribution of VC types among the two investigated subzones.

Table 1. Relative frequency of mating type alleles among sampling areas.

	Relative frequency (%)		
allele	Valli Natisone 1	Valli Natisone 2	Total
Mat-1	60.9	40.9	50.9
Mat-2	39.1	59.1	49.1

***Suceptibility of local chestnut cultivars to* C. parasitica**

The smallest average lesion lengths, considering all five isolates at 28 dpi, were found for 'Bouche de Bétizac' (3.9 cm ± 1.7 SD) and the local cultivar 'Marujac' (3.9 cm ± 1.3 SD). The two other local cultivars, 'Obiacco' and 'Muron', showed average lesion lengths of

(4.8 cm ± 1.4 SD) and 4.4 cm (± 1.4 SD), respectively. Among the local varieties, 'Marujac' exhibited the lowest susceptibility to four of the tested isolates of *C. parasitica*, while one isolate induced significantly longer lesions in most of the cultivars.

Hypovirulence occurrence

Preliminary data based on the appearance of the bark cankers from which the 58 samples were collected, inicated that 20 represented healing cankers, resulting in an estimated hypovirulence rate of 34 %. This preliminary finding has to be supported by the molecular analyis on the presence of CHV-1 in the obtained *C. parasitica* isolates, which is currently ongoing.

Possible implementation of a biocontrol strategy in Friuli Venezia Giulia

The implementation of an effective biocontrol strategy in Friuli Venezia Giulia could be challenging. The susceptibility of both commercial and local varieties to *C. parasitica* infection promotes the spread of chestnut blight in the area. Furthermore, choosing potential hypovirulent strains of *C. parasitica* is difficult due to the low frequency of hypovirulent strains and the large variability of VC types. To perform an effective biocontrol strategy in the area, a consortium of hypovirulent *C. parasitica* strains should be developed.

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References

Ahmad, F., and Baric, S. 2022. Genetic diversity of *Cryphonectria parasitica* causing chestnut blight in South Tyrol (northern Italy). Eur. J. Plant Pathol. 162: 621-635.

Ahmad, F., Tomada, S., Poonsiri, T., and Baric, S. 2024. Molecular genetic variability of *Cryphonectria hypovirus* 1 associated with *Cryphonectria parasitica* in South Tyrol (northern Italy). Front Microbiol. 15: 1291542.

Cortesi, P., and Milgroom, M. G. 1998. Genetics of vegetative incompatibility in *Cryphonectria parasitica*. Applied Environ. Microbiol. 64(8): 2988-2994.

Donno, D., Fabro, M., Mellano, M. G., Gamba, G., Fioccardi, A., and Beccaro, G. L. 2022. Integrating traditional wheat-based foods with high health value flours: *Castanea* spp. agro-biodiversity in bakery products. Agriculture 12(7): 946.

McGuire, I. C., Marra, R. E., Turgeon, B. G., and Milgroom, M. G. 2001. Analysis of mating type genes in the chestnut blight fungus, *Cryphonectria parasitica*. Fung. Gen. Biol. 34(2): 131-144.

Mlinarec, J., Ježić, M., Ćosić, J., and Ćurković-Perica, M. 2018. Multilocus PCR assay reveals high diversity of vegetative compatibility types in populations of *Cryphonectria parasitica* in Croatia. Plant Path. 67(3): 741-749.

Rigling, D., and Prospero, S. 2018. *Cryphonectria parasitica*, the causal agent of chestnut blight: invasion history, population biology and disease control. Mol. Plant Pathol. 19: 7-20.

Waldboth, M., and Oberhuber, W. 2009. Synergistic effect of drought and chestnut blight (*Cryphonectria parasitica*) on growth decline of European chestnut (*Castanea sativa*). For. Pathol. 39: 43-55.

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Exploring the multifunctional potential of indigenous Brazilian *Trichoderma* isolates and developing an innovative, sustainable bioreactor-in-a-granule formulation

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Abstract: In a series of studies, we explored the multifunctional potential of two indigenous Brazilian strains, *Trichoderma asperelloides* CMAA 1584 and *Trichoderma lentiforme* CMAA 1585, for controlling *Sclerotinia sclerotiorum* and for promoting growth in cotton plants. Additionally, we proposed an innovative approach to develop a rice flour-based formulation of the biocontrol agent *T. asperelloides* CMAA 1584, designed to simulate a micro-bioreactor within the full biorefinery context. This approach enables *in situ* conidiation, extended shelf-life, and effective control of *S. sclerotiorum*. Our findings revealed that *T. asperelloides* CMAA 1584 was more effective in parasitizing *S. sclerotiorum*, while *T. lentiforme* CMAA 1585 demonstrated a more pronounced effect on cotton growth. Furthermore, an environmentally friendly bioprocess using rice flour as the main feedstock was successfully implemented to develop waste-free granular formulations of *Trichoderma* conidia. Such formulations successfully suppressed *Sclerotinia* and improved the shelf-life of the biofungicide.

Key words: bioprotectant, biofertilizer, solid-state fermentation, formulation

Introduction

Trichoderma spp. is the most successful microbial antagonist for the biological management of Sclerotinia stem rot (Faria et al., 2022) through various mechanisms (Monte et al., 2019; Monte and Hermosa, 2021). However, the effectiveness of *Trichoderma* in suppressing plant pathogens and promoting plant growth varies among species and strains (Woo et al., 2023).

In this context, we explored the multifunctional potential of two indigenous Brazilian strains, *T. asperelloides* CMAA 1584 and *T. lentiforme* CMAA 1585, for controlling *S. sclerotiorum* and promoting growth in cotton plants. Additionally, we proposed an innovative approach to develop a rice flour-based formulation of the biocontrol agent *T. asperelloides* CMAA 1584, designed to simulate a micro-bioreactor within the full biorefinery context, enabling *in situ* conidiation, extending shelf life, and providing effective control of *S. sclerotiorum*.

Materials and methods

Biocontrol of **Sclerotinia sclerotiorum** *and biostimulant effects on cotton plants*

We explored the multifunctional potential of two indigenous Brazilian *Trichoderma* strains, *T. asperelloides* CMAA 1584 and *T. lentiforme* CMAA 1585, for controlling *S. sclerotiorum* and for promoting cotton growth. In this study, we evaluated the ability of these *Trichoderma* strains to solubilize phosphate and inhibit the mycelial growth of *S. sclerotiorum*. Additionally, we investigated their capacity to parasitize *S. sclerotiorum* sclerotia. Furthermore, we assessed the effects of these *Trichoderma* strains on cotton seed germination and seedling vigor, as well as their biostimulant effects on cotton plants. A detailed description of the methodology can be found in Silva et al. (2022).

Innovative granule formulation of **Trichoderma asperelloides** *CMAA 1584*

We developed a novel rice flour-based formulation for the biocontrol agent *T. asperelloides* CMAA 1584. This formulation, mimicking a micro-bioreactor, enables efficient production of conidia, extends product shelf-life, and effectively controls *S. sclerotiorum*. Rice flour, a low-cost byproduct of rice milling, was used as the primary substrate for conidial yields through our optimized fermentation-formulation process. A Plackett-Burman design was employed to optimize solid-state fermentation of *T. asperelloides* in rice flour. Five variables were investigated, including substrate moisture (40 and 60%), inoculum density (1×10^5 and 1×10^6 conidia/g of substrate), substrate weight (100 g and 150 g), fermentor type (polypropylene bags and Erlenmeyer flasks), and nitrogen content (0.1 and 1 % w/w of autolyzed yeast, ammonium sulfate, corn steep liquor, cottonseed flour, and hydrolyzed yeast).

After mass production, five formulations were prepared using the inoculated rice flour with additional components: bentonite ($G_{Bentonite}$), soy lecithin ($G_{Lecithin}$), Break-Thru S301 ($G_{Break\text{-}Thru}$) and Break-Thru S301 with organic compost (Ribumin®) ($G_{Organic\ compost\ +\ Break\text{-}Thru}$). Formulations were processed into granules using a 2.5-mm extruder cylinder and then air-dried at room temperature inside an exhaust hood until reaching constant final moisture of 5 ± 1 % (w/w), measured with a moisture analyzer. The storage stability of the formulations, conidiation capacity of the granules after 12 months of storage, and their effectiveness against *S. sclerotiorum* were evaluated. Detailed experimental procedures are described in Silva et al. (2023).

Results and discussion

Biocontrol of **Sclerotinia sclerotiorum** *and biostimulant effect on cotton plants*

Trichoderma asperelloides CMAA 1584 and *T. lentiforme* CMAA 1585 demonstrated the ability to control *S. sclerotiorum* and promote cotton growth. *Trichoderma asperelloides* was more effective in suppressing *S. sclerotiorum*, while *T. lentiforme* enhanced plant growth (Table 1). These findings suggest that combining these strains could provide a sustainable approach to managing white mold in cotton, reducing the need for synthetic inputs.

Table 1. Phosphate solubilization and antagonistic activity of *Trichoderma asperelloides* CMAA 1584 and *Trichoderma lentiforme* CMAA 1585 against *Sclerotinia sclerotiorum* through dual culture test, production of volatile organic compounds (VOC), inhibition of sclerotia myceliogenic germination, and cotton growth promotion.

Treatments	Phosphate solubilization (%)	Inhibition of myceliogenic germination (%) at 10^8 conidia/ml	Cotton root fresh weight (g) at 10^8 conidia/ml	Cotton aboveground fresh weight (g) at 10^8 conidia/ml
CMAA 1584	5.2 ± 0.79 b	100	19.2 ± 1.4	19.8 ± 2.0
CMAA 1585	31.7 ± 4.08 a	69	30.3 ± 2.1	27.1 ± 3.3
Control	-	-	16.2 ± 0.9	16.0 ± 1.8

***Innovative granule formulation of Trichoderma asperelloides* CMAA1584**

Conidial yield was primarily influenced by the nitrogen content (0.1 % w/w) added to the rice meal, along with the type of fermentor used. Hydrolyzed-yeast was the best nitrogen source, yielding 2.6×10^9 colony-forming units (CFU)/g within 14 days when using Elernmeyer flask as solid-state fermentation reactor.

All conidia-based granule formulations lost viability more rapidly at ambient temperature (25 ± 2 °C) if compared to cold storage (4 °C). Notably, $G_{Control}$ and $G_{Bentonite}$ showed formulations the best shelf-life performance at ambient temperature, with no significant viability loss ($P < 0.05$) after three months. At time zero, the $G_{Bentonite}$ formulation contained 1.98×10^8 CFU/g, and after 12 months of storage, its viability declined to 1.04×10^6 CFU/g.

$G_{Lecithin}$, $G_{Organic\ compost\ +\ Break\text{-}Thru}$, and $G_{Bentonite}$ formulations maintained high viability for 24 months under cold storage. At time zero, their initial viability was 2.06×10^8, 1.48×10^8 and 2.09×10^8 CFU/g, and after 24 months in refrigeration, 1.40×10^8, 1.87×10^8 and 1.79×10^8 CFU/g, respectively.

None of the formulations retained conidial viability after 12 months of storage at room temperature. However, $G_{Lecithin}$, $G_{Bentonite}$, and $G_{Organic\ compost\ +\ Break\text{-}Thru}$ stood out when kept under cold storage by maintaining viability above 1.5×10^8 CFU/g after 24 months, outperforming other formulations that exhibited less than 1×10^8 CFU/g.

All formulations demonstrated comparable efficacy in suppressing the myceliogenic germination of *Sclerotinia* sclerotia, regardless of the concentration tested (5×10^4 to 5×10^6 CFU/g of soil), achieving a relative inhibition of 79.2 % to 93.7 %. Remarkably, formulations stored under cold conditions for 24 months retained their ability to suppress sclerotia germination (Figure 1).

This study underscores an environmentally sustainable bioprocess that utilizes rice flour as a cost-effective substrate to produce granular *Trichoderma* conidia formulations for agricultural bioinputs.

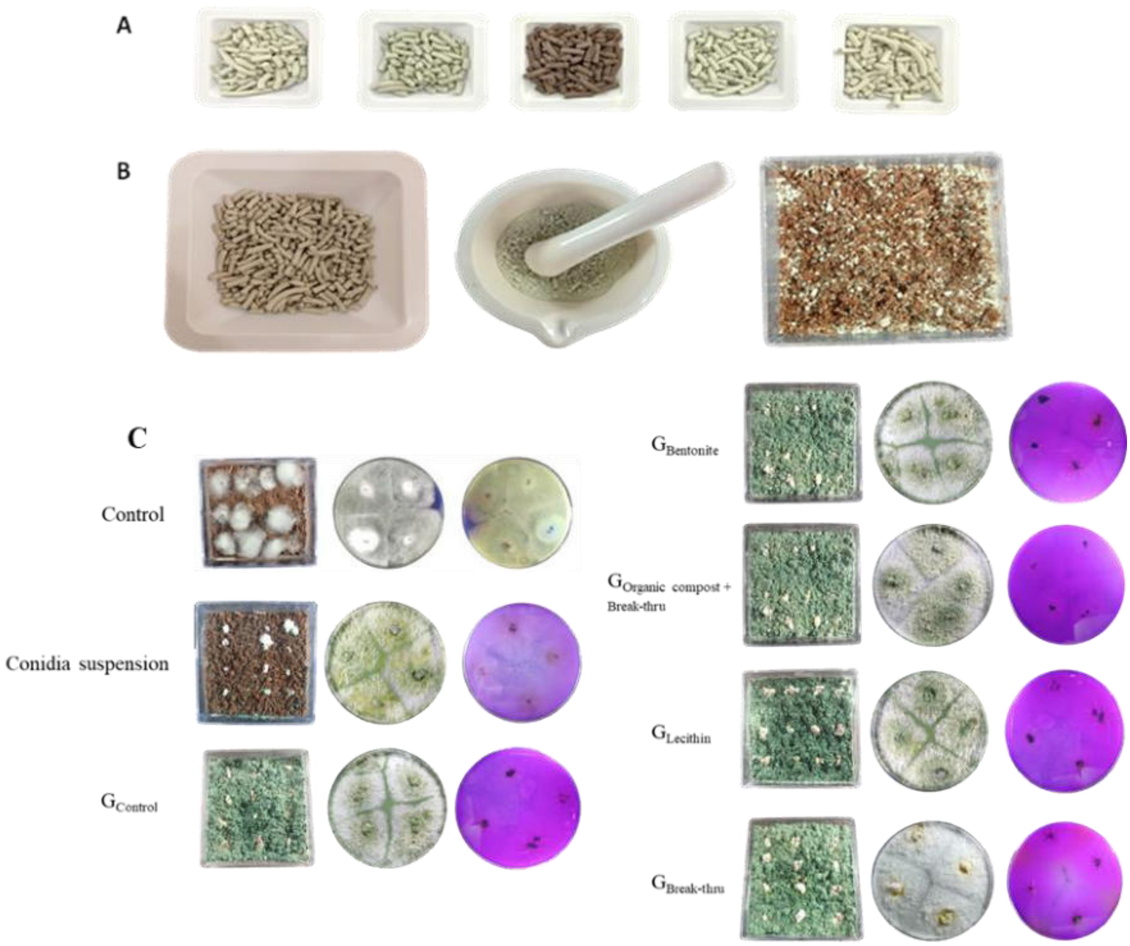

Figure 1. Granular formulations of *Trichoderma asperelloides* CMAA 1584 after 24 months of storage at 4 °C (A), preparation and incorporation into the soil (B), and suppressiveness effect on the myceliogenic germination of *Sclerotinia sclerotiorum* sclerotia in soil (C). Non-viable sclerotia are indicated by the purple color.

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References

Faria, A. F., Schulman, P., Meyer, M. C., Campos, H. D., Cruz-Magalhães, V., Godoy, C. V., Guimarães, R. A., Silva, L. H. C. P., Goussain, M. M., Martins, M. C., Nunes Júnior, J., Venancio, W. S., Fantin, L., Brustolin, R., Jaccoud Filho, D. S., Carneiro, L. C., Juliatti, F. C., and Medeiros, F. H.V. 2022. Seven years of white mold biocontrol product's performance efficacy on *Sclerotinia sclerotiorum* carpogenic germination in Brazil: A meta-analysis. Biol. Control 176: 105080.

Monte, E., and Hermosa, R. 2021. The use of *Trichoderma* spp. to control plant diseases. In: Köhl, J., and Ravensberg, W. (eds.): Microbial bioprotectants for plant disease management, pp. 401-428. Burleigh Dodds, Cambridge.

Monte, E., Bettiol, W., and Hermosa, R. 2019. *Trichoderma* e seus mecanismos de ação para o controle de doenças de plantas. In: Meyer, M. C., Mazaro, M. C., Silva J. C. (eds.): *Trichoderma:* Usos na agricultura. Brasília: Embrapa Soja, pp. 181-200.

Silva, L. C., Camargo, R. C., Mascarin, G. M., Nunes, P. S. O., Dunlap, C. and Bettiol, W. 2022. Dual functionality of *Trichoderma*: biocontrol of *Sclerotinia sclerotiorum* and biostimulant of cotton plants. Front. Plant Sci. 13: 983127.

Woo, S. L., Hermosa, R., Lorito, M., and Monte, E. 2023. *Trichoderma*: A multipurpose, plant-beneficial microorganism for eco-sustainable agriculture. Nat. Rev. Microbiol. 21: 312-326.

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Rhizosphere bacteria from the Bolivian highlands improve growth and drought tolerance in quinoa (*Chenopodium quinoa*) Willd.

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Abstract: Drought is one of the most destructive abiotic factors for agricultural production, causing considerable yield losses. Quinoa (*Chenopodium quinoa* Willd.) is cultivated worldwide in different environmental conditions due to its nutritional characteristics and ability to grow in harsh environments. This study aims to select drought stress tolerant rhizosphere bacteria from the Bolivian altiplano to evaluate their quinoa growth-promoting capacity, including *in vitro* germination, seedling growth under drought stress in greenhouse conditions and field studies.

Rhizosphere soil from the southern highlands of Bolivia was collected to isolate 164 drought-stress tolerant bacteria. From these, 28 strains were shown to produce indole acetic acid, and/or to possess nitrogen-fixing or phosphate solubilizing capacity under *in vitro* conditions. Furthermore, all strains were evaluated for improvement of *in vitro* quinoa seed germination. Based on these properties, nine bacterial strains were formulated in three different matrixes and evaluated for quinoa seedling growth promotion during drought stress in a three-month greenhouse experiment. Three strains were shown to significantly ($P < 0.05$) increase root length of the quinoa seedlings. One strain was selected and shown to significantly ($P < 0.05$) increase leaf number in a field trial under semi-arid conditions in the southern altiplano in Bolivia. DNA sequencing and phylogenetic analyses of the 16S locus putatively identified the three strains with growth-promoting potential under drought stress as members of the genera *Bacillus*, *Pseudomonas* and *Serratia*.

Microorganisms from the arid Bolivian altiplano constitute a potential biological source of bioinoculants to improve quinoa productivity and provide sustainable mitigation of climate change effects.

Key words: *Bacillus*, drought tolerance, PGPR, *Pseudomonas*, quinoa, *Serratia*

Session II
Biocontrol targeting One Health

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Exploring rhizosphere microbiomes in Mediterranean vineyards through metabarcoding analysis: insights from Italy, Portugal, Morocco, and Turkey

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Abstract: The Mediterranean ring is a unique area directly influenced by the presence of the Mediterranean Sea. Since ancient times, grapevine cultivation has been one of the primary agricultural activities in this region. The viticultural sector in the Mediterranean basin includes vineyards for wine production and table grapes, that became distinctive products and trademarks of the landscape and environment of the entire region. Vineyard agronomic practices are directly shaped by the *Vitis vinifera* cultivars, the environmental and soil characteristics, and, certainly, the agricultural management systems. All these factors, along with others, constitute the so-called *terroir*, a term that comprises the set of factors characterizing the product of a specific geographical area. Among these factors, the composition of the microbial communities inhabiting soil plays a crucial role. These communities are responsible for numerous bio-geochemical cycles that affect the nutritional status of the plants. Single entities in the rhizosphere also influence plant health by interacting with one another and contributing to the balance of microbial and nutrient dynamics.

In this study, we focused on unrevealing the biodiversity of microbial communities, both bacterial and fungal, through a metabarcoding approach. Three different vineyards were sampled from four countries in the mediterranean basin, namely Italy, Morocco, Portugal and Turkey. Our results demonstrate that, despite originating from a relatively confined area, significant variations in the composition of rhizosphere microbial communities in grapevine soil can be observed. Different variables are involved in shaping both bacterial and fungal communities. Each *terroir* displayed unique features, driven by either environmental, edaphic and possibly managemental differences that shape what might be referred to as microbial *terroir*. Despite differences in overall microbial community structure, less clear differences were noticed at functional level, revealing functional redundancy, especially for fungal communities.

These results could be further exploited to define microbial community/function requirements for vineyards in the Mediterranean region. This may help both the scientific community and farmers to adopt approaches to optimize soil health, increase grapevine resilience in a challenging biome that is increasingly under the effects of climate change.

Key words: metabarcoding, grapevine, Mediterranean basin

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Use in a controlled environment of *Trichoderma asperellum* ICC012 and *Trichoderma gamsii* ICC080 to manage FHB on common wheat

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Abstract: *Fusarium* head blight (FHB) represents a significant threat for wheat production due to the risk for food security and safety. Despite the huge number of biofungicides on the market, only one is available at European level to control *Fusarium* infections on cereals. The present work aimed to assess the possible use of *Trichoderma asperellum* strain ICC012 and *Trichoderma gamsii* strain ICC080, active ingredients of the commercial biofungicide Remedier®, to manage FHB on common wheat *Triticum aestivum* cv. Apogee. Initially, the capability of ICC012 and ICC080 to endophytically colonize wheat roots, a prerequisite very often correlated with the induction of resistance in the host plant, was investigated. It resulted in 100 % of roots internally colonized by the two strains, followed by a significant up-regulation of the defense-related genes encoding for pathogenesis-related protein 1 (*pr1*), superoxide dismutase (*sod*), polygalacturonase inhibitor protein 2 (*pgip2*) and phenylalanine ammonia-lyase 1 *(pal1*). When the expression of the same genes was investigated in spikes treated at the flowering stage with the two strains, applied individually or co-inoculated, a significant up-regulation of only *pal1* was registered 24 hours post inoculation (hpi) in spikes treated with ICC080. To check if a systemic defense response was induced, the expression of the same genes was analyzed in leaves collected at 7 and 14 days post inoculation (dpi) of roots, resulting in a significant up-regulation of *sod* at 7 dpi in leaves collected from plants inoculated with ICC012. Even if induction of resistance is probably not the main mode of action of the two strains, ICC012 and ICC080 applied on spikes at anthesis significantly reduced, in greenhouse conditions, the Disease Incidence (DI) caused by the inoculation mix of *Fusarium graminearum*, *Fusarium culmorum*, *Fusarium langsethiae* and *Fusarium sporotrichioides*, four of the most important FHB casual agents. This reduction in disease symptoms was observed when the two beneficial strains were applied both individually and co-inoculated on the spikes. Finally, ICC012 and ICC080 demonstrated a good competitive ability for substrate possession. The amount of *F. graminearum* (as DNA and number of perithecia) on wheat straw pieces was significantly reduced after 6 months of incubation in presence of the two beneficial strains, applied individually and co-inoculated. Being cultural debris used to overwinter, this competitive behaviour of ICC012 and ICC080 is an important trait to reduce the potential inoculum of the pathogen. The results collected here lay the groundwork for the use of ICC012 and ICC080 in managing FHB on common wheat.

Key words: FHB, *Trichoderma asperellum*, *Trichoderma gamsii*, common wheat

Acknowledgements

This work was performed within the research contract "Evaluation of the mechanisms of action of *Trichoderma gamsii* ICC080 and *Trichoderma asperellum* ICC012 (active ingredients of the commercial formulation Remedier®) against the causal agents of *Fusarium* head blight on wheat" between GOWAN Crop Protection Limited and the University of Pisa.

References

Cesarini, M., Petrucci, A., Hotaj, E., Venturini, G., Liguori, R., Sarrocco, S. 2025. Use in a controlled environment of *Trichoderma asperellum* ICC012 and *Trichoderma gamsii* ICC080 to manage FHB on common wheat. Microbiol. Res. 290: 127941. https://doi.org/10.1016/j.micres.2024.127941.

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Combinations of beneficial microbes and lignin copper nanoparticles for sustainable control of *Plasmopara viticola* on Falanghina grapevines

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Abstract: Downy mildew, caused by the oomycete *Plasmopara viticola*, is one of the most severe diseases of grape (*Vitis vinifera* L.) worldwide. As an obligate biotrophic pathogen, *P. viticola* grows only on living plant tissues including leaves, tendrils, shoots, and clusters, thus, is a primary causal agent of grape production losses, having a significant economic impact. In organic agriculture, pathogen control relies mainly on the application of copper (Cu) based fungicides. However, continuous use has caused the accumulation of the metal in soil/water matrices, thus negatively impacting micro- and macroorganism diversity and health. With limitations programmed for the use of Cu based products in the European Union, research for alternative sustainable products is strongly needed.

This study aimed to reduce copper doses for the control of *P. viticola* on grapevine by applying biological control agents (BCAs) belonging to *Trichoderma* (*T. virens, T. asperellum*) and *Bacillus* (*B. subtilis, B. amyloliquefaciens*), alone and in combination with lignin copper nanoparticles (LC_NP) containing reduced Cu concentrations (~30 g/ha) compared to recommended doses for commercial formulations (~300 g/ha). LC_NP, derived from the combination of lignin with brochantite [$Cu_4(OH)_6SO_4$], was successfully applied for the control of different bacterial and fungal phytopathogens on numerous crops.

A three-year field experiment (from 2022 to 2024) was conducted on grafted grapevine plants (*Vitis vinifera* L. cultivar Falanghina) cultivated in 30 l, soil filled vases, inserted in the ground, and grown in natural environmental conditions, at the Department of Agricultural Sciences of the University of Naples, Italy. Plants were treated with 21 different formulations, consisting of BCAs and/or LC_NP components, used singly or in combinations. Applications were performed mainly by foliar spray, with the exception of the *Trichoderma* treatments which were applied as a soil drench watering. Disease incidence and severity indices of natural *P. viticola* infections were assessed visually. In addition, multispectral data were acquired by Unmanned Aerial Vehicles and analyzed to identify high performing vegetative indices that discriminate between healthy and infected plants and provide indications of treatment effectiveness.

In general, varying levels of disease pressure were observed during the three-year period of the experiment. The integrated application of the microbial BCAs, *B. subtilis* or *T. virens,* in combination with LC_NP resulted in a consistent reduction in disease severity compared to the untreated plants, which was analogous to the efficacy obtained with the full dose of the chemical Cu based fungicide. The utilization of vegetation indices, such as NDVI and TVI obtained from multispectral data, permitted the development of an accurate field map corresponding to the indications of plant health.

These results suggested that beneficial microbes can be used in combination with innovative copper-based nanoparticles for the effective management of *P. viticola* on grapevine, drastically reducing the use of Cu based fungicides and the potential release of the element in the environment. Vegetation indices, highly correlated to disease severity, represent promising, rapid and high throughput tools for disease monitoring, determining provisional interventions, evaluation of treatment effectiveness, and guiding efficient interventions in time and space for crop protection management.

Key words: microbial consortia, bioformulations, Integrated Pest Management (IPM), *Trichoderma, Bacillus,* vegetation indices, precision agriculture

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From compost microbiomes to potentially disease suppressive bacterial isolates

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Abstract: Composting helps closing nutrient cycles, and applying composts improves soil structure. Even more, composts application can suppress soilborne diseases, most likely driven by microorganisms present in the compost. A disease-suppressing effect has been demonstrated for many microorganisms (MO) isolated from composts, or effective biocontrol strains isolated from other environments can also be found in composts. In practice, composts are often more effective than individual biocontrol strains when applied to pathogen-infested soils. Yet, the disease-suppressive activity varies among different composts, and cannot yet be predicted or reliably reproduced, even though this would be crucial for optimizing compost use in disease control.

In a study, we analyzed 37 composts from 7 large-scale compost producers to determine whether suppressiveness in three different plant – pathogen systems (cress – *Globisporangium ultimum*, cucumber – *G. ultimum*, cucumber – *Rhizoctonia solani*) is associated with certain bacterial or fungal communities, and whether these could be used as indicators of suppressiveness. We were able to confirm that the suppressiveness of composts is strongly pathogen- and, to a lesser extent, host-plant specific. Suppressiveness did neither correlate with physicochemical parameters of the composts nor with microbial parameters such as microbial activity, alpha- and beta-diversity. However, specific bacterial and a few fungal sequences could be identified that were associated with the most suppressive composts in the respective plant-pathogen system. Bacteria of the genera *Luteimonas*, *Sphingopyxis* and *Algoriphagus* were promising candidates for *G. ultimum* suppressiveness. We isolated bacteria belonging to the last two and to 14 additional genera from compost, and selected those which matched an indicative sequence. They are now being analyzed for their function (e.g., in vitro inhibitory effect against *G. ultimum*, in vivo effect of single strains/consortia of selected strains).

Our findings emphasize the need for more nuanced approaches to identify compost traits that promote disease suppression, particularly by investigating specific microbial taxa that may serve as indicators of disease-suppressive composts.

Key words: soil amendments, compost, *Globisporanigum ultimum, Pythium ultimum, Rhizoctonia solani,* biocontrol, biofertilizer

The genetic architecture underlying plant-microbiome interactions is altered under increasing environmental temperature

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Abstract: Host-microbiome interactions are essential drivers of the ecology and evolution of most organisms. Plants recruit their microbial associates from the environment, particularly in response to biotic and abiotic stressors. In this context, global changes are predicted to influence the outcome of plant-microbiome interactions with consequences on the ecology and evolution of plant communities. In this study, we grew a panel of 99 duckweed (*Spirodela polyrhiza*) genotypes together with a synthetic microbial community and exposed them to two different temperatures: 23 °C simulating control conditions and 27 °C simulating warming conditions. We then characterized changes in the microbiome and fitness for each individual plant genotype. Using a GWAS approach we identified the loci involved in the variation of specific microbial taxa, the overall microbiome, and the microbiome-driven changes in plant fitness. These loci are associated to genes involved in different functions, including resistance to stressors. Within a plant population, these loci can influence selection under changing climate, ultimately driving the rapid evolution of plant populations with ecological consequences on ecological communities.

Key words: GWAS, climate change

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Crop residue management to decrease *Alternaria solani*, *Cercospora beticola* and *Alternaria dauci* inoculum in arable crop rotation systems

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Abstract: Crop residues, defined as the above-ground biomass of the crop (i. e., dead leaves and stems) left in the field after harvesting, can act as a source of inoculum being colonized saprophytically by necrotrophic plant pathogens. These pathogens may survive until the following season, when conditions are favourable again to replicate. First insights have shown that plant pathogens in arable crops are present in crop residues of sensitive host plants. Additionally, these pathogens have been found in residues from weeds, non-susceptible cover crops and other crops within the rotation system (Köhl et al., 2024). Properly managing crop residues can hamper pathogen cycles that initiate the infection in the following seasons. This is why in this work we have tested different strategies to be applied for the management of crop residues against *Alternaria solani* (causal agent of potato early blight), *Cercospora beticola* (causal agent of leaf spot in sugar beet) and *Alternaria dauci* (causal agent of *Alternaria* leaf blight in carrot). We used the biological control agent *Clonostachys rosea* J1446, well- adapted microorganism to the ecological niche of crop residues, sugar beet digestate and different side streams rich in chitin from the mushroom and insect industry.

Efficacy trials were performed using residue material of potato, sugar beet and carrot, collected from naturally infected plots. Net bags (size 25 × 30 cm) were filled with approximately 250 g of crop residues and placed on the soil, at the WUR research station located in Vredepeel, the Netherlands, in a randomized block design. Bags containing crop residues were treated with: *C. rosea* J1446, using 1 application or 2 applications within 1 month interval at a concentration of 500 g/ha (following manufacturer's instructions), and different side streams, namely sugar beet digestate, mushroom feet of *Agaricus bisporus* and insect frass and exuviae of *Hermetia illucens* and *Tenebrio molitor*. All side streams were applied at the maximum concentration allowed, taking into account their N content, in order to comply with the Dutch regulations for N applications into the field. At the start of the experiment (T0), ten bags were collected per crop, and one, two and six months after application (T1, T2 and T3, respectively) five bags were collected per crop and treatment.

The efficacy of the different treatments is being evaluated as follows: the amount of crop residues recovered are weighed to evaluate the decomposition rate and DNA is extracted from the same samples. DNA is analysed for the presence and quantification of the targeted pathogens, using qPCR assays that have been/ will be designed and available at WUR. Microbial community profiling will be performed to assess how the different treatments are steering the resident microbiome. Chitin-rich organic side streams have been proven to change the amended soil microbial communities (Andreo-Jimenez et al., 2021). The effect of the treatments on the population dynamics (survival, increase, decrease) of pathogens and also on the microbial community composition is determined and compared statistically. Datasets are being elaborated containing the pathogens quantification and the development of microbial

communities along time. The most promising treatments to outcompete the pathogenic populations, enhance the colonization of crop residues by naturally occurring non-pathogenic micro-organisms and speed up the decomposition will be integrated in future field experiments.

Key words: crop residues management, arable crop diseases, *Clonostachys rosea*, side streams

References

Andreo-Jimenez, B., Schilder, M. T., Nijhuis, E. H., Te Beest, D. E., Bloem, J., Visser, J. H. M., van Os, G., Brolsma, K., de Boer, W., and Postma, J. 2021. Chitin- and keratin-rich soil amendments suppress *Rhizoctonia solani* disease via changes to the soil microbial community. Appl. Environ. Microbiol. 11: 87(11): e00318-21. doi: 10.1128/AEM.00318-21.

Köhl, J., Elena, G., Hanse, B., Houwers, I., Groenenboom-de Haas, L., de Lange, E., Verstegen, H., and Evenhuis, A. 2024. Population dynamics of *Alternaria solani*, *Cercospora beticola*, *Ramularia beticola*, and *Stemphylium beticola* in residues of host crops, non-host crops, and weeds in Dutch rotation systems. Front. Agron. 6: 1470598. doi: 10.3389/fagro.2024.1470598.

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3D platforms made from biopolymers for the sustained release of microorganisms in biological control applications

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Abstract: The aim of this abstract focuses on developing extrusion-based bioprinted 3D platforms using biopolymer-based bioinks, such as gellan gum (GG), enhanced with reinforcement particles like clays to form composite hydrogels (CH). These platforms aim to encapsulate biological control agents (ACBs), such as *Trichoderma* spp. fungi, for sustained release in soil as biodefensives. Physicochemical characterizations and viability tests were carried out. The goal is to create formulations that ensure microorganism viability, reduce agrochemical use, and offer controlled release of encapsulated agents.

Key words: 3D bioprinting, composite hydrogel, biological control, biopolymers

Introduction

The indiscriminate use of pesticides in agriculture has caused serious environmental impacts, such as soil degradation, environmental contamination and risks to human health (Vandenberghe et al., 2017). As a sustainable alternative, biological control agents (ACBs) emerge as a promising solution for natural pest control (Lucon et al., 2014). In this context, the development of CH based on gellan gum and clays, to encapsulate and release ACBs in a controlled manner, represents an innovation with the potential to promote more sustainable and balanced agriculture.

Materials and methods

Hydrogels development and characterization

Different CHs (GGLP and GGHNT) were developed and fungi of the species *Trichoderma harzianum* were incorporated. The composites were processed by extrusion using a 3D printer. Cell viability tests were carried out to evaluate the survival of the fungus both in the hydrogel matrix and during the 3D printing process, in addition to complementary physicochemical characterizations.

Results and discussion

Rheology results indicated that the CH (GGLP and GGHNT) exhibited a $G' > G''$ behavior, characterizing a gel suitable for 3D printing. FT-IR and TG analyzes confirmed the incorporation of clays into the GG matrix. Cell viability assays demonstrated that the fungi remained viable in some CH samples. Additional tests will be conducted to evaluate the material behavior under exposure to temperature and UV radiation.

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References

Lucon, C. M. M., Chaves, A. L. R., Bacilieri, S. 2014. *Trichoderma*: o que é, para que serve e como usar corretamente na lavoura. Chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://biologico.agricultura.sp.gov.br/uploads/files/pdf/cartilhas/trichoderma.pdf

Vandenberghe, L. P., Garcia, L. M. B., Rodrigues, C., Camara, M.C., de Melo Pereira, G. V., de Oliveira, J., Soccol, C. R. 2017. Potential applications of plant probiotic microorganisms in agriculture and forestry. AIMS microbiology 3(3): 629.

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Selection of antagonistic yeasts for the control of strawberry postharvest rots and their effect on the fruit microbiome

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Extended abstract: Strawberries are highly perishable fruits and reducing postharvest decay is a major challenge. The main pathogen is *Botrytis cinerea*, the causal agent of grey mould, which causes significant production losses (Feliziani and Romanazzi, 2016). There is a need to develop alternative management strategies to synthetic fungicides, effective and safer for consumers. The use of yeasts as biocontrol agents (BCAs) is emerging as a promising approach for postharvest disease control. Their remarkable ability to adapt to the fruit microenvironment and environmental stresses, combined with their safety to humans, makes them ideal candidates (Spadaro and Droby, 2016). In this study we selected some antagonistic yeasts by evaluating their efficacy in controlling postharvest rots of strawberries. The effects of the treatments on fruit quality and on the fungal and bacterial microbiome were also evaluated.

A protocol was developed to isolate endophytic yeasts from healthy strawberries, which were then screened for their potential antagonistic activity along with yeasts preserved in the collection of the University of Turin. Screening trials were conducted *in vivo* using naturally infected strawberries. Treatments were performed by spraying the yeast cell suspensions onto the fruit surface. Strawberries treated with a commercial biofungicide based on *Metschnikowia fructicola* were included in the trials, while untreated strawberries served as control. Rot incidence was assessed after 7 days of storage at 1 ± 1 °C and after 2 days of shelf-life at 19 ± 1 °C. Tested yeasts demonstrated varying levels of effectiveness. Four strains (FR4A, FR9B, MS and PL5) showed the highest antagonistic activity and were selected to conduct further analyses. These strains were molecularly identified as *Metschnikowia pulcherrima* (MS) and *Aureobasidium pullulans* (PL5, FR4A, and FR9B).

An efficacy trial was performed on a larger number of fruits to confirm their effectiveness in reducing postharvest rots. Strawberries were stored at 1 ± 1 °C for 10 days, followed by a 2-day shelf-life period at 19 ± 1 °C. Rot incidence and disease severity were assessed after storage and shelf-life. Disease severity was evaluated using an empirical scale, that allowed the calculation of the McKinney index.

Treated strawberries consistently exhibited significantly lower rot incidence and severity compared to the untreated control. Average rot incidence for the selected strains after storage ranged from 16.4 % (*A. pullulans* FR4A) to 19.1 % (*A. pullulans* FR9B), significantly lower than the untreated control (32 %) and comparable to the commercial biofungicide (13.1 %). Similarly, McKinney index values after storage ranged from 4.9 % (*A. pullulans* FR4A) to 8.1 % (*A. pullulans* FR9B), compared to 3.7 % for the commercial biofungicide and 14.4 % for the untreated control. All treatments maintained a significantly lower rot incidence and severity than the untreated control after shelf-life.

The effect of the treatments on fruit quality parameters, including firmness, total soluble solids and titratable acidity, was assessed. None of the tested strains negatively impacted these parameters, either after storage or following shelf-life.

Metabarcoding was used to evaluate the impact of the treatments on the fruit microbiome. Sampling and DNA extraction were conducted after storage. The ITS2 and 16S rRNA regions were sequenced to analyse fungal and bacterial communities, respectively. Changes in response to the treatment application were observed. Analysis of the fungal microbiome showed a good colonization of the treated fruits by the applied yeasts, along with a reduction in the abundance of *Botrytis* spp. compared to the untreated control. In the bacterial microbiome, higher abundance of the genus *Achromobacter* was observed in all treated fruits. Moreover, changes in the abundance of other microbial genera were detected.

Results showed that antagonistic yeasts represent a promising tool for reducing postharvest diseases in strawberries, while preserving fruit quality.

Key words: metabarcoding, biocontrol, *Fragaria* x *ananassa*

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References

Feliziani, E., and Romanazzi, G. 2016. Postharvest decay of strawberry fruit: Etiology, epidemiology, and disease management. J. Berry Res. 6: 47-63.

Spadaro, D., and Droby, S. 2016. Development of biocontrol products for postharvest diseases of fruit: The importance of elucidating the mechanisms of action of yeast antagonists. J. Trends Food Sci. Technol. 47: 39-49.

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Enhancing the biocontrol potential of *Lysobacter capsici* AZ78 against *Pythium ultimum* in soil utilising organic amendments

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Abstract: Soil disease suppressiveness has been linked to *Lysobacter* species, known for their antagonistic activity against soilborne plant pathogens (Postma et al., 2010). Organic and protein-rich materials can also stimulate *Lysobacter* populations, enhancing soil suppressiveness (Postma and Schilder, 2015). Recently, *Lysobacter capsici* AZ78 (AZ78) showed detrimental effects on soil-borne plant pathogens, including *Pythium ultimum* under *in vitro* conditions (Vlassi et al., 2020). Building on this knowledge, this study investigated the role of organic amendments, namely chitin, feather meal, fish meal, hoof meal, and yeast, in enhancing the biocontrol efficacy of AZ78 against *P. ultimum* in tomato seedlings. Sandy loam soil was incorporated with organic amendments at 0.3 % (w/w), followed by inoculation with AZ78 (1×10^6 CFU/ml). *Pythium ultimum* inoculum was prepared by culturing the plant pathogen on saturated millet seeds for 21 days and introduced into the soil at 0.3 % (w/w) dry weight. Control treatments received non-inoculated millet. The experiment consisted of four treatment groups: (1) soil only, (2) soil + AZ78, (3) soil + *P. ultimum*, and (4) soil + *P. ultimum* + AZ78, each group tested under both amended and unamended conditions. Tomato seeds (five seeds per treatment) were sown equidistantly in the soil and incubated in a greenhouse at 25 ± 1 °C. The seedling germination rate was recorded after 14 days. Results showed that soil inoculated with *P. ultimum* alone exhibited the lowest germination rate. In contrast, treatment with AZ78 significantly increased germination rates, surpassing even the untreated control, highlighting its potential as a plant growth-promoting rhizobacterium. Organic amendments influenced the AZ78 effectiveness, with hoof, feather, and fish meal yielding the highest germination rates. In contrast, yeast amendment hindered germination. These findings suggest that specific organic amendments can enhance the AZ78 protective effects. This is the first study to demonstrate the protective capability of AZ78 against *P. ultimum* under semi-natural soil conditions. Furthermore, using organic amendments aligns with sustainable agricultural practices and has promising implications for field applications.

Key words: *Lysobacter capsici* AZ78, *Pythium ultimum*, organic amendments, soil-suppressiveness

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References

Postma, J., and Schilder, M. T. 2015. Enhancement of soil suppressiveness against *Rhizoctonia solani* in sugar beet by organic amendments. Appl. Soil Ecol. 94: 72-79.

Postma, J., Nijhuis, E. H., and Yassin, A. F. 2010. Genotypic and phenotypic variation among *Lysobacter capsici* strains isolated from *Rhizoctonia* suppressive soils. Syst. Appl. Microbiol. 33: 232-235.

Vlassi, A., Nesler, A., Perazzolli, M., Lazazzara, V., Büschl, C., Parich, A., Puopolo, G., and Schuhmacher, R. 2020. Volatile organic compounds from *Lysobacter capsici* AZ78 as potential candidates for biological control of soilborne plant pathogens. Front. Microbiol. 11: 1748.

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Application of multifunctional *Bacillus velezensis* CMC-6 for straw degradation

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Abstract: Livestock manure and agricultural straw waste disposal have become a major issue for agriculture in Inner Mongolia. In this study, the strain CMC-6 was screened by Congo red staining, aniline blue decolorization and filter strip, and it was identified as *Bacillus velezensis*. The degradation ability of strain CMC-6 was verified by laboratory degradation test, cellulase activity determination and scanning electron microscope observation. The results showed that the hydrolysis value of CMC-6 Congo red was 7.78, the diameter of aniline blue decolorization ring was 3.6 cm, and the strain had a strong ability to degrade the filter paper. After 30 days of liquid fermentation, the degradation rate of corn stalk reached 55.55 %, and the cellulase activity reached a maximum of 216.06 U/ml after 4 days of fermentation. Meanwhile, scanning electron microscopy (SEM) observation showed that corn stalk treated by the strain CMC-6 collapsed and broke. After 4 days of inoculation, the fermentation center temperature reached 47.5 °C, and the cumulative high temperature period lasted for more than 8 days. After 27 days of inoculation, the water content of corn stalks inoculated with the strains decreased by 20.35 %, the organic matter content decreased by 27.10 %, the C/N decreased to 17.69, and the total nitrogen increased by 27.17 %. *Bacillus velezensis* CMC-6 demonstrated strong capabilities in straw degradation, shortening the compost fermentation cycle and improving compost quality. These findings provide a foundation for the application and development of strain resources for efficient straw degradation and composting.

Key words: straw degradation, *Bacillus*

Uncovering the potential of *Bacillus* strains in promoting wheat growth under biotic and abiotic stresses

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Abstract: Drought stress and *Fusarium* diseases are yield limiting factor in wheat production. Fungal diseases are mainly managed with chemical pesticides; however, their use poses risks of environmental contamination and the emergence of resistant strains. Recently, the use of drought-tolerant plant growth promoting bacteria with biocontrol potential has been promoted as a promising and ecofriendly practice. In this sense, the *Bacillus* genus is of particular interest due to its stress tolerance, production of antifungal metabolites, and plant growth promoting traits. This research evaluated the potential of three *Bacillus* strains to promote wheat growth under combined drought and *Fusarium* infection stresses. Three *Bacillus* strains (TII-19, TCG-6, and TMG-6), isolated from Cuban wheat varieties, were characterized *in vitro* for their drought tolerance by growth under different concentration of PEG-6000 and biofilm production under drought stress. Moreover, the biocontrol potential of theses strains was assessed by evaluating their effect on reducing *Fusarium* symptoms on wheat spikelets and the effect of their metabolites on the germination of *Fusarium graminearum* and *Fusarium culmorum*. The potential of *Bacillus* strains and a bacterial consortium formed by two of the strains was assessed for wheat growth promotion under stress conditions in pot experiments. The bioprotection activity of the bacterial strains against *F. graminearum* was analyzed using a detached leaf bioassay in bacterized plants. Results showed the bacteria's ability to grow in Tryptone Soy Broth amended with 30 % (w/v) PEG-6000, exhibiting higher biofilm production under these conditions. *Bacillus* strains reduced the severity of *F. graminearum* and *F. culmorum* on wheat spikelets. In vitro test, bacterial metabolites delayed the germination of both fungal strains. In planta, bacterized plants showed the highest average plant size, under both irrigated and drought stress conditions, along with increased leaf and root production. Under drought stress, plants treated with TMG-6 and the bacterial consortium had a size similar to control plants under irrigated conditions. Detached leaf bioassay confirmed that *Bacillus* strains reduced *F. graminearum* lesions on wheat leaves. These findings highlight the positive effect of bacterial strains in promoting wheat growth and providing bioprotection under stress conditions.

Key words: *Bacillus*, drought stress, *Fusarium*, combined stress, wheat

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Potential of probiotics in the control of coffee leaf rust

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Abstract: Considering the benefits that probiotics confer on the health of various hosts, as well as the availability of commercial products in the global market, this study aimed to evaluate the potential of probiotics in controlling coffee leaf rust caused by *Hemileia vastatrix*. We evaluate the potential of Colostrum™ BS Liquid, Colostrum™ BS Powder, Colostrum™ BIO 21 Liquid, and Colostrum™ BIO 21 MIX Powder (designed for animal use), along with Enterogermina™ (designed for human use), in inhibiting the germination of *H. vastatrix* uredionospores, and in controlling coffee leaf rust in coffee seedlings. The probiotics Colostrum™ BIO 21 Liquid, Colostrum™ BIO 21 MIX Powder, Colostrum™ BS Powder, and Enterogermina™ inhibited the urediniospores germination in 100 %, 31.1 %, 23.0 %, and 14 %, respectively. Colostrum™ BS Powder reduced the severity of coffee leaf rust by 77.6 % and 98.5 %, while Colostrum™ BIO 21 MIX Powder reduced the severity by 77.6 % and 78.8 % and 87.5 % in two different experiments.

Key words: probiotics, *Hemileia vastatrix*

Introduction

Coffee leaf rust, caused by *Hemileia vastatrix,* significantly impacts coffee yields worldwide, particularly in Brazil. While coffee cultivars resistant to coffee leaf rust are available to Brazilian growers, most of the high-yielding cultivars currently planted remain susceptible to this disease. Consequently, chemical fungicides have been extensively used for this disease management (Meira et al., 2009; Zambolim, 2016; Sera et al., 2022). However, growing consumer demand for sustainable practices in coffee production had driven interest in alternative approaches, such as biological control (Bettiol et al., 2021).

Probiotics, well-documented for their benefits to human and animal health, are increasingly being explored for their potential to enhance plant health and protect against diseases (Harutyunyan et al., 2022). Several species of bacteria commonly used in probiotic formulations, such as *Bacillus, Lactobacillus*, and *Bifidobacteria*, have shown potential to control plant diseases (Daranas et al., 2019; Raman et al., 2022). By applying probiotics to plants or soil, it is possible to manipulate the microbiome and its functions in a targeted manner.

Considering the benefits that probiotics confer on the health of various hosts, as well as the availability of commercial products on the global market, this study aimed to evaluate the potential of commercial probiotics in controlling coffee leaf rust.

Materials and methods

The ability of probiotics to inhibit *H. vastatrix* urediniospores germination was evaluated *in vitro*. Microplates containing 24 wells were filled with water agar medium. The probiotics Colostrum™ BS Liquid, Colostrum™ BS Powder, Colostrum™ BIO 21 Liquid, and Colostrum™ BIO 21 MIX Powder (commercialized for animal use), and Enterogermina™ (liquid formulation) were prepared at concentrations of 1×10^7 Colony Forming Units (CFU)/ml for liquid products and 1×10^6 CFU/g for powder products. Each well received 150 µl of the respective probiotic concentration. Urediniospores were applied to wells using a brush. The microplates were incubated in the dark at 22 °C for 6 hours. A total of 100 urediniospores per well were examined, and the number of germinated and non-germinated urediniospores was recorded. The percentage of germination was calculated, and the means were compared by Tukey's test at 5 % probability. The experiment was conducted in a completely randomized design twice.

Two experiments were conducted to assess the efficacy of probiotics in controlling coffee leaf rust on coffee seedlings cv. Topazio containing four or five pairs of leaves. Seedlings were treated with Colostrum™ BS Liquid, Colostrum™ BS Powder, Colostrum™ BIO 21 Liquid, Colostrum™ BIO 21 MIX Powder, and Enterogermina™ at a concentration of 1×10^7 CFU/ml until runoff. Each seedling was sprayed with 16 mL of the probiotic solutions seven days and twenty-four hours before and seven days after pathogen inoculation. A suspension containing 1×10^5 urediniospores/mL of *H. vastatrix* was sprayed onto the abaxial surface of the three youngest leaf pairs of each coffee seedling. After inoculation, seedlings were placed in a humid chamber (100 % relative humidity in the dark) for 72 hours before being transferred to a nursery with automated micro-sprinkler irrigation system. Weekly evaluations of coffee leaf rust were performed in each experiment. Infected leaves were photographed, and the leaf area affected by the disease quantified using the Pliman package in R platform. Disease severity data were transformed using arcsine root (×/100) and subjected to Shapiro-Wilk test to assess normality of errors. Means were compared using the Tukey test ($p < 0.05$) in R platform.

Results and discussion

The probiotic Colostrum™ BIO 21 Liquid completely inhibited the germination of the *H. vastatrix* urediniospores. Colostrum™ BIO 21 MIX Powder and Colostrum™ BS Powder inhibited the urediniospores germination in 31.1 % and 23.0 %, respectively, while Enterogermina™ achieved a 14.0 % inhibition. Colostrum™ BS Liquid had not inhibitory effect on urediniospore germination.

Colostrum™ BIO 21 MIX Powder and Colostrum™ BS Powder were the most efficient probiotics in reducing coffee leaf rust severity. In the first experiment, at 73 days after inoculation, the leaf area affected by coffee leaf rust was 14.0 % in the control, compared to 0.2 % in Colostrum™ BS Powder, and 0.7 % in Colostrum™ BIO 21 Powder, representing a reduction of 98.5 % and 87.8 %, respectively (Table 1). In the second experiment, at 73 days after inoculation, the severity in the control was 11.0 %, while 2.5 % in Colostrum™ BS Powder, and 2.3 % in Colostrum™ BIO 21 Powder, corresponding to reduction of 77.6 % and 78.8 %, respectively (Table 1).

Originally developed for poultry production, the probiotics Colostrum™ BS Powder and Colostrum™ BIO 21 MIX Powder reduced the severity of coffee leaf rust. Preventive application of probiotics, such as Colostrum™ BS Powder and Colostrum™ BIO 21 MIX Powder, emerges as a promising and sustainable strategy for the management of coffee leaf rust.

Table 1. Effect of probiotics on the severity (%) of coffee leaf rust, caused by *Hemileia vastatrix*, evaluated by the leaf area affected by the disease 73 days after pathogen inoculation, with coffee seedlings cultivar Topazio.

Treatment	1st experiment	2nd experiment
Control	14.0a	11.0a
Colostrum™ BS Liquid	6.3a (54.6)	9.5a (13.9)
Colostrum™ BS Powder	0.2c (98.5)	2.5b (77.6)
Colostrum® BIO 21 Liquid	7.2ab (51.3)	8.1ab (26.0)
Colostrum™ BIO 21 MIX Powder	0.7bc (87.8)	2.3b (78.8)
Enterogermina™	14.6a	4.8ab (56.5)
CV (%)	38.9	25.8

Severity estimated by the leaf area affected by coffee leaf rust. Means followed by the same letter in the columns do not differ by the Tukey test ($p < 0.05$).

Based on the results presented in this study, coupled with the observations of Daranas et al. (2019), Harutyunyan et al. (2022) and Raman et al. (2022), the utilization of commercial probiotics or even isolated microorganisms derived from these probiotics could serve as a feasible alternative for managing plant diseases. This approach presents a promising strategy for sustainable agriculture, as suggested by Faria et al. (2020).

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References

Bettiol, W., Medeiros, F. H. V., Chiaramonte, J. B., and Mendes, R. 2021. Advances in screening approaches for the development of microbial bioprotectants to control plant diseases. In: Köhl, J., and Ravensberg, W. J. (eds.): Microbial bioprotectants for plant disease management, pp. 33-86. Burleigh Dodds, Cambridge.

Daranas, N., Roselló, G., Cabrefiga, J., Donati, I., Francés, J., Badosa, E., and Bonaterra, A. 2019. Biological control of bacterial plant diseases with *Lactobacillus plantarum* strains selected for their broad-spectrum activity. Ann. Appl. Biol. 174: 92-105.

Faria, M. R., Costa, L. S. A. S., Chiaramonte, J. B., Bettiol, W., and Mendes, R. 2020. The rhizosphere microbiome: functions, dynamics, and role in plant protection. Trop. Plant Pathol. 46: 13-25.

Harutyunyan, N., Kushugulova, A., Hovhannisyan, N., and Pepoyan, A. 2022. One health probiotics as biocontrol agents: One health tomato probiotics. Plants 11: 1334.

Meira, C. A. A., Rodrigues, L. H. A., and Moraes, S. A. D. 2009. Modelos de alerta para o controle da ferrugem-do-cafeeiro em lavouras com alta carga pendente. Pesq. Agropecu. Bras. 44: 233-242.
Raman, J., Kim, J. S., Choi, K. R., Eun, H., Yang, D., Ko, Y. J., and Kim, S. J. 2022. Application of lactic acid bacteria (LAB) in sustainable agriculture: Advantages and limitations. Inter. J. Molec. Sci. 23(14): 7784.
Sera, G. H., Carvalho, C. H. S., Abrahão, J. C. R., Pozza, E. A., Matiello, J. B., Almeida, S. R., Bartelega, L., and Botelho, D. M. S. 2022. Coffee leaf rust in Brazil: Historical events, current situation, and control measures. Agronomy 12: 496.
Zambolim, L. 2016. Current status and management of coffee leaf rust in Brazil. Trop. Plant Pathol. 41: 1-8.

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Exploring soil microbiomes for early detection of soilborne pathogens in tomato cultivation

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Abstract: Microbiome-based plant disease diagnostic tools hold great promise for sustainable agriculture by enabling early pathogen detection and improving disease management strategies. In this study, we used an amplicon-based metagenomics method to detect tomato soilborne pathogens. We collected samples in three different locations: a tomato field, the field margin, and an adjacent uncultivated area. Following DNA isolation, the fungal communities were characterized using amplicon sequencing. We found differences in fungal community structure across the three sampling sites. Among the most abundant taxa (relative abundance > 1 %), we identified multiple amplicon sequence variants (ASVs) belonging to the genus *Fusarium*, a well-known tomato pathogen. We also detected *Alternaria alternata*, another major fungal pathogen affecting tomato crops. These findings underscore the potential of utilizing amplicon metagenomics for comprehensive pathogen detection and monitoring in agricultural soils, providing valuable insights for disease management strategies in tomato cultivation.

Key words: metagenomics, amplicon sequencing, *Fusarium* spp., *Alternaria alternata*, soil, plant-disease biomarkers

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Use of wild garlic volatile organic compounds to suppress the growth of soil-borne plant pathogenic fungi

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Abstract: Soil-borne diseases are posing a significant threat to global food security. Among soil-borne pathogens, plant pathogenic fungi are particularly aggressive and their control often relies on the use of fungicides whose application has raised environmental and public health concerns. As a result, there is a strong demand for sustainable alternatives for disease management. Antifungal volatile organic compounds (VOCs) offer a promising biocontrol approach, as their ability to diffuse through the water- and air-filled pores of the soil, allow for long-distance pathogen suppression. Wild garlic (*Allium ursinum*) is a widely distributed plant known for its strong antimicrobial properties, a result of the distinctive sulfur containing VOCs emitted from its bulbs. In this study we examined the effects of the wild garlic-derived VOCs on the growth of the soil-borne pathogens, *Pythium violae* and *Rhizoctonia solani*. Our results demonstrate a strong inhibitory effect on both pathogens with the volatile analysis revealing a complex blend, rich in sulfur compounds. Future trials will focus on evaluating the wild garlic's potential to suppress disease emergence in susceptible plants and its impact on the soil microbial community.

Key words: *Allium ursinum*, volatile organic compounds, soil-borne phytopathogenic fungi, biocontrol

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Erwinia amylovora specific bacteriophages distribution during the fire blight epidemic in central Serbia

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Abstract: Since first detection of *Erwinia amylovora* on apple and pear in Serbia, in 1989, the fire blight (FB) disease occurred periodically depending mostly on weather conditions. Rainy weather during blooming period induced severe symptom development, resulting in reduced yield, plant damage and eradication. During the spring of 2024, fruit growers reported frequent occurrence of apple, pear and quince FB in several localities of Central Serbia. We assume that unusual high average monthly temperatures for February, March and April significantly contributed to a high disease incidence. Typical symptoms included blackening along the leaf midrib and veins or full necrosis of leaves as if scorched by fire, shepherd's crook and occasional occurrence of bacterial exudate on infected tissue. The etiology of the symptoms was confirmed by isolation of bacteria from the diseased tissue. The isolates showed growth and biochemical characteristics typical for *E. amylovora*. Pathogenicity of the isolates was confirmed by prick-inoculation of young pear fruits. After 48 h at high humidity, the tissue necrosis developed around the inoculation site followed by oozing of bacterial exudate one day later. The identity of the isolates with *E. amylovora* was confirmed by real-time PCR analysis using Ams116F/Ams189R primers and Ams141T hydrolysis probe and by conventional PCR.

Due to lack of efficient bactericides, control of fire blight is one of major challenges in pome fruit production. Being widespread natural bacterial enemies, simple for cultivation, host-specific, suitable for integration with other control practices, human and environment friendly, bacteriophages became a promising alternative for FB control.

Samples for bacteriophage isolation were collected from different localities in Serbia, primarily from the regions where FB presence was observed, during 2024 and 2025. Isolation was attempted from leaves of host plants with or without FB symptoms (apple, pear, quince and medlar), rhizosphere soil, and from irrigation water by standard procedure for phage isolation from environmental samples. Presence of phages in samples was detected by "spot test", pipetting 10 µl of suspensions onto NYA medium surface, previously inoculated with *E. amylovora* strains. Following purification, preliminary screening for phage specificity was performed to 35 *E. amylovora* strains originating from Serbia.

Bacteriophages specific to *E. amylovora* were isolated from 8 out of 154 samples. The low percentage of successful phage isolation may be due to high temperature and UV index, and long dry periods during the season. Six samples originated from plant material with characteristic symptoms of FB, while two positive samples originated from rhizosphere soil. All phages were specific to all *E. amylovora* strains but some differences in their virulence were revealed. Our results showed that bacteriophages specific to *E. amylovora* were isolated more frequently from diseased plant material than from rhizosphere soil. Since they lysed *E. amylovora* strains originating from different hosts and localities, they represent good candidates for future use in biological control of FB.

Key words: pome fruits, fire blight, biocontrol, phage therapy, host range

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Plant extract (PE2) for optimum efficacy of LALSTOP G46 WG, for the control of *B. cinerea* and *Erysiphe necator* on grapes

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Abstract: Until recently, microbial-based biofungicides have offered uneven results in the control of *Botrytis cinerea and Erysiphe necator* on grapes and the reputation of biofungicides has suffered as a result. Critical parameters could impact the variation in efficacy of LALSTOP G46 WG, based on the fungus *Clonostachys rosea* strain J1446 such as UV-B radiation, very high T °C and lack of water. The aim of this study was to find a determinant parameter to regularize and enhance LALSTOP G46 WG efficacy in open field conditions. Following several trials conducted in different grapes aeras in Europe we were able to conclude that a specific plant extract (PE2) in addition to LALSTOP G46 WG improves the effectiveness of the product against those diseases.

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Screening and functional characterization of *Bacillus subtilis* YJBS-26: a salt-alkali tolerant and plant growth-promoting strain

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Abstract: A salt-alkali-tolerant strain of *Bacillus subtilis* YJBS-26 was isolated from saline-alkali soil. The strain demonstrated significant tolerance to both salt and alkali, being capable of growth in LB medium with a salt concentration of 16 % (w/v) and at a pH of 10.0. Under salt-alkali stress, YJBS-26 exhibited notable plant growth-promoting (PGP) characteristics compared to the control conditions, including the production of 11.45 µg/ml of indole-3-acetic acid (IAA). Inoculation of oat seeds or seedlings with YJBS-26 fermentation broth significantly improved plant growth parameters, including plant height, as well as enhanced root length and fresh weight. Additionally, inoculation with YJBS-26 led to a marked improvement in oat yield, with a 41.22 % increase in grain yield, a 35.32 % rise in thousand-kernel weight. Strain YJBS-26 exhibited strong tolerance to both salt and alkali with the capability to grow in LB medium containing a 16 % NaCl mass fraction and at a pH of 10.0, and it demonstrated significant growth-promoting effects under saline-alkali stress conditions. Treatment of oat seeds or seedlings with its fermentation broth led to increased plant height, root length, fresh weight, and enhanced yield compared to the control group.

Key words: *Bacillus subtilis*, saline-alkali tolerance, growth promotion, oat

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Trichoderma-based bioformulation for biocontrol and biostimulation of forage crops

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Abstract: Traditional agricultural crop protection methods primarily rely on synthetic chemicals. However, there is an urgent need to reduce or eliminate chemical applications in agriculture. Biostimulants and biological control agents (BCAs) represent effective alternatives for sustainable farming. This study aimed to evaluate the effects of *Trichoderma*-based bioproducts on sorghum (*Sorghum bicolor* L. Moench) using a metabolomic approach to assess their impact on plant metabolism and mycotoxin contamination.

The experiment was conducted in a field trial of 1 ha located in Pannarano (BN, Italy). Sorghum seeds were coated with 10^7 spores/ml suspension of *Trichoderma harzianum* strain M10, *T. afroharzianum* strain T22, or a combination of both. The formulations included also a natural adhesive derived from carob (*Ceratonia siliqua*), primarily composed of polygalactomannans. Untreated seeds served as the control group. At harvest, representative grain and plant samples were collected and analyzed for mycotoxin contamination using LC-MS.

Only slight yield differences were observed at harvest among treated plants and control. The plants exhibited minimal fungal pathogen attacks, prompting an evaluation of mycotoxin contamination. Analysis of plant and grain samples revealed significant lower contents of mycotoxins in sorghum treated with M10 and T22 compared to controls. The combination of T22 and M10 reduced the concentration of all analyzed mycotoxins, except for fumonisin B1 (45.04 ppb). Additionally, chemical composition of sorghum grain samples was also analyzed. Crude protein content was significantly higher in untreated control and T22-treated samples (8.49 % and 8.47 % DM, respectively), whereas these treatments exhibited the lowest starch content (57.03 % and 59.43 % DM, respectively).

The results suggest that the application of formulations based on BCAs and natural adhesive substances constitutes a promising strategy for forage treatment and the mitigation of mycotoxin contamination in forage crops and derived products.

Key words: sorghum, forage, mycotoxins, *Trichoderma harzianum, Trichoderma afroharzianum*, carob, metabolomics

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Session III
Development of single strains, synthetic consortia, and microbiomes

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Designing a healthy plant microbiome by customized single strains, synthetic consortia, and microbiome transplants

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Abstract: Intensive agriculture is an important factor contributing to changes in the Anthropocene. The use of pesticides, synthetic fertilizers, and monocultures of high-yield crops have not only led to the crossing of planetary boundaries but have also changed the plant microbiome. This man-made epoch is characterized by decreased biodiversity, evenness, and host specificity and increased abundance of copiotrophic microbes, multi-resistant pathogens, and hypermutators. The plant microbiome is connected to both the soil and the human gut, influencing health outcomes throughout the food chain. Therefore, it is crucial to understand which criteria are important for a healthy microbiome and how we can manage plant microbiomes to improve host health.

Key words: biocontrol, biologicals, plant diseases, microbiome management

Introduction

Human activities substantially affect the environment; especially agriculture causes long-lasting anthropogenic environmental impacts, characterizing the human-dominated Anthropocene epoch. The Haber-Bosch process, utilized for converting atmospheric nitrogen to ammonia for fertilizer, has altered the global nitrogen cycle fundamentally. The overuse of agrochemicals leads to the development of resistant plant and human pathogens, which are difficult to control. In fact, we have already crossed planetary boundaries for biogeochemical flows, land system change, and the integrity of the biosphere (Gupta et al., 2024). In addition, climate change is contributing to increasingly frequent and severe incidents of flooding and drought and the introduction of novel entities to the environment. This is also reflected in the plant microbiome; here, human activities are commonly linked to shifts in diversity and evenness of the plant microbiota, which is also characterized by a decrease of host specificity and an increase of r-strategic microbes, pathogens, and hypermutators (Berg and Cernava, 2022). This trend is particularly evident in the increasing occurrence of plant diseases and resistant pathogens due to microbial diversity loss, highlighting the urgent need for environmentally friendly solutions in plant production (Berg et al., 2017). In general, microbiomes can be managed either directly by applying (i) single strains with beneficial properties, (ii) customized consortia, (iii) microbiome transplants, or indirectly (iv) by changing abiotic environmental conditions in a way that microbiomes also shift their structure and function from dysbiosis to a healthy state.

The first question that needs to be answered relates to the definition of a healthy plant microbiome. This fundamental question is not yet fully answered (Sessitsch et al., 2024); however, a healthy plant microbiome should be functionally diverse, rich, and evenly structured with members known for beneficial as well as for pathogenic interaction (pathobionts) and keystone species (Banerjee et al., 2018). The balanced ratio of copiotrophic and oligotrophic strains is of importance as well. Another detail that should be considered is the host-specificity of the plant microbiome. More than 475 million years of co-evolution resulted in a diversified plant kingdom accompanied by specific microbiota. Since not all members of the microbiome have the same impact on their host and other microbes within the same community, specific attention should be given to pathobionts and soterobionts. While the former group is well-known and has been studied for centuries, the latter was recently defined as those microorganisms that cause disease-suppressive effects within the plant holobiont (Cernava and Berg, 2022). The members of the plant microbiome fulfill important functions, e. g., nutrient uptake, mineral, co-factor, vitamin, and hormone supply, as well as protection against biotic and abiotic stress. Therefore, intrinsic plant microbiota are an important reservoir for selecting inocula. Over time, many different plant sources have been evaluated, yielding successful approaches from the following categories: i) wild relatives of crops, ii) healthy individuals within a diseased population, iii) indigenous plants from the same environment, iv) endemic plants, v) medicinal plants, vi) "ancient" plants like mosses, vii) suppressive soils, and viii) plants from extreme environments. However, the isolation methods should be as well adapted to the desired microbes; enrichment cultures, low-nutrient agar containing plant extracts, and "bait" plants were used to catch beneficial biologicals.

The second objective pertains to the selection of plant-beneficial strains. The plant microbiome contains more than 10^{10} microbes. Although not all these microbes are cultivable, there are plenty to choose from. Again, the selection method is crucial: *in vitro*-selected strains have a high risk of failure. Microbial synthetic communities, in short Syncoms, have emerged as a promising tool to investigate microbial-mediated functions more realistically to natural conditions and to harness microbial capability to promote multiple and diverse ecosystem services (Delgado-Baquerizo et al., 2025). Still, the application of single strains, consortia, and microbiome transplants each have their advantages and disadvantages, which are summarized in Table 1. For commercial applications, strategies for single strains and small consortia are well-defined, therefore, the majority of products available on the market are based on single strains, mainly *Bacillus* spp., which are easy to produce and formulate. Microbiome transplants show a high efficiency, but their way into the market is not clearly defined, especially if a specific purpose is targeted.

Microbiome transplants are mostly available as fertilizers, e. g., as compost, vermicompost, or manure. Single strains, consortia, and microbiome transplants (biologicals) based on beneficial microbes can complement/replace chemical fertilizers and pesticides. In fact, the exceptional ability of microbes to enhance stress tolerance supports plant growth under drought conditions and helps to save water. Currently, most commercialized bacteria are spore-formers, representing only a minor proportion (less than 10 %) of the beneficial microbiome. Despite the enormous potential of microorganisms, their use at a commercial scale is currently strongly limited due to the lack of appropriate technologies to provide reliable microbial products (Fadiji et al., 2024). Moreover, the efficacy of microbe-based tools remains inconsistent under field conditions and appears to be influenced by time, climate, soil type, and other environmental factors. Another often overlooked factor is the compatibility of the individual inocula with the respective plant species and genotype. The plant microbiome is tightly linked to host genetics and controlled by so-called *Microbiome* genes, or *M* genes (Su et al., 2024).

Table 1. Overview of different microbiome management options.

Procedure for development of biologicals	**Single strains**	**Consortia (SynComs)**	**Microbiome transplants**
Selection	easy	Needs an additional strategy (functional, synergistic, etc.) and validation experiments	easy
Characterization	easy	Depending on the number of consortium members, many possibilities	Difficult, natural variation expected
Production	easy	Difficult but possible	Very difficult, "harvest from nature" is the main strategy
Formulation	Gram+ bacteria: spores Gram- bacteria: freeze drying, BFC tech[1]	Similar to single strains or BFC tech[1]	difficult
Product development	Standardization and quality control possible	Standardization and quality control possible	variable quality
Registration	According to applicable legislation, product category and region	According to applicable legislation, product category and region based on each included strain	Not regulated

[1]BFC technology (Berg and Müller, 2022)

Increasing the compatibility of plants and biologicals via M gene breeding is a promising avenue to improve the efficacy of microbe-based solutions in plant production. Overall, mechanistic knowledge and scientific guidance are urgently needed to develop customized solutions and improve their efficiency and efficacy under field conditions. Moreover, it is necessary to identify the fundamental ecological and evolutionary principles that govern the successful establishment and subsequent trait manifestation in new environments (Delgado-Baquerizo et al., 2025). The integration of ecological theories, advanced formulation technologies, and real-time field diagnostics will be critical to bringing next-generation biologicals into mainstream agriculture.

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References

Banerjee, S., Schlaeppi, K., van der Heijden, M. G. A. 2018. Keystone taxa as drivers of microbiome structure and functioning. Nature Reviews Microbiology 16: 567-576.

Berg, G., and Cernava, T. 2022. The plant microbiota signature of the Anthropocene as a challenge for microbiome research. Microbiome 10(1): 54.

Berg, G., Müller, H. 2022. Polymeric particles containing microorganisms. Patent No. UA 126082.

Berg, G., Köberl, M., Rybakova, D., Müller, H., Grosch, R., Smalla, K. 2017. Plant microbial diversity is suggested as the key to future biocontrol and health trends. FEMS Microbiol Ecol. 93(5).

Berg, G., Kusstatscher, P., Abdelfattah, A., Cernava, T., Smalla, K. 2021. Microbiome Modulation-Toward a Better Understanding of Plant Microbiome Response to Microbial Inoculants. Front Microbiol. 12: 650610.

Cernava, T. 2024. Coming of age for *Microbiome* gene breeding in plants. Nature Communications 15: 6623.

Cernava, T., Berg, G. 2022. The emergence of disease-preventing bacteria within the plant microbiota. Environ. Microbiol. 24(8): 3259-3263.

Delgado-Baquerizo, M., Singh, B. K., Liu, Y. R., Sáez-Sandino, T., Coleine, C., Muñoz-Rojas, M., Bastida, F., Trivedi, P. 2025. Integrating ecological and evolutionary frameworks for SynCom success. New Phytol. Epub ahead of print. PMID: 40177999.

Fadiji, A. E., Xiong, C., Egidi, E., Singh, B. K. 2024. Formulation challenges associated with microbial biofertilizers in sustainable agriculture and paths forward. Journal of Sustainable Agriculture and Environment 3: e70006.

Gupta, J., Bai, X., Liverman, D. M., Rockström, J., et al. 2024. A just world on a safe planet: a Lancet Planetary Health-Earth Commission report on Earth-system boundaries, translations, and transformations. Lancet Planet Health. 8(10): e813-e873.

Sessitsch, A., Wakelin, S., Schloter, M., Maguin, E., Cernava, T., Champomier-Verges, M. C., Charles, T. C., Cotter, P. D., Ferrocino, I., Kriaa, A., Lebre, P., Cowan, D., Lange, L., Kiran, S., Markiewicz, L., Meisner, A., Olivares, M., Sarand, I., Schelkle, B., Selvin, J., Smidt, H., van Overbeek, L., Berg, G., Cocolin, L., Sanz, Y., Fernandes, W. L. Jr., Liu, S, J,, Ryan, M., Singh, B., Kostic, T. 2023. Microbiome Interconnectedness throughout Environments with Major Consequences for Healthy People and a Healthy Planet. Microbiol Mol Biol Rev. 26: 87(3): e0021222.

Su, P., Kang, H., Peng, Q., Wicaksono, W. A., Berg, G., Liu, Z., Ma, J., Zhang, D., Cernava, T., Liu, Y. 2024. Microbiome homeostasis on rice leaves is regulated by a precursor molecule of lignin biosynthesis. Nature Communications 15: 23.

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From microbiome to SynComm: developing biocontrol agents for the strawberry phyllosphere

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Abstract: Strawberries are found to contain the highest chemical pesticide residues of all tested fruit. As soft fruit, strawberries are particularly susceptible to infections by pathogens such as *Botrytis cinerea*. Biocontrol agents using single strains of bacteria have been introduced to the strawberry market but are not widely used. This is likely due to cost, lack of knowledge about microbial-based products and limited usability when compared to conventional chemical pesticides (Pressecq et al., 2024). Use of multiple microorganisms as part of a synthetic community (SynComm) is a potential tool to create more consistent and effective biocontrol products. We aim to use bacteria naturally found in the phyllosphere (the aboveground portion of the plant) to create SynComms for future applications as biocontrol products.

To achieve this goal, a sampling campaign was undertaken to collect samples from 27 strawberry farms in Belgium. Leaves, fruits and flowers were collected from strawberry plants and the bacteria from the surface of the plant matter was washed off and the bacterial community was identified using 16S rRNA sequencing. Over 650 samples were sequenced to provide the most comprehensive view of the strawberry phyllosphere microbiome to date. Farms using different cultivation techniques (organic vs. conventional) and levels of agricultural intensity (open-field, tunnels and greenhouses) were sampled and compared to assess the impact cultivation techniques have on the microbiome. We have identified overlapping genera shared between most samples regardless of cultivation strategy indicating a core community that can be used as the scaffolding for a SynComm. The core community (> 80 % occupancy) was primarily made up of members of Alphaproteobacteria, common phyllosphere inhabitants, with members belonging to genera such as *Methylobacterium*, *Pseudomonas*, *Sphingomonas* and *Pantoea*.

We have employed topic models using Latent Dirichlet allocation (LDA) to find co-occurring taxa that served as a guide for designing SynComms (Tackmann, Matias Rodrigues and von Mering, 2019). This analysis has provided insight into co-occurring taxa but needs to be complemented with laboratory experiments. Co-inoculation and competition assays are currently ongoing to provide more information about the interbacterial interactions. Using co-occurrence networks and the core community, we have designed a scaffold SynComm that recapitulates community structures found in the phyllosphere.

Microbiome analysis identified that greenhouse grown plants have a less diverse microbiome compared to those grown in open-field environments. This finding corroborates previous research from our group which has observed underdeveloped phyllosphere microbiomes of tomatoes and strawberries grown in greenhouses (Legein et al., 2022; Temmermans et al., 2025). Often, the development of microbiomes of greenhouse-grown plants are the result of stochastic processes due to a lack of diversity and opportunity for inoculation

with natural bacteria that would typically be present, leaving the phyllosphere open to colonization by opportunistic adverse microbes. We will take advantage of this gap through the development and application of SynComms containing strawberry-adapted bacteria that should form stable communities when applied to the phyllosphere. The communities from open-grown organic farms show the most species richness and diversity providing a rich selection of bacteria to incorporate into SynComms.

Further work using laboratory-based experiments has refined our SynComm design to incorporate niche occupancy of individual isolates, antipathogenic abilities and co-occurring taxa.

Key words: strawberry, phyllosphere, synthetic community, greenhouse, open field

References

DeAngelis, K. M. (ed.) 2022. Associations with Introduced Bumblebees and Predatory Mites. Microbiol. Spectr. 10: e01755-22.

Pressecq, T., Nicot, P. C., Bourgeay, J. F. et al. 2024. Using microbial biocontrol for disease control in French vegetable production: An analysis of the perspectives of farmers and farm advisors. Crop Protection 180: 106648.

Tackmann, J., Matias Rodrigues, J. F., von Mering, C. 2019. Rapid Inference of Direct Interactions in Large-Scale Ecological Networks from Heterogeneous Microbial Sequencing Data. Cell Systems 9: 286-296.e8.

Temmermans, J., Legein, M., Checcia, I. et al. 2025. Agricultural practices and pollinators modulate the anthosphere microbiome. ISME Communications [Accepted].

Identification of new biocontrol agents based on microbiome analysis of the peach fruit epidermis

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Abstract: Peach fruit undergo rapid soft ripening after harvest and are susceptible to fungal diseases, which often result in severe losses during storage. Microorganisms on the peach epidermis are complicated, dynamically changed, and highly related to fruit postharvest preservation. In addition, the trichome on the peach epidermis forms a specified structure that is different from many other fruit. In the present study, we investigated the microbial community and diversity of the peach epidermis and the correlations among postharvest disease, the structure of the peach epidermis and the microbial communities. The fungal and bacterial diversity on the peach epidermis showed a decreasing trend during storage. Beta diversity analysis revealed that the microbial communities of the peach epidermis and trichomes show different change trends during storage. Trichome removal decreased the relative abundance of *Monilinia* spp., increased the relative abundance of potential yeast and bacterial biocontrol agents, and reduced postharvest decay. This study suggested that trichomes might modulate the microbial communities on fruit surfaces, and trichome removal technology after harvest might be developed to control peach postharvest decay.

Key words: *Prunus persica*, *Monilinia fructicola*, microbial community, trichome

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Biocontrol of grapevine downy mildew: A Synthetic Microbial Community (SynCom) approach

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Abstract: Culturomic approaches, involving the use of a range of different culture conditions coupled with a high-throughput method of identification, are recommended to isolate and identify microorganisms in environmental samples. In the case of plant samples, a number of studies have shown that microorganism cultivability can be increased by using plant-based culture media rather than conventional media that contain chemically synthesized or animal-based extracts. Being able to access this microbial biodiversity is crucial to the development of microbial biocontrol for plant diseases. Particularly in vineyards, where the majority of fungicide treatments target two major pathogens, the oomycete *Plasmopara viticola* that causes downy mildew and the fungus *Erysiphe necator* that causes powdery mildew, the development of new microbial biocontrol products is expected to reduce fungicide use. Tapping into the existing reservoir of naturally occurring disease biocontrol agents and growth-promoting microorganisms associated with grapevines is the first step to formulate effective biocontrol products and move towards more sustainable viticulture.

This study aimed to use a culturomic approach to build a collection of grapevine foliar microorganisms which can subsequently be used to assemble Synthetic Microbial Communities (SynComs) for biocontrol of downy mildew. Alongside three conventional culture media, we developed a grapevine leaf-based culture medium to cultivate epiphytic and endophytic bacteria, yeast and filamentous fungi. Overall, we isolated 1090 microorganisms, including 543 bacteria, 243 yeast/yeast-like fungi and 304 filamentous fungi. Using MALDI-TOF MS and Sanger sequencing, we were able to identify 88 % of the isolates with 91 total genera identified across the 4 culture media used. Using the grapevine leaf-based culture medium enabled the cultivation of 4 genera of bacteria, 1 yeast genus and 12 genera of filamentous fungi that were not seen on the other media. We proceeded to build a SynCom of 42 isolates, selecting candidates from the collection that were either known to be representative of the natural grapevine leaf microbiota or to have biocontrol activity against downy mildew or other plant diseases. *In vitro* confrontation tests on leaf discs revealed that the SynCom significantly reduced downy mildew symptoms. Over a hundred subsets of the whole SynCom were then tested against *Plasmopara viticola* to test hypotheses on the effect of community diversity and composition on leaf colonization as well as resistance to invasion by the pathogen. These findings could be useful in informing the design of eventual commercial multi-strain biocontrol products.

Key words: Culturomics, plant-based culture media, grapevine microbiome, downy mildew, MALDI-TOF MS, culture collection, biocontrol, synthetic microbial community (SynCom)

References

Duran, P., Thiergart, T., Garrido-Oter, R., Agler, M., Kemen, E., Schulze-Lefert, P., et al. 2018. Microbial Interkingdom Interactions in Roots Promote *Arabidopsis* Survival. Cell 175(4): 973-983.e14.

Eitzen, K., Sengupta, P., Kroll, S., Kemen, E. and Doehlemann, G. 2021. A fungal member of the *Arabidopsis thaliana* phyllosphere antagonizes *Albugo laibachii* via a GH25 lysozyme. Elife 10.e65306.

Lagier, J.-C., Hugon, P., Khelaifia, S., Fournier, P.-E., La Scola, B., and Raoult, D. 2015. The Rebirth of Culture in Microbiology through the Example of Culturomics To Study Human Gut Microbiota. Clin. Microbiol. Rev. 28(1): 237-264.

Sarhan, M. S., Mourad, E. F., Hamza, M. A., Youssef, H. H., Scherwinski, A.-C., El-Tahan, M., Fayez, M., Ruppel, S. and Hegazi, N. A. 2016. Plant powder teabags: A novel and practical approach to resolve culturability and diversity of rhizobacteria. Physiol. Plant. 157(4): 403-413.

Youssef, H. H., Hamza, M. A., Fayez, M., Mourad, E. F., Saleh, M. Y., Sarhan, M. S., Suker, R. M., Eltahlawy, A. A., Nemr, R. A., El-Tahan, M., Ruppel, S. and Hegazi, N. A. 2016. Plant-based culture media: Efficiently support culturing rhizobacteria and correctly mirror their in-situ diversity. J. Adv. Res. 7(2): 305-316.

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Dual-action bacterial consortium: antagonistic effects against *Fusarium oxysporum* and growth promotion in tomato crops

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Abstract: Chemical control has been the primary method for managing crop pests and diseases, but its negative impact on agroecosystem biodiversity has led to a shift towards biological control. The use of soil bacteria secondary metabolites has emerged as a promising alternative to counteract phytopathogenic microorganisms. This study evaluated the antagonistic effectiveness of a consortia comprising *Pseudomonas mediterranea* and *Pantoea ananatis* against *Fusarium oxysporum*, demonstrating up to 86 % inhibition in *vitro*. Additionally, the consortia's growth-promoting capacity was assessed in tomato crops, revealing significant improvements during flowering and fruiting stages. This research contributes to the growing body of evidence supporting the use of bacterial consortia as an efficient and environmentally friendly approach to crop management, offering potential benefits in both disease control and plant growth promotion.

Key words: diseases, biological control, tomato crop

Introduction

In the face of growing environmental concerns and the need for sustainable agricultural practices, the field of crop protection is undergoing a significant transformation. For decades, chemical control methods have dominated pest and disease management strategies in agriculture. However, the detrimental effects of these practices on biodiversity and ecosystem health have become increasingly apparent, prompting an urgent need for alternative approaches (Maitra et al., 2022; Wang et al., 2021).

Biological control, particularly the use of beneficial soil microorganisms, has emerged as a promising solution to this challenge. Among these, Plant Growth-Promoting Rhizobacteria (PGPR) have garnered considerable attention due to their dual ability to suppress pathogens and enhance plant growth. Of particular interest is the potential of bacterial consortia, which can offer synergistic benefits beyond those of individual strains (Khoso et al., 2024; Sriwati et al., 2023).

Materials and methods

Compatibility testing
To determine the ability of the bacteria to grow with each other without antagonism between them, the Kirby Bauer test was used. The absence of an inhibition halo revealed a positive interaction (compatible), the presence of an inhibition halo, not compatible.

Inhibition of Fusarium oxysporum growth
Place 160 µl of the consortium mixture (*Pseudomonas mediterranea* and *Pantoea ananatis)* on a PDA plate (90 × 15 mm) previously prepared with a sterile cellophane disc. Incubate the plate at 28 °C for 48 hours to allow for metabolite production. Subsequently, remove the cellophane disc to obtain a PDA plate impregnated with secondary metabolites and/or enzymes. Place a ~ 1 cm^2 disk of PDA medium containing fungal structures of *F. oxysporum* at the center of this plate. As a positive control, place a similar disk of mycelium on a fresh PDA plate (uninoculated with consortium). Incubate all plates at 28 °C for 7 days.

Effect of consortium on tomato crop growth
The growth-promoting effect of the PGPR consortia was evaluated by inoculating 10 ml of the consortia weekly. This application continued until the plants reached the flowering and fruiting stages. The following parameters were assessed: plant height, root length, fresh and dry weight of the plant, number of flowers, and number of fruits. Each treatment consisted of 30 replicates ($n = 30$).

Results and discussion

The biochemical profiles of two PGPR strains, *Pseudomonas mediterranea* and *Pantoea ananatis*, were previously analyzed in separate studies. The results revealed that both species possess significant plant growth-promoting fitness and the ability to solubilize phosphates and potassium, in addition, they produce hydrogen cyanide (HCN) and volatile organic compounds (VOCs), both of which can contribute to plant growth stimulation and pathogen suppression. Furthermore, when tested individually, both *P. mediterranea* and *P. ananatis* exhibited antibiosis mechanisms capable of inhibiting the growth of *Fusarium oxysporum*, a notorious plant pathogen responsible for vascular wilt disease in many crops.

Inhibition of Fusarium oxysporum growth
The consortium's metabolites exhibited a potent inhibitory effect on *F. oxysporum*, resulting in an 84% reduction in growth compared to the control. Significant morphological changes were observed in the treated fungus. While the control sample displayed optimal growth with typical mycelial development under the incubation conditions, the treated *F. oxysporum* showed a markedly altered appearance. The fungal structure appeared rigid and compact, with a notable absence of visible mycelial growth. This stark contrast in morphology and growth pattern underscores the effectiveness of the bacterial consortium's metabolites in suppressing *F. oxysporum* development.

Effect of consortium on tomato crop growth
The application of Plant Growth-Promoting Rhizobacteria (PGPR) offers a promising and sustainable alternative to chemical compounds in crop management. The data collected and analyzed in this study provides robust evidence suggesting that PGPR exert a significant

influence on plant hormonal pathways, resulting in a notable acceleration of the phenological cycle. This beneficial effect is primarily attributed to the unique ability of these bacteria to establish symbiotic associations with the plant root system, functioning as epiphytic bacteria.

Improved nutrient absorption PGPR facilitates more efficient uptake of essential elements for plant growth. Particularly relevant is their ability of solubilizing phosphates (PO_4^{3-}), increasing the availability of this crucial macronutrient for various plant metabolic processes. On the other hand, an increased production of phytohormones stimulates the synthesis or modifies the levels of important plant growth regulators. The modulation of these compounds by PGPR significantly contributes to optimizing plant growth and development. The multifaceted benefits of PGPR highlight their potential as a key component in integrated crop management strategies, aligning with the growing global emphasis on sustainable and environmentally friendly agricultural practices.

References

Khoso, M. A., Wagan, S., Alam, I., Hussain, A., Ali, Q., Saha, S., Poudel, T. R., Manghwar, H., and Liu, F. 2024. Impact of plant growth-promoting rhizobacteria (PGPR) on plant nutrition and root characteristics: Current perspective. Plant Stress 11: 100341. https://doi.org/10.1016/j.stress.2023.100341

Maitra, S., Brestic, M., Bhadra, P., Shankar, T., Praharaj, S., Palai, J. B., Shah, M. M. R., Barek, V., Ondrisik, P., Skalick, M., and Hossain, A. 2022. Bioinoculants – Natural Biological Resources for Sustainable Plant Production. Microorganisms https://doi.org/10.3390/microorganisms10010051

Sriwati, R., Maulidia, V., Intan, N., Oktarina, H., Syamsuddin, Khairan, K., Skala, L., and Mahmud, T. 2023. Endophytic bacteria as biological agents to control *Fusarium* wilt disease and promote tomato plant growth. Physiological and Molecular Plant Pathology 125. https://doi.org/10.1016/j.pmpp.2023.101994

Wang, Z., Chen, Z., Kowalchuk, G. A., Xu, Z., Fu, X., and Kuramae, E. E. 2021. Succession of the Resident Soil Microbial Community in Response to Periodic Inoculations. Applied and Environmental Microbiology 87(9): e00046-21. https://doi.org/10.1128/AEM.00046-2

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Extremophilic yeasts as biological control agents of *Penicillium expansum* and their role in patulin reduction

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Abstract: The increasing challenges posed by the global climate change and the need to ensure food security have prompted a growing interest in sustainable agricultural practices, including the possible use of extremophilic yeasts as biocontrol agents (BCAs) (Ali et al., 2024).

Usually, microorganisms that need extreme environments to grow are called "true extremophiles" (Buzzini et al., 2018). These microorganisms, which thrive in extreme ecosystems such as deserts and glaciers, have unique adaptations that allow them to survive under harsh conditions, including low and high temperatures, high salinity, osmotic stress, radiation, and drought. This ecological versatility has made extremophilic yeasts valuable sources for novel biotechnological applications (Ferreira et al., 2023).

The aim of this study was to assess the potential of newly isolated extremophilic yeasts as BCAs of a postharvest fungal pathogen, *Penicillium expansum*. This fungal pathogen is known to be the causal agent of green mould of apples and a producer of the mycotoxin, patulin. To date, the use of fungicides has been an effective strategy to reduce the pathogen incidence and patulin contamination in apples. BCAs are a promising environmentally friendly alternative to chemical fungicides, so several commercial products based on microorganisms have been developed.

In the present study, four psychrophilic and thermophilic yeasts that were isolated from the Algerian Desert and Sweden Coast during winter 2023 were evaluated as potential BCAs of *P. expansum* and their role in apple patulin reduction was investigated.

Microorganisms were identified with molecular techniques, namely sequencing of ITS (internal transcribed spacer) and LSU (large subunit). Phylogenetic trees revealed that the isolates belonged to *Naganishia* spp. and *Papiliotrema* spp.

Sixteen strains belonging to these genera were tested as BCAs against *P. expansum* in a dual culture assay to evaluate their inhibitory effect on the pathogen mycelial growth. Four strains were selected as the most active: *Naganishia albida* strain RDSH(1), *Naganishia diffluens* strain S6P2(4) and the strains SP6(4) and S1BSC2(1), both belonging to the species *Papiliotrema wisconsinensis.*

Non-volatile and volatile compounds (No-VOCs and VOCs, respectively) produced by the selected strains were tested by *in vitro* and *in vivo* assays to assess their involvement in antagonism.

The SP6(4) and S1BSC2(1) strains were selected as the best producers of active non-volatile compounds, as well as RDSH(1) and S6P2(4) for volatiles.

Based on the efficacy of their metabolites, the strains were tested on Pink Lady apples, artificially wounded and inoculated with *P. expansum* conidial suspension (20 µl, 1×10^5 conidia/ml). The SP6(4) and S1BSC2(1) strains completely suppressed apple green mould thanks to the production of No-VOCs. The VOCs production by the strains RDSH(1) and S6P2(4), only reduce pathogen severity by 10 %.

Apple rotten tissues were collected and used for DNA extraction. Specific primers pair for the *patF* gene involved in patulin biosynthesis was used. The antagonism of all selected strains was further demonstrated by the very low amount of pathogen DNA in treated fruits detected by qPCR.

Biochemical characterization of the extremophilic yeasts metabolites was carried out by Fourier Transform Infrared Spectroscopy (FTIR) and Solid Phase Micro Extraction Gas Chromatography-Mass Spectrometry (SPME-GC-MS), respectively for No-VOCs and VOCs, unveiling interesting differences between the strains.

These results highlighted the antagonistic potential of the extremophilic yeasts, emphasizing their promising applications in agriculture and food technology.

By harnessing the adaptive strategies to extreme conditions demonstrated by these microorganisms, innovative and sustainable solutions for improving fruit postharvest management could be developed.

Key words: extremophiles, postharvest, mycotoxin, BCAs, secondary metabolites

References

Ali, I., Qaiser, H., Abdullah, R., Kaleem, A., Iqtedar, M., Iqbal, I. and Chen, X. 2024. Prospective roles of extremophilic fungi in climate change mitigation strategies. J. Fungi 10: 385.

Buzzini, P., Turchetti, B. and Yurkov, A. 2018. Extremophilic yeasts: the toughest yeasts around? Yeast 35: 167-183.

Ferreira, E. M. S., Garmendia, G., Gonçalves, V. N., da Silva, J. F. M., Rosa, L. H., Vero, S. and Pimenta, R. S. 2023. Selection of Antarctic yeasts as gray mold biocontrol agents in strawberry. Extremophiles 27: 16.

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The Apple Sooty Blotch pathobiome: Composition and microbial interactions

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Abstract: The Sooty Blotch and Flyspeck (SBFS) complex consists of superficial dark blemishes and spots on trees, crops, and grasses caused by over 100 fungal species. SBFS fungi are non-pathogenic ectophytes: they thrive on surfaces and rely on leachates from underlying plant tissues and external carbohydrate sources (e. g., honeydew from aphids and other insect species). Their success as ectophytes derives from their austere genome and recalcitrant metabolism.

Such recalcitrance is a significant issue, particularly in apple orchards during the growing season and fruit storage. Unfortunately, due to massive restrictions of active substances, it is more and more observed in integrated pest management systems, leading to economic losses due to reduced fruit quality. Currently, no specific control strategies exist for SBFS. Furthermore, the chemical treatments used during the growing season and the controlled atmospheric storage conditions exert selective pressures that alter microbial communities on the apple surface, with unknown consequences for SBFS outbreaks.

This study aims to determine whether SBFS fungi influence the composition of the apple microbiome, whether certain microbiome compositions promote or inhibit SBFS growth, and which microbial species could serve as potential antagonists. To address these research questions, a large-scale microbial survey was conducted. Rosy Glow – Sekzie/ Cripps Pink apples were sampled from two experimental fields of Laimburg Research Centre (South Tyrol, Italy) at harvest and subsequently after 6 months ultra-low oxygen (ULO) storage. During the growing season, the orchards were separated into random plots, subjected to three different spraying programs based on active substances approved for organic production and two actives approved for integrated apple production.

Apple peels were excised from 144 apples, evenly distributed among the treatments. Total DNA was extracted from pooled samples taken from the calyx, equator, and stem of the apple peels. The fungal internal transcribed spacer (ITS3-4) region and the bacterial V3-V4 16S rRNA region were amplified following Illumina 16S Metagenomic Sequencing Library Preparation protocol and sequenced with AVITI instrument (Element Biosciences, San Diego, CA) using 300-bp paired-end mode (Igatech, Udine, Italy). Thus, microbiomes will be correlated with SBFS incidence surveys conducted at the two sampling times, enabling the characterization of macro-shifts from healthy to infected apples based on the different treatments. Furthermore, SBFS colonies are being isolated and identified from symptomatic apples using modified Koch's postulates to determine the primary local fungal species that cause symptoms.

Initial results from a small pilot study comparing healthy and SBFS-symptomatic apples show that SBFS-associated genera and other ectophytes are present, including *Cladosporium*, *Paraphoma*, *Entyloma, Golubevia, Uwebraunia and Tilletiopsis*. Moreover, symptomatic apples exhibited reduced fungal diversity compared to healthy apples, and the specific causes are yet to be determined. Furthermore, early sequencing efforts of the bacterial locus were inconclusive, as up to 99 % of reads corresponded to chloroplast and mitochondrial sequences.

To address this issue, peptide nucleic acid (PNA) blockers were incorporated into the large-scale experiment, successfully reducing host reads by up to 50 % and allowing for a more reliable bacterial characterization.

Preliminary results from the manual isolation experiments of SBFS colonies led to the identification of previously known genera that had not yet been reported in Italy, such as *Schizothyrium*, *Microcyclospora*, and *Ramichloridium*.

This study contributes to the development of biobased, environmentally friendly control strategies for SBFS outbreaks and symptom development during fruit storage. By integrating abiotic and previously uncharacterized biotic factors, it provides a comprehensive understanding of the ecological conditions that favour SBFS fungi.

Key words: Sooty Blotch and Flyspeck, holobiont, apple, metabarcoding, biocontrol

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Fungal isolates reduce *Phytophthora infestans* infection in tomato

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Abstract: This collaborative research project between the Julius Kühn-Institute (Institute for Biological Control, Dossenheim, Germany) and the University of Embu, Kenya (DAAD grant), employs a synergistic approach towards the search for microbial antagonists against *Phytophthora infestans* in tomato production. To do so, culture-dependent data were combined with microbiome analyses of bacteria and fungi in different tissues and environments and evaluated in the context of tomato plant health. We investigated two soil origins from organic tomato growers from the Wiesbaden and Rüsselsheim regions of Germany. Here, we present first-hand results on the fungal work, including the isolation of fungi from the rhizosphere, the endosphere, and the phyllosphere of tomato plants, and characterization regarding their potential as antagonists against *P. infestans,* in combination with microbiome assessment of the same plant tissues. In the culture-dependent approach, 246 fungal isolates were screened for antagonistic activity against two destructive fungal pathogens, *P. infestans* and *Alternaria solani,* through *in-vitro* assays. The isolation effort yielded more fungal antagonists from the rhizosphere than the phyllosphere and endosphere. It was also intriguing to recover more fungal antagonists from diseased phyllosphere tissues compared with healthy phyllosphere tissues. The genera *Penicillium, Trichoderma, Chlonostachys, Mortierella,* and *Pseudogymnoascus* were the most abundant among the promising candidates from the screening. Promising candidates from *in-vitro* tests were selected for use in plant trials following a stepwise screening strategy that combines growth features, ecological aspects, taxonomic data, potential health risks, and commercial properties with antagonistic efficacy. Five fungal candidates subjected to *ad-planta* trials, including *Chaetomium* sp., *Chlonostachys* sp., *Ctenomyces* sp., *Pseudogymnoascus* sp., and *Trichoderma* sp. demonstrated varied but potential suppressive effects against *P. infestans* in growth room conditions. In particular, an isolate of *Chaetomium* sp. significantly suppressed *P. infestans*, resulting in more than 70 % disease reduction compared to the non-treated control. In the microbiome analysis approach, we performed high throughput sequencing of the ITS region using total DNA extracted from the micro-compartments from healthy and diseased tomato plants. The aim was to reveal the degree of alteration of fungal communities in the two soil origins after infection with *P. infestans* and to characterize differences between individual plant tissues. Diversity analysis revealed that the pathogen did not significantly influence fungal microbiota composition between *P. infestans*-infected and healthy tomato plants grown in the two soils. However, the fungal community differed significantly between the two soil origins. The synergy between the two methods is demonstrated by the fact that 70% of our antagonistic fungal isolates were found in the tomato microbiome when ITS sequences were mapped to the amplicon sequence variant (ASV) sequencing database. Among the top 15 most prevalent taxa in the complete data set were fungal antagonists associated with the genera *Penicillium and Cladosporium*. Overall, this work demonstrates that combining functional screening and microbiome profiling is efficient in

exploring fungal biocontrol agents for *P. infestans* and other plant pathogens. Our findings will contribute to reduced synthetic chemical pesticide use, in which microbial antagonists can play a significant role.

Key words: tomato, fungi, microbiome, *Phytophthora infestans,* microbial antagonists

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Engineering resilient microbial communities to control postharvest diseases

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Abstract: The increasing focus on plant microbiomes is driven by the awareness of their critical role in mitigating plant diseases, including postharvest diseases of fruits and vegetables. This study aimed to engineer microbial communities capable of effectively inhibiting postharvest diseases. Initial microbial communities sourced from lichens, apples, pears, oranges, and quinces were used to inoculate wounds on apple fruits. Microbial communities were then selected for their ability to quickly colonize the wound without causing disease through a 10-cycle successive passaging. Microbial communities from the first and the last cycle were then tested to evaluate their efficacy in preventing infection and development of *Botrytis cinerea* and *Penicillium expansum* on apples. Amplicon sequencing analyses revealed shifts in the structure and diversity of the microbiome across the cycles, resulting in a reduction in disease incidence by 90 and 70 % for *B. cinerea* and *P. expansum*, respectively. To further validate the robustness of our engineered communities, we conducted additional tests using three synthetic microbial communities (SynComs) designed to mimic the microbiome we recovered in wounds after ten re-inoculation cycles. The efficacy of SynComs proved comparable to that of the sourcing selected microbiomes, thereby reinforcing the validity of our approach for designing functional SynComs and developing sustainable and effective solutions for enhancing postharvest disease management in fruit production.

Key words: plant microbiomes, amplicon sequencing, *Botrytis cinerea*, *Penicillium expansum*, microbiome engineering, agricultural sustainability

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Evaluation of the impact of storage protocols on complex soil communities in agricultural pathosystems

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Extended abstract: In recent years, the necessity to institute structures to preserve soil microbial communities, such as biobanks, microbiome vaults and soil repositories, has grown, especially as an answer to threads such as climate change and heavy soil usage (Manter et al., 2017). The importance of storing whole communities, as opposed to axenic cultures, is linked to the observation that microbial biodiversity is linked to ecosystemic functions, such as pathogen development, nutrient provisioning and element cycling (Jiao et al., 2022). So far, studies performed on soil storage have provided contrasting results, with the effect of different storage protocols changing based on soil type and considered parameters (Fromin, 2025).

To assess the impact of storage protocols on microbial communities associated with the rhizosphere of different crops, rhizospheric soil was collected from grapevine, kiwivine and strawberry fields, each with two different conditions based on health status (grapevine and kiwivine) or field treatments (strawberry). For each soil, storage at 4 °C, storage at -80 °C and freeze-dried lyophilisation were tested.

The effect of storage protocol was evaluated using an integrated analysis based on metagenomics, culturomics and functional characterization, with checkpoints set at soil collection, six months of storage and twelve months of storage. For the metagenomics analysis, fungal and bacterial communities were characterized by metabarcoding with primer couples ITS3_kyo2/ITS4ngs and Pro341f/Pro805r, respectively. For the culturomic analysis, 10 g soil aliquots were resuspended in PBS solution and serially diluted in NaCl 0.9 %. For fungi the solutions were plated on MEA additioned with 100 µg/ml streptomycin and amoxicillin, as well as on DRBCA. Plates were incubated for three days at 15 °C, then colony count was performed. For bacteria, the solutions were plated on 0.1 TSA with 100 µg/ml cycloheximide. Colony count and morphology were noted at 48 h and 6 d time point to highlight eco-physiological differences between r/k strategists. For the characterization of metabolic activities and functions of soil microbial communities, the Biolog EcoPlate™ technique was used. The Community Level Physiological Profiles (CLPPs) were assessed by 95 carbon substrate utilization. Absorbance was read at OD590 and OD750 every 24 hours until complete saturation. Plates were incubated in dark conditions at 25 °C and 50 r. p. m. during the experiment.

The results for the metabarcoding analysis indicated the presence of statistically significant differences in microbiota composition compared to the harvested soil already at 6 months of storage and highlighted differences in storage protocol impact based on host crop, health condition and investigated taxon. With the exception of fungal communities in grapevine, a molecular signal could be recovered for all storage treatments, both in bacteria and fungi. For fungi, in grapevine soil, selected storage protocol did not result in significant differences for microbiota composition after six months, although -80 °C had the best performance. In kiwivine

soil, storage at 4 °C was better, regardless of health condition. Finally, in strawberry soil, both storage at 4 °C and storage at -80 °C had comparable performance. For bacteria, both storage at 4 °C and -80 °C resulted in the least divergence of stored samples compared to the harvested soil for most crops and health conditions, with the exception of healthy grapevine soil (only 4 °C storage) and diseased kiwivine soil (only -80 °C storage).

The culturomic analysis indicated the presence of the significant differences between storage protocols, with effects already observed at 6 months of storage. For fungi, lyophilization resulted in almost total loss in the number of colony forming units, regardless of host crop and health status. In healthy soil associated with grapevine and kiwivine, both 4 °C and -80 °C storage preserved CFU number, while in the diseased soil the number of CFU decreased regardless of storage protocol. In strawberry, number of CFU for the control soil was preserved only at 4 °C, while in the solarized soil both 4 °C and -80 °C preserved CFU number compared to the control. Total bacterial counts were unaltered in soil samples stored both at 4 °C and -80 °C for all cultivars and soil type, while strongly affected by the lyophilization technique. The eco-physiological population distribution in strawberry samples shifted significantly from fast growers and less adaptable bacteria, r-strategists, to oligotrophs organisms, k-strategists, in all storage conditions. The trend was observed also in grapevine and kiwivine samples stored at 4 °C.

The BIOLOG analysis confirmed that lyophilization resulted in a complete loss of function of the microbial communities within the samples. The other preservation techniques showed an overall integrity of the microbial populations in terms of metabolic activity at time 6 months of storage although shifted towards alternative carbon sources utilization when compared to fresh samples.

Key words: soil microbiome, metabarcoding, culturomics, Biolog, strawberry, kiwivine, grapevine

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References

Fromin, N. 2025. Impacts of soil storage on microbial parameters, Soil 11: 247-265. doi: 10.5194/soil-11-247-2025

Jiao, S., Lu, Y. and Wei, G. 2022. Soil multitrophic network complexity enhances the link between biodiversity and multifunctionality in agricultural systems. Glob. Chang. Biol. 28(1): 140-153.

Manter, D. K., Delgado, J. A., Blackburn, H. D., Harmel, D., Pérez De León, A. A., and Honeycutt, C. W. 2017. Opinion: Why we need a National Living Soil Repository. Proc. Natl. Acad. Sci. U. S. A. 114 (52): 13587-13590.

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Metagenomic analysis of epiphytic microorganisms of broccoli with LED and UV-C treatments

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Abstract: Foodborne diseases caused by pathogens on fresh products pose a severe threat to public health, and some opportunistic pathogens of broccoli also cause head rot. The analysis of the microbial taxa on the surface of broccoli head can be used to identify pathogenic microorganisms and evaluate the impact of management practices. In this study, broccoli was treated with UV-C, LED red light (at a rate of 50 µl/m^2/s), and a combination of UV-C and LED red light, respectively, either before or during storage. Subsequently, the epiphytic microorganisms of broccoli heads were analyzed by metagenome. The preservation efficacy of three different light treatments on broccoli, from the most effective to the least effective, is as follows: red LED + UV-C, LED red light, and UV-C. Proteobacteria and Firmicutes were the main epiphytic bacterial phyla of broccoli heads under three light treatments, while Ascomycota and Basidiomycota were the main epiphytic fungal phyla. Three light treatments decreased the relative abundance of foodborne pathogens and bacterial communities responsible for broccoli rot. The UV-C treatment can inhibit growth of *Botrytis cinerea*, while the light treatments led to an increase in the relative abundance of *Pseudomonas fluorescens*. The above results indicate that light treatments can prolong the shelf life of broccoli through the maintenance of its storage quality, and the inhibition of foodborne pathogens that cause broccoli decay.

Key words: metagenome, microorganism, LED red light, UV-C, broccoli

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Ramularia mali and dry lenticel rot in apples: Preliminary assessment of storage conditions and their effects on the microbiome

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Abstract: *Ramularia mali* has emerged as a significant postharvest pathogen affecting apples, with an increasing number of cases reported since 2017 in North Italy (Primisser et al., 2024). While the fungal infection remains asymptomatic in the field, symptoms of dry lenticel rot appear after prolonged storage under low-oxygen conditions (1 % O_2) at 1 °C. First epidemiological studies, using a metabarcoding approach, revealed a shift in the pathogen's lifestyle from an endophytic presence during the early growing season to an epiphytic phase closer to harvest (Garello et al., 2023). As preliminary data indicated, a significantly reduced incidence of dry lenticel rot when apples are stored under atmospheric conditions at 4 °C could be observed. The present study further investigates the microbial composition and diversity (alpha and beta diversity) under different pre-harvest treatments and storage conditions to assess whether microbiome shifts contribute to disease incidence. Apples cv. Golden Delicious from an orchard, with historical disease incidence of ~ 80 % fruit affected with *R. mali* were taken: Fifty fruits originated from untreated control and fifty from plots with a standard fungicide treatment. Samples were subjected to five treatments: (1) untreated, stored at 4 °C; (2) untreated, stored at 1 % O_2 at 1 °C; (3) hot water treatment (120 s, 43 °C) stored at 1 % O_2 at 1 °C; (4) 1-Methylcyclopropene (1-MCP) treatment, stored at 1 % O_2 at 1 °C; and (5) hot water treatment and 1-Methylcyclopropene (1-MCP) treatment, stored at 1 % O_2 at 1 °C. Sampling was conducted at harvest (t0), after storage (t1), and shelf life (t2, 10 days at 20 °C). For each sample six apple plugs of 1 cm diameter were shock-frozen in liquid nitrogen, and DNA was extracted for metabarcoding analysis using ITS3F/ITS4R and 16S-341f/16S-805R primers together with PNA (peptide nucleic acid) clamps during the first amplification step to minimize host-derived sequences. The microbial composition and diversity metrics will be analyzed to determine microbiome shifts associated with dry lenticel rot with regards to different pre- and post-harvest conditions. These findings will enhance the understanding of disease development and inform improved apple storage strategies.

Key words: post harvest, apple pathogen, microbial communities

References

Garello, M., Piombo, E., Prencipe, S., Schiavon, G., Berra, L., Wisniewski, M., Droby, S., Spadaro, D. 2023. Fruit microbiome: A powerful tool to study the epidemiology of dry lenticel rot and white haze – Emerging postharvest diseases of apple. Postharvest Biology and Technology 196 (112163).

Primisser, S., Spadaro, D., Deltedesco, E., Oettl, S. 2024. *Ramularia mali* associated with symptoms of dry lenticel rot, an emerging postharvest disease on apples in Italy. Plant Dis. 2024 Jun 17. doi: 10.1094/PDIS-04-24-0826-PDN

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In vitro ecophysiological insights into apple microbiome biocontrol agents isolated from mountain and valley

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Abstract: The adaptation of biocontrol agents to environmental conditions is crucial for their success in real-world applications. This study focused on the ecophysiological characterization of four potential biocontrol agents isolated from the apple microbiome: two bacterial strains (*Pantoea* spp.) from mountain (BM) and valley (BV) orchards, and two yeast strains (*Vishniacozyma* spp.) from mountain (LM) and valley (LV) environments. *In vitro* growth of BM, BV, LM and LV was evaluated under six temperature conditions (0 °C, 4 °C, 10 °C, 25 °C, 30 °C and 35 °C) and four water activity (a_w) levels (0.94, 0.96, 0.98 and 0.998 a_w) using LB and PDB media modified with glycerol to achieve the target a_w. Periodic optical density measurements (600 nm) provided growth curves to assess the optimal conditions for each microorganism. Results revealed significant differences in adaptation among the strains evaluated considering mountain and valley. In general, the optimal growth temperature ranged between 10 °C and 25 °C for all microorganisms, except for the valley yeast (LV), which was recorded at 30 °C. Above 30 °C, reduced growth was observed for bacterial strains (BV and BV) and the mountain yeast (LM), with the latter being unable to grow beyond this temperature. Additionally, neither of the yeast strains was able to grow at 36 °C. Regarding the water activity (a_w) results, it was identified as a limiting factor for bacterial growth, particularly evident in BM, which failed to grow at all temperatures tested when a_w evaluated was 0.94 and 0.96. In contrast, the valley strain (BV) was able to grow under these conditions at temperatures of 25 °C and 30 °C. These findings enhance our understanding of the environmental adaptability of biocontrol agents, paving the way for tailored applications for postharvest disease management (pre or postharvest). By selecting strains with specific environmental adaptations, we can optimize their efficacy under diverse conditions, contributing to developing robust and sustainable biocontrol strategies such as microbial consortia combining strains from distinct environments. Such consortia could leverage the complementary adaptations of these isolates, enhancing biocontrol resilience and efficacy across diverse environmental conditions.

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Key words: apple microbiome, ecophysiological characterization, postharvest, biocontrol agents

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Antagonistic effects of plant growth-promoting rhizobacteria consortium against the tomato wilt disease caused by *Clavibacter michiganensis* subsp. *michiganensis*

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Abstract: Bacterial canker, caused by *Clavibacter michiganensis* subsp. *michiganensis* (Cmm), is a major threat to tomatoes, leading to significant global losses. With no commercially available resistant seeds and limited progress in breeding, control relies on preventive measures, pesticides, and antibiotics. However, environmental and health concerns drive the search for safer, more effective alternatives to manage the disease and improve crop yield. The application of plant growth-promoting rhizobacteria (PGPR) as biocontrol agents is an ecologically sustainable method of protecting plants and offers a promising alternative to chemical pesticides. In this study, several antagonistic bacterial strains were isolated from the rhizosphere soil and tissues of healthy tomato plants. The isolated bacteria were screened for antibacterial activity against *Cmm* and identified through 16S rRNA gene sequencing. Nine strains exhibited inhibition rates of 18 % to 22.25 %, belonging to distinct genera such as *Pseudomonas*, *Bacillus*, and *Microbacterium*. In plant trials, two-week-old tomato plants were treated with *Pseudomonas* and *Microbacterium* strains through soil drenching. Seven days later, the plants were infected with *Cmm* infection via needle injection into the stem. Results showed the individual efficacy of *Microbacterium* and *Pseudomonas* strains against *Cmm*, with treated plants showing a significant reduction in disease incidence of 58.90 % and 38.76 %, respectively. Remarkably, combining the two strains led to a substantial 78.34 % reduction in disease severity of 78.34 %, demonstrating their efficacy as a consortium against *C. michiganensis* subsp. *michiganensis*. Additionally, these strains reduced canker lesions size and vascular browning caused by *Cmm*. Besides disease suppressing, they effectively promoted tomato plant growth by enhancing the root system, as reflected in improved vegetative parameters compared to untreated plants. The real-time quantitative PCR (qRT-PCR) analysis showed that treatment with the consortium, followed by *Cmm* infection, significantly upregulated pathogenesis-related (PR) genes, particularly *PR1* and *PR5*, compared to plants treated with individual strains.

Key words: *Clavibacter michiganensis* subsp. *michiganensis*, tomato, bacterial canker, sustainable agriculture, PGPR, biocontrol

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Biocontrol of emerging and complex plant diseases

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From grapevine to grapevine: microbial community from table grape to manage grapevine trunk diseases

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Abstract: Grapevine trunk diseases (GTDs) include a group of grapevine diseases caused by several fungal pathogens colonizing the vine wood, which causes wood discoloration and necrosis, vascular infections, and white rot decays, affecting the productivity and grapevine longevity (Fontaine et al., 2016; Mondello et al., 2018). The simultaneous presence of multiple trunk pathogens even in asymptomatic plants and the irregular expression of GTDs symptoms make management difficult. To date, most research has addressed wine grapes, and few available plant protection products with significant efficacy are reported (Leal et al., 2024). This study highlights the potential of members of the microbial communities colonizing table grapes wood as a promising approach to control fungal pathogens associated with GTDs on table grapes. The antagonistic activity of already characterized strains of *Aphanocladium album*, *Clonostachys rosea*, *Trichoderma* spp., *Bacillus*, *Streptomyces* and *Pseudomonas* isolated from GTDs asymptomatic and symptomatic table grapes in Apulia and Sicily was *in vitro* evaluated in dual culture assay against a panel of GTDs pathogens. Among the isolates assessed, 16 different *Trichoderma* species affected the growth of *Neofusicoccum parvum* (37-62 %), *Eutypa lata* (34-63 %), *Diplodia seriata* (50-64 %), *Cylindrocarpon destructans* (22-79 %), *Phaeomoniella chlamydospora* (37 %) and *Diaporthe viticola* (47-71 %). *A. album* showed a deadlock at mycelial contact during the interactions with *Fomitiporia mediterranea* and *Phaeoacremonium minimum*. The growth of pathogens was inhibited when they came into contact with *C. rosea* GP80. Among the assessed bacterial strains, *Bacillus amyloliquefaciens* (D747) and *Bacillus subtilis* (QST 713) were active against *N. parvum* (34 and 33 %) and *E. lata* (13 % and 16 %). Furthermore, evaluations on the ability of the most promising biocontrol agent (BCA) candidates to produce inhibitory substances, enzymes and/or volatile organic compounds are in progress as well as *in planta* effectiveness tests. The new BCAs investigated in this study might represent a useful tool for the sustainable control of GTDs on table grapes.

Key words: antagonistic activity, dual culture, microbial community, grapevine trunk diseases

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References

Fontaine, F., Gramaje, D., Armengol, J., Smart, R., Nagy, Z. A., Borgo, M., Rego, C., and Corio-Costet, M.-F. 2016. Grapevine Trunk Diseases: A Review. 1st Edition. OIV Publications, Paris.

Leal, C., Bujanda, R., López-Manzanares, B., Ojeda, S., Berbegal, M., Villa-Llop, A., Santesteban, L. G., Palacios, J., and Gramaje, D. 2024. Evaluating Treatments for the Protection of Grapevine Pruning Wounds from Natural Infection by Trunk Disease Fungi. Plant Disease 108(10): 3052-3062. DOI: 10.1094/PDIS-02-24-0473-RE.

Mondello, V., Songy, A., Battiston, E., Pinto, C., Coppin, C., Trotel-Aziz, P., Clément, C., Mugnai, L., and Fontaine, F. 2018. Grapevine Trunk Diseases: A Review of Fifteen Years of Trials for Their Control with Chemicals and Biocontrol Agents. Plant Disease 102: 1189-1217. DOI: 10.1094/PDIS-08-17-1181-FE.

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From lab to field: Insights into the use of biological control agents against *Fusarium* root and crown rot in durum wheat

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Abstract: *Fusarium* root and crown rot (FRR/FCR), primarily caused by *Fusarium culmorum* and *Fusarium graminearum,* are fungal diseases that affect wheat production worldwide. These diseases result in significant yield and quality losses, particularly under the constraints of organic farming, where effective sustainable solutions are limited. This study explores biological strategies to enhance durum wheat resilience against FCR/FRR, focusing on beneficial bacteria. *In vitro* screening identified promising candidates from the *Bacillus* and Lactic Acid Bacteria (LAB) genera. LAB candidates demonstrated high effectiveness also in growth chamber seed priming tests, significantly reducing disease caused by *Fusarium culmorum* (strain Fc1126) by 44.4 % and *Fusarium graminearum* (strain Fg566) by 49.2 %. Genomic analyses were conducted on the most promising bacterial strains to identify genes involved in the biosynthesis of antifungal metabolites. *In vitro* metabolite production confirmed their ability to produce key compounds, such as lactic acid, surfactin, fengycin, and benzoic, hydrocinnamic, and phenyllactic acids. Open field trials were conducted over two consecutive years (2023-24 and 2024-25) in Ravenna area (Northen Italy) to assess the performance of bacterial treatments under natural conditions. Durum wheat seeds (cv San Carlo), treated with different bacterial strains, were co-sown with barley inoculated with the pathogen Fc1126. Seedling emergence was monitored throughout the trials, with initial counts recorded manually. A follow-up evaluation was conducted at BBCH stages 30-31 using drone technology. Results from the first year demonstrated the effectiveness of seed treatments, particularly the *Bacillus amyloliquefaciens* CAAd strain, which increased germination by 50 % compared to the untreated control. Treatments with LAB strains also showed notable improvements, with increases of up to 37.3 %. The evaluation of the second year is still ongoing.

This research was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – M4 C2, Investimento 1.4 – D.D. 1032 17/06/2022, CN00000022) and the project PRIN 2022 PNRR (M4 C2. Investment 1.1.) – D.D. n. 1409 of 14/09/2022-BICONTRARIUM.

Key words: durum wheat, FCR, FFR, *Fusarium culmorum*, *Fusarium graminearum*, biological control agents, beneficial bacteria, *Bacillus*, LAB

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Bacillus velezensis NM-24 as a sustainable plant growth promoter and biocontrol agent against fungal diseases of potato

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Abstract: Potato fungal diseases such as black scurf caused by *Rhizoctonia solani*, early and late blight caused by *Alternaria solani* and *Phytophthora infestans*, black dot caused by *Colletotrichum coccodes*, silver scurf caused by *Helminthosporium solani*, and potato tuber rot caused by *Fusarium* spp. represent ongoing challenges to global potato production and are often difficult to manage The traditional reliance on non-selective inorganic pesticides and fertilizers raises significant concerns regarding environmental sustainability and human health. In response to global initiatives aimed at reducing pesticide use and promoting eco-friendly agricultural practices, there is an increasing demand for sustainable solutions to combat crop diseases. This study investigates the potential of *Bacillus velezensis* NM-24, isolated from the rhizosphere soil of healthy potato plants, as a sustainable plant growth promoter and biocontrol agent against soilborne pathogens. *B. velezensis* NM-24 exhibited broad-spectrum antifungal activity, inhibiting the growth of key pathogens, including *Alternaria alternata*, *Fusarium* spp., *Colletotrichum* spp., and *Rhizoctonia solani* AG-3PT, also demonstrating growth-promoting effect in pot trials. The NM-24 genome was sequenced using the Illumina HiSeq 4,000 platform, revealing eight gene clusters involved in the synthesis of non-ribosomal lipopeptides, ribosomal genes for extracellular lytic enzymes, biofilm formation, volatile organic compounds (VOCs), and other secondary metabolites with antifungal and plant growth-promoting properties. This study highlights the application of *B. velezensis* NM-24 as an effective biocontrol agent against fungal pathogens and a promoter of plant growth. These findings position NM-24 as a promising candidate for sustainable agricultural practices, providing a viable alternative to chemical pesticides and fertilizers in potato production.

Key words: biocontrol, potato, fungal diseases, *Bacillus velezensis*

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Effects of microbial biocontrol agents on tomato physiology, productivity and response to biotic and abiotic stresses

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Abstract: In agriculture, food losses caused by pests and plant pathogens are estimated between 20-40 % on a global scale (Savary et al., 2019; FAO, 2019). These losses may be effectively limited by using chemical-based plant protection products. However, chemicals represent a major threat to human health, and they are responsible for the loss of biodiversity in the environment (Goulson, 2014). Thus, there is an urgent need to find more sustainable alternatives to ensure safe food production for the growing human population (UNEP, 2016). The use of microbial biocontrol agents (mBCAs) in agriculture is increasingly acknowledged as a sustainable strategy to enhance crop growth and resilience to biotic and abiotic stresses.

In this study, the effects of root treatments with selected mBCAs on tomato (*Solanum lycopersicum* L. cv. Moneymaker) were investigated, focusing on increased tolerance to water stress, improved plant growth promotion, and biocontrol of two plant pathogens: *Rhizoctonia solani* and *Fusarium oxysporum* f. sp. *lycopersici*. Model mBCAs, including the fungus *Trichoderma asperellum* (C1 and C2) and three bacterial strains (*Lysobacter capsici* AZ78, *Bacillus velezensis* PSE31B, and *Pseudomonas salmasensis* POE54) were applied individually and in combinations. In pot bioassays were conducted on plants in full (100 %) or reduced (50 %) irrigation regimes to evaluate water stress tolerance.

Assessment of biometric parameters revealed that treatments containing *B. velezensis* PSE31B, either alone or combined with *Trichoderma* strains, significantly increased plant growth and chlorophyll content. Enhanced plant development was also observed under the water stress condition, indicating that mBCA treatments could counteract the negative effects of reduced water availability.

Tomato plants were treated with mBCAs for biocontrol of the root pathogen *F. oxysporum* f. sp. *lycopersici*, by watering with microbial suspensions at seedling emergence and one week later. After 5 days, plants roots were soaked in a pathogen spore suspension for 1 h and then transplanted to soil-vases. Plant mortality was evaluated 28 days post-inoculation (dpi). Plants treated with mBCAs, e. g., *Bacillus* sp., either applied alone or in combination with *T. asperellum* C2, showed a significant reduction in mortality, up to 35 % compared to 90 % mortality, observed in infected untreated plants.

mBCAs were also tested on tomato by watering with microbial suspensions at 5 days after planting into soil infected with the root pathogen *R. solani*. Plant mortality was evaluated at 25 dpi. Overall, when applied singly, mBCAs significantly reduced the mortality of *Rhizoctonia*-infected tomato plants to 10 %, compared to controls where mortality exceeded 50 %. Surviving mBCA-treated plants showed a substantial increase in height and fresh weight,

compared to either infected or uninfected controls, highlighting the beneficial effects of mBCAs on plant growth and development.

The effects of mBCA application on plant productivity and fruit qualitative parameters were also analysed. No significant differences were observed on tomato yield (i. e., number of fruits/plant, fruit fresh weight/plant), total polyphenol, antioxidant activity, carotenoid and ascorbic acid contents between the inoculated and control plants. Interestingly, statistical differences ($p \leq 0.05$) were observed in terms of fruit colour and total soluble solid (TSS) content, depending upon the treatment.

In conclusion, mBCAs, particularly *Bacillus* sp., significantly enhanced tomato growth, chlorophyll content, and resilience to water stress. Additionally, both *Bacillus* sp. and *T. asperellum* C2 effectively reduced plant mortality and disease symptoms caused by *R. solani* and *F. oxysporum*. These results increase the knowledge of the impact that mBCA may have on crops, highlighting the potential of selected microorganisms to mitigate biotic and abiotic stresses, and affect plant physiology and productivity, that influence the complex plant-microbe interactions occurring within the plant holobiont able to promote sustainable crop production.

Key words: plant holobiont, microbial biocontrol agents, *Bacillus, Pseudomonas, Lysobacter, Trichoderma, Solanum lycopersicum,* water stress, pathogen infection

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References

FAO 2019. http://www.fao.org/plant-health-2020/about/en/

Goulson, D. 2014. Pesticides linked to bird declines. Nature 511(7509): 295-296.

Savary, S., Willocquet, L., Pethybridge, S. J., Esker, P., McRoberts, N. and Nelson, A. 2019. The global burden of pathogens and pests on major food crops. Nature Ecology & Evolution 3(3): 430-439.

UNEP 2016. https://www.resourcepanel.org/sites/default/files/documents/document/media/food_systems_summary_report_english.pdf

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Assessment of efficacy and side effects of a new sustainable fungicide against grapevine diseases

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Abstract: Downy mildew (caused by *Plasmopara viticola*) and powdery mildew (caused by *Erysiphe necator*) are responsible for negative impacts on grapevine. Disease management typically relies on conventional fungicide applications, raising concerns about environmental sustainability and human health. This study aimed to evaluate the efficacy of a new fungicide, based on choline pelargonate (CP, patent BE1026779B1), against *P. viticola* and *E. necator* and to assess potential side effects on phyllosphere microorganisms under field conditions. Greenhouse experiments demonstrated that CP reduced the disease severity of both pathogens with no phytotoxic effects on grapevine leaves. Field trials confirmed the efficacy of CP in two experimental vineyards and showed high efficacy against powdery mildew. To assess potential side effects on non-target microorganisms, phyllosphere microbial communities were collected from leaves and bunches of CP-treated and control plants. DNA was extracted, bacterial and fungal communities were analyzed by amplicon sequencing, and statistical analysis will highlight possible changes between CP-treated and control samples. This study demonstrated that CP is an effective fungicide for managing grapevine diseases for further development as an alternative to conventional treatments.

Key words: grapevine, fungicide, downy mildew, powdery mildew, phyllosphere microorganisms

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Control and antifungal mechanism of protocatechuic acid ester on postharvest grey mold disease of strawberry fruit

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Abstract: The extensive application of synthetic fungicides has progressively exacerbated drug resistance, adversely impacted human health and lead to environmental contamination. The control of postharvest disease with plant-derived biologically active compounds is a promising alternative. This study aimed to investigate the efficacy and mechanisms of action of two simple esterified derivates of protocatechuic acid, i. e., methyl protocatechuate and ethyl protocatechuate, against grey mold of postharvest strawberry fruit infected by *Botrytis cinerea*. Both esters dose-dependently suppressed *in vitro* the mycelial growth of *B. cinerea* and effectively inhibited grey mold decay in strawberry fruit, with methyl protocatechuate exhibiting superior antifungal activity compared to ethyl protocatechuate at equivalent dosages. Treatments with these eaters increased the cellular leakage, including nucleotide, soluble protein and soluble sugar, indicating altered membrane permeability in *B. cinerea*. RNA sequencing analysis of *B. cinerea* exposed to ethyl protocatechuate for 0 h, 0.5 h, 2 h or 8 h revealed significant alterations in oxidoreductase activity during early exposure, consistent with observations in *B. cinerea* treated with methyl protocatechuate. Prolonged exposure primarily affected metabolic processes involving organonitrogen compounds and small molecules. Additionally, protocatechuic acid esters treatment upregulated the expression of antioxidant enzymes such as catalase (*CAT*), Cu/Zn-superoxide dismutase (*Cu/ZnSOD*), peroxidase 12 (*POD12*), and polyphenol oxidase (*PPO*), as well as defense-related proteins like beta-1,3-glucanase (*GLU*) and chitinase (*CHI*), in *B. cinerea*-infected strawberries. Collectively, these findings suggest that protocatechuic acid esters suppress grey mold decay in postharvest strawberry fruit through direct antifungal effects and induced disease resistance. Therefore, protocatechuic acid ester can be considered as novel alternatives to traditional fungicides for controlling postharvest diseases in strawberry fruit.

Key words: *Botrytis cinerea*, strawberry fruit, methyl protocatechuate, ethyl protocatechuate, antifungal mechanism

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Evaluation of the antifungal activity of endophytic organisms against *Fusarium* fruit rot in tomato

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Abstract: The tomato plant (*Solanum lycopersicum* L.) is one of the most widely cultivated horticultural crops globally. However, it suffers significant post-harvest losses, estimated at 50 %, due to biotic and abiotic factors during shipping, storage, and retail. Tomato fruit rots compromise both the quality and commercial value of the produce. Fusarium fruit rot, caused by *Fusarium oxysporum*, is a major post-harvest disease affecting tomato crops in various regions of Pakistan. In this study, symptomatic tomato fruit samples were collected, and the pathogen was successfully isolated. Pathogenicity tests revealed that the fungus infects tomato fruits primarily through wounds, underscoring the critical role of fruit injury in facilitating fungal invasion. Management strategies, both *in vitro* and *in vivo*, were assessed with a focus on the antifungal potential of endophytic organisms. Among the tested biocontrol agents, *Bacillus subtilis* demonstrated the highest efficacy, inhibiting mycelial growth by 60 %, followed by *Trichoderma harzianum*, which achieved a 40 % inhibition rate. This study highlights the potential of endophytic organisms as environmentally sustainable alternatives for managing *Fusarium* fruit rot in tomatoes.

Key words: Post-harvest losses, *Fusarium oxysporum*, *Bacillus subtilis*, *Trichoderma harzianum*

Evaluation of *Pseudomonas chlororaphis* M71 and *Trichoderma atroviride* SC1 movement and persistence after their injection in the trunk of grapevine plants affected by Esca

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Abstract: The increasing difficulties in protecting plant health due to climate change pave the way for integrating endotherapy as a sustainable alternative, especially in contexts where conventional treatments are complex and/or ineffective, such as Esca Complex, one of the main wood diseases of grapevine. Despite numerous benefits, endotherapy is rarely used due to high application costs and the limited availability of specific formulations that guarantee good results (Ferreira et al., 2023). The unclear outcomes of tests conducted over the years with different compounds are attributed not so much to the ineffectiveness of the active ingredient but rather to the injection method and the chosen endotherapeutic formulation (Berger and Laurent, 2019). Microbial Biocontrol Agents can significantly contribute to plant protection, and using plant-beneficial microorganisms for endotherapy could be a promising method for combating plant diseases. This approach benefits from the plant's active and persistent colonization of these microorganisms. It allows them to reach areas otherwise inaccessible to the passive movement of injected compounds, with no need for multiple applications throughout the year. This work aims to verify if the plant-beneficial microorganisms *Pseudomonas chlororaphis* M71 and *Trichoderma atroviride* SC1 can systemically establish themselves when injected, both in rooted cuttings in the greenhouse and grapevine plants in the vineyard. Experiments were conducted to evaluate the colonization efficiency, translocation within the grapevine plant, long-term persistence, and protection against new infections caused by the main Esca pathogens. Results indicated successful colonization by both microorganisms, with significant translocation observed throughout the grapevine plant tissues, providing reassurance about the effectiveness of the method. Long-term colonization results showed that these plant-beneficial microorganisms could persist in the trunk for at least 60 days. Further research will be needed to optimize injection protocols and assess this method's practical applications under field conditions.

Key words: beneficial microorganisms, endotherapy, grapevine

References

Berger, C., and Laurent, F. 2019. Trunk injection of plant protection products to protect trees from pests and diseases. Crop Protection 124: 104831.

Ferreira, J. A., Esparraguera, L. B., Queiroz, S. C. N., and Bottoli, C. B. G. 2023. Vegetative Endotherapy – Advances, Perspectives, and Challenges. Agriculture 13(7): 1465.

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Development of new biological management strategies to control 'Mal dello stacco' disease of hazelnut

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Abstract: Hazelnut is one of the most economically significant nut crops on a global level, representing an excellent Italian production. "Mal dello stacco", caused by the fungus *Anthostoma decipiens*, is a t fungal trunk disease affecting this crop and causing considerable damage. The symptoms are characterized by internal dark necrotic lesions and elongated cankers, which can break branches, with reddish-brown conidia masses on the bark. In recent years, the disease incidence has increased in Piedmonta significant decrease in production and plant death. Currently, management strategies are lacking due to the limited number of registered active ingredients, and it is necessary to develop innovative approaches. Thus, effectiveness tests of biocontrol products were performed on detached twigs and potted plants using commercial biological products. Twigs and plants were wounded with a scalpel, treated twice with selected products, and inoculated with mycelial plugs of *A. decipiens*. The twigs were incubated in moist chambers for 20 days, and plants were kept in the greenhouse for four months. The length of the internal necrotic lesion was then measured. ANOVA and Tukey's post hoc test (p-value > 0.05) analysis showed promising results. Biological commercial products containing *Bacillus* spp., *Trichoderma* spp., and *Pythium oligandrum* demonstrated a statistically comparable level of effectiveness to the chemical fungicide used as positive control, with a reduction in lesion length of 40-50 %.

Key words: hazelnut, "Mal dello stacco", *Anthostoma decipien*s, biological control

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Preharvest application of *Bacillus velezensis* 12Y improves strawberry fruit quality and storability

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Abstract: *Bacillus velezensis* is a beneficial microbe that can effectively control plant diseases, but its impact on the entire harvest chain of strawberries from pre-harvest to post-harvest has not been fully elucidated. In this study, *B. velezensis* 12Y was applied to the strawberry rhizosphere, and several agronomic traits before harvest, fruit quality at harvest, and fruit storability after harvest were determined. The results showed that *B. velezensis* 12Y could control preharvest diseases with an efficacy of 78.35 %. At harvest, *B. velezensis* 12Y significantly improved the quality of fruits and enhanced the content of aldehydes and esters, representing fresh aroma and fruity aromas, respectively. The preharvest application of *B. velezensis* 12Y resulted in a significant enrichment of the strawberry surface epiphytes with various potential biocontrol microbes, including *Cryptococcus, Rhodotorula*, *Verticillium*, *Sphingomonas*, *Pantoea*, *Hannaella*, etc. During storage, the decay of strawberries was delayed with the decay index reduced by 30.26 %. The storage quality, antioxidant activity, and shelf life of fruits were enhanced, thus maintaining the freshness of fruits. This study elucidated the influence of *B. velezensis* 12Y on the entire harvest chain of strawberries and provided a basis for the use of beneficial microbes with dual effects of plant protection and better fruit quality and shelf life.

Key words: strawberry, *Bacillus velezensis*, aromatic compounds, preharvest, microbial community

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Promising approaches for the management of kiwifruit vine decline syndrome by biocontrol agents

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Abstract: Kiwifruit vine decline syndrome (KVDS), firstly reported in Veneto region has significantly affected the Italian kiwifruit sector since 2012. While the exact aetiology of KVDS still remains unclear, several studies have demonstrated the association of KVDS with the presence of soil-borne pathogens, primarily belonging to the *Phytophthora* and *Phytopythium* genera (Donati et al., 2020; Savian et al., 2020). In new established orchards, the use of KVDS-resistant rootstocks appears to be the most promising tool. Meanwhile, a holistic approach is suggested to prevent the spread of KVDS in existing orchards. Specifically, this work aimed to explore the feasibility of using biocontrol agents to manage KVDS. An experimental trial was conducted in *in vivo* on *Actinidia deliciosa* cv. Hayward plants under controlled conditions. The study tested the following commercial products: Amylo-X® LC (*Bacillus amyloliquefaciens* strain D747), Remedier (*Trichoderma gamsii* ICC 080 and *T. harzianum* ICC 012), and the microbiological consortium Micosat F® (hereinafter referred as T1, T2, and T3). Additionally, the trial assessed three isolates from the University of Udine microbiological collection: *Pseudomonas asplenii* strain 4A7 (T4), *Pseudomonas protegens* strains 4 and 11 (T5 and T6, respectively). Vermicompost soil amendment (T7), foliar applications of potassium phosphite (T8) and untreated control plants (T9) completed the experimental set-up. In March, before sprouting, eight kiwifruit plants for each treatment were transplanted in KVDS inducing soil (soil sampled nearby roots of decaying plants in an orchard with presence of KVDS since 2017). At transplanting, test plants of treatments T1, T2, T3, T4, T5 and T6 were drenched by a solution of biocontrol agents (BCAs) at the maximum label dosage, while in T7 1 % W/W of cow dung based vermicompost was amended into the soil. Drenching of the pots with BCAs was repeated one month after transplanting. In T8, foliar treatments with potassium phosphite were applied three times, every 10 days starting one month after sprouting. The plants were grown in greenhouse under optimal conditions for two months; then, three 24-hour waterlogging events were subsequently applied once a week. The plants have been constantly monitored for symptoms appearance, the experiment ended in June when 80 % of the most affected group of plants showed typical KVDS symptoms. At the end of the experiment, symptoms on the canopy were assessed according to a visual score: 0 = no symptoms, 1 = symptoms on less than 50 % of leaves, 2 = symptoms on more than 50 % of leaves, 3 = irreversible plant wilting.

First symptoms on the canopy appeared at the end of May, ten days after the first event of waterlogging. Irreversible wilting occurred in 88 % of T1 plants, 75 % of T2, T3 and untreated control, 62.5 % of T6, 50 % of vermicompost-treated plants, and 37.5 % of T4. Interestingly, neither T4 nor T8 plants displayed KVDS symptoms on the canopy. Biometric data of canopy growth and canopy dry weight suggested that vermicompost and all microbiological based products enhanced the aerial growth of the plants in the following order: vermicompost > T1 = T2 = T3 > T4 = T5 > T6 = T8= untreated plants. Visual inspection of the

roots evidenced the lower percentage of damaged roots in T8 and T4 (mean % of damaged roots: 11 and 18.6, respectively). Treatments T1, T2, T3 and T6 did not show different levels of root protection compared to the untreated control (% of damaged roots: 57.1, 50, 62, 61.4, 61.5, respectively). In T7 and T5 37.1 % and 46.2 % of roots were compromised. Overall, the best levels of protection were achieved with the potassium phosphite and *Pseudomonas asplenii* strain 4A7 treatments. Specifically, foliar treatments with a potassium phosphites-based product led to the best control of KVDS, confirming indirectly the pivotal role of oomycetes in KVDS aetiology. In fact, as reported in literature, phosphite anions are active compounds involved in the suppression of *Phytophthora* spp. in several pathosystems (Nyoni et al., 2021). Anyway, it will be necessary to evaluate the feasibility of using phosphite-based products (not allowed in organic farming) against KVDS both to confirm their efficacy in the field and for their residual persistence in kiwifruits. The commercial BCA-based products, despite their positive effects on plant growth under optimal conditions, did not outperform the untreated control under KVDS-predisposing conditions. Furthermore, for some of them, the imbalance caused by an enhanced growth of the canopy and a negligible protective effect against root rot, probably accelerated the onset of symptoms, highlighting the need to identify specific BCAs with improved activity for KVDS pathosystem. In this regard, the perspectives for the use of *Pseudomonas asplenii*, a microorganism isolated as an endophyte in kiwi, is a promising tool against KVDS. Its ability to colonize micropropagated *Actinidia deliciosa* plantlets through the roots and to remain inside the plant for several weeks after inoculation had already been demonstrated in the past (Ferrini et al., 2019).

Key words: Oomycetes, KVDS, *Actinidia deliciosa*, endophytes

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References

Donati, I., Cellini, A., Sangiorgio, D., Caldera, E., Sorrenti, G., and Spinelli, F. 2020. Pathogens associated to kiwifruit vine decline in Italy. Agriculture 10: 119.

Ferrini, F., Borselli, S., Martini, M., Frausin, C., Zanini, G., Fanna, G., Loi, N., and Ermacora, P. 2019. First attempts to obtain *Actinidia deliciosa* plants inoculated with endophytes antagonistic against Psa. Acta Hortic. 1243: 29-32. DOI: 10.17660/ActaHortic.2019.1243.5

Nyoni, M., Mazzola, M., Wessels, J. P. B., and McLeod, A. 2021. Phosphonate treatment effects on *Phytophthora* root rot control, phosphite residues and *Phytophthora cactorum* inoculum in young apple orchards. Plant Dis. 105: 3835-3847.

Savian, F., Ginaldi, F., Musetti, R., Sandrin, N., Tarquini, G., Pagliari, L., Firrao, G., Martini, M., and Ermacora, P. 2020. Studies on the aetiology of kiwifruit decline: interaction between soil-borne pathogens and waterlogging. Plant and Soil 456: 113-128.

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Antagonistic interactions between maize seeds microbiome species and the late wilt disease agent, *Magnaporthiopsis maydis*

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Abstract: *Magnaporthiopsis maydis* late wilt disease (LWD) causes severe damage to commercial corn fields in the late growth stages. This study uncovered *M. maydis* interactions with newly identified maize endophytes. To this end, six fungi were isolated from the seeds of three sweet corn cultivars having varying susceptibility to LWD. These isolates were identified using colony morphology and microscopic characterization, universal internal transcribed spacer (ITS) molecular targeting, and phylogenetic analysis. Most of them belonged to pathogenic species. Compared to three previously identified bioprotective microorganisms, the new species were tested for their ability to secrete metabolites that repress *M. maydis in vitro* and to antagonize it in a solid media confront test and a seedlings pathogenicity assay. The opportunistic fungal species *Aspergillus flavus* (ME1), *Aspergillus terreus* (PE3), and the reference biocontrol bacteria *Bacillus subtilis* (R2) achieved the highest *M. maydis* inhibition degree in the plates' tests (74-100 % inhibition). The seedlings' pathogenicity assay that predicts the seeds' microflora resistance to *M. maydis* highlighted the bio-shielding potential of most species (23 % or more epicotyl elongation over the infected control). *Fusarium* sp. (ME2) was the leading species in this measure (43 % enhancement), and *B. subtilis* gave the best protection in terms of seeds' germination (50 %) and sprouts' biomass (34 %). The results of this study could enhance our understanding of the pathobiome's role in the context of LWD and represent a first step in using the seeds' natural protective microflora to develop novel management strategies.

Key words: crop protection, endophytes, fungus, plate confrontation, secreted metabolites

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Exploring the possibility of incorporating *Ganoderma* biomass to improve the efficacy of *Scytalidium parasiticum* and *Clonostachys rosea* in reducing basal stem rot (BSR) disease

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Abstract: *Scytalidium parasiticum* (Sp) and *Clonostachys rosea* (Cr) were reported to be associated with *Ganoderma boninense*, with the former found in culture and the latter in infected substrate. Additionally, Sp and Cr have been observed to be mycoparasitic and antagonistic fungi, respectively. Studies have reported that the tissues of insect and plant hosts improve the pathogenicity of entomopathogenic fungi and plant pathogens. This study aims to explore the possibility of mixing inactivated *Ganoderma* biomass with maize substrate to formulate inocula of Sp and Cr to establish the efficacy of these selected biocontrol agents in reducing basal stem rot (BSR) disease in the nursery. Autoclaved *Ganoderma* biomass was mixed with non-inoculated maize at different ratios, namely 1 %, 5 %, 10 %, 15 %, and 20 %, prior to inoculating Sp and Cr for two weeks. Both Sp and Cr formulations were screened in the nursery experiment with oil palm seedlings challenged with artifically-inoculated *Ganoderma* inoculum. *Ganoderma* disease incidence and severity indices were recorded at monthly intervals. At 6.5 months after transplanting (MAT), treatments with Sp (5 % *Ganoderma* biomass) and Sp (without *Ganoderma* biomass) showed a 99.3 % and 85.3 % disease reduction compared to the control with non-inoculated maize, respectively. Sp (5 % *Ganoderma* biomass) demonstrated a better disease reduction compared to Sp with 0 %, 10 %, and 15 % *Ganoderma* biomass. Conversely, treatments with Cr mixed with 5 %, 10 %, and 15 % *Ganoderma* biomass showed no observed disease reduction. The results indicate that the addition of *Ganoderma* biomass improves the efficacy of Sp in reducing *Ganoderma* BSR disease, but not Cr.

Key words: antagonist, *Ganoderma boninense*, inoculum, mycoparasite

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First biological control tests of *Neopestalotiopsis clavispora* in Costa Rica

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Abstract: Crown rot of strawberry caused by *Neopestalotiopsis clavispora* is a serious disease. This pathogen infects several crops, including apple, crabapple, sweet cherry, grape, peach, pear and ornamental plants. The use of synthetic pesticides contributes to loss of biodiversity and risks to both aquatic and terrestrial environments, poses risks for human health. Biological control using antagonistic fungi is a promising strategy for managing *N. clavispora*. Three strains of *Trichoderma asperellum*, *T. viride*, and *T. longibrachiatum* were provided by Garsol Biological Laboratory. In the dual tests, substrate competition was classified as class II according to the scale used for the three *Trichoderma* species. The contact time was 96 hours for *T. asperellum* and *T. viride*, but it was faster (72 hours) for *T. longibrachiatum*. Growth inhibition percentages (PCI) for antibiotics ranged from 70.55 % to 81.38 %, with *T. longibrachiatum* showing the highest values (81.38 %) and significant differences in all confrontations. This is the first biological control test of *N. clavispora* causing crown rot of strawberry in Costa Rica. This pathogen could pose a serious and emerging threat to several crops, including blackberry, strawberry, and ornamental plants.

Key words: crown rot of strawberry, biological control, *Trichoderma*

Introduction

Strawberry (*Fragaria* x *ananassa* Duch.) is cultivated worldwide, with Costa Rica being one small producer in Latin America. In Costa Rica, fruit is primarily grown in areas with volcanic soils around the Central Valley, with plantations typically covering less than one hectare (ha). The provinces with the largest number of producers involved in this activity are Cartago (39 %), Alajuela (22 %), San José (21 %), Heredia (16 %), and Puntarenas (3 %). However, the occurrence of plant pathogens poses a significant challenge to the sustainable and healthy development of the local strawberry industry.

In recent years, this crop has been affected by a disease known as "crown rot" which is considered devastating because it causes the progressive death of plants from the early stages (Mariscal et al., 2017). The most common symptoms of this disease include wilting of the plant, a reddish interveinal coloration of the leaves, which eventually turns brown to coffee-colored in older leaves, as well as poor vegetative development (Hidrobo et al., 2021). In September 2022, we observed the following symptoms in three-month strawberry plantations (cv. San Andreas) in three Costa Rica provinces: Alajuela, Cartago and San José; a crown rot and dieback disease and a brown vascular discoloration on the edges of the leaflets of older leaves, with the formation of generalized necrotic lesions. *N. clavispora* has been reported as the causal organism of strawberry root rot in Italy (Gilardi et al., 2019). Seven representative isolates were selected for further study. Based on morphological characteristics and phylogenetic analyses of the combined internal transcribed spacer (ITS) region and the transcription elongation factor 1-α (tef1), all isolates were identified as *N. clavispora*. In this study, a root rot disease accompanied by plant wilting in strawberry was observed in Costa Rica. The objective of the study was to identify the causal agent of the disease using morphological features and molecular analysis, and to evaluate *Trichoderma* as a biocontrol agent against this pathogen.

Materials and methods

Pathogen isolation

Samples were taken from symptomatic plants, the crowns were cut into small pieces (5 × 5 mm), disinfected with 75 % ethanol for 30 s, 0.5 % 22 NaClO for 1 min and rinsed twice with sterile distilled water. The pieces were plated on potato dextrose agar (PDA) plates, which were incubated at 26 °C (± 2 °C) in the dark. Single spore isolation was performed to obtain pure culture as described by Chakravarthi et al. (2020).

Pathogen identification

Based on morphological characteristics and phylogenetic analyses of the combined internal transcribed spacer (ITS) region and the transcription elongation factor 1-α (tef1). Extraction, purification and polymerase chain reactions (PCR) were carried out in the Molecular Biology Laboratory. The PCR products were purified and sequenced by Macrogen (Korea). Sequences were deposited in GenBank under accession numbers PP957625, PP957624, PP957623, PP957622, PP957621, PP957619 and PP957620.

Trichoderma isolates

Three *Trichoderma* isolates, *T. asperellum*, *T. viride*, and *T. longibrachiatum*, were collected from Garsol Biological Laboratory.

Screening by dual culture method

One method was followed for dual culture technique. A disc (5 mm) was taken from 7-day-old PDA culture plates of each *Trichoderma* isolate and placed at the periphery of the PDA plates (9 mm). Another agar disc of the same size of *N. clavispora* was also placed at the periphery but on the opposing end of the same Petri dish. As a control, *N. clavispora* was placed in a simi-manner on a fresh PDA plate. All pairings were carried out in quadruplicate and incubated at 26 °C ± 2 °C. Antagonistic activity was tested 7 days after incubation by measuring the radius of the *N. clavispora* colony in the direction of the antagonist colony (R2) and the radius of the *N. clavispora* colony in the control plate (R1). The two readings were transformed into percentage inhibition of radial growth (PIRG) using the formula developed by Skidmore and Dickinson (1976):

$$\text{PIRG} (\%) = [(R1-R2) / R1] \times 100$$

The growths of the *N. clavispora* and *Trichoderma* isolates were observed and their antagonistic activities were evaluated based on 5 class scale proposed by Bell et al.,1982.

Results and discussion

N. clavispora isolates

Seven were obtained from infected strawberry plants identified. Based on cultural and morphological characteristics, including whitish colonies with smooth and black globular acervuli with slimy spore masses. Colonies reached 90 mm diameter after 8 days of incubation. Conidia were fusiform to clavate with 5-celled segmentation with darker median cells and hyaline end cells (Prasannath et al., 2020). Molecular identification was performed using the partial region of translation elongation factor 1-alpha (tef1) with its corresponding primers EF1-526/EF1-1567 (Maharachchikumbura et al., 2012).

Screening by dual culture technique.

Each *Trichoderma* isolate inhibited the radial mycelial growth of *N. clavispora*. The percentage inhibition of radial growth (PIRG) values ranged from 70.55 to 81.38 % (Table 1). The highest PIRG values (81.38) were observed with *T. longibrachiatum* and the lowest recorded (70.55 %) were observed with *T. viride*. Colony overgrowth times varied from 6 to 9 days (Table 1). The minimum colony overgrowth time recorded was for *T. longibrachiatum*.

Table 1. Mean PIRG values and colony time contact of *Trichoderma* isolates against *N. clavispora* by dual culture method.

***Trichoderma* isolates**	**Mean % inhibition of radial growth (PIRG)**	**No. of days to contact *N. clavispora* colony**	**No. of days to overgrow *N. clavispora* colony**
T. asperellum	76.66 b	4	8
T. viride	70.55 c	4	8
T. longibrachiatum	81.38 a	3	6

The *N. clavispora* strain was given 1 day advantage because of its slow growth compared to *Trichoderma.* Most of the *Trichoderma* isolates showed a visible overgrowth zone with the hyphae of *N. clavispora*; the greater the area of overgrowth, the greater the aggressiveness of the antagonistic fungus (Benhamou and Chet, 1993).

In this sense, Olivares-Rodriguez et al., 2024, reported antagonism II and III of *Trichoderma* sp. against *Neopestalotiopsis* sp.

The findings indicated that all strains of *Trichoderma* inhibited the growth of *N. clavispora* to varying extents. Olivares-Rodriguez et al. (2024) observed that the fifteen isolates of *Trichoderma* spp. had differing capabilities to suppress *N. clavispora*, likely due to competition and/or the production of antifungal metabolites. Our study demonstrated that *Trichoderma* spp. effectively reduced *N. clavispora* growth in dual culture assays by growing rapidly and covering the pathogen colonies. Competition for nutrients and space emerged as a key mechanism in their antifungal activity. The rapid growth of *Trichoderma* spp. allowed them to dominate resources *in vitro*, as shown by the dual culture assay, a common method for evaluating pathogen antagonism. This suggests that *T. asperellum, T. longibrachiatum*, and *T. viride* exhibited competitive mechanisms to inhibit *N. clavispora*, which causes crown rot in strawberries. Additionally, Athinuwat et al. (2024) identified *T. virens* as a potential biological control agent against *N. clavispora*, employing mechanisms such as antibiosis through volatile antifungal compounds. Our study highlights the potential of *Trichoderma* in managing *N. clavispora* and supporting plant health.

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References

Athinuwat, D., Ruangwong, O. U., Harishchandra, D. L., Pitija, K., and Sunpapao, A. 2024. Biological Control Activities of Rhizosphere Fungus *Trichoderma virens* T1-02 in Suppressing Flower Blight of Flamingo Flower (*Anthurium andraeanum* Lind.). Journal of Fungi 10(1): 66. https://doi.org/10.3390/jof10010066

Benhamou, N., and Chet, I. 1993. Hyphal interactions between *Trichoderma harzianum* and *Rhizoctonia solani*: Ultrastructure and gold cytochemistry of the mycoparasitic process. Phytopathology 83: 1062.

Chakravarthi, B. V., Singh, S., Kamalraj, S., Gupta, V. K., and Jayabaskaran, C. 2020. Evaluation of spore inoculum and confirmation of pathway genetic blueprint of T13αH and DBAT from a Taxol-producing endophytic fungus. Scientific Reports 10(1): 21139.

Gilardi, G., Bergeretti, F., Gullino, M. L., and Garibaldi, A. 2019. First report of *Neopestalotiopsis clavispora* causing root and crown rot on strawberry in Italy. Plant Disease 103(11): 2959-2959.

Hidrobo, J., Ramírez-Villacis, D., Barriga-Medina, N., Herrera, K., and León-Reyes, A. 2021. First Report of *Neopestalotiopsis mesopotamica* causing root and crown rot on strawberry in Ecuador. Plant Disease. https://doi.org/10.1094/PDIS-06-21-1278-PDN

Maharachchikumbura, S. S., Guo, L. D., Cai, L., Chukeatirote, E., Wu, W. P., Sun, X., and Hyde, K. D. 2012. A multi-locus backbone tree for *Pestalotiopsis*, with a polyphasic characterization of 14 new species. Fungal Diversity 56: 95-129. https://doi.org/10.1007/s13225-012-0198-1

Mariscal-Amaro, L. A., Rivera-Yerena, A., Dávalos-González, P. A., and Ávila-Martínez, D. 2017. Situación actual de hongos asociados a la secadera de la fresa (*Fragaria* × *ananassa* Duch.) en Guanajuato, México. Agrociencia 51(6): 673-681.

Olivares-Rodriguez, G., Angeles-Núñez, J. G., Mondragón-Rojas, F., Rivas-Valencia, P., et al. 2024. Control *in vitro* de *Neopestalotiopsis* sp. aislada de fresa empleando *Trichoderma* y fungicidas comerciales. Revista Mexicana de Fitopatología 42(4): 52. https://doi.org/10.18781/R.MEX.FIT.2024-28

Prasannath, K., Galea, V. J., and Akinsanmi, O. A. 2020. Characterisation of leaf spots caused by *Neopestalotiopsis clavispora* and *Colletotrichum siamense* in macadamia in Australia. European Journal of Plant Pathology 156(4): 1219-1225.

Skidmore, A. M., and Dickinson, C. H. 1976. Colony interactions and hyphal interference between *Septoria nodorum* and phylloplane fungi. Transactions of the British Mycological Society 66(1): 57-64.

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Impact of BVOCs from *Lysinibacillus fusiformis* and LED irradiation on pigment metabolism in stored broccoli

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Abstract: Volatile organic compounds (BVOCs) produced by bacteria have been shown to influence the postharvest metabolism of fruits and vegetables. In this study, the quality, color, and antioxidant properties of broccoli stored under treatment with BVOCs from *Lysinibacillus fusiformis*, combined with white LED light irradiation, were effectively preserved. A combined transcriptomic and metabolomic analysis of broccoli exposed to this LED-BVOC treatment revealed 49 differentially expressed genes (DEGs) and 13 differentially abundant metabolites (DAMs) associated with photosynthesis, chlorophyll, carotenoid, and flavonoid metabolism. Specifically, 32 DEGs related to photosynthesis were upregulated, while chlorophyll metabolism saw 7 DEGs and 1 DAM related to chlorophyll, and 5 DEGs and 1 DAM related to carotenoid metabolism. The preservation of the green color in harvested broccoli treated with LED-BVOC was linked to DEGs and DAMs that suppressed chlorophyll breakdown and promoted carotenoid accumulation. These findings offer valuable insights into the potential of BVOC-LED technology for delaying the senescence of broccoli during storage.

Key words: BVOCs, LED, broccoli, *Lysinibacillus fusiformis*, metabolism

On the hunt – Identifying endophytic bacteria against apple proliferation disease

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Abstract: Apple proliferation (AP) is one of the most serious apple (*Malus domestica*) diseases caused by phytoplasmas in Europe. The disease is associated with the bacterium '*Candidatus* Phytoplasma mali' (AP phytoplasma) belonging to the class of Mollicutes. An infection causes a wide range of symptoms, including so called witches'-brooms, rosettes, enlarged stipules, leaf reddening, reduced growth and reduced fruit size. To date, in the absence of varieties resistant to this disease, management of AP relies mainly on the use of insecticides to control vector insects and eradication of infected plants. However, these interventions incur significant economic costs and negative environmental impacts. Endophytic bacteria are a promising phytosanitary tool in agriculture. These bacteria can enhance plant growth and health, e. g., by improving nutrient uptake, modulating growth-related hormones, and controlling pathogen growth. We hypothesize that AP phytoplasma infected, but asymptomatic apple trees -i.e. trees in which the phytoplasma are only present in the roots but not in the upper parts of the tree- contain certain microorganisms that prevent the phytoplasma to colonize the apple tree's canopy. To analyze this, bacteria were isolated from roots, shoots, leaves and the rhizosphere of AP infected, but asymptomatic apple trees. These bacteria were then identified via 16S rRNA gene sequencing. The four most abundant bacteria genera in samples from asymptomatic trees were *Bacillus*, *Micromonospora*, *Pseudomonas* and *Streptomyces*. No dominant bacterial genera could be determined in samples from symptomatic trees. The aim of this study is to identify endophytic bacteria and analyze their potential as biocontrol agents against AP phytoplasma. To test their plant-growth-promoting abilities, more than hundred bacterial isolates from asymptomatic trees were analyzed for their biochemical properties such as auxin production, phosphate solubilization and siderophore production. For this reason, the potential inhibitory activity of isolated endophytes was tested on a phylogenetically related, but cultivatable Mollicutes, i. e., *Spiroplasma melliferum*. A sensitive and reliable assay based on alamarBlue® was established to screen the antagonistic activities of the filtrates produced by the isolated endophytes. Based on our results and in combination with a thorough literature review we identified five endophytes that i) produce a filtrate with marked ability to inhibit the growth of the model-microorganism *S. melliferum* and ii) have been documented in the literature to have microbial biocontrol activity. Due to their promising characteristics, these isolates will be further analyzed regarding their potential as microbial biocontrol agents against AP phytoplasma.

Key words: phytoplasma, endophytes, apple, spiroplasma, microbial biocontrol

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Control strategies combining calcium oxide and biochar for effective management of *Phytophthora nicotianae* in tomato

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Abstract: *Phytophthora nicotianae* is a highly significant plant pathogen worldwide, known for its polyphagous nature and its ability to cause severe economic losses across various crops. In Italy, it is particularly damaging to tomato production, posing a substantial threat to both yield and quality. Currently, the management of this pathogen relies predominantly on chemical control methods, but their continuous and repeated use can have negative effects on both the environment and human health.

Consequently, there is an increasing demand for more sustainable and eco-friendly alternatives to mitigate the damage caused by this pathogen.

Among these alternatives, calcium oxide and biochar have shown promise due to their ability to stimulate plant defense mechanisms. Calcium oxide and biochar have demonstrated efficacy against various plant pathogens; however, their potential against *Phytophthora nicotianae* on tomato remains unexplored.

This study evaluated their potential through three application methods: (1) nursery treatment on seedlings, (2) pot application at transplanting, and (3) a combination of both approaches. Experimental trials were conducted under controlled greenhouse conditions. A first set, consisting of three trials, assessed the efficacy of calcium oxide and biochar applied individually. A second set of three trials evaluated potential synergistic effects by combining the two treatments across the three application methods.

Tomato plants were grown in 1.5 l pots containing a peat substrate, 2 plants were placed in each pot and replicated 5 times for each treatment. The substrate was infested with the pathogen by mixing 1 g/l of infested kernels.

Disease incidence and severity were recorded to evaluate the efficacy of treatments. Results revealed that the use of calcium oxide or biochar individually was effective in reducing disease incidence and severity. However, the efficacy of the products in controlling *P. nicotianae* was significantly influenced by the method of application. No evident synergistic effect was observed when the two treatments were combined.

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Key words: root rot, greenhouse, disease control, suppressiveness

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Characterization of *Stemphylium vesicarium* isolates from pear orchards in Emilia Romagna (Italy) and assessment of potential microbial biocontrol agents

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Abstract: Brown spot of pear caused by *Stemphylium vesicarium* is one of the most important fungal diseases in Europe, as it may cause more than 90 % yield losses. A set of ten *S. vesicarium* isolates from diseased pear fruits cv. Abbé Fétel, collected in Emilia-Romagna orchards, was molecularly identified, showing high genetic similarity. At pathogenic level, *S. vesicarium* isolates showed two main degrees of virulence (i. e., "low" and "high"). The most virulent *S. vesicarium* isolate was then used for assessing the biocontrol of six bacterial isolates, by means of *in vitro* tests and detached pear fruit assays. The two most promising bacteria, belonging to the genera *Bacillus* and *Pseudomonas,* highlighted their potential as putative microbial biocontrol agents for implementing a sustainable control of *S. vesicarium*.

Key words: brown spot of pear, bacterial antagonists, biological control

Introduction

Stemphylium vesicarium (Wallroth) (Simmons, 1969), the causal agent of brown spot of pear (BSP), is responsible for important economic losses in the main producing areas of Italy, such as in the Emilia Romagna region, on the highly susceptible pear cultivar Abbé Fétel (Bugiani, 2022). Climatic changes, such as increased average temperatures and an altered rainfalls pattern, can foster *S. vesicarium* inoculum build-up and disease severity, therefore leading to an increased economic impact of this fungal disease (Moragrega et al., 2018). BSP integrated management includes sanitation practices (e. g., leaf litter removal), aimed to decrease the inoculum potential, and multiple fungicides sprays during the cropping season. However, the disease pressure and the development of resistance traits to chemical fungicides by *S. vesicarium*, call attention to the need for sustainable complementary or alternative strategies in the management of BSP (Soriato et al., 2024). Our study aimed to characterize *S. vesicarium* isolates from Emilia Romagna orchards, at a molecular and pathogenic level, and to search for carposphere epiphytes as possible microbial biocontrol agents (mBCAs).

Materials and methods

***Stemphylium vesicarium* isolation and identification**

Stemphylium spp. were isolated from diseased pear fruits, cv. Abbé Fétel, collected in summer 2023 and 2024 in four Emilia Romagna orchards. The isolates were morphologically characterized and molecularly identified by means of the partial amplification and sequencing of the internal transcribed spacer (*ITS*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and elongation factor (*EF*) regions (Cortiello et al., 2023). All sequences were subjected to BLASTn search (https://blast.ncbi.nlm.nih.gov/), followed by phylogenetic analyses through multiple-sequence alignment using the MEGA11 software.

***Stemphylium vesicarium* strains bioassay on detached fruits**

The BSP severity was assessed for each *S. vesicarium* isolate on detached fruits, according to a bioassay described by Kohl et al. (2009), with modifications. Three biological replicates were used, each consisting of two fruits.

Dual and double culture assays

The antimicrobial activity of a set of four bacterial isolates from the pear carposphere was tested *in vitro* against *S. vesicarium* Sv 2021, both in dual culture and double plate assays for direct antagonism and emission of volatile organic compounds (VOCs), respectively (Xhemali et al., 2023).

Bioassay on detached fruits

In a second bioassay on detached fruits, the efficacy of bacterial isolates to control BSP severity was assessed. Experimental conditions and procedures were similar to the first bioassay. Briefly, 40 µl droplets of bacterial suspension (1×10^8 CFU/ml) were spotted four times on the fruit surface; sterile distilled water was used as a control. After 24 hours, 40 µl of *S. vesicarium* Sv 2021 conidial suspension were spotted on each mBCAs pre-treated point on fruits.

Statistical analysis

The collected data were subjected to ANOVA and Tuckey's tests at $p \leq 0.05$, using SPSS 15.0 for Windows® (SPSS Inc., Chicago, IL).

Results and discussion

Identification and pathogenic characterization of Stemphylium vesicarium isolates

A total of 10 fungal isolates were collected from symptomatic pear fruits. For all isolates, (*i*) morphological characteristics of both colonies and spores were consistent with *S. vesicarium* (Simmons, 1969); (*ii*) BLAST analysis for ITS, GPDH and EF gene sequences showed 99-100 % identity with *S. vesicarium* ATCC 18521. Phylogenetic analysis revealed a high genetic similarity among the *S. vesicarium* strains, with nine isolates that clustered together with the ex-type strain *S. vesicarium* ATCC 18521$^{EX\text{-}T}$. The strain Sv 2261 clustered separately in a second clade, despite having a very low genetic distance (Figure 1 A). All ten *S. vesicarium* strains were able to reproduce the typical symptoms of the BSP on detached pear fruits, with 100 % incidence. The pathogenic characterization tests showed the presence of two *S. vesicarium* groups with "high" and "low" virulence (Figure 1 B). Three strains displayed a significantly lower virulence ($p < 0.05$) in comparison to others. The Sv 2021, belonging to a "high" virulence group and showing the higher disease score (13.84 ± 2.12), was selected for further experiments with mBCAs.

The genetic similarity and the presence of different degrees of virulence observed among our *S. vesicarium* isolates from pear were consistent with previous studies (Temperini et al., 2022).

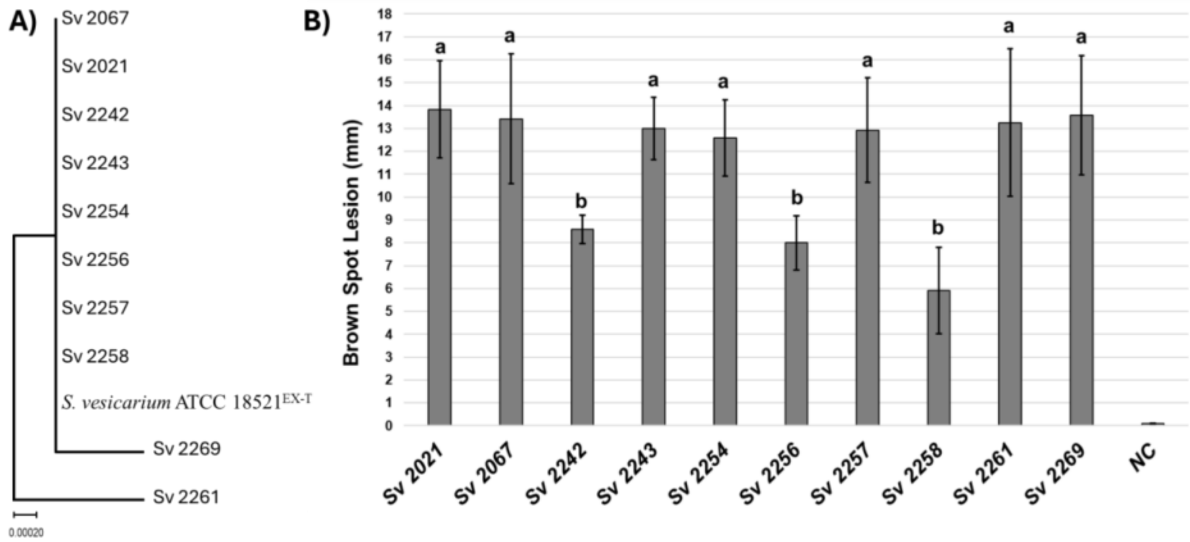

Figure 1. A) Phylogenetic tree based on combined ITS, GAPDH and EF sequences of the 10 *Stemphylium* isolates with the ex-type strain *S. vesicarium* ATCC 18521$^{EX\text{-}T}$. B) Evaluation of BSP disease severity on detached pear fruits infected with *S. vesicarium* isolates. The different letters indicate statistically significant differences among isolates according to the one-way ANOVA test and Tukey's HSD test ($p < 0.05$).

Biocontrol potential of bacterial isolates

The selected bacterial isolates were identified as members of the genera *Bacillus* (DLS188, DLS321 and DLS323) and *Pseudomonas* (DLS329) (Table 1). Regarding their direct antagonistic ability, dual plate assay showed that *Bacillus* sp. DLS321 was the most effective in reducing *S. vesicarium* growth (74.29 %). Meanwhile, *Pseudomonas* sp. DLS329 was the most performing in both double plate assay (VOCs inhibiting mycelial growth), with a reduction rate up to 29.89 % and bioassay on detached fruits (80.37 %).

Intriguingly, for these bacterial isolates the *in vitro* and pear fruit bioassay results were not always closely correlated. Therefore, further studies are needed to investigate the mechanisms by which these mBCAs might exert their adverse effects against *S vesicarium* (Kohl et al., 2020). Overall, these findings highlight the potential of strains *Bacillus* sp. DLS323 and *Pseudomonas* sp. DLS329 as promising biocontrol agents. Future experiments will focus on *in planta* validation to confirm their effectiveness in controlling BSP.

Table 1. Identity and effect of 4 bacterial isolates on *S. vesicarium* Sv 2021 mycelia growth inhibition (MGI) *in vitro* (dual and double plate assays) and BSP disease severity reduction on detached pear fruits. The different letters indicate statistically significant differences among treatments according to the one-way ANOVA test and Tukey's HSD test ($p < 0.05$).

Strain	**Identity (BLASTn 16S rRNA)**	**Dual plate MGI (%)**	**Double plate MGI (%)**	**BSP severity reduction on detached fruits (%)**
DLS188	*Bacillus* sp.	72.41[a]	16.67[c]	26.30[c]
DLS321	*Bacillus* sp.	74.29[a]	24.46[b]	11.48[c]
DLS329	*Pseudomonas* sp.	21.00[b]	29.89[a]	80.37[a]
DLS323	*Bacillus* sp.	70.11[a]	22.96[b]	33.33[b]

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References

Bugiani, R. 2022. Maculatura bruna del pero, Bilancio Fitosanitario 2020-21. Available at: https://www.giornatefitopatologiche.it/UserFiles/File/Bilancio_fitosanitario/Bilancio_fitosanitario_20202021/PERO_20_gennaio_2022/Bilancio-Pero-Maculatura-Bruna-Nord-Italia.pdf (retrieved on January 24th, 2025).

Cortiello, M., Prodi, A., Stefani, E., and Giovanardi, D. 2023. First report of *Stemphylium* leaf blight of onion (*Allium cepa*) caused by *Stemphylium vesicarium* in Italy. Plant Disease. https://doi.org/10.1094/PDIS-10-22-2398-PDN.

Köhl, J., Groenenboom-de Haas, B., Goossen-van de Geijn, H., Speksnijder, A., Kastelein, P., de Hoog, S., and van den Ende, B. G. 2009. Pathogenicity of *Stemphylium vesicarium* from different hosts causing brown spot in pear. Eur. J. Plant Pathol. 124: 151-162.

Moragrega, C., Puig, M., Ruz, L., Montesinos, E., and Llorente, I. 2018. Epidemiological features and trends of brown spot of pear disease based on the diversity of pathogen populations and climate change effects. Phytopathology 108(2): 223-233.

Simmons, E. G. 1969. Perfect states of *Stemphylium*. Mycologia 61: 1-26.

Soriato, E., Gatta, M., Danzi, D., Casagrande, M., Cerrato, A., Cucchi, F., and Vandelle, E. 2024. Characterization of autochthone biological control agents for pear protection against the brown spot of pear disease caused by *Stemphylium vesicarium*. Journal Plant Pathology. https://doi.org/10.1007/s42161-024-01788-9.

Temperini, C. V., Tudela, M. A. A., Gimenez, G. N., Di Masi, S. N., Pardo, A. G., and Pose, G. N. 2022. Brown spot of pear, an emerging disease in Argentina: identification and pathogenicity characterization of Argentinean *Stemphylium vesicarium* isolates. Eur. J. Plant Pathol. 163: 529-544.

Xhemali, B., Cortiello, M., Gjinovci, G., Bresilla, B., Modica, F., Stefani, E., and Giovanardi, D. 2023. First finding in Europe of *Colletotrichum scovillei*, a new agent of pepper anthracnose and assessment of potential bacterial biocontrol agents. In: Proceeding of the XIV International Scientific Agriculture Symposium "AGROSYM 2023", Jahorina, Bosnia and Herzegovina, October 05-08, 2023, pp. 712-717.

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Grapevine endosphere as a source of potential novel biocontrol agents of grapevine trunk disease pathogens

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Abstract: Grapevine trunk diseases (GTDs), caused by different fungal pathogens, are major threats to grapevine (*Vitis vinifera*) cultivation in European countries, causing severe economic losses. Despite significant knowledge about GTD etiology and epidemiology, no curative methods are available, and prevention remains the most effective approach for managing these diseases. Grapevine microbiome can be a source of novel biocontrol agents (BCA) that can antagonize GTD agents. Endophytic microorganisms could establish a mutually beneficial interaction with the plant host and may act as grapevine pruning wound protectants against GTD pathogens. Fungal and bacterial endophytic strains were isolated from grapevine trunk samples of cv. Arneis, Barbera, Cabernet Sauvignon, Sauvignon Blanc and Nebbiolo. The antagonistic potential of 37 fungal and 42 bacterial endophytic strains, was preliminary assessed through an *in vitro* dual culture assay to inhibit the main causal agents of GTD such as *Botryosphaeria dothidea, Diplodia seriata, Neofusicoccum parvum* and *Phaemoniella chlamydospora*. The effectiveness of the most promising strains was further tested through a detached cane assay to control the same pathogen species and was compared with the biofungicide Vintec® (*Trichoderma atroviride* SC1, Certis Belchim) and the fungicide Signum® (pyraclostrobyn + boscalid, BASF). This study provides new tools to sustainably control GTD.

Key words: *Vitis vinifera*, Esca disease, *Botryosphaeria* dieback, biological control, endophytes

Integrating hot water treatment and biocontrol agents: Impacts on grapevine trunk diseases and wood microbiome dynamics

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Abstract: Grapevine trunk diseases (GTDs) are caused by different pathogens, such as *Neofusicoccum parvum* (Np) and *Phaeomoniella chlamydospora* (Pch), representing a threat to viticulture (Mondello et al., 2018; Dardani et al., 2023). This study investigates a novel integrated approach combining hot water treatment (HWT) with the application of commercial biocontrol agents (BCAs) including *Trichoderma* and *Pythium* species to manage GTDs. Barbera grapevine cuttings grafted on Kober 5BB were used. Two plant groups were established: thermo-treated (T) and non-thermo-treated (NT). Both groups received BCA treatments and were compared to a chemical treatment (pyraclostrobyn + boscalid) and a positive control (pathogen-inoculated, untreated plants). Cuttings were inoculated with Np and Pch under controlled greenhouse conditions. To evaluate disease severity (DS), final disease assessment was performed by measuring necrotic lesion length caused by Np and Pc five months post-inoculation. Preliminary results indicate that HWT-BCA combinations may significantly reduce GTD severity compared to the positive control. Specifically, *Trichoderma* and *Pythium* based treatments showed significant efficacy in reducing disease severity, particularly when applied to thermo-treated plants, suggesting a synergistic effect between HWT and BCAs. Metagenomic analysis of the wood microbiome at inoculation sites revealed alterations in microbial community composition in response to treatments. These findings suggest that the integrated approach may not only directly suppress pathogens, but also promote the establishment of a protective microbiome, enhancing plant health and disease resistance. This study has significant implications for sustainable grapevine disease management by promoting the use of eco-friendly and integrated control strategies.

References

Dardani, G., Mugnai, L., Bussotti, S., Gullino, M. L., and Guarnaccia, V. 2023. Grapevine dieback caused by Botryosphaeriaceae species, *Paraconiothyrium brasiliense*, *Seimatosporium vitis-viniferae* and *Truncatella angustata* in Piedmont: characterization and pathogenicity. Phytopathologia Mediterranea 62(2): 283-306. doi: 10.36253/phyto-14673

Mondello, V., Songy, A., Battiston, E., Pinto, C., Coppin, C., et al. 2018. Grapevine Trunk Diseases: A Review of Fifteen Years of Trials for Their Control with Chemicals and Biocontrol Agents. Plant Disease 102(7): 1189-1217. doi: 10.1094/PDIS-08-17-1181-FE.

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Assessment of biological products as promising solutions against apple bitter rot and *Glomerella* leaf spot in apple orchards

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Abstract: Apple bitter rot (ABR) and *Glomerella* leaf spot (GLS), caused by *Colletotrichum* spp., are two destructive apple tree (*Malus domestica*) diseases, affecting both fruits and leaves. Although *Colletotrichum* species are widely spread in all apple growing areas, ABR and GLS are emergent in Europe context. In Italy, they are particularly damaging apple orchards with huge economic losses, mainly in organic farms where the use of several chemical products is restricted or denied. In this perspective, this study aims to evaluate the *in vitro* and *in vivo* efficacy of three commercial biological products (based on *Bacillus amyloliquefacens* strain D747, potassium bicarbonate and sulphur), compared with chemical ones, against five *Colletotrichum* strains (*C. chrysophilum* C101*, C. grossum* C38 and *C. siamense* C32 in *Gloeosporioides* SC, *C. fioriniae* C125 in *Acutatum* SC and *C. sojae* C116 in *Orchidearum* SC) generally involved in ABR and GLS. *In vitro* screening of the products tested at field concentration revealed interesting growth inhibition rates (GI %) of the colonies. The most performing were *Bacillus amyloliquefacens* and potassium bicarbonate, with GI rates between 87.3 % and 93 % for the latter, while *B. amyloliquefacens* reported more variability among species belonging to different complexes, with a GI of 67.4 % in *C. siamense* C32 and 91.3 % in *C. fioriniae* C125. Conversely the sulphur exhibited lower GI values, with the maximum rates in *C. siamense* C32 and *C. grossum* C38 (47 % and 47.6 %, respectively) and the minimum one in *C. fioriniae* C125 (21.4 %). In 2024, the same products were evaluated in open field trial in Tresigallo (FE) on Gala apple trees. The objective was to determine their efficacy under natural conditions by assessing disease incidence (%) on both fruit and leaves. Four trees for each thesis were inoculated with two different *Colletotrichum* species at 10^5 spores/ml and treated with 10 applications of the products between BBCH 74 and BBCH 79 stages. On apple fruits, all biological products showed an incidence below 10 %, compared to the control at 40 %, while on apple leaves the incidence was around 40 % and 70 % on the untreated thesis. These preliminary results demonstrate that biological products could be a promising solution to contain diseases in organic apple orchards.

Key words: *Malus domestica*, apple bitter rot, *Glomerella* leaf spot, biological control

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Biological control of black rot (*Guignardia bidwellii*) with microbial antagonists in fungal resistant grapevine cultivars

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Abstract: *Guignardia bidwellii* (anamorph: *Phyllosticta ampelicida*) causes sporadic outbreaks of black rot in grapevine, but the disease is of relatively low economic importance in European viticulture – so far. It is commonly suppressed as a side effect of fungicides targeting the economically more important grapevine pathogens *Plasmopara viticola* and *Erysiphe necator*, the causing agents of downy and powdery mildew, respectively. In recent years, the increasing cultivation of fungal resistant grapevine cultivars has led to a reduction in fungicide applications, enabling the emergence of other fungal pathogens for which no specific resistance loci have been introduced into the grapevine cultivars, such as *G. bidwellii*. However, resistance to black rot has been identified in some grapevine cultivars, with mapped resistance loci and observed reduced susceptibility in both greenhouse trials and in the field.

However, especially for integrated crop protection concepts that rely on preventive measures such as the cultivation of resistant varieties, effective yet environmentally friendly crop protection solutions are needed to prevent black rot infections in susceptible cultivars.

Therefore, the objectives of the present study were (1) to investigate the susceptibility of different fungal resistant grapevine cultivars to black rot under greenhouse conditions, (2) to screen for biocontrol agents with sufficient efficacy against black rot, with (3) a focus on fungal resistant grapevine cultivars of high and low black rot susceptibility.

In order to identify grapevine cultivars of different susceptibility to black rot, the disease symptoms of white and red fungal resistant grapevine cultivars with increased tolerance against downy and/or powdery mildew have been assessed in artificially inoculated potted plants in the greenhouse after 14 days of incubation. In accordance with observations in the field, *Vitis vinifera* 'Souvignier gris' and 'Satin noir', as high and low susceptible cultivars, respectively, have been chosen for the subsequent bioprotection experiments. In in-vitro dual culture assay, the direct inhibitory effect of commercially available living microbial biocontrol agents on mycelial growth and conidia germination of *P. ampelicida*, the asexual form of black rot, was evaluated. *Trichoderma atroviridae* and *Bacillus amyloliquefaciens* showed the highest inhibitory effect and have been selected for in-planta greenhouse trials.

Besides the living biocontrol agents, another series of experiments aimed at evaluating the biocontrol effect of non-living agents based on plant extracts.

Ongoing in-planta experiments in the greenhouse investigate whether the selected biocontrol agents show different biocontrol effects on differently susceptible grapevine cultivars and whether the agents are viable control measures against black rot.

This study contributes to the increasing interest in implementing microbial-based biocontrol solutions into integrated and sustainable plant protection concepts.

Key words: *Phyllosticta ampelicida*, integrated plant protection, biocontrol agents, microbial antagonists, grapevine pathogens, plant pathogenic fungi

Kosakonia cowanii SH2 and its application in growth promotion and enhanced tolerance to drought and root rot in soybean

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Abstract: Soil salinization, compaction, and drought severely influence agricultural production and food security. Root rot is a worldwide disease decreasing soybean yield and quality. In this study, we isolated and screened a multifunctional strain SH2, which was identified as *Kosakonia cowanii*. It produced indole-3-acetic acid (IAA) and showed high drought tolerance. Pot experimental results revealed that soybean growth was improved and drought tolerance enhanced by application of strain SH2 under drought stress conditions. Plant height, root length, and fresh weight increased by 38.44 %, 9.06 %, and 58.66 %, respectively. Furthermore, strain SH2 enhanced soybean tolerance to root rot with control efficacy of 64.40 %. This study lays the foundation for utilizing microorganisms to enhance drought resistance and root rot tolerance in soybean, and it provides a theoretical basis for the development and application of functional microbes. *K. cowanii* SH2 demonstrated the ability to produce indole-3-acetic acid (IAA), significantly promoting soybean growth and enhancing tolerance to drought and root rot.

Key words: *Kosakonia cowanii*, drought resistance, root rot, growth promotion

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Developing a systems approach for the control of pear scab – how to implement biocontrol?

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Extended abstract: European pear scab (*Venturia pirina,* also written as *V. pyrina*) is a significant economic concern in many regions worldwide where European pears (*Pyrus communis*) are grown, particularly in organic pear orchards. One of the main challenges in organic pear cultivation is the prevalence of scab-susceptible cultivars like 'Conference' (Sokolova et al., 2014; Timmermans and Jansonius, 2014). Furthermore, the management of pear scab is challenging due to limited availability of effective biological crop protection products and the pathogen's complex life cycle. Unlike the well-studied, *V. inaequalis*, the causal agent of apple scab, *V. pirina* has an additional inoculum source – conidia from twig lesions – that further contributes to the disease cycle.

Currently, fungicide applications are the primary strategy for managing pear scab in both organic and integrated pear cultivation systems. The timing and frequency of fungicide treatments depend on various factors, including weather conditions, the type of fungicides used, and the pear cultivar's susceptibility to scab. Protective fungicide sprays are typically applied in spring to prevent primary infections, mainly from ascospores. The early release of *V. pirina* ascospores may have a more significant impact on the disease's spread compared to *V. inaequalis*, the cause of apple scab (Creemers et al., 2013). Disease prediction systems are used to assess the infection risk and determine when protective fungicide applications are needed. While systems like RIMpro are based on current knowledge of *V. inaequalis*, they may not fully account for the earlier release of *V. pirina* ascospores. This difference may lead to underestimating or misjudging the infection risk when relying solely on apple scab models (Creemers et al., 2013). Therefore, reliable disease prediction systems specifically designed to predict *V. pirina* infections are essential for sustainable disease management and reducing fungicide use.

This research project aims to review the current understanding of pear scab biology and epidemiology, identify knowledge gaps, and conduct further studies to address these gaps. Ultimately the data produced during this research project will aid the validation and optimisation of a pear scab prediction model for the Dutch pear cultivation scenario. However, even with appropriate timing of fungicide application, the limitations of current crop protection products are unavoidable. A systems approach combines multiple management strategies, realizing that a single management strategy is not durable. Therefore, the overall objective of this research project is to create a toolbox of control measures, which appreciates a resistant cultivar as a basis to build on, takes into account sanitation practices and offers and optimised prediction model to aid efficient timing of crop protection products. Within such a systems approach, the potential of biocontrol strategies are appreciated.

In contrast to pear scab, biocontrol strategies have been investigated for various stages of the apple scab disease cycle. Given the parallels in the disease cycles, research on pear scab can benefit from insights gained on *V. inaequalis*. A common approach involves applying biocontrol agents in autumn to disrupt overwintering pseudothecia, leading to reduced ascospore numbers and lower disease severity in spring (Okoro et al., 2024). Control of conidia

has also shown promise, with studies highlighting the efficacy of compounds such as chitosan, laminarin-based Vacciplant (Rusevski et al., 2018), and *Yucca schidigera* extract (Bengtsson et al., 2009) in activating plant defences and inhibiting fungal development. While these findings offer a strong foundation, also challenges remain regarding the implementation of biocontrol strategies against apple scab. These challenges include application timing, translation of research findings into field application, commercialization, and investment constraints (Wenneker and Thomma, 2020). Nevertheless, biocontrol is increasingly recognized as a sustainable alternative to conventional fungicides (Belete and Boyraz, 2017). This contribution seeks to stimulate discussion on bridging the gap between biocontrol research and practical application for pear scab management.

Key words: fungal disease, pome fruit, bio-control, antagonists, *Venturia*

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References

Bautista-Baños, S., Hernandez-Lauzardo, A. N., Velazquez-Del Valle, M. G., Hernández-López, M., Barka, E. A., Bosquez-Molina, E., and Wilson, C. L. 2006. Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities. Crop protection 25(2): 108-118.

Belete, T., and Boyraz, N. 2017. Critical review on apple scab (*Venturia inaequalis*) biology, epidemiology, economic importance, management and defense mechanisms to the causal agent. J. Plant Physiol. Pathol. 5(2): 2.

Bengtsson, M., Wulff, E., Lyngs Jørgensen, H. J., Pham, A., Lübeck, M., and Hockenhull, J. 2009. Comparative studies on the effects of a yucca extract and acibenzolar-S-methyl (ASM) on inhibition of *Venturia inaequalis* in apple leaves. European Journal of Plant Pathology 124: 187-198.

Creemers, P., Vanmechelen, A., and Hauke, K. 2006. Sanitation practices to reduce apple scab inoculum in orchards. IOBC-WPRS Bulletin 29(1): 15-23.

Creemers, P., Hemelrijck, W. van, Keulemans, W., and Martens, D. 2013. Epidemiologisch onderzoek naar de etiologie van takschurft op peer als basis voor een rationele en ecologisch verantwoorde bestrijding. EINDVERSLAG IWT PROJECT 080496. Proefcentrum Fruitteelt Vzw Unit TWO Mycologie Fruittuinweg En Katholieke Universiteit Leuven Fruitteeltcentrum, 93 pp.

Croes, E., van Hemelrijck, W., de Landtsheer, A., Keulemans, W., and Creemers, P. 2010. Epidemiological research of twig scab on pear as basis for a rational and ecological disease management. Communications in Agricultural and Applied Biological Sciences 75: 621-625.

Okoro, C. A., El-Hasan, A., and Voegele, R. T. 2024. Integrating Biological Control Agents for Enhanced Management of Apple Scab (*Venturia inaequalis*): Insights, Risks, Challenges, and Prospects. Agrochemicals 3(2): 118-146.

Rusevski, R., Kuzmanovska, B., Petkovski, E., and Bandzo, K. 2018. Biological control of *Venturia inequalis* – the cause of apple scab in apple. Journal of Agricultural, Food and Environmental Sciences, JAFES 72(3): 16-18.

Sokolova, O., Moročko-Bičevska, I., and Bankina, B. 2014. Review of the pear scab caused by *Venturia pyrina*. Research for Rural Development 1: 26-33.

Timmermans, B. G. H., and Jansonius, P. J. 2014. Influence of infection parameters on pear scab dynamics in organic orchards in The Netherlands, 2010-2013. In: Ecofruit. Proceedings of the 16th International Conference on Organic-Fruit Growing, pp. 17-19. February 17-19, 2014, Hohenheim, Germany. Fördergemeinschaft Ökologischer Obstbau, Weinsberg.

Wenneker, M., and Thomma, B. P. H. J. 2020. Latent postharvest pathogens of pome fruit and their management: From single measures to a systems intervention approach. European Journal of Plant Pathology 156(3): 663-681.

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Streptomyces strains as biocontrol agents against fungal soilborne pathogens

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Abstract: The *Streptomyces* genus represents a good source of microorganisms, bioactive compounds, antibiotics, and extracellular enzymes active against phytopathogens (BCAs) and for promotion of plant growth (PGPAs). A specific strain of *Streptomyces albidoflavus*, CARA17, used as BCAs by colony and by culture filtrate (containing putative bioactive crude extracts), provided good abilities to contrast soilborne fungi, such as *Athelia rolfsii*, *Fusarium oxysporum*, *Plectosphaerella ramiseptata*, *Sclerotinia sclerotiorum* and *Verticillium dahliae*, both in *in vitro* and *in vivo* conditions. CARA17 strain, in dual culture, was significantly able to inhibit all fungi used with different degrees except *A. rolfsii*. Greenhouse experiments further demonstrated that CARA17 strain significantly reduced disease severity and promoted better plant growth in lettuce seedlings artificially inoculated with the above mentioned fungal pathogens.

Key words: biocontrol, soilborne fungi, *Streptomyces* sp.

Introduction

The *Streptomyces* genus represents an important source of bioactive compounds, antibiotics, and extracellular enzymes. It is undoubtedly the most abundant and important actinomycete used in agriculture, showing promise as both a biological control agent (BCA) (Colombo et al., 2019) and a plant growth promoting agent (PGPA) (Kunova et al., 2021; Basu et al., 2021). *Streptomyces* species have been reported to produce several phytohormones, such as auxins, gibberellins, ethylene, cytokinin-like chemicals, salicylic acid, jasmonic acid, abscisic acid, which play a vital role in promoting plant growth and helping plants respond to biotic threates (microbial diseases) and abiotic stresses (salinity, drought, and contaminants) (Etesami et al., 2018). Additionally, *Streptomyces* spp. have been studied for the production of secondary metabolites as active biocompounds. Since the use of chemicals (fungicides and fertilizers) in agriculture is limited in Europe due to public concern for the environment and human health, in recent years our studies have focused on the evaluation of alternative means to control serious fungal pathogens responsible for important diseases of horticultural crops such as fennel and lettuce.

Materials and methods

Microbial antagonist

Among a cospicous number of Actinomycetes isolates obtained from soil isolations, a strain of *Streptomyces* was selected to perform the essays carried out in *in vitro* conditions and then in greenhouse. This strain, called CARA17, was subjected to molecular identification by the following primer pairs 16SAct1F (50 CGC GGC CTA TCA GCT TGT TG 30) and 16SAct1R (50 CCG TAC TCC CCA GGC GGG G 30) of 16S rDNA (Shariffah-Muzaimah et al., 2018). The PCR products allowed to confirm the preliminary morphological identification and to ascertain the sequence similarity searches on GenBank database.

Inhibitory activity against fungal pathogens in dual cultures

To perform its ability to inhibit the fungal growth, dual cultures were prepared using an agar-mycelium disc (5 mm diameter) of CARA17 colony was put in a PDA Petri dish at 15 mm from the center and was left to grow at 25 ± 3 °C in darkness. After 14, 21 and 28 days incubation, an agar-mycelium disc (5 mm diameter) from five fungal pathogens, *Athelia rolfsii, Fusarium oxysporum, Plectosphaerella ramiseptata, Sclerotinia sclerotiorum* and *Verticillium dahliae* was put at 15 mm from the center in front of the agar disc with the CARA17 strain. Five replicates were performed for each fungal strain, and the plates only inoculated with the pathogen and the sterile agar disk were used as a control. The dual cultures were kept at 21 ± 3 °C for 15 days in darkness. The inhibitory activity (IA) was calculated as the percentage of mycelium growth inhibition compared to the control by the formula [(R1-R2)/R1] × 100, according to Kunova et al. (2016). One-way ANOVA analysis was performed using Statistica, version 6 (StatSoft, Hamburg, Germany) to assess the significant differences of inhibition activity values. Fisher's test was used as a post-hoc test (p = 0.01).

Inhibitory activity against fungal pathogens as bioactive crude extracts (BCEs) from CARA17 strain

To assess the inhibitory activity of culture filtrate of CARA17 strain, a broth GYM medium was prepared and inoculated with 1 × 10^6 cfu per ml of CARA17 propagules, incubated at 28 ± 3 °C at 75 rpm for 21 days. The culture filtrate was added to GYMA medium at concentration of 15, 30 and 50 % (v:v). On the Petri dishes was inoculated the CARA17 strain 7 days before fungal pathogens inoculation. The Petri plates obtained were inoculated with an agar-mycelium disc (5 mm diameter) from both phytopathogens fungi, and incubated at 28 ± 3 °C for six days to evaluate the inhibition activity played from CARA17 filtrate. Five replicates were performed for soilborne fungal pathogens. Plates inoculated with phytopathogens and the sterile agar discs were used as controls. The inhibitory activity (IA) was calculated as the percentage of mycelium growth inhibition compared to the control as reported above. The data obtained were subjected to one-way ANOVA analysis as desribd above.

Assessment of CARA17 strain as biological agent in vivo

The inoculum solution with the CARA17 strain and fungal soil-borne pathogens propagules was prepared by collecting spores and small fragments of mycelium scraped from surface of 21-day-old *Streptomyces* colonies grown on PDA medium at 28 ± 3 °C in darkness until reaching a concentration of 1 × 10^7 cfu/ml in sterile Tween 20 solution (0.2 %). The experimental design was performed dipping the root of 30-day-old seedlings of fennel and lettuce in the inoculum solution of the CARA17 strain for 30 min, before transplanting them in a pot containing 1.5 kg of soil and peat (3:1). Then, 50 ml of inoculum solution of each fungal soil-borne pathogen was poured into the soil of each pot around the collar of the seedlings. Control trials were included. Each trial was replicated fifteen times. The pots with fennel and lettuce seedlings were placed in a greenhouse with temperature and humidity not conditioned. After 100 days, the fennel and lettuce plants were gently removed from the pots, the roots and collars were carefully washed, and the presence/absence of browning symptoms observed on the root and collar were evaluated and described using an empiric scale from 0 to 5 classes in order to assess the disease severities (DS) on the roots and collar. All fungi underwent re-isolation from the root, collar and stem of the inoculated plants to fulfil Koch's postulates.

Assessment of CARA17 strain as plant growth promotion agent in vivo

To assess putative plant growth promotion by the CARA17 strain on fennel and lettuce plants, all plants were cut at the basis of the collar, and all tissues of epigeal (collar, stem and leaves) and hypogeal portions (root), after a careful washing, were separately weighed and put into a stove at 105 °C until reaching a constant dry weight. For all trials, the average weight of 15 target plants (epigeal and hypogeal portions) were calculated. One-way ANOVA was performed to determine the significant differences in fresh and dried weights.

Results and discussion

One-way ANOVA demonstrated that significant differences in inhibitory activity (IA) by the CARA17 strain against fungal pathogens were observed. The IAs played by the CARA17 strain in dual cultures with fungal pathogens is reported in Table 1. In general, the *Streptomyces* strain was able to inhibit the mycelial growth of all fungal pathogens tested with variable percentage values of IA. *Sclerotina sclerotiorum* was the most sensible to antagonistic activity played by the CARA17 strain, as the IAs recorded were 100 % at 14, 21 and 28 days after its inoculation. *Athelia rolfsii* resulted to be the least inhibited by the CARA17 strain at all inoculation times. The remaining fungal soil-borne pathogens, *V. dahliae*, *F. oxysporum* and *P. ramiseptata*, resulted to be well controlled with percentage values of IA, such as 71.30 % (*V. dahliae*), 82.73 % (*P. ramiseptata*) and 84.71 % (*F. oxysporum*) after 28 days of inoculation (Table 1; Figure 1). In this study, *S. albidoflavus* CARA17 was able to significantly inhibit the growth of all soil-borne fungal pathogens in dual culture except *A. rolfsii* (IA = 0.0 %) (Figure 1 b).

Table 1. Inhibition on colony growth played by CARA17 strain in dual culture.

	Inhibition Activity (IA) %					
	14 Days [a]				21 Days	
Fungal pathogens	Mean	Min–Max [c]	Mean	Min–Max	Mean	Min–Max
Athelia rolfsii	15.08 D [b]	14.92-15.25	4.31 D	4.12-4.50	0.00 D	0.00-0.00
Verticillium dahliae	87.42 C	86.31-86.54	77.15 BC	76.96-77.34	71.30 C	71.28-71.32
Plect. aerella ramiseptata	96.35 B	96.33-96.74	84.82 B	84.58-85.07	82.73 B	82.27-83.19
Fusarium oxysporum	88.26 D	88.63-87.89	84.91 B	82.32-87.50	84.71 B	80.82-88.60
Sclerotinia sclerotiorum	100.00 A	100.00-100.00	100.00 A	100.00-100.00	100.00 A	100.00-100.00

[a] Exposition of fungal strains vs. CARA17 grown on medium at different colony ages; [b] Data followed by different capital letters within the column are significantly different (Fisher's tests; $p < 0.01$); [c] Minimum and maximum values detected (five observations).

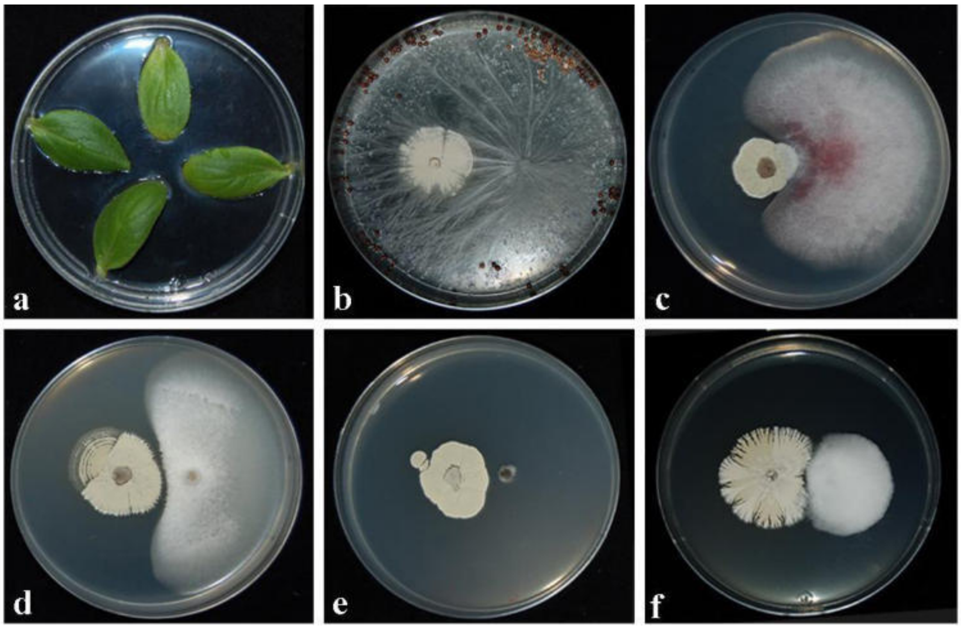

Figure 1. Phytotoxicity assays carried out on young leaf cotyledones of cucumber (a), and inhibition activity by CARA17 strain against *Athelia rolfsii* (b), *Fusarium oxysporum* (c), *Plectosphaerella ramiseptata* (d), *Sclerotinia sclerotiorum* (e), and *Verticillium dahliae* (f).

Table 2 shows that CARA17 dipping significantly preserve the root of fennel seedlings from fungal pathogens. Moreover, dip treatment with CARA17 propagule inoculum protected 98.3 and 88.3 % of lettuce plants (healthy seedlings) against *S. sclerotiorum* and *A. rolfsii*, respectively, while dip treatment with filtered inoculum (BCEs) showed a less effective protection of 86.7 and 80.0 % of the lettuce plants, respectively, although the results obtained were significant (data not shown). Finally, The CARA17 strain protected fennel seedlings from soil-borne fungal pathogens better by dip inoculation before transplanting than by pouring the inoculation into pots after transplanting. Probably, the filtered culture of the CARA17 strain, used for dip inoculation, contained important antifungal compounds immediately adsorbed by the roots, enhancing the "hyperparasitism" mechanism and inducing systemic resistance. Moreover, the same experiment demonstrated that the CARA17 strain promoted the growth of fennel seedlings and facilitated tolerance to biotic (phytopathogens used in in vivo conditions) and abiotic (no organic and mineral fertilizers were provided) stresses. This study showed that the culture filtrates were also able to inhibit *Athelia rolfsii*, while when added to soil (lettuce seedlings) were able to control both soil pathogens under *in vivo* conditions, demonstrating significant protection of the root and crown, without actinomycete propagules, from fungal infection.

Table 2. Effectiveness of CARA17 strain as antagonist for controlling disease severity (DS) against fungal soil-borne pathogens on fennel seedlings.

Treatment with CARA17 strain	Fungal soilborne pathogen	Description Symptoms recorded Leaves	DS	Root	DS	Fresh Biomass* (gr)	Dried Biomass (gr)
Dipping	*F. oxysporum*	Growth reduction/ yellowing	0.6	Light root browning	0.9	85.07 ± 3.93 A	6.90 ± 0.53 A
Dipping	*V. dahliae*	Growth reduction	0.7	Root growth reduction	0.8	85.15 ± 2.91 A	6.92 ± 0.44 A
Dipping	*P. ramiseptata*	Light apical yellowing	0.4	Root growth reduction	0.6	85.57 ± 6.20 A	7.15 ± 0.32 B
Dipping	*S. sclerotiorum*	No disease symptoms	0.0	No disease symptoms	0.0	87.27 ± 1.37 C	8.23 ± 0.15 BC
Dipping	*At. rolfsii*	No disease symptoms	0.0	No disease symptoms	0.0	89.11 ± 6.96 C	10.69 ± 1.01 C
-	CTRL – No treatment	No disease symptoms	0.0	No disease symptoms	0.0	102.61 ± 5.53 CD	10.67 ± 0.91 C
Dipping	CTRL treated with only Cara17	No disease symptoms	0.0	No disease symptoms	0.0	175.03 ± 9.35 E	16.43 ± 1.10 D

References

Basu, A., Prasad, P., Das, S. N., Kalam, S., Sayyed, R. Z., Reddy, M. S., El Enshasy, H. 2021. Plant Growth Promoting Rhizobacteria (PGPR) as Green Bioinoculants: Recent Developments, Constraints, and Prospects. Sustainability 13: 1140.

Colombo, E. M., Kunova, A., Pizzatti, C., Saracchi, M., Cortesi, P., Pasquali, M. 2019. Selection of an endophytic *Streptomyces* sp. strain DEF09 from wheat roots as a biocontrol agent against *Fusarium graminearum*. Front. Microbiol. 10: 2356.

Crumble, R. D. and Notsch, B. G. 1999. Variation in host range, systemic infection and epidemiology of *Leptosphaeria maculans*. Plant. Pathol. 43: 269-277.

Etesami, H., Maheshwa, D. K. 2018. Use of plant growth promoting rhizobacteria (PGPRs) with multiple plant growth promoting traits in stress agriculture: Action mechanisms and future prospects. Ecotoxicol. Environ. Safe. 156: 225-246.

Kunova, A., Bonaldi, M., Saracchi, M., Pizzatti, C., Chen, X., Cortesi, P. 2016. Selection of *Streptomyces* against soil borne fungal pathogens by a standardized dual culture assay and evaluation of their effects on seed germination and plant growth. BMC Microbiol. 16: 272.

Kunova, A., Cortesi, P., Saracchi, M., Migdal, G., Pasquali, M. 2021. Draft genome sequences of two *Streptomyces albidoflavus* strains DEF1AK and DEF147AK with plant growth promoting and biocontrol potential. Ann. Microbiol. 71: 2-8.

Shariffah-Muzaimah, S. A., Idris, A. S., Madihah, A. Z., Dzolkhifli, O., Kamaruzzaman, S., Maizatul-Suriza, M. 2018. Characterization of *Streptomyces* spp. isolated from the rhizosphere of oil palm and evaluation of their ability to suppress basal stem rot disease in oil palm seedlings when applied as powder formulations in a glasshouse trial. World J. Microb. Biot. 34: 15.

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Influence of the biofungicide, Esquive®WP, on grapevine wood microbial communities after a 6-year period of applications

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Abstract: Esquive®WP, is the first biocontrol product registered in France to control Esca, a grapevine trunk disease (GTD). Esquive®WP, based on the fungus *Trichoderma atroviridel*-1237, was applied annually on the pruning wounds of grapevines over a 6-year-period. Our aim was to assess this biofungicide's ability to reduce wood internal necroses and evaluate the induction, of shifts in grapevine wood native microbial communities. Healthy wood samples were collected at two and ten months after Esquive®WP applications in 4 vineyards. For one vineyard, 24-year-old, Chardonnay vines were used, for the three others, the vines were about 15 years old, with three different varieties: Chardonnay, Cabernet Savignon and Melon de Bourgogne. Image analyses showed that the internal wood necrosis sizes did not differ between grapevines treated or not with Esquive®WP. Based on high throughput sequencing (MiSeq) analyses, data pointed out that microbial communities were mainly affected by vineyard factor (geographical location, cultivar, grapevine age, etc.). Indeed, when vineyards were compared, the significant differences in bacterial and fungal diversities indicated a major effect of sampling location/cultivar. Moreover, grapevine age may contribute to the observed variability. The beta diversity of fungal communities in young and old Chardonnay plants were significantly differents. This result has not been observed in bacterial communities. However, following Esquive®WP applications, only few temporary changes in grapevine wood microbial communities were observed. In Cabernet Sauvignon and Melon de Bourgogne vineyards, a significant modification of OTU richness and evenness was observed only at 2 months after Esquive®WP treatment. The same result was observed on abundance of fungal and bacterial taxa. The application of the biocontrol product decreased the abundance of certain fungal (*Malassezia*, *Sclerostagonospora* and *Filobasidium*) and bacterial (*Bradyrhizobium*, *Amycolatopsis*, *Pantoea* and *Dyadobacter*) genera. All these modified taxa are not known to be involved in grapevine trunk diseases or as biocontrol agents. Globally, the application of the biocontrol product does not induce perturbations of fungal and bacterial communities of healthy grapevine wood, even after 6 years of treatment. Only a transient effect was observed in richness and abundance of certain microbial communities 2 months after the sixth application of the biocontrol product. This demonstrates the natural capacity of fungal and bacterial communities to be resilient, and their return to a balanced state.

Key words: Esquive®WP, grapevine, Esca, wood necroses, microbial communities

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Trichoderma asperellum as a potential biocontrol agent to reduce the level of *Geotrichum candidum* inoculum on stone fruit orchards soil

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Abstract: Sour rot (SR) is an emerging disease that has been affecting stone fruit in Catalonia-producing areas since 2016. While it is not the main postharvest disease, if the conditions are favourable, a SR outbreak could result in important reduction of production and economic losses. The common symptoms of the disease are watering, soft rot, white mycelial, and sour smell. *Geotrichum candidum* is the main pathogen causing SR in peaches and nectarines, even though some yeasts have been associated with the disease. Currently, common pre- and postharvest fungicides are not effective in controlling SR in stone fruit. Therefore, considering that *G. candidum* inoculum is primarily found in soil surface and could be dispersed by wind, our efforts focused on reducing *G. candidum* inoculum in the soil using alternative strategies, such as the biocontrol agent *Trichoderma asperellum* T34. First, two *in vitro* assays were performed, a dual culture test to evaluate the interaction between *G. candidum* and T34, and hyphal interaction to observe the T34 mode of action. For both assays, a disk of an active colony of each microorganism was placed in the same media. Diameters of colonies from the first assays were measured daily. For the second assay, a block of medium was observed under a microscope after 48 hours to determine the percentage of *G. candidum* germination. Second, sterile soil was inoculated with (a) *G. candidum*, (b) T34 and (c) both microorganisms, and incubated all at 10, 30 and 40 ºC. Periodically, samples of soil were analysed to determine the concentration of each microorganism. Finally, T34 efficacy was tested in field conditions: (i) H_2O (control), (ii) 250 g/ha of T34 plus the same dose after 15 days, (iii) 500 g/ha of T34. The concentration of microorganisms in the soil was determined before treatments and after 0, 7, 15, and 30 days of application. Results of *in vitro* studies showed that *G. candidum* colonies suffered an invasion of T34, whose growth was not affected by the presence of *G. candidum*. Besides, the presence of T34 in the same medium as *G. candidum* drastically reduced its conidia germination. Regarding soil assays, after 2 days of inoculation, T34 reduced the level of *G. candidum* inoculum in the soil by 76 %, and after 7 days, the reduction was 47 %, which remained stable for a month. Lastly, T34 was successfully established in the field soil, although the concentration of *G. candidum* inoculum in the soil was not high enough to observe significant reductions. Despite the last results, this study provided a potential tool to control SR by reducing the inoculum of *G. candidum* in the soil, and, consequently, its dispersion to the fruit. This work has been financed by 'Generalitat de Catalunya' (CERCA Programme, 2021 SGR 01477 and 16.01.01.PDR2014-2022) and by 'Ministerio de Universidades' (grant FPU22/03902).

Key words: emerging disease, inoculum reduction, biological control, plant pathology

Session V
Mass production, formulation, and application of biocontrol agents

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Multifunctional products based on biocontrol agents from microbiome: new solution to control postharvest diseases

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Abstract: Postharvest biocontrol as a viable alternative to the use of synthetic chemicals has been the focus of scientists and some commercial companies during the last 30 years. However, although successful results have been obtained at laboratory and commercial assays, the number of products in the market is scarce. Different approaches, whether used individually or in combination, are proposed in this presentation in order to achieve the objective of having new tools based on biocontrol agents available and with good efficacy. The first one is the host microbiome knowledge as a promising source of potential biocontrol agents adapted to specific environments. The second strategy consists in combining isolates from different environments into microbial consortia to enhance the final product's biocontrol resilience and stability under varying conditions that may occur in the field. The third one is to develop multifunctional products that address not only disease control but also physiological disorders, thereby improving the overall quality of fruit. Finally, an adequate process of production and formulation of microorganisms and all the other components is necessary to ensure the development of competitive and effective products.

Key words: antagonists, coatings, formulation, microbial consortia, production

Introduction

Over the last decades, research on postharvest diseases in fruit has focused on developing alternative strategies to chemical ones. Postharvest biocontrol has demonstrated remarkable advances in terms of research and publications; however, its commercialization has been generally limited and scarce for several reasons (Droby et al., 2009). Technical shortcomings such as lack of consistency under practical conditions, compatibility problems with commonly used processes and equipment, narrow range of activity and limited potential market in the case of postharvest, as well as, economic and regulatory constraints, have been the main limitations for successful implementation of these products in the market. Low-toxicity products have also been widely studied to control the postharvest disease of fruit (Palou, 2018), but their efficacy is not as good as synthetic chemical products, and often there is a need to combine them with other antifungal strategies to achieve the desired level of efficacy (Usall et al., 2016).

However, for current fruit production, it is essential to go toward more sustainable strategies, prioritizing alternative treatments to synthetic chemicals. This is a consequence of several factors, such as the increased social pressure created by consumer demands for environmentally friendly fruit, but also other considerations, such as stricter legislation on authorized active ingredients, their allowable presence on fruit, and the risk of these pesticides developing resistant strains. In addition, the new European plant health regulation (EU 2016/2031) aims to strengthen compliance with health and safety standards throughout the agri-food chain, highlighting the importance of using safer products, reducing the impact of chemical pesticides to 50 % and increasing organic production by 25 %. All these facts have generated the emergence of a need and interest in integrating alternative tools to synthetic chemicals in fruit production strategies.

Microbiome knowledge as a valuable source of potential biocontrol agents adapted to specific environments

Fruit microbiome is a rich reservoir of microorganisms, offering a valuable source of potential biocontrol agents (BCAs) against major postharvest diseases. The idea of recovering and using native microorganisms from the surface of the fruit as BCAs is still particularly advantageous (Droby et al., 2016), as these microbes are naturally adapted to the specific conditions of the fruit and its environment (Berg et al., 2020), improving their stability and therefore their effectiveness. Our group has used a metabarcoding approach to understand the composition and structure of the apple microbiome from the Pyrenees Mountain and Ebro Valley in order to see the differences between these two environments (Sánchez et al., 2025). Later, it has been used a culturomics approach, a cutting-edge methodology that facilitates the recovery of a wide range of cultivable microorganisms (Sarhan et al., 2019), with the aim of obtaining a collection of cultivable microorganisms from these two distinct studied regions. Recent advances have highlighted the importance of not only identifying effective individual isolates but also exploring their capacity to adapt to different environmental conditions. This collection of microorganisms has been isolated, and their efficacy against main postharvest diseases has been studied, obtaining various strains with promising potential as BCAs. These strains also exhibited resilience to varying temperature and water activity stresses, highlighting their potential adaptability to diverse environmental conditions.

Combination of isolates from distinct environments into microbial consortia

Traditionally, strategies for microbe-based biocontrol aimed at managing postharvest diseases have relied on the application of a single microorganism. In recent years, the development of microbial consortia aimed at enhancing the reliability of existing biocontrol products has become a significant trend within the field of biotechnology, becoming a product more stable compared to those with single-strain inoculation (Minchev et al., 2021).

Our approach combines selected microbial isolates from diverse environmental origins and ecological niches into defined consortia. Additionally, these isolates not only represent different environments but are also part of the core microbiome (Sánchez et al., 2025), suggesting that these microorganisms may have co-evolved with the host throughout the domestication process and therefore could suggest natural ecological adaptability (Abdelfattah et al., 2021). This strategic assembly aims to leverage their combined biocontrol capabilities and also exhibit increased resilience and adaptability to changes in abiotic conditions that occur in field and storage situations, particularly under fluctuating environmental conditions encountered in agricultural practices.

Development of multifunctional products for agricultural enhancement

This approach focuses on the development of eco-friendly multifunctional products (MPs) for controlling both, fungal diseases and physiological disorders, while also improving fruit quality on multi-host. Nano-based edible coatings are studied as the main component of the MPs to preserve fruit quality and prevent physiological disorders such as scald or internal browning (Gago et al., 2020). They minimize quality losses by forming a semi-permeable barrier that regulates moisture and gas exchange between the fruit and environment (Kumar et al., 2017). Moreover, recent advancements in the application of nanotechnology for coatings development have shown efficacy in enhancing fruit quality and extending postharvest storage (Maringgal et al., 2020). Our group has been working on the development of MPs based on edible coatings, which enhance postharvest shelf-life by retarding ripening, delaying physicochemical changes and controlling fungal diseases. Our studies center on nano-systems, which offer advantages over macro and micro-sized dispersions or emulsions, including higher gravimetric stability, and may increase antimicrobial activity due to their increased surface area ratio. Furthermore, incorporating BCAs or antifungal low-toxicity substances into edible coating matrices may improve antifungal efficacy while also enhancing microorganism stability during storage (Miranda et al., 2024). This strategy revolutionises agriculture by integrating advanced technologies with sustainable practices to minimise food losses and drive innovation in the agri-food sector.

Adequate production and formulation process

For the commercial development of BCAs‘ products, microorganisms should be mass-produced at large-scale, and efficiently formulated, in order to have a final product long-term stable, easily handled and, of course, consistently effective in controlling target diseases. This complex process to obtain biocontrol products, with particular attention to production, formulation, packaging, and shelf-life of BCAs – suitable for pre- and postharvest applications for controlling fungal diseases of fruit has to be addressed (Teixidó et al., 2022). If a multifunctional product or biocontrol agent‘s consortia are the objective, the process becomes more challenging, requiring key considerations.

After determining which operating conditions allow the large-scale production of the microorganism, a detailed plan regarding the formulation of BCAs by multidisciplinary approaches (liquid or solid) is required to optimize the yield, efficacy, and storage of the developed product. Unfortunately, not all microorganisms are able to survive the conditions imposed during formulation. Improved stability can be achieved by either, using special conditions during growing or adding protecting substances to the formulation medium. Moreover, formulation must help the microorganism to survive and on the target under adverse environmental conditions which provide improved consistency and efficacy of the antagonist in the field. A wide variety of formulation types have been developed for BCAs. However, a general procedure does not exist, and the process must be optimized for each strain: liquid or solid formulation, dehydration system (freeze-drying, spray-drying, fluidized bed-drying, and fluidized bed-spray drying) and adjuvants addition if necessary. To be practical, BCAs or MPs must ensure long-term stability and ease of application. Thus, selecting appropriate packaging materials and conditions is essential (Gotor-Vila et al., 2019).

Conclusions

To be more successful in the development of effective products based on BCAs to control not only fungal diseases but also physiological disorders and improve fruit quality, different approaches are proposed: microbiome knowledge to obtain the suitable BCAs candidates, the possibility to use microorganisms adapted to different environmental conditions and consortia of them, to develop MPs with coating matrices and perform an adequate production and formulation process.

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References

Abdelfattah, A., Freilich, S., Bartuv, R., Zhimo, V. Y., ... and Droby, S. 2021. Global analysis of the apple fruit microbiome: Are all apples the same? Environ. Microbiol. 23(10): 6038-6055.

Berg, G., Rybakova, D., Fischer, D., Cernava, T., Vergès, M.-C. C., Charles, T., Chen, X., … and Schloter, M. 2020. Microbiome definition re-visited: Old concepts and new challenges. Microbiome 8(1): 103. https://doi.org/10.1186/s40168-020-00875-0

Droby, S., Wisniewski, M., Macarisin, D., and Wilson, C. 2009. Twenty years of postharvest biocontrol research: Is it time for a new paradigm? Postharvest Biol. Technol. 52(2): 137-145.

Droby, S., Wisniewski, M., Teixidó, N., Spadaro, D., and Jijakli, M. H. 2016. The science, development, and commercialization of postharvest biocontrol products. Postharvest Biol. Technol. 122: 22-29.

Gago, C., Antão, R., Dores, C., Guerreiro, A., Miguel, M. G., Faleiro, M. L., Figueiredo, A. C., and Antunes, M. D. 2020. The Effect of Nanocoatings Enriched with Essential Oils on 'Rocha' Pear Long Storage. Foods 9(2): 240. https://doi.org/10.3390/foods9020240

Gotor-Vila, A., Usall, J., Torres, R., Solsona, C., and Teixidó, N. 2019. Enhanced shelf-life of the formulated biocontrol agent *Bacillus amyloliquefaciens* CPA-8 combining diverse packaging strategies and storage conditions. Int. J. Food Microb. 290: 205-213.

Kumar, P., Sethi, S., Sharma, R. R., Srivastav, M., and Varghese, E. 2017. Effect of chitosan coating on postharvest life and quality of plum during storage at low temperature. Sci. Hortic. 226: 104-109.

Maringgal, B., Hashim, N., Mohamed Amin Tawakkal, I. S. and Muda Mohamed, M. T. 2020. Recent advance in edible coating and its effect on fresh/fresh-cut fruits quality. Trends Food Sci. Technol. 96: 253-267.

Minchev, Z., Kostenko, O., Soler, R., and Pozo, M. J. 2021. Microbial Consortia for Effective Biocontrol of Root and Foliar Diseases in Tomato. Front. Plant Sci. 12: 756368.

Miranda, M., Bai, J., Pilon, L., Torres, R., Casals, C., Solsona, C. and Teixidó, N. 2024. Fundamentals of edible coatings and combination with biocontrol agents: A strategy to improve postharvest fruit preservation. Foods. 13: 2980. https://doi.org/10.3390/foods13182980

Palou, L. 2018. Postharvest treatments with GRAS salts to control fresh fruit decay. Horticulturae 4(4): 46. https://doi.org/10.3390/horticulturae4040046

Sánchez, A., Oliva, J., Abdelfattah, A., Torres, R., Vilanova, L. and Teixidó, N. 2025. Exploring the impact of altitude variability and apple genotype on the epiphytic microbiome. Int. J. Agric. Sust. 23: 1, 2480955. https://doi.org/10.1080/14735903.2025.2480955
Sarhan, M. S., Hamza, M. A., Youssef, H. H., Patz, S., Becker, M., ElSawey, H., Nemr, R., ... and Hegazi, N. A. 2019. Culturomics of the plant prokaryotic microbiome and the dawn of plant-based culture media – A review. J. Adv. Res. 19: 15-27.
Teixidó, N., Usall, J., and Torres, R. 2022. Insight into a successful development of biocontrol agents: production, formulation, packaging, and shelf life as key aspects. Horticulturae 8(4): 305. https://doi.org/10.3390/horticulturae8040305
Usall, J., Torres, R., and Teixidó, N. 2016. Biological control of postharvest diseases on fruit: A suitable alternative? Curr. Opin. Food Sci. 11: 51-55.

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Can bee-vectored bacteria remediate the degenerated strawberry flower microbiome in intensively managed greenhouses?

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Extended abstract: Strawberries are one of the most widely cultivated small fruit crops with worldwide cultivation of over 350,000 ha. However, strawberries are prone to microbial diseases responsible for heavy economic losses. The agricultural industry relies on chemical measures to control this problem. However, pathogens acquiring resistance and environmental and health concerns, have amplified the need for safe and effective alternatives. To address this challenge, could we harness the plant microbiome in its defence? After all, the plant microbiome plays a pivotal role in a plant's growth, health, and overall fitness, with extensive research focusing on the rhizosphere and phyllosphere. Surprisingly, the anthosphere (flower microbiome) remains understudied, despite its direct impact on a plant's reproductive success and being a major infection site for plant pathogens. This knowledge gap is particularly relevant in agriculture, where practices such as greenhouse cultivation, tunnel systems, and using plant protection products (PPPs) are common. Our research indicates the pant microbiome functions differently than in natural conditions and these practices could hamper the plant's resistance against plant pathogens.

By adopting a citizen science approach, we investigated the bacterial composition of the strawberry anthosphere in agricultural contexts across various commonly used cultivation practices, focusing on cultivation type (open field, tunnel systems and greenhouse) and pollinator management. Thirty strawberry growers in Flanders and the Netherlands participated in this study by sampling flowers and providing insight into their cultivation practices through a questionnaire. The bacterial composition of the strawberry flowers was determined using 16S rRNA gene sequencing, revealing that more sheltered cultivation systems led to decreased diversity, the loss of core taxa, and greater variability, potentially hampering the plant's resistance against pathogens. Beyond cultivation type, various practices such as PPP use, field size, substrate type, and the use of commercial pollinators significantly influenced the strawberry anthosphere community composition. Nevertheless, the greatest factor determining the anthosphere microbiome was cultivation type, with a strongly degenerated community in greenhouses.

Additional exclusion experiments focusing on the role of large pollinators in the anthosphere microbiome highlighted that the effect of pollinators was stronger in a conventionally maintained greenhouse compared to an organically maintained open field. This

further demonstrates that strawberry flowers cultivated in sheltered cultivation systems lack a stable community, leaving them more susceptible to incoming microbes (both pathogenic and beneficial). The potential of rewilding strategies, including bee vectoring with typical plant-surface microbes such as *Sphingomonas*, *Pseudomonas*, and *Methylobacterium*, to influence disease resistance and fruit quality requires further investigation to provide growers with accurate guidance. When determining optimal cultivation practices, it is essential to balance trade-offs among yield, year-round production, PPP usage and regulations, and fruit quality.

To further investigate the potential of pollinators to introduce beneficial microbes into intensively managed systems, we conducted bee vectoring trials. Microbial strains, isolated from strawberry flowers during the Sabofleur citizen science project, were screened for antimicrobial activity against *Botrytis cinerea*, with several reducing fungal growth by over 70 %. The top-performing strains were spray-dried, loaded into hive-mounted dispensers, and effectively vectored by bees in a net-covered greenhouse. Notably, one strain significantly reduced fungal incidence on stored ripe fruit.

In conclusion, our findings emphasise the impact of agricultural practices on the anthosphere community composition, revealing an important opportunity for microbe-based agricultural strategies to optimise plant health and growth. Integrating these insights into sustainable farming practices holds the potential to enhance agricultural yield while decreasing dependence on chemical inputs.

Key words: strawberry, anthosphere, 16S rRNA, microbiome, biocontrol, intensification, bee vectoring, *Botrytis*

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WheatSimpCom project: From metagenomic data to plant health-identifying microbial candidates and designing a microbial simplified community for effective biocontrol in wheat

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Abstract: Plant-associated microbes establish functional relationships with their hosts, conferring beneficial traits that enhance stress tolerance, performance, and productivity of crops. Inoculating plants with a microbial consortium offers greater potential for plant growth promotion and biological control, as multiple microbial types can interact synergistically to enhance various physiological traits of the plant. These interactions promote stable plant phenotypes and increase robustness in terms of biostimulation and induced resistance against pathogens. As a result, such interactions have gained significant attention for their potential to improve agricultural productivity, reduce environmental impact, and foster sustainable practices. Therefore, developing methods for (i) identifying bacterial biocontrol candidates through microbiome data analysis using network inference techniques and (ii) designing, formulating, and optimizing effective bacterial consortia is of considerable interest. Advances in sequencing technologies, metagenomics, and microbial profiling enable the characterization of microbiome structure and function. Consequently, microbiome data can be leveraged to discover novel biocontrol agents and design consortia that exhibit traits such as robust colonization, high prevalence, and specific beneficial functions. In the WheatSimpCom project, network inference methods were applied to metabarcoding data from symptomatic and asymptomatic wheat leaves during *Zymoseptoria tritici* infection. This approach identified microbial taxa forming network hubs and potential antagonists of *Z. tritici*, which may contribute to stabilizing the wheat phyllosphere microbiome. To validate theoretical predictions and improve the congruence between theoretical and experimental results, 200 isolates each of *Bacillus* and *Pseudomonas* were isolated from wheat phyllosphere samples across three locations, each with three cultivars and two treatments (treated and untreated plots) in the North East of France. Their antagonistic activity against *Zymoseptoria* tritici was evaluated in vitro and in planta. The project aims to experimentally confirm these interactions and develop a multifunctional bacterial consortium to enhance wheat resilience against *Z. tritici*. The project will provide outreach guidelines with recommendations on applying microbial network models for screening biocontrol candidate agents.

Key words: WheatSimpCom project, co-occurrence networks, biocontrol agents, wheat, *Zymoseptoria tritici*, *Bacillus*, *Pseudomonas*

Challenges in scaling, stabilizing, and formulating biocontrol agents for pest management from an industrial perspective

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Abstract: The global reduction in the use of chemical pesticides has led to an increasing demand for microbial biocontrol agents. The biopesticide market has been growing at an annual rate of 15 % for nearly two decades, reflecting a shift towards environmentally friendly pest management solutions. However, it is important to note that the industry is struggling with limited production capacity, making it difficult to meet the rising demand for biocontrol agents. Scaling from small laboratory cultures to large-scale bioreactors (up to 80,000 l) is technically complex and requires maintaining the stability and efficacy of microorganisms. Furthermore, the development of effective formulations for field application is also a challenge.

The path from laboratory research to marketable biocontrol products involves several steps, including optimization of microorganism growth on a large scale, stabilization during storage, and ensuring field application success. These challenges are critical to ensuring that biocontrol agents perform effectively in agricultural systems. Companies like e-nema, founded in 1997, play a key role in the development and production of nematode-based biopesticides, addressing both production and formulation challenges while supporting the global transition to more sustainable pest management strategies.

Moreover, it is important that industrial production possibilities should be considered early in the development of a new biocontrol agent. Not taking in account these factors in the initial stages of development can result in major production challenges later on, making the scalability and commercial success of the product harder. Addressing these industrial production challenges early in the process might ensure that a product is not only scientifically viable but also commercially scalable and effective in the field.

Key words: biocontrol agents, production up-scaling, product development, microorganism stabilization, formulation

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From *in vitro* selection to field evaluation of endophytic bacteria against *Fomitiporia mediterranea*, a key pathogen of Esca, a grapevine trunk disease

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Abstract: The complex grapevine trunk disease, Esca, is a global challenge for viticulture. Since the banning of sodium arsenite, the last registered pesticide, there is no curative treatment available to control Esca. In this context, our objectives were (i) to select bacterial biological control agents (BCAs) effective against one of the main pathogens of Esca, *Fomitiporia mediterranea*, (ii) to study the modes of action of selected strains and (iii) to evaluate their efficacy under field conditions.

A stepwise screening of increasing complexity was performed *in vitro* to evaluate the ability of 58 grapevine endophytic bacteria to inhibit *F. mediterranea* growth. Three strains, *Pseudomonas lactis* SV9, *Pseudomonas paracarnis* S45 and *Paenibacillus polymyxa* SV13, were selected as being highly effective in inhibiting *F. mediterranea in vitro*. Whole genome and metabolome analyses of the three strains showed that these bacterial strains act via both direct and indirect modes of action against pathogens. The efficacy of the *P. paracarnis* S45 and *P. polymyxa* SV13 strains was evaluated in a vineyard trial by injecting them into the trunks of Sauvignon Blanc Esca-symptomatic grapevines. Bacterial injections reduced the severity of Esca without affecting grape quality. The abundance of *Bacillus* spp. increased in grapevines injected with *P. polymyxa* SV13, which correlated negatively with the abundance of *Phaeomoniella* spp. and *Diplodia* spp., a negative correlation between *Pseudomonas* spp. and *Fomitiporia* spp. was observed in grapevines injected with *P. paracarnis* S45. Furthermore, *P. paracarnis* S45 improved the defence mechanisms of the grapevine. These results highlight the potential of both strains as effective biological control agents for the management of Esca.

Key words: biological control, endotherapy, *Vitis vinifera*, plant microbiome, network analysis

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Nanoparticle-based strategies for controlling *Alternaria alternata*: A step towards sustainable crop protection

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Abstract: Fungal diseases are a major cause of crop losses worldwide, contributing significantly to global food insecurity. Among these, *Alternaria alternata* is particularly harmful, causing extensive damage to crops like sunflower, chili, and wheat, affecting both pre- and post-harvest yields. Current control methods for this pathogen are inadequate, highlighting the need for innovative solutions. This study explores the use of nanotechnology, specifically biogenic zinc oxide nanoparticles (ZnO-NPs) and silver-doped zinc oxide nanoparticles (Ag/ZnO-NPs), as potential control agents against *A. alternata*.

In a series of experiments, these nanoparticles exhibited concentration-dependent inhibition of *A. alternata* mycelial growth. Microscopic examination of treated fungal mycelia revealed notable alterations in cell wall structure and destruction of cytoplasmic organelles. Biochemical assays further demonstrated that the nanoparticles affected the fungus's polysaccharide and chitin content, along with reducing the activity of key enzymes such as β-1,4-glucanase and extracellular lipase. Moreover, the nanoparticles induced oxidative stress in *A. alternata*, leading to increased levels of hydrogen peroxide and malondialdehyde, which contributed to the fungus's deterioration.

In addition, proteomic analysis offered deeper insights, showing changes in the expression of essential proteins involved in fungal growth and development. The study concludes that ZnO-NPs and Ag/ZnO-NPs are promising tools for controlling *A. alternata* and could be applied in developing nanofungicides for sustainable agriculture. These findings lay the groundwork for future nanopesticide formulations to reduce crop pathogen damage, potentially transforming plant disease management strategies. This research marks a crucial step toward integrating nanotechnology into agriculture, offering a sustainable and effective approach to managing harmful plant-microbe interactions.

Key words: nanotechnology, zinc oxide nanoparticles, *Alternaria alternata,* crop protection, fungal pathogen control

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Prototype formulation of *Aureobasidium pullulans* strains: efficacy against grey mould of table grape

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Abstract: The market is demanding new products for a more sustainable control of postharvest pathogens. Among the several fungi that can affect crops during cold storage, *Botrytis cinerea* is one of the most common and difficult to control. In the study, prototype oil dispersion (OD) formulations containing *Aureobasidium pullulans* as active ingredient were developed. Two different strains, namely UC14 and AP1 were used for prototype development. The efficacy of both formulations was evaluated in *in vitro* and *in vivo* experiments against *B. cinerea.* The AP1 OD formulation was the most effective, showing 67 % inhibition of fungal mycelial growth. In *in vivo* assay, the highest reduction of pathogen incidence (-50 %) was obtained with AP1 OD as curative treatment (800 mg/l). Our results showed the potential of a new formulation prototype to be applied against grey mould and its possible commercial product development perspectives.

Key words: BCAs, *Aureobasidium* strains, formulation, metabolites, oil dispersion

Introduction

Increasing restrictions on the use of synthetic pesticides, the risk of the development of resistant pathogens and the market's demand for more sustainable fruit production are driving a change in plant defence management (Casals et al., 2022). Yeasts are the most studied BCAs thanks to their multiple mechanisms of action against fungal pathogens. Among all BCAs, *A. pullulans* stands out for its excellent antagonistic activity against *Botrytis cinerea* (Di Francesco et al., 2018). This pathogen heavily affects fruits and in particular table grape during the postharvest phase, causing important economic losses (Zhao et al., 2023)

Once an active strain has been selected as a BCA, various criteria need to be evaluated in order to formulate an effective commercial product, such as testing its performance and assessing its relative environmental impact, as well as cost and marketing aspects (Teixidó et al., 2022). For these reasons, the formulation is the most important and challenging step in the development and commercialization of a bio-based product (Carbó et al., 2019).

Materials and methods

Biocontrol agents and prototype formulations

Aureobasidium pullulans strains AP1 and UC14 (mycological collection of Department of Agriculture, Food, Environmental and Animal Sciences, Udine University) were used as active ingredient for an oil dispersion (OD) formulation prototype. The production started with biomass fermentation (starting inoculum: 200 ml at 1×10^8 cells/ml) for 24 h at 25 °C on

NYDB medium (8 g nutrient broth, 5 g yeast extract, 10 g dextrose in 1 l of distilled water) (Oxoid, UK) in a MiniIO reactor (Solaris, Italy), where sterile air insufflation and the pH were set to 100 % (O_2) and to 6.5, respectively. The biomass was recovered by centrifugation, suspended in a cryoprotectant and freeze dried. Finally, the dry biomass was grinded (grid meshes: 80 µm) and mixed with the other ingredients and dispersed in the oil matrix (not disclosed co-formulants for industrial reasons).

Pathogen and fruits

Botrytis cinerea strain Bc1 was isolated from grapefruit cv 'Sugraone' and identified by morphological analysis. *In vitro* and *in vivo* experiments were carried out with 7-days-old colonies cultured on PDA medium (Oxoid, UK) at 20 °C. *In vivo* assays were performed on table grape cv 'Black Magic', bought in the local market at the right maturity stage (18 °brix) and without any visible diseases or defects.

In vitro *formulations effectiveness and determination of the EC_{50} values*

The two formulated prototypes (AP1OD and UC14OD) and Botector® New, used as reference, were inoculated in sterile flasks containing two media: NYDB (NYDA without agar) and Grape Juice Broth (GJB; 250 ml of grape juice diluted in 750 ml of distilled water) at the final concentration of 1×10^7cells/ml (Cignola et al., 2024). After two days, cell filtrates were used to amend technical agar (15 g/l), poured into sterile Petri plates and a mycelial plug (6 mm Ø) of a 7-days-old colony of *B. cinerea* (Bc1) was inoculated in the centre. Also, the volatile metabolites (VOCs) produced by the tested formulations were evaluated using a double Petri dish assay according to the method of Roussi et al. (2013), using the NYDA/PDA and GJA/GJA media coupling.

The EC_{50} value of each bioproduct for both assays was calculated using the probit analysis applied to the percentage of mycelial colony growth inhibition (Lesaffre and Molenberghs, 1991). Five different concentrations (600 mg/l, 400 mg/l, 200 mg/l, 100 mg/l, and 50 mg/l) were used to calculate the inhibition values (Chen and Dai, 2012). All the Petri plates were incubated at 20 °C and the pathogen colony diameter was measured after 3 days by using a caliber.

Formulations effectiveness on table grape

I*n vivo* experiments were carried out on table grape, evaluating both curative and preventative application of treatments against *B. cinerea* Bc1. For each experiment, fruits were washed carefully in sodium hypochlorite solution (1 %) and then rinsed with tap water. For the curative assay, grapes were inoculated with 15 µl of Bc1 (1×10^5 conidia/ml) and for the preventative assay, with 15 µl of three different bioproduct concentrations (800 mg/l, 400 mg/l, and 200 mg/l), defined previously on the bases of the EC_{50} values. After 2 h, fruits were inoculated with the same volume and in the same wound, with the different bioproduct concentrations and Bc1 suspension, respectively for the curative and the preventative assay. Grapes were stored in sterile plastic boxes at 20 °C and the incidence of the disease was evaluated after 3 days for both experiments. Both assays were conducted twice. As negative control, grapes were inoculated with sterile distilled water, instead of treatments. As positive control, Botector® New (distributed by Manica S.p.a., 0.4 mg/l, as reported by the distributor for bunch application) was used. Twenty berries of table grape cv "Black magic" per 3 independent replicates were used.

Results and discussion

In vitro *formulations efficacy and EC₅₀ values*

The *in vitro* assay highlighted the different efficacy of *A. pullulans* formulations metabolites against Bc1. The assay performed with formulates filtrates (Figure 1) resulted more effective in controlling the target pathogen compared to the VOCs assay, where the higher inhibition (25 %) was achieved by AP1OD grown on NYDA medium (data not reported). Conversely, AP1OD cell filtrates, obtained from GJB medium, reduced by 67 % the mycelial growth of Bc1. The effect of AP1 OD and UC14 OD filtrates obtained from NYDB medium, showed an inhibition of 40 %. No inhibitory effect was observed with Botector® New filtrates. Regarding the EC_{50}, values were 518 mg/l and 368 mg/l for UC14 OD and AP1 OD, respectively.

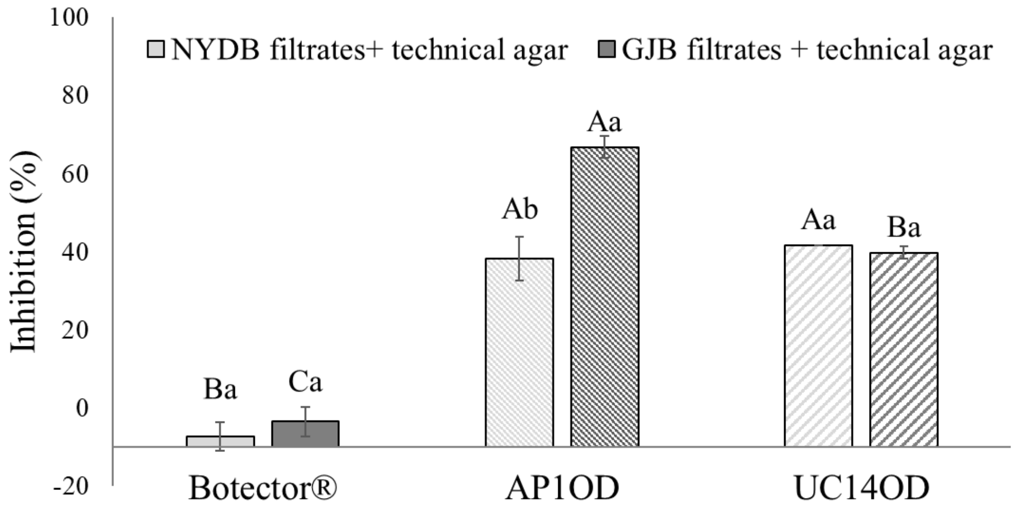

Figure 1. Inhibition (%) of *Botrytis cinerea* (Bc1) mycelial growth with cell filtrates of the two prototype formulations AP1 OD and UC14 OD, and Botector® New. Each value is the mean of 5 plates (replicates) ± standard error. Different uppercase letters indicate significant differences within the same medium and different lowercase letters within the same treatment according to Fisher LSD Test ($\alpha = 0.05$).

In vivo *assay*

Curative application (Figure 2) resulted the most effective treatment compared with the preventative one (data not repoted). The AP1 OD at the dose of 800 mg/l determined the highest grey mould incidence reduction with respect to the control (-50 %). Also, the concentration 200 and 400 mg/l showed both a significant reduction of grey mould incidence by 25 %, with respect to the control. The UC14 OD displayed significantly lower effectiveness.

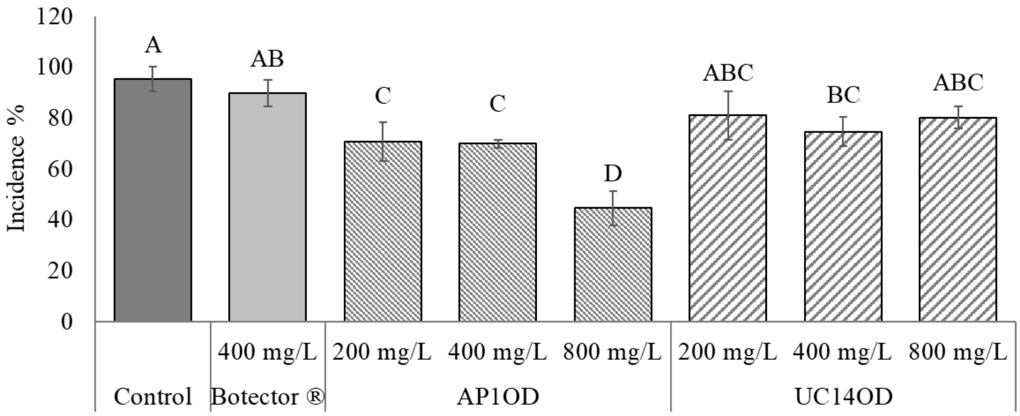

Figure 2. Incidence (%) of grey mould on table grape cv "Black magic" after curative application of the treatments. Values are the mean of 10 berries ± standard error. Each experiment was repeated three times. Different letters indicate significant differences according to Fisher LSD Test ($\alpha = 0.05$).

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References

Casals, C., Guijarro, B., De Cal, A., Torres, R., Usall, J., Perdrix, V., Hilscher, U., Ladurner, E., Smets, T., and Teixidó, N. 2021. Field validation of biocontrol strategies to control brown rot on stone fruit in several European countries. Pest Manag. Sci. 77: 2502-2511.

Cignola, R., Firrao, G., Freschi, G., and Di Francesco, A. 2024. *Aureobasidium pullulans* formulations: Evaluation of the effectiveness against grey mould of table grape. J. Plant Pathol. 106: 1259-1268.

Di Francesco, A., Mari, M., Ugolini, L., and Baraldi, E. 2018. Effect of *Aureobasidium pullulans* strains against *Botrytis cinerea* on kiwifruit during storage and on fruit nutritional composition. Food Microbiol. 72: 67-72.

Lesaffre, E., and Molenberghs, G. 1991. Multivariate probit analysis: a neglected procedure in medical statistics. Stat. Med. 10: 1391-1403.

Rouissi, W., Ugolini, L., Martini, C., Lazzeri, L., and Mari, M. 2013. Control of postharvest fungal pathogens by antifungal compounds from *Penicillium expansum*. J. Food Prot. 76: 1879-1886.

Zhao, Y., Jin, Q., Wang, Z.-J., Tao, X.-Y., and Luo, X.-D., 2023. Quality assurance of postharvest grapes against *Botrytis cinerea* by terbinafine. Nat. Prod. Biopros. 13: 25.

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Encapsulation of entomopathogenic fungus using spray drying: sustainable biological control of agricultural pests and diseases

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Abstract: The aim of this work was to investigate the encapsulation of *Beauveria bassiana* by spray drying using biopolymers (maltodextrin, starch, and cellulose nanofibers) as protecting agents. FTIR confirmed that the polymer backbone was not modified after the fungus incorporation, while SEM revealed spherical microparticles (± 3 µm) with increased roughness in CNF-containing samples. Viability tests showed 2.9 × 10^9 CFU/gram, confirming the encapsulation's efficacy. The results point to a promising advance in developing sustainable, agricultural biopesticides.

Key words: entomopathogenic fungus; biological control; encapsulation; sustainability

Introduction

One of the main challenges in the development of biopesticides is to increase the shelf life of the formulations, ensuring the stability of the microorganisms. In this context, spray drying emerges as a promising technique, allowing the encapsulation of biocontrol agents in protective matrices. This work aims to develop formulations based on biopolymers, characterized by physicochemical analyses and cell viability, aiming to reduce the dependence on chemical pesticides. The proposal seeks to combine efficiency in the control of agricultural pests with the promotion of sustainable agriculture (de Farias et al., 2009; Mahmood et al., 2016; Muñoz-Celaya et al., 2012).

Materials and methods

Samples containing the entomopathogenic fungus *B. bassiana* were prepared using biopolymers such as maltodextrin, starch and cellulose nanofibers as encapsulating agents. Through the spray drying process with an outlet temperature set at 70 °C. The obtained formulations were characterized through thermal, structural and viability analyses, evaluating the functionality of the encapsulated fungi and the efficiency of the technique employed.

Results and discussion

Formulations containing *B. bassiana* demonstrated improved thermal stability, as shown by TG/DSC analyses, which highlighted the protective role of the biopolymers. FTIR confirmed chemical interactions between the biopolymers and the fungus, while SEM images revealed spherical particles with increased roughness in samples containing CNF. Furthermore, viability tests showed an average of 2.9×10^9 CFU/gram, also confirming the efficacy of the encapsulation. These findings emphasize the protection provided to the fungus and its potential to develop innovative agricultural biopesticides.

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References

De Farias, V. L., Maciel, T. C., Fernandes, F., and Pinto, G. 2009. Secagem de conídios de Trichoderma harzianum LCB47 por atomização: efeito da temperatura de entrada e de saída do ar. XVII Simposio Nacional de Bioprocesos, Natal/RN, 02-05 de agosto 2009. chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://www.alice.cnptia.embrapa.br/alice/bitstream/doc/577197/1/AT09045.pdf

Mahmood, I., Imadi, S. R., Shazadi, K., Gul, A., and Hakeem, K. R. 2016. Effects of pesticides on environment. Plant, soil and microbes, vol. 1: Implications in crop science, pp. 253-269. https://doi.org/10.1007/978-3-319-27455-3_13.

Muñoz-Celaya, A. L., Ortiz-García, M., Vernon-Carter, E. J., Jauregui-Rincón, J., Galindo, E., and Serrano-Carreón, L. 2012. Spray-drying microencapsulation of *Trichoderma harzianum* conidia in carbohydrate polymers matrices. Carbohydrate polymers 88(4): 1141-1148. https://doi.org/10.1016/j.carbpol.2011.12.030.

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Optimizing growth conditions for *Metschnikowia pulcherrima*: a comprehensive approach

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Abstract: The increasing demand for sustainable agricultural practices has led to the exploration of biocontrol microorganisms like *Metschnikowia pulcherrima*, known for its ability to combat fruit pathogens. Despite its potential, the optimization of its growth conditions remains underexplored. This study utilized various experimental design approaches to optimize the media composition and growth parameters for *M. pulcherrima* strain APC 1.2. Initially, a screening was conducted to identify the best carbon source for the yeast's growth. Then, using shake flask tests, the Full Mixed Factorial Approach was used to find the optimal ratio of sucrose to yeast extract. Central Composite Design (CCD) was utilized to optimize the pH and temperature conditions in 1 l bioreactors under aerobic conditions. The Crabtree effect of the strain was assessed, showing that the *M. pulcherrima* APC 1.2 is Crabtree-negative. Finally, the Taguchi experimental design was employed in deep well plates to assess the impact of incorporating essential micro and macro nutrients into the complex medium. By systematically varying key factors such as nutrient concentrations, pH, and temperature, we identified the optimal conditions that maximize the yeast's growth. The study provides a comprehensive framework for future research, enabling further refinement of growth conditions to fully harness the potential of *M. pulcherrima* in various agricultural settings.

Key words: *Metschnikowia pulcherrima*, biocontrol, biomass, growth optimization, experimental design

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After planting microbial nematicide application reduced *Meloidogyne incognita* reproduction in soybean

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Abstract: Root-knot nematode caused by *Meloidogyne incognita* is a cosmopolitan pathogen that compromise the plant root system and it overwinters as eggs in the soil. Therefore, the reduction in egg viability, reduces plant infection and assures grain yield. The use of microbiological nematicides has become an important tool in the management of this soil parasite as soil drench or seed treatment prior to planting but the validation of its potential use as an after-planting soil drench application is scarce. Thus, this study aimed to evaluate the efficacy of biological products in the control of *M. incognita* in soybean. The trial was conducted in 5 l-pots and 4 plants per pot in natural soil. At 21 days after emergence, different combinations of commercially-available biocontrol products were amended to the plant collar as drench at the rate recommended by the manufacturer's. The treatments encompassed: *Bacillus amyloliquefaciens*, *B. subtilis* (Baccure, Satis) at 400 ml/ha, *Purpureocillium lilacinum* and *Cordyceps fumosorosea* (Fungardil, Satis) at 400 ml/ha, *B. amyloliquefaciens*, *B. velezensis*, *B. thuringiensis* (Baccure + Fungardil), *Bacillus velezensis, B. amyloliquefaciens, B. thuringiensis* (Biomagno, Biotrop) at 400 ml/ha, cadusafos (Rugby, FMC) 4.0 l/ha and control (water). After 5 days of treatment amendment, each pot was infested with 5,000 *M. incognita* eggs. The efficacy of the treatments were determined at 30 days after plant treatment as root and shoot fresh weight, plant height, number of eggs and number of eggs per gram of root. There was no significant effect for the plant-related growth-related variables: root and shoot fresh weight as well as plant height ($p < 0.05$). On the other hand, the products contributed to the reduction in egg numbers. The products Rugby and Biomagno reduced both the number of eggs and number of eggs/gram of root in 98 and 100 % compared to the water control. The products Baccure and Fugardil + Baccure had an intermediate efficacy with 82 and 90 % reduction while Fungardi use resulted in 48 % reduction. These results suggest that the amendment of biocontrol for nematode management can be performed after plant emergence with a reduction of up to 100 %, a result similar to a chemical nematicide but a commonly reported growth promotion benefit could not be detected within the considered timeframe of the experiment. Although field experiments should be carried out to confirm the efficacy of the technologies, the after-planting microbial nematicide application is a plausible alternative to be considered in the management of root knot nematode.

Key words: *Glycine max* (L.) Merr, nematodes, biological control, plant disease management

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Development of a plant protection agent based on an isolate of the bacterial organism *Lysobacter enzymogenes*

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Extended Abstract: Sustainable plant protection through biological control is a key approach that offers effective solutions for both integrated pest management and organic farming. By utilizing natural resources such as beneficial insects, microbial agents, and plant derived substances we can achieve long term pest control while preserving the environment. This approach not only reduces the reliance on chemical synthetic pesticides and copper-based products but also supports biodiversity and soil health. Biological control agents (BCAs) and specifically microorganisms represent candidates for the reduction and potentially replacement of problematic chemical plant protectants. They possess a variety of benefits including novel and often multiple modes of action combating the rise of resistances and high environmental safety, as they are generally more specific for a given pest organism. Versatile BCA candidates can be found in the bacterial genus *Lysobacter* (Family: Xanthomonadaceae) and especially in the species *Lysobacter enzymogenes* which is well characterized regarding its ability to synthesis a broad variety of lytic enzymes and secondary metabolites with antimicrobial activity. Antimicrobial metabolites produced by *L. enzymogenes* include lysobactin, tripropeptins, cyclic lipodepsipeptides (e. g., WAPs), polycyclic tetramate macrolactams (e. g., Alteramide A & B, HSAF), among others (Lin et al., 2021).

To develop a marketable plant protection agent based on *L. enzymogenes* the projects `mikroPraep´ and `OptiLyso´ were launched. Both are joint projects between research institutes, the Julius Kühn-Institut (Institute for Biological Control, Dossenheim, Germany), and the Hochschule Geisenheim University (Department of Crop Protection, Geisenheim, Germany) and industrial partners e-nema GmbH (Schwentinental, Germany) and BioProtect GmbH (Konstanz, Germany). An isolate of *L. enzymogenes* (LEC) was identified, exhibiting a significantly reproducible suppressive activity against different plant pathogenic fungi and oomycetes on different crops when applied as foliar spray, including *Venturia inaequalis* on apples, *Phytophthora infestans* on potato and tomato, *Pseudoperonospora cubensis* on cucumber, and *Plasmopara viticola* on grape both in *in vitro* and *ad planta* experiments (Drenker et al., 2023; Drenker et al., 2024). Furthermore, it was demonstrated by combining LEC and copper-based products the application quantity of both could be reduced while conferring protection levels equal to respective full quantity treatments. However, at the end of the project `microPraep´ challenges remained. The agent, displaying stable and reproducible results in growth chamber trials, showed a considerable reduction in protection under greenhouse and open field conditions due to (sun)light instability. The follow up project `OptiLyso´ focusses on the improvement of the environmental stability and investigates the agent's miscibility with common fungicides and insecticides, among others. To improve the light stability different additives are tested in combination with LEC. Common crop protection

agents and LEC are mixed together to examine their compatibility and determine whether they reduce LEC's effectiveness.

The ATLAS Suntest XXL + FD weathering machine mimicks natural sunlight and therefore can be used for light stability testing. In that system, different additives were analyzed regarding their ability to improve light stability of LEC using the pathosystem cucumber *P. cubensis*. The polyelectrolyte polymer Calcium lignosulfonate significantly improved the agent's durability and extended its protection time. In addition, *in vitro* miscibility assays using a 24 well plate assay and *P. infestans* spore suspensions demonstrated the combinability of several common plant protection products and LEC without any significant reduction of LEC efficacy. In addition to tackling issues related to practical application in the field, the agent's mode of action is further investigated by looking at the production of antimicrobial volatile organic compounds (aVOCs) and potential plant defense regulation. *In vitro* split plate experiments characterizing the ability of LEC to produce aVOCs against a variety of phytopathogenic fungi showed a reproducible growth repression for *P. infestans* and *P. lingam* (*L. maculans*), among others. Furthermore, the ability of LEC to trigger potential beneficial effects on the level of gene expression in cucumber plants when spray applied to leaves and subsequently challenged with *P. cubensis* will be assessed in a time resolved manner via mRNA Sequencing.

References

Drenker, C., El Mazouar, D., Bücker, G., Weißhaupt, S., Wienke, E., Koch, E., Kunz, S., Reineke, A., Rondot, Y., and Linkies, A. 2023. Characterization of a disease-suppressive isolate of *Lysobacter enzymogenes* with broad antagonistic activity against bacterial, oomycetal and fungal pathogens in different crops. Plants 12(3): 682. https://www.mdpi.com/2223-7747/12/3/682

Drenker, C., Mazouar, D., Seib, T., Weißhaupt, S., Stephan, D., Kunz, S., Reineke, A., Rondot, Y., Linkies, A., and Koch, E. 2024. Biocontrol of plant pathogens by *Lysobacter enzymogenes* isolate LEC: efficacy of shake flask broth, fermentation broth and dried preparations. Biocontrol Science and Technology 35(1): 1-20. https://doi.org/10.1080/09583157.2024.2425931

Lin, L., Xu, K., Shen, D., Chou, S.-H., Gomelsky, M., and Qian, G. 2021. Antifungal weapons of *Lysobacter*, a mighty biocontrol agent. Environmental Microbiology 23(10): 5704-5715. https://doi.org/https://doi.org/10.1111/1462-2920.15674

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Development of the microbial antagonist *Lysobacter enzymogenes* as a plant protection product for soil application against *Pythium ultimum* in cucumber

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Abstract: Bacterial biocontrol agents (BCAs), such as *Lysobacter enzymogenes,* can contribute to fungicide reduction in agriculture through the production of antifungal metabolites. *L. enzymogenes* is a ubiquitous, gram-negative bacterium that exhibits antagonistic properties against various oomycetes due to its production of the heat-stable antifungal factor (HSAF). Drenker et al. (2023) have shown that *L. enzymogenes* is effective against downy mildew in cucumber and grapevine when applied on leaves.

The overall aim of the present study was to convert *L. enzymogenes* isolate JKI-BI-6432 into a biocontrol agent against soilborne plant pathogens for soil application. Therefore, in a first experiment a suitable colony morphology with the highest biocontrol efficacy should be identified. *L. enzymogenes* splits into two different colony morphologies after repeated subculturing on solid media, which can be divided into "large colonies" and "small colonies". In dual culture assays the large colony form and the mixture of large and small colonies inhibited mycelial growth of *Pythium ultimum* (syn. *Globisporangium ultimum*). The strongest inhibitory effect was observed for the mixture of the large and small colonies, with an inhibition zone of 72 mm (± 1.3). With 62 mm (± 0.7), the inhibition zone of the large colonies was smaller compared to the inhibition zone of the mixture. The small colonies alone did not show any inhibitory effect on *P. ultimum*.

In the next step, *L. enzymogenes* was cultivated in shake flasks in soy medium for 72 h, at 200 rpm and 26 °C, in order to provide a sufficient amount of viable biomass and antibiotic metabolites for subsequent bioassays. In in-planta bioassays using cucumber seedlings, the biocontrol efficacy of the unformulated *L. enzymogenes* culture against *P. ultimum* was evaluated. Subsequently, to enable an easy handling and soil application, different formulations (liquid, powder, capsules) and carrier materials have been tested and their efficacy was evaluated in bioassays using cucumber seedlings. Ongoing experiments investigate whether the biocontrol efficacy of *L. enzymogenes* against *P. ultimum* can further be improved by formulation additives.

The results of the present study show that formulated *L. enzymogenes* is a promising agent for the biological control of soilborne phytopathogens such as *P. ultimum*. Its potential for integrated and organic farming is substantial, as it offers an environmentally friendly and sustainable approach to reducing the use of conventional plant protection products.

Key words: bacterial antagonist, *Globisporangium ultimum*, HSAF, biocontrol, liquid cultivation, formulation, damping-off disease

Reference

Drenker, C., El Mazouar, D., Bücker, G., Weißhaupt, S., Wienke, E., Koch, E., Kunz, S., Reineke, A., Rondot, Y. and Linkies, A. 2023. Characterization of a Disease-Suppressive Isolate of *Lysobacter enzymogenes* with Broad Antagonistic Activity against Bacterial, Oomycetal and Fungal Pathogens in Different Crops. Plants 12: 682.

Session VI Round Table
A winding road to biopesticides registration: bottlenecks and opportunities

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The need of biocontrol alternatives to synthetic pesticides: the case of basic substances

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Abstract: Pathogens, pests and weeds induce severe loss of production, and the application of control measures is mandatory to reduce food loss, occurring from the production to the meeting with consumers, and waste, occurring from buying to consumer home. Nowadays most of measures to control pathogens, pests and weeds rely on the use of plant protection products derived by chemical synthesis, although a long list of alternatives is available and more or less widely used by growers. Registration of a new plant protection product is costly, around 300 million Euro, and can take up to 13 years. The consumers are becoming increasingly sensitive to the presence of pesticide residues on fresh produce, and retailers address this fear asking a reduced number and amount of residues as compared to the allowed maximum residue level (Romanazzi et al., 2022). Therefore, there is a high demand of alternatives to synthetic plant protection products, including biocontrol agents and natural compounds, It should be desirable to have a dedicated path for registration of biocontrol solutions, with decreased cost and time, since a list of safety aspects may be not needed for such compounds. Among alternatives to synthetic plant protection products covered by Regulation 2009/1007, article 23 deals with basic substances, that if compounds used as food or feed, then the risk for human and environment is mild and so registration is less costly (around 50000 Euro) and time consuming (1-2 years) (Romanazzi et al., 2022). Today we have a list of 28 basic substances registered for use in plant protection, and a good number of them have been applied to control plant diseases in the field (e. g., grapevine downy and powdery mildews) or postharvest decay of fresh fruit (e. g., gray mold of strawberries, brown rot of sweet cherries). Among the most widely tested basic substances for plant disease management there are chitosan hydrochloride, chitosan, vinegar, *Urtica* extracts and sodium bicarbonate. Research is very active in this field, and it follows an important interest of growers, grower associations, policy makers, agricultural technicians, researchers, companies, retailers and consumers, that may lead to an increasing number of basic substances available on the market in the near future and standardised protocols for application.

References

https://food.ec.europa.eu/plants/pesticides/eu-pesticides-database_en

Romanazzi, G., Orçonneau, Y., Moumni, M., Davillerd, Y., Marchand, P. A. 2022. Basic substances, a sustainable tool to complement and eventually replace synthetic pesticides in the management of pre and postharvest diseases: reviewed instructions for users. Molecules 27(11): 3484. https://doi.org/10.3390/molecules27113484

Session VII
Unexplored sources
of potential biocontrol agents

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Fungal diversity: From isolation and characterisation of populations to utilisation of fungal strains in plant disease control

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Abstract: The inconsistency of biocontrol agents (BCAs) applied to manage disease problems in field crops remains a major constraint to the development of novel BCAs. Many organisms from plants are adapted to their specific habitat, making it difficult for introduced BCAs to find a niche. We search for novel BCAs in habitats where they potentially interact with pathogens of interest, predicting that organisms isolated from environments where they are intended for use are more likely adapted to environmental conditions that favour development of specific diseases. Focusing on endophytic fungi may be advantageous since they colonise plants internally and are thereby potentially protected better from environmental stresses. In wheat and tomato plants, we combined cultivation dependent and independent approaches to obtain a deep understanding diversity and structure of endophytic mycobiomes, aiming at identification and isolation of novel reliable BCAs. Assessing the lifestyle of fungal endophytes originating from healthy tomato roots on tomato seedlings revealed the presence of both beneficial endophytes including *Pseudeurotium* sp. and four latent pathogen taxa. These tomato root pathogens were also the most dominating taxa based on community meta-barcoding. Intriguingly, *Pseudeurotium* species, not previously reported as beneficial to plants, were also highly abundant, indicating that this genus may hold novel potential BCAs. Taken together, complex interactions between members of the microbiome are suggested to maintain an equilibrium in the community, preventing pathogens from causing disease. In wheat spikes, endophyte communities were dominated by basidiomycete yeasts before anthesis while shifting towards a more opportunistic ascomycete-rich community during kernel development. These dynamics were interrupted when *Fusarium* spp. colonised wheat spikes and the pathogens excluded other fungi from floral tissues and reduced the community diversity. However, the presence of several endophytes correlated negatively with *Fusarium* spp. and linked with spikes staying healthy despite exposure to the pathogen. These endophytes belonged to the genera *Cladosporium* and the yeasts *Itersonillia* and *Holtermanniella,* suggesting they may represent an unexplored source of potential BCAs in wheat. Based on a community analysis of the wheat mycobiome from seed to plant to seed again in one growth season, it is evident that the structure of the endophytic microbiome is highly dependent on *e. g.* organ specific interactions between host genotype and abiotic climate condition. This emphasises the importance of searching for potential BCA in habitats matching both biotic and abiotic factors conducive to pathogen infection and disease development. Finally, the screening approach is in our opinion equally important to finding candidate strains. We firstly strive to evaluate their biocontrol potential in plant bioassays mimicking some natural environmental conditions. From our experience, *in vitro* inhibitory activities often do not correlate with *in planta* biocontrol efficacy. In fact, the best performing BCA strains against two important wheat diseases showed no growth inhibition of their causal organisms, *Fusarium* spp. and *Zymoseptoria tritici,* in dual cultures. This may reflect that mechanism of induced resistance cannot be discovered in the absence of the plant.

Key words: fungal endophytes, meta-genomics, biocontrol agents, biocontrol mechanisms

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Spinach seed-inhabiting microorganisms are associated with suppression of seedling disease in the spinach – *Globisporangium ultimum* pathobiome

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Extended Abstract: Seed microbiota are gaining recognition by the scientific community for their beneficial role in plant health and crop yield (Simonin et al., 2022). Understanding how the seed microbiome can support plant performance and mitigate yield losses offers a promising new tool for the production of healthy seeds and, consequently, for securing global food supply. While several chemical seed-applied phytosanitary products have been or are expected to be phased out in the near future, the underexplored biological control potential of seed microbiota may be a stepping stone towards addressing some of the current and future challenges that agriculture faces.

We hypothesized that, in the absence of a phytosanitary treatment, an intact seed microbiome may be able to prevent dysbiosis and maintain the health status of the seed and emerging seedling. To explore this, 11 different pathosystems were tested for seed microbiome suppressive potential in a large-scale screening which included 260 seed lots from seven different crops, namely *Beta vulgaris* (beetroot), *Allium cepa* (onion), *Spinacia oleracea* (spinach), *Capsicum annuum* (pepper), *Coriandrum sativum* (coriander), *Festuca rubra* (red fescue) and *Lolium perenne* (perennial ryegrass) (Diakaki et al., 2022). Each crop was challenged with one or two of the following six pathogens: *Globisporangium ultimum* (or *Globisporangium* sp.), *Setophoma terrestris*, *Fusarium oxysporum*, *Phytophthora capsici*, *Laetisaria fuciformis* and *Puccinia* sp. Consequently, we screened 420 seed lot – pathogen combinations. We confirmed our hypothesis and demonstrated that certain spinach and beetroot seed lots suffer less from *G. ultimum* damping-off in the presence of an intact microbiome and become significantly more infected when seed disinfection reduces the microbiome. *Globisporangium ultimum* (previously known as *Pythium ultimum*), is an oomycete pathogen that can infect crops of high importance including maize, soybean and wheat as well as numerous vegetable crops (Rai et al., 2020).

The stage of seed germination is an opportunity for *G. ultimum* infection (Rai et al., 2020) but could be prevented by antagonistic microbes inhabiting seeds and seedlings. Although soil-derived microbiota can colonise the young plant (Rochefort et al., 2021), the same is true for seed-associated bacteria and fungi, with positive implications for plant health (Jack and Nelson, 2018; Nelson, 2018). The importance of seed-associated microbiota, especially during germination, for seed health is further supported by our recent results which point at the suppressive potential of seed microbiomes (Diakaki et al., 2022).

In a follow-up study, we focused on eight spinach seed lots of different levels of damping-off suppressiveness and performed a second spinach – *G. ultimum* bioassay, with which we confirmed our previous findings (Diakaki et al., 2025). In order to elucidate the characteristics

of seed microbiota with a suppressive potential against *G. ultimum*, we then analysed the relative abundance and taxonomical composition of the bacterial and fungal fractions of seed microbiota based on 16S and ITS1 amplicon sequences, respectively, for the same eight seed lots. We hypothesised that their microbial communities differ in taxonomical composition and that these differences correlate with damping-off suppressive potential. We were able to confirm our hypothesis and showed that 9.8 % of bacterial and 7.1 % of fungal community variance correlated with disease suppression, as well as that fungal diversity correlated with suppressiveness. Additionally, a higher relative abundance of the bacterial genus *Massilia* was a key feature of suppressive seed microbiomes, a fact that is not surprising given that this genus has been found in suppressive systems and is known for its ability to suppress plant pathogens (Andreo-Jimenez et al., 2021). The same was true for *Vishniacozyma*, *Filobasidium* and *Papiliotrema*, three genera which belong to the *Tremellomycetes* class of dimorphic basidiomycetous fungi. While yeasts have been commercialised as biological control agents for use in agriculture, little is known about their role in seed microbiomes. Our data indicate their ubiquity in spinach seed microbiomes and their potential beneficial roles for seed health. We are now working on the identification of viable seed microbiota as a step towards validating their importance in mitigating *G. ultimum* infection.

Key words: seed microbiome, bacteria, fungi, yeasts, *Pythium ultimum*, *Spinacia oleracea*, disease suppression, seed disinfection, plant-pathogen bioassay

References

Andreo-Jimenez, B., Schilder, M. T., Nijhuis, E. H., et al. 2021. Chitin-and keratin-rich soil amendments suppress *Rhizoctonia solani* disease via changes to the soil microbial community. Appl. Environ. Microb. 87(11): e00318-21.

Barret, M., Briand, M., Bonneau, S., et al. 2015. Emergence shapes the structure of the seed microbiota. Appl. Environ. Microb. 81(4): 1257-1266.

Diakaki, M., van der Heijden, L., Lopez-Reyes, J. G., et al. 2022. Beetroot and spinach seed microbiomes can suppress *Pythium ultimum* infection: results from a large-scale screening. Seed Sci. Res. 32(4): 1-9.

Diakaki, M., Jimenez, B. A., de Lange, E., et al. 2025. Spinach Seed Microbiome Characteristics Linked to Suppressiveness Against *Globisporangium ultimum* Damping-Off. FEMS Microbiol. Ecol. 101(2): fiaf004.

Jack, A. L. H., and Nelson, E. B. 2018. A seed-recruited microbiome protects developing seedlings from disease by altering homing responses of *Pythium aphanidermatum* zoospores. Plant Soil 422: 209-222.

Nelson, E. B. 2018. The seed microbiome: origins interactions and impacts. Plant Soil 422: 7-34.

Rai, M., Abd-Elsalam, K. A., Ingle, A. P. 2020. *Pythium* diagnosis diseases and management. CRC Press, Boca Raton.

Rochefort, A., Simonin, M., Marais, C., et al. 2021. Transmission of seed and soil microbiota to seedling. mSystems 6(3).

Simonin, M., Briand, M., Chesneau, G., et al. 2022. Seed microbiota revealed by a large-scale meta-analysis including 50 plant species. New Phytol. 234(4): 1448-1463.

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Endophytes as rice seed dressing for the biological control of *Fusarium fujikuroi*

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Extended Abstract: Rice is one of the most important staple food worldwide, and Italy is the largest producer in the EU, where bakanae disease poses an increasingly significant threat to rice seed producers (Mongiano et al., 2021). To limit the spread of this disease, restrictive thresholds for certified seed production were introduced under the Commission Implementing Directive 2012/1/EU. *Fusarium fujikuroi*, the causal agent of bakanae disease, is a seedborne hemibiotrophic pathogen that causes systemic symptoms in rice plants, including internode elongation and crown necrosis. Since seeds are the primary source of inoculum, chemical seed dressing has traditionally been the main control strategy. However, the Farm to Fork Strategy, in the framework of the European Green Deal, aims at the progressive reduction of chemical fungicide use, highlighting the necessity of eco-friendly bakanae disease management strategies. Biological control using microbial antagonists offers a promising sustainable alternative, but inconsistent efficacy under field conditions represents a notable limitation. Endophytes, due to their close association with plant hosts, represent a promising source of biocontrol agents (BCAs) with the potential to perform better under field conditions (Collinge et al., 2022). This study aimed to select rice endophytes with biocontrol potential against bakanae disease.

Seeds from 24 rice experimental and commercial lines, as well as shoots from five genotypes, were surface sterilized using 1-2 % sodium hypochlorite, 70 % ethanol, and five washes with sterile distilled water. A total of 135 endophytes were isolated, including 81 bacteria, 36 filamentous fungi, and 18 yeasts. Traditional BCA selection often relies on mass screenings based on *in vitro* pathogen inhibition, which rarely correlates with *in vivo* efficacy (Rojas et al., 2020). To avoid this limitation, all isolates were tested *in planta* under controlled conditions against *F. fujikuroi*. Rice seeds of the bakanae-susceptible cultivar Galileo were treated with a microbial suspension (10^7 CFU/ml) and subsequently inoculated with the pathogen (10^5 conidia/ml) before sowing. Eighteen isolates significantly reduced disease severity by up to 40 % compared to the control. The selected endophytes were molecularly identified, and their growth rates were compared at 25 °C and 37 °C to exclude potential plant or human pathogens, respectively (Latz et al., 2020). The five remaining strains belonged to *Epicoccum layuense*, *Epicoccum catenisporum*, *Microbacterium testaceum* and *Methylobacterium oryzae* species.

The ability of the selected endophytes to inhibit *F. fujikuroi* was further tested *in vitro* through dual culture assay and sandwich plate method, to assess the production of agar diffusible or volatile antimicrobial compounds respectively (Sipiczki, 2023). Four strains significantly inhibited the pathogen's growth, although with limited efficacy (7-25 %). *M. oryzae* did not show statistically significant inhibition compared to the control. The selected endophytes' abiotic stress tolerance was evaluated by testing their growth rates on Petri dishes across a range of temperatures (4, 10, 22, 28, and 37 °C) and KCl concentrations (mimicking osmotic pressures of 0, -1, -2, -3, and -4 MPa). Four strains demonstrated optimal growth

between 22 °C and 28 °C and tolerated conditions as low as 10 °C and -4 MPa, while *M. oryzae* exhibited low stress resilience, thriving only at 22 °C and without salt stress. The biocontrol efficacy of the selected endophytes was confirmed *in vivo* by assessing disease severity, incidence and total fresh biomass. All strains significantly reduced disease severity, similarly to the standard chemical fungicide for rice seed dressing against bakanae. Additionally, strains of *M. testaceum* and *E. catenisporum* significantly decreased disease incidence, while strains of *E. catenisporum*, *E. layuense*, and *M. oryzae* significantly increased total fresh biomass compared to the control.

These findings suggest that the biocontrol mechanisms of these strains are closely linked to their interaction with the plant host, highlighting the importance of *in planta* screening methods, which prevent the exclusion of potential BCAs with low *in vitro* inhibition potential. Future studies will investigate the specific modes of action, including the effects of the most promising strains on rice defense-related gene expression and resident microbiota, with the goal of developing biofungicides for field testing.

Key words: rice, *Fusarium fujikuroi*, endophytes, biocontrol

References

Collinge, D. B., Jensen, D. F., Rabiey, M., Sarrocco, S., Shaw, M. W., and Shaw, R. H. 2022. Biological control of plant diseases – What has been achieved and what is the direction? Plant Pathol. 71(5): 1024-1047.

Latz, M. A., Jensen, B., Collinge, D. B., and Jørgensen, H. J. L. 2020. Identification of two endophytic fungi that control *Septoria tritici* blotch in the field, using a structured screening approach. Biol. Control 141: 104128.

Mongiano, G., Zampieri, E., Morcia, C., Titone, P., Volante, A., Terzi, V., and Monaco, S. 2021. Application of plant-derived bioactive compounds as seed treatments to manage the rice pathogen *Fusarium fujikuroi*. Crop Prot. 148: 105739.

Rojas, E. C., Jensen, B., Jørgensen, H. J., Latz, M. A., Esteban, P., Ding, Y., and Collinge, D. B. 2020. Selection of fungal endophytes with biocontrol potential against *Fusarium* head blight in wheat. Biol. Control 144: 104222.

Sipiczki, M. 2023. Identification of antagonistic yeasts as potential biocontrol agents: Diverse criteria and strategies. Int. J. Food Microbiol.: 110360.

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Known antagonistic microbiota are key drivers in suppressive soils against *Sclerotinia sclerotiorum*, a widespread soilborne fungal plant pathogen

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Abstract: Disease-suppressive soils, where suppressive activity is conferred by the soil microbiome, have been extensively studied for many soil-borne fungal plant pathogens. However, little is known about soils suppressive to *Sclerotinia sclerotiorum* which causes the economically devastating disease *Sclerotinia* stem rot (SSR). The pathogen produces long-term survival structures called sclerotia that germinate carpogenically via ascospores infecting above-ground plant parts or myceliogenically to directly infect the plant stem base and roots. The aim of this study was therefore to identify soils suppressive to *S. sclerotiorum*, decipher the associated microbiomes, and verify the microbial drivers conferring disease suppressiveness. We identified a soil suppressive to SSR through the reduction of basal infection in seedling assays and carpogenic germination of sclerotia. Disease suppressiveness was further confirmed to be mediated by soil microbiomes that were transferable to soils conducive to the disease. Microbiome analysis revealed a significant difference in the microbiota community between SSR-suppressive and -conducive soils with a higher abundance and significant enrichment of known biocontrol taxa in suppressive soils. Microbial association network analysis further confirmed several known biocontrol genera as potential keystone microbial drivers for soil suppressiveness, typically *Bacillus* (family Bacillaceae, phylum Firmicutes). Suppressive soils also had a denser microbial co-occurrence network and higher communication between the resident microbiota. Further, we confirmed a significantly higher number of cultivable biocontrol bacteria in suppressive soils with species of the bacterial genera *Bacillus* and *Streptomyces* as the main groups to suppress *S. sclerotiorum* growth and reduce SSR in plant assays. These validated our results on identified microbial drivers of disease suppressiveness in microbiome analysis. Overall, this study demonstrated that high abundance and dominance of known antagonistic microbiota may confer suppressive activity to mycelial growth and germination of *S. sclerotiorum*. This knowledge could be used to improve the field management of SSR by enhancing the antagonistic microbiota in soils, leading to improved microbiological soil health and sustainable management of SSR.

Key words: soil microbiomes, soil health, disease suppressive soils, biological control, antagonistic microorganisms, *Sclerotinia* stem rot

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Cultivar-specific root exudates enhance the resistance of susceptible cucumber cultivar to *Fusarium* wilt through recruitment of beneficial microbiota

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Extended Abstract: *Fusarium* wilt, caused by *Fusarium oxysporum* f. sp. *cucumerinum* (Foc), significantly threatens cucumber (*Cucumis sativus*) production, leading to substantial yield losses worldwide. Traditional breeding approaches emphasize genetic resistance, yet increasing evidence suggests that cultivar-specific root exudates play a pivotal role in shaping the rhizosphere microbiota, which can influence disease suppression. Understanding the interplay between root exudates and microbial recruitment is essential for developing novel and sustainable strategies for disease management. This underscores the potential of leveraging natural plant processes to reduce reliance on external inputs and enhance agricultural sustainability. This study builds upon previous research that highlights the intricate relationships between plant-microbe interactions and disease suppression, further emphasizing the importance of understanding root exudate dynamics. This study investigates how root exudates of wilt resistant (CL11) and wilt susceptible (ZN6) cucumber cultivars planted in continuous cropping soil modulate microbial communities and whether exogenous addition of specific metabolites can enhance the cultivar resistance to *Fusarium* wilt. Four sample groups, asymptomatic (CL11H, ZN6H) and symptomatic (CL11D, ZN6D) plants of resistant and susceptible cultivars CL11 and ZN6 were collected, and root exudate metabolites were profiled using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS), while rhizosphere bacterial communities were assessed via Illumina HiSeq sequencing. Exogenous additions of specific root exudate metabolites were tested for their abilities to reprogram rhizosphere microbial communities and enhance disease resistance. Statistical analyses, including Principal Coordinate Analysis (PCoA), diversity indices, and correlation analysis, were performed to elucidate relationships between root exudate composition, microbial community shift, and disease suppression. Based on metabolomics analyses, 10,280 metabolites were identified across all groups. Resistant (CL11) and susceptible (ZN6) cultivars exhibited distinct root exudate profiles. The comparison on the root exudates of symptomatic and asymptomatic resistant CL11 indicated that high relative abundance of metabolites were related to the stress mitigation and signaling pathways. For example, compounds such as methylmalonic acid, leucyl-isoleucine, L-3-cyanoalanine, succinic acid and indole-3-acetyl-L-glutamic acid were significantly elevated in asymptomatic plants, suggesting their potential roles in plant resistance. In contrast, susceptible ZN6 exhibited pronounced metabolic fluctuations, demonstrated by high relative abundance of some metabolites in asymptomatic plants, such as citric acid, L-tryptophan, indoleacrylic acid and corchorifatty acid F, which may reflect a reactive rather than a preventative response to pathogen attack. Illumina sequencing revealed significant differences in the rhizosphere bacterial community composition between resistant and susceptible cultivars. Resistant cultivars maintained higher abundances of *Streptomyces*, *Cellvibrio*, *Ensifer*, and *Bacillus*, as well as the phylum Proteobacteria, family

Comamonadaceae and Bacillaceae while susceptible cultivars ZN6 had greater abundances of *Amycolatopsis*, *Flavobacterium*, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, and *Pseudomonas,* as well as the phylum Actinobacteria and Bacteroidota. Correlation analyses indicated that leucyl-isoleucine is strongly correlated with the abundance increase of *Streptomyces* ($r = 0.72$, $p < 0.01$), while hexadecanoic acid was positively correlated with *Ensifer* ($r = 0.68$, $p < 0.01$). Conversely, citric acid and L-tryptophan were correlated with *Amycolatopsis* and *Flavobacterium*, respectively, suggesting that susceptible cultivars naturally recruit distinct microbial communities that may aid in pathogen suppression. Exogenous addition of leucyl-isoleucine (LISO) significantly restructured the rhizosphere microbial community of susceptible ZN6 to resemble that of resistant CL11, enhancing the relative abundance of Proteobacteria, Comamonadaceae, *Pseudomonas*, and *Bacillus*, and reducing disease severity by 79.84 % ($p < 0.05$). Similarly, L-tryptophan (LT) increased the abundance of *Flavobacterium* and Proteobacteria in ZN6, strengthening its microbial defense potential. *In vitro* assays showed that LISO and LT significantly inhibited *Fusarium oxysporum* mycelial growth at the concentration of 5 mM and 10 mM, respectively, providing evidence for their antifungal properties. This study highlights the significant role of cultivar-specific root exudates in shaping the rhizosphere microbiota and their potential in controlling *Fusarium* wilt in cucumber. Our findings demonstrate that:

i) resistant and susceptible cucumber cultivars exude distinct metabolites that differentially recruit beneficial microbes;

ii) exogenous addition of specific metabolites can successfully reprogram the rhizosphere microbiota of susceptible cultivars, mimicking the disease-suppressive environment of resistant cultivars;

iii) leucyl-isoleucine and L-tryptophan represent promising biostimulants for microbiome-based disease management strategies in cucumber production.

These results further suggest that metabolite applications may serve as a viable alternative to traditional chemical treatments, paving the way for microbiome-driven disease control strategies. Future research should explore metabolite – microbe interactions in more cultivars and other cropping systems, and investigate synthetic microbiome engineering approaches for improving disease resistance. These insights would contribute to the advancement of microbiome-informed agricultural practices for sustainable plant disease management.

Key words: cucumber *Fusarium* wilt, root exudates, rhizosphere microbiome, disease resistance, microbial recruitment

Effect of root rot infection on rhizosphere microbial community of *Panax notoginseng*

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Abstract: The health status of *Panax notoginseng* (Sanqi) is closely related to its rhizosphere microbial community. Exploring the differences in the structure of the rhizosphere soil microbial community before and after the occurrence of root rot in Sanqi can provide a scientific basis for the ecological regulation of soil microorganisms to control Sanqi root rot. Using high-throughput sequencing technology, the diversity and community structure differences of rhizosphere soil microorganisms between healthy Sanqi and Sanqi infected with root rot were compared and analyzed at four different locations in Wenshan City, Yunnan Province, a major production area of Sanqi, with varying planting durations. The results showed that the richness and diversity of both bacterial and fungal communities in the rhizosphere of Sanqi significantly decreased after the occurrence of root rot. Moreover, the number of microbial organisms in the rhizosphere soil of healthy Sanqi was generally higher than that in the rhizosphere soil of diseased Sanqi at different taxonomic levels. Community composition analysis revealed that the dominant bacterial families in the Sanqi rhizosphere were Sphingomonadaceae, Xanthobacteraceae, Micrococcaceae, and Gemmatimonadaceae, while the dominant fungal genera were *Mortierella*, *Plectosphaerella*, *Fusarium* and *Ilyonectria*. Characteristic microbial community analysis indicated that the characteristic bacteria in the rhizosphere soil of healthy Sanqi included *Streptomyces*, *Gemmatimonas*, *Nodella*, and *Pedomicrobium*; and the characteristic fungi included *Penicillium* and *Talaromyces*. In contrast, the characteristic bacteria in the rhizosphere soil of Sanqi infected with root rot included *Rhodococcus*, *Pseudarthrobacter*, *Microbacterium*, *Flavobacterium*, *Leifsonia*, and *Sphingobacterium*, while the characteristic fungi included *Ilyonectria*, *Plectosphaerella* and *Colletotrichum*, which showed significantly increased relative abundance in the rhizosphere soil of Sanqi with severe root rot after long-term cultivation. Among these, bacteria such as *Streptomyces* and *Sphingomonas* could be further explored as new resources for the biological control of Sanqi root rot, while several fungal genera such as *Plectosphaerella*, *Ilyonectria*, and *Colletotrichum* could serve as characteristic pathogenic fungi for the diagnosis and detection of Sanqi root rot based on the rhizosphere microbiome. These results would provid important references for early monitoring and warning of Sanqi root rot and the exploration of probiotic resources.

Key words: *Panax notoginseng*, root rot, rhizosphere microbial diversity

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Exploring endophytic bacteria as biological control agent for *Ganoderma* basal stem rot infection in oil palm: Integrated disease management in the field

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Abstract: *Ganoderma* basal stem rot (BSR) is a soil-borne disease that significantly impacts oil palm cultivation in Malaysia. The disease causes severe yield losses in the crop which is currently entering the 4th generation planting in Malaysia. One effective method of managing this disease is through Integrated Disease Management (IDM), a combination of cultural and sustainable and green practices such as the application of biological control agents (BCA). This study investigates the potential of endophytic bacterial (EB) species isolated from oil palm roots in protecting the crop from the deadly disease, assessed through greenhouse and field trials. The isolated EB species were identified using molecular techniques and screened for antifungal properties through multiple *in vitro* assays, *in vivo* and finally followed by field evaluations. Scanning electron microscopy (SEM) revealed that culture filtrates from the four selected EB species caused structural damage to the hyphae of *Ganoderma boninense*, the pathogen responsible for BSR. This suggests that the endophytic bacteria release antifungal compounds and lytic enzymes, which disrupt the hyphal structure of the pathogen. Some of the EB strains also tested positive for antibiotic genes (bacillomycin D and iturin A) through PCR-based detection and sequencing. Additionally, the EB strains were found to promote the vegetative growth of oil palm seedlings, showing significant improvements in several growth parameters. The EB strains reduced disease severity by 66-68 %, as compared to untreated oil palm seedlings at 45 % at the end of 6 months of assessment. At present, selected bacterial endophyte is being studied through a research collaboration between MPOB and CJ Bio Malaysia Sdn. Bhd., by supplementing their amino acid-based liquid product, produced via fermentation technology, as a fortified biofungicide (GANOSHIELD™) against BSR infection in the field.

Key words: basal stem rot, oil palm, biological control, endophytic bacteria, integrated disease management

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Isolation and screening of endophytes as potential biocontrol agents against latent and wound postharvest pathogens of apples

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Abstract: Apple is one of the most cultivated fruit crops worldwide. After harvest, fruits can be stored at low temperatures under controlled atmosphere for several months, ensuring year-round availability. However, they are susceptible to fungal infections, resulting in significant economic losses. The most effective control strategy is the application of synthetic fungicides with preventive action. However, their use raises concerns about environmental and human health risks, as well as the development of resistant fungal strains. In light of these concerns and recent European guidelines, there is an increasing need for alternative management strategies. The use of biological control agents (BCAs) is an effective approach for managing fruit postharvest diseases. Particularly, endophytes are interesting potential BCAs, due to their close relationship with the host plant.

This work aimed to isolate endophytic yeasts and bacteria from apples and to evaluate their potential as biocontrol agents against apple pathogens. A protocol was developed to isolate endophytes from healthy apples of different cultivars (Crimson Snow, Golden Delicious, Story Inored, Gala and Red Delicious) and geographical origins. A total of 137 strains were obtained. All the strains were tested *in vivo* for their potential antagonistic activity, using *Botrytis cinerea* as target pathogen. The screening aimed to identify endophytes able to reduce grey mold severity to levels comparable to thiabendazole. The most effective strains (13 bacteria and 11 yeasts) were molecularly identified and selected for further analyses. Selected yeasts belonged to the genera *Aureobasidium*, *Bullera*, *Metschnikowia* and *Wickerhamomyces*, while bacteria belonged to the genera *Pantoea*, *Pseudomonas*, *Rahnella* and *Stenotrophomonas*.

These strains were further tested for their growth at different temperatures, to evaluate their ability to survive under field and storage conditions. Moreover, their ability to control additional apple pathogens was assessed. *In vivo* trials were conducted using *Phlyctema vagabunda*, the causal agent of bull's eye rot, and two *Colletotrichum* species, causal agents of apple bitter rot, as target pathogens. Most strains significantly reduced disease severity compared to the inoculated control for all pathogens tested.

The most effective ones will be further investigated to clarify their mechanisms of action and assess their potential for field and postharvest applications.

Key words: BCAs, yeasts, bacteria, postharvest, *Malus* x *domestica*

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Impact of stump treatments based on *Pseudomonas protegens* against *Heterobasidion irregulare* on non-target microbes as determined by metagenomics

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Abstract: The fungal species belonging to the *Heterobasidion annosum* s.l. complex score among the most damaging pathogens of conifer trees worldwide, causing wood decay and root rots in association with high mortality rates and relevant economic losses (Garbelotto and Gonthier, 2013). Three *Heterobasidion* species are native to Europe, where they coevolved with their hosts, while *H. irregulare* Garbel. and Otrosina was accidentally introduced from North America to Central Italy during WWII, where it established and spread in *Pinus pinea* L. (Italian stone pine) forest stands along the Tyrrhenian coast (Gonthier et al., 2014). Based on its current and potential impact, the European and Mediterranean Plant Protection Organisation (EPPO) included *H. irregulare* in the A2 list of pests recommended for regulation at the continental scale. *Heterobasidion* species are characterized by a typical infection pattern: airborne basidiospores landing on stump surface after tree felling germinate and the mycelium colonizes the wood tissues spreading to neighboring healthy trees through root contacts or grafts (Garbelotto and Gonthier, 2013). Hence, an effective control option often used in practical forestry is to treat the stump surface immediately after cutting with chemical or biological products to prevent the establishment and germination of basidiospores (Garbelotto and Gonthier, 2013). Previous studies were conducted in the field to test the efficacy against *H. irregulare* airborne stump infections of a suspension of *Pseudomonas protegens* (strain DSMZ 13134, Proradix®) and of its cell-free filtrate (CFF) (Pellicciaro et al., 2021). However, whether and to what extent such treatments may impact on non-target microbes inhabiting stumps is currently unknown.

In this work, a metagenomic approach was used to investigate the short-term impact of such treatments on both bacterial and fungal communities inhabiting the stump surfaces. In a forest stand located in the zone of infestation of *H. irregulare*, stumps of *P. pinea* were treated immediately after felling with Proradix®, CFF or sterile water, the latter used as controls. Wood slivers were taken from the surface of 15 stumps per treatment four months after treatments. Amplicon metagenomic sequencing of 16S and ITS genes was performed to investigate the effects of treatments on bacterial and fungal communities, respectively. The effects of Proradix® and CFF were comparable. In fact, Simpson, Shannon and Chao1 diversity indices of fungal communities associated with Proradix® (0.88, 4.29 and 213.02, respectively), CFF (0.91, 4.82 and 227.56) and control (0.92, 4.68 and 211.02) were not significantly different ($P > 0.05$). Similar results were obtained for the diversity indices of bacterial communities ($P > 0.05$).

Overall, our results suggest a limited impact of treatments with Proradix® and with the *P. protegens* cell-free filtrate against *H. irregulare* on the microbial communities inhabiting *Pinus pinea* stumps. This aspect should be accounted for in the design of sustainable and integrated control strategies.

Key words: bacteria, biodiversity, forestry, fungi

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References

Garbelotto, M., and Gonthier, P. 2013. Biology, epidemiology, and control of *Heterobasidion* species worldwide. Annu. Rev. Phytopathol. 51: 39-59.

Gonthier, P. Anselmi, N., Capretti, P., Bussotti, F., Feducci, M., Giordano, L., Honorati, T., Lione, G., Luchi, N. Michelozzi, M., Paparatti, B., Pollastrini, M., Sillo, F., Vettraino, A. M., and Garbelotto, M. 2014. An integrated approach to control the introduced forest pathogen *Heterobasidion irregulare* in Europe. Forestry 87: 471-481.

Pellicciaro, M., Lione, G., Ongaro, S., and Gonthier, P. 2021. Comparative efficacy of state-of-the-art and new biological stump treatments in forests infested by the native and the alien invasive *Heterobasidion* species present in Europe. Pathogens 10: 1272.

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Exploring the grapevine microbial endosphere in the context of flavescence dorée

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Extended Abstract: Flavescence dorée (FD) is one of the most important grapevine yellows in Europe. The microbiota of the grapevine endosphere comprises prokaryotic and eukaryotic endophytes, which may establish diverse relationships with the host plant from symbiotic to pathogenic. A sustainable strategy and promising tool against FD could be the use of grapevine endophytic microorganisms with known biocontrol or biofortification properties.

To explore the interaction between grapevine endophytic bacteria and fungi with FD phytoplasma, the endomicrobiome associated with grapevine wood was characterized using next-generation Illumina sequencing. In particular, a meta-barcoding approach was undertaken to investigate and compare the structure and composition of bacterial and fungal endophyte communities associated with three groups of plants with different FD-phytosanitary status with the aim of identifying potential strains that could be applied as biocontrol agents useful to control phytoplasma diseases.

Since 2018 the health status of grapevines cv. Glera grafted on Kober 5BB in a vineyard located in north-eastern Italy (FVG region, 46°10’35.57”N; 13°11’03.54”E) was monitored by an annual survey for symptom development and by molecular analyses as described in Martini et al. (2019), which evidenced the diffusion of FD phytoplasma strains of *map*-genotype M54 in the vineyard. In September 2023 wood chips (including vascular tissues) were collected from trunks of 9-11 symptomatic, recovered and healthy grapevines. For metabarcoding analyses, 50 mg of the previously obtained wood powder was used to extract DNA following the manufacturer’s instructions of the Plant/fungi DNA isolation kit (Norgen Biotech Corp., Thorold, ON, Canada) as previously reported (Nerva et al., 2024).

Total DNA was quantified using a NanoDrop One spectrophotometer (Thermo Fisher Scientific) and sent to Macrogen Inc. (South Korea) for library preparation and sequencing by Illumina MiSeq technology. To analyse the bacterial community, the V3-V4 hypervariable region was targeted by the universal primers 319F/806R; whereas for the fungal community, the internal transcribed spacer (ITS) 2 region was analysed using the primers ITS3/ITS4. *Ad hoc*-designed peptide nucleotide acid (PNA) blocker oligos for *V. vinifera* were used to inhibit plant material amplification (Nerva et al., 2024). Bioinformatics and statistics were conducted as previously reported.

In this study 426 bacterial and 254 fungal ASVs (amplicon sequence variants) were identified and taxonomically classified extending the knowledge on the diversity of the endophytic microbiota of grapevine wood tissue suggesting that it is composed primarily of Firmicutes and Ascomycota with Paenibacillales and Pezizales as the main bacterial and fungal orders, respectively.

When looking at diversity indexes of the bacterial and fungal communities across the different health status of grapevines, it was observed that the fungal communities are more stable than bacterial communities which showed a significantly lower bacterial diversity in healthy grapevines compared to recovered and diseased plants. These data are in contrast with those reported by Bulgari et al. (2011, 2014) in which a lower richness of bacterial species is registered in FD-affected grapevine plants and in those that underwent spontaneous recovery from FD, compared to healthy grapevine plants. This could be mainly attributed to the different grapevine tissues used for the analysis (trunk *vs* leaves).

Some significant differences between the plant groups were found in the composition of the bacterial and fungal communities. In particular, some bacterial (i. e., *Anaerocolumna*, *Parafilimonas*, *Novosphingobium*, Solirubrobacterales_gen_67-14) and fungal (i. e., *Phaeomoniella*, *Puccinia*) genera varied significantly in recovered and symptomatic samples, in a similar way compared to healthy ones. Moreover, both Didymellaceae and *Erysiphe* increased significantly in symptomatic grapevines, compared to healthy ones.

Interesting to note that in the samples from recovered grapevines there is a significant increase in some bacterial genera like *Aurantisolimonas*, *Mucilaginibacter*, *Peredibacter*, *Edaphobaculum*, *Rhizobacter*, *Leuconostoc*, some of which may be related to the presence of potential biocontrol strains acting via antibiosis against phytoplasmas or via induced resistance in plant. There is also a significant increase in all those saprotrophic fungi associated with wood degradation (i. e., *Diplodia*, *Ceratobasidium*, *Paraphaeosphaeria*) compared to symptomatic and healthy grapevines.

These results confirm that the sanitary status of the plant can influence the composition of the bacterial and fungal endophyte communities present in a plant and that phytoplasma infection affects the whole microbiome composition.

Key words: phytoplasma disease, endophytes, potential biocontrol agents

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References

Bulgari, D., Casati, P., Crepaldi, P., Daffonchio, D., Quaglino, F., Brusetti, L., and Bianco, P. A. 2011. Restructuring of endophytic bacterial communities in grapevine yellows-diseased and recovered *Vitis vinifera* L. plants. Appl. Environ. Microbiol. 77: 5018-5022.

Bulgari, D., Casati, P., Quaglino, F., and Bianco, P. A. 2014. Endophytic bacterial community of grapevine leaves influenced by sampling date and phytoplasma infection process. BMC Microbiol. 14: 198.

Martini, M., Pavan, F., Bianchi, G. L., Loi, N. and Ermacora, P. 2019. Recent spread of the "flavescence dorée" disease in north-eastern Italy. Phytopathogenic Mollicutes 9(1): 207-208.

Nerva, L., Gambino, G., Moffa, L., Spada, A., Falginella, L., De Luca, E., Zambon, Y., and Chitarra, W. 2024. Conjoined partners: efficacy and side effects of grafting and dsRNA application on the microbial endophyte population of grapevine plants inoculated with two esca-related fungal pathogens. J. Exp. Bot.: erae461.

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Functional characterization and metabolomic analysis of *Salvia* spp. extracts to control grapevine downy mildew

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Abstract: *Plasmopara viticola*, the causal agent of grapevine downy mildew, is one of the major threats to viticulture, especially in areas with warm and humid climate conditions. *Plasmopara viticola* infections are controlled by frequent application of copper and synthetic fungicides, with possible negative impacts on human health and the environment. Thus, alternative products are required to develop sustainable plant protection strategies. Extracts of medicinal plants and herbs contain bioactive compounds for possible applications against plant diseases. For example, alcoholic extracts of *Salvia officinalis* previously showed strong inhibitory activity against *P. viticola*. This study aims to characterize the activity of *Salvia* spp. extracts against grapevine downy mildew and to identify bioactive metabolites responsible for the inhibitory activity. Alcoholic extracts of *Salvia* spp. shoots, leaves, and flowers showed strong disease reduction in grapevine leaf-disk assays, while stem extract was only partially active against *P. viticola*. Moreover, fractions of shoot extracts were obtained using preparative liquid chromatography, and the most efficient fractions were selected by inhibitory activity tests on leaf disks. Active fractions were subjected to untargeted metabolomic analysis with liquid chromatography-high resolution mass spectrometry (LC-HRMS) and 30 putative bioactive compounds belonging to classes of terpenoids were annotated, such as nine abietane diterpenoids, five icetexane diterpenoids, and two secoabietane diterpenoids. The compound annotation will be validated by LC-HRMS analysis using reference standards, and the inhibitory activity of the identified bioactive compounds will be tested on leaf disks using pure substances. The identification of novel bioactive compounds from *Salvia* spp. and the characterization of their mode of action will pave the way for the development of new sustainable alternatives for grapevine protection.

Key words: *Plasmopara viticola*, plant extracts, bioactive compounds, anti-oomycete compounds

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Microbial allies for sustainable viticulture: The case of *Pseudomonas* sp. 714A

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Abstract: Grapevine is one of the most significant crops in Italy, particularly in the Veneto region. Till now, grape cultivation has still largely relied on the extensive use of fungicides and chemical fertilizers to face various fungal diseases and improve yields. Therefore, alternative, sustainable, and eco-friendly approaches should be investigated to reduce the application of chemical products in the field. The rhizosphere is a nutrient-rich environment characterized by large microbial complexity. Thus, this niche could be used as a reservoir of microbial biological control agents well adapted to a certain agroecosystem, especially with a view of conservative biocontrol application.

The aim of this study was to identify and characterize microbial strains, isolated from grapevine rhizosphere of local vineyards, to be applied as alternatives to chemical products to improve the resilience of vineyards to biotic and abiotic stresses. Out of 105 bacterial isolates, obtained from the rhizosphere of three different vineyards located in the Verona province, we selected an isolate of *Pseudomonas* sp. that displayed interesting traits related to the biological control of grapevine fungal pathogens as well as plant growth promotion. In particular, *Pseudomonas* sp. 714A showed promising PGPR behavior and a significant biological control capacity, especially through the secretion of volatile organic compounds.

This isolate was further subjected to whole-genome sequencing, to unravel the genomic features related to its activities.

The results confirm that vineyard microbial biodiversity represents an important reservoir of endogenous microbe-based potential alternatives to chemicals to improve viticulture sustainability.

Key words: BCA, PGPR, grapevine, Veneto

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Endogenous *Trichoderma* strains from vineyard rhizosphere: a step toward eco-friendly viticulture practices

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Abstract: Grapevine is one of the most important crops in Italy, particularly in the Veneto region, which possess various DOC and DOCG areas, with a remarkable variety of wine styles and types. In the field, agronomical practices still largely rely on the extensive use of fungicides and chemical fertilizers to face various grapevine fungal diseases and improve yields. Thus, alternative, sustainable, and eco-friendly approaches should be explored to minimize agrochemical applications. The rhizosphere is a nutrient-rich environment characterized by a large microbial complexity. This niche could be used as a reservoir of microbial biological control agents well adapted to a certain agroecosystem. *Trichoderma* sp. is referred to as a fungal saprophyte that can prevent diseases, promote plant growth, improve nutrient utilization efficiency, enhance plant resistance, and improve agrochemical pollution of the environment. In recent years, strains of the *Trichoderma* genus have been extensively and efficiently used to control plant pathogens, either directly through antimicrobial activity or indirectly by promoting plant defenses.

The aim of this study was to isolate, identify and characterize fungal strains that belong to the well-known *Trichoderma* genus. In this study, we collected 17 *Trichoderma* sp. isolates, obtained from the rhizosphere of three different vineyards located in the Verona province. These isolates were evaluated for their biocontrol activity against different local grapevine fungal pathogens. Direct antagonism, the production of volatile organic compounds displaying antifungal activity and the effect of secreted metabolites were assessed. The strains were also evaluated for different plant growth promotion traits, like the solubilization of inorganic phosphate and the production of IAA. Furthermore, each isolate was evaluated for its ability to resist different concentrations of copper, one of the main antifungal products being used in vineyards. Altogether, these results indicate that vineyard microbial biodiversity represents an important reservoir of endogenous fungal-based potential alternatives to chemicals to improve viticulture sustainability.

Key words: BCA, *Trichoderma*, grapevine, Veneto

Inhibitory effect *in vitro* and action mechanism of *Piper divortans* Trel. & Yunck. metabolites on the *Fusarium* spp. in banana crops

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Abstract: Musaceae are the fourth most important food worldwide. It is a crop susceptible to phytopathogens that cause oxidative stress in the plant, which are controlled with agrochemicals. Currently, transitional strategies are being established for control with products obtained from plant extracts (Mesa et al., 2019). *Piper* spp. present amide-type compounds, phenylpropanes and terpenes that serve as protectors of phytopathogens (Rahman et al., 2011; Suaza et al., 2023). In this work, the activity of *Piper divortans* Trel. & Yunck. on *Fusarium* spp. isolates in banana crops in Colombia was determined. The phenolic content and the percentage of free radical inhibition were determined by the DPPH•, ABTS•+ methods of the ethanolic extract of leaves and stems of *P. divortans*. Metabolites were characterized by 1H-NMR spectroscopic techniques on a Bruker Ascend II HD 600 MHz and mechanisms of action were established by SEM microscopy. In vitro biological tests were performed using the disk diffusion method with the extract on *Fusarium* spp. (1000-312.5 ppm) with inhibition percentages greater than 50 % at 7 days in all treatments.

The most representative metabolites in the extracts presented spectroscopic signals of flavonoids and neolignans. *Piper* extracts may be an efficient strategy for the control of *Fusarium* spp. with antioxidant protective potential in Musaceae crops.

Key words: extracts, *Piper*, phytopathogens

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References

Mesa, V. A. M., Marín, P., Ocampo, O., Calle, J., and Monsalve, Z. 2019. Fungicidas a partir de extractos vegetales: una alternativa en el manejo integrado de fitopatógenos. RIA. Revista de Investigaciones Agropecuarias 45(1): 23-30.

Rahman, A., Al-Reza, S. M., and Kang, S. C. 2011. Antifungal activity of essential oil and extracts of *Piper chaba* Hunter against phytopathogenic fungi. JAOCS, Journal of the American Oil Chemists' Society.

Suaza, G. V., Mesa, V. A. M., Ocampo, J. O., and Monsalve, F. Z. I. 2023. Antioxidant activity and phytopathogenic control of extracts and fraction from *Struthanthus calophyllus* AC Sm. (Loranthaceae). Chemistry and Biodiversity 20(2): e202200830.

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Preliminary assessment of the usefulness of magnesium hydroxide and selected yeast strains for protecting potatoes against potato blight in organic farming systems

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Abstract: The pathogen *Phytophthora infestans* is a very important pathogen that causes significant losses in potato yields, especially in organic farming. Many alternative methods and products are being tested to limit losses and extend the growing plant to allow tuber production. *In vitro* tests *P. infestans* was evaluated for its sensitivity to magnesium hydroxide. The concentration of 125 µg $Mg(OH)_2$ in ml PDA did not inhibit mycelial growth, while concentrations of 250 and 500 µg/ml were effective. At the concentration of 250 µg/ml, the coefficient of inhibition of linear growth of *P. infestans* was over 16 %, and at the concentration of 250 µg/ml almost 30 %. Yeast species selected in other tests and potentially useful in limiting growth of mycelium pathogen, in the presence of magnesium hydroxide developed less intensively than on the control plate. The concentration of 125 µg $Mg(OH)_2$/ml of PDA was neutral for colonies of all yeast species, while the concentration of 250 and 500 µg/ ml caused that yeasts *Saccharomyces cerevisiae* were completely inhibited, and *Cryptococcus albidosimilis* 117/10 isolate and *Torulaspora delbrueckii* grew less intensively.

Key words: basic substances, *P. infestans*, potato, organic farming, integrated protection, yeast

Introduction

Organic farming (OF) requires specific methods and protective measures that can be applied to achieve yields. The entire farm management process is based on agro-technical, preventive and non-chemical methods that should be implemented comprehensively. Plant protection products (PPPs), other natural substances such as basic substances (BSs), biological methods and agricultural practices that provide good plant nutrition and stimulate the plant's defense system can be used as direct treatments. Potato (*Solanum tuberosum* L.) is an important food crop, but is difficult to grow in organic farming due to the limited range of fungicides that can be used to control late blight and early blight of potatoes. Currently, only copper fungicides can be used to control *P. infestans*, but there have been discussions for several years about removing copper from organic production in the EU. It is clear that many different methods and substances need to be combined and used to maintain yields. The basis of protection in OF is crop rotation and variety selection, but this is not always possible or sufficient. Therefore, research has been carried out using basic substances (BSs), defined as compounds that are not primarily used as plant protection products but can be useful in crop protection and must be qualified by EFSA (Marchand, 2017). European pesticide database (https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/active-substances). BSs have no toxicological effects and do not cause adverse effects to humans, animals or the environment. No residue limits have been established for the basic substances. To increase efficacy, BS can be used in combination with other BS, PPPs or other agents such as yeast. (Kowalska et al., 2022; Kowalska, 2024 a; b). To develop and evaluate the efficacy of different BS-based strategies used to reduce the symptoms of two major potato plant diseases to keep them below the economic loss level has been presented in some papers and conferences (Kowalska and Krzymińska, 2024, Toffolatti et al., 2023). BS also have the potential to replace synthetic pesticides in the management of pre- and postharvest diseases (Romanazzi et al., 2022).

Magnesium hydroxide [$Mg(OH)_2$] is an inorganic chemical compound belonging to the hydroxide group. It is widely used as an additive in food and feed, in medicine as an osmotic laxative and gastric acid neutralizer, in cosmetics as a deodorant ingredient, and in agriculture as a fertilizer. As a basic substance, it has been approved under Commission Regulation 2024/836 on the basic substance E528 magnesium hydroxide in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council and amendments to Commission Implementing Regulation (EU) No 540/2011. As a basic substance, magnesium hydroxide is used to protect crops such as cereals (e. g., wheat, oats, rye), grapes, tomatoes, peppers, potatoes and fruit trees (e. g., peaches, cherries, apricots) against plant diseases. In potato protection, the substance is proposed to reduce symptoms caused by *P. infestans*.

The objective of the experiments was to evaluate the efficacy of magnesium hydroxide at different concentrations as an in vitro growth inhibitor of *P. infestans*, and then to evaluate the effect of magnesium hydroxide on the growth of commercial yeast strains with potential use against *P. infestans*. The results obtained will serve as a basis for the design of greenhouse and field experiments, taking into account the simultaneous or alternating use of magnesium hydroxide and selected yeast strains in the protection of potatoes in relation to varieties with different susceptibility.

Materials and methods

Effect of magnesium hydroxide addition on the inhibition of linear growth of P. infestans on PDA medium in vitro

The effect of the addition of magnesium hydroxide at different concentrations (from 125 to 500 µg/ml of medium PDA) on the inhibition of linear growth of *P. infestans in vitro* was evaluated. In the study, a strain of *P. infestans* derived from an in-house culture (isolated from the surface of a potato leaf) was used. 10 ml of PDA, pH 6.5, was poured into 90-mm-diameter Petri plates, and a 5-mm-diameter disk of an intensively growing 10-day-old culture of *P. infestans* was applied to each dish. The plates thus prepared were placed in a thermostat at a constant temperature of 21 °C. The control consisted of plates with filamentous fungi on medium without magnesium hydroxide. The experiment was carried out in 5 replicates. The diameter of mycelium was measured after 14 days. The results were converted into percentage of inhibition - growth stimulation according to Abbott's formula:

$$I = \frac{K - A}{K} * 100\ \%$$

Where: I – linear growth inhibition factor of the fungus, K – diameter of the fungus colony on the control petri dish, A – diameter of the fungus colony on the petri dish growing in the presence of magnesium hydroxide

Effect of magnesium hydroxide on yeast colony count in vitro tests

The effect of magnesium hydroxide concentration on the growth of selected yeasts on artificial medium (PDA) was tested. Yeasts belonging to the species: 1) *Saccharomyces cerevisiae* (Saflager W 34/70 and Coobra strains), which have shown antagonistic activity against various plant pathogens in own experiments, 2) *Torulaspora delbrueckii*, which belongs to the so-called killer yeasts, which produce toxins that limit the presence of other microorganisms, 3) *Cryptococcus albidosimilis*, isolate 117/10, which has shown antagonistic effects against various plant pathogens and has retained antagonistic abilities after freeze-drying. One hundred µl of the suspension of the yeast species were applied to PDA medium at pH 6.5 in Petri dishes with a diameter of 90 mm, with an optical density of the cell suspension of 0.5 on the McFarland scale, and the experiment was carried out in 4 replicates for each combination. After 5 days of incubation at 21 °C, fungal colonies were counted.

Results and discussion

Effect of magnesium hydroxide addition on the inhibition of linear growth of P. infestans on PDA medium in vitro.

The fungus *P. infestans* showed a sensitivity to the presence of magnesium hydroxide that was directly proportional to its concentration. For combinations using a concentration of 125 µg/ml of medium, no inhibition of mycelial growth was observed (mycelia measured an average of 41 mm) compared to control objects (mycelia measured an average of 45 mm), whereas concentrations of 250 and 500 µg/ml had an inhibitory effect on mycelial growth (mycelia measured an average of 37.6 mm and 31.6 mm, respectively) (Table 1).

Table.1. Effect of the presence of magnesium hydroxide at different concentrations (µg/ml of medium) on the linear growth of *P. infestans* mycelium on Petri dishes [mm] on day 14 of the experiment.

Concentration of magnesium hydroxide	125	250	500	Non-modified medium
Diameter of mycelium	41 a	37.6 b	31.6 b	45a

Test Anova, one factor analysis, $p < 0.05$

With the 250 µg/ ml PDA concentration, the linear growth inhibition rate of *P. infestans* was over 16 % and with the 250 µg/ ml PDA concentration it was almost 30 % (Figure 1).

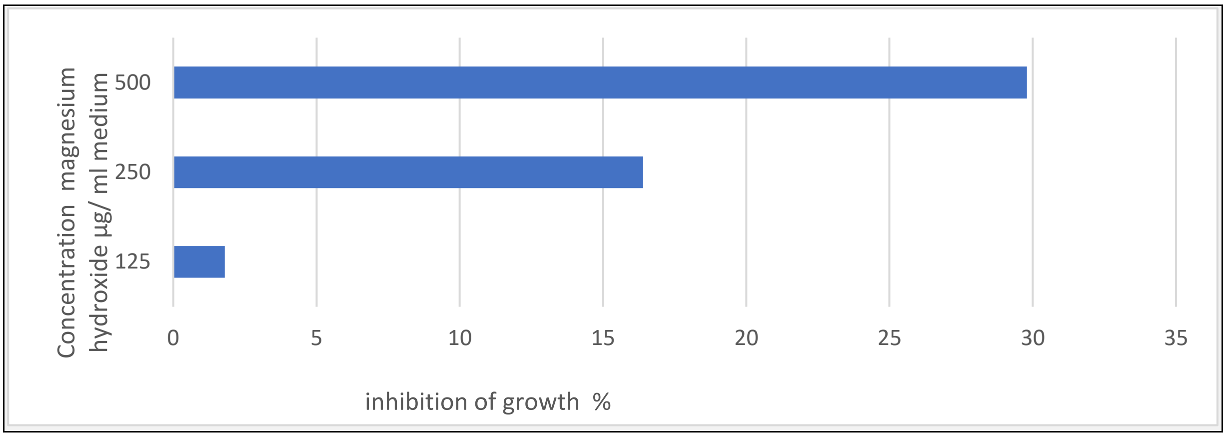

Figure 1. Percentage inhibition of linear growth of *P. infestans* by magnesium hydroxide at different concentrations (µg/ ml of medium) on day 14 of the *in vitro* experiment.

Effect of magnesium hydroxide on yeast colony count in vitro

All yeast species developed less intensely in the presence of magnesium hydroxide than on the controls. At a concentration of 125 µg/ml of medium, colonies of all yeast species were observed, whereas at concentrations of 250 and 500 µg/ml of medium, yeast of the species *S. cerevisiae* did not grow and yeast of the other two species grew less intensively than at a concentration of 125 µg/ml of medium.

Table 2. Effect of magnesium hydroxide concentration in PDA medium on growth colony of tested yeast strains/isolates.

Concentration of magnesium hydroxide	Yeast			
	Coobra	Saflager W 34/70	117/10	*T. delbrueckii*
Non-modified medium	196.50 a	93.75 a	216.00 a	231.50 a
125 µg/ ml medium	57.50 b	23.75 b	127.75 b	168.50 b
250 µg/ ml medium	0 c	0 c	38.75 c	43.25 c
500 µg/ ml medium	0 c	0 c	20.25 c	23.50 c

Magnesium hydroxide is patented for use as an agricultural contact fungicide by Macaigne and Duclos (2020). Magnesium hydroxide [$Mg(OH)_2$] nanoparticles have been shown to disrupt the cell surface of the plant pathogen *P. infestans*. This is toxic to the pathogen and can inhibit metabolic pathways and membrane transport activity (Wang et al., 2022; Battaglia et al., 2023). The development of an effective strategy of $Mg(OH)_2$ application with biological agents, taking into account the economic point of view and the additional positive effect of magnesium as a fertilizer, can be a promising solution to the problems of potato cultivation under OR rules.

Conclusions

The newly approved active ingredient – magnesium hydroxide – is promising and can be used (at a dose of 125 g/100 l of water) to protect potatoes against late blight, especially since it can be combined with the *C. albidosimilis* 117/10 isolate and *T. delbrueckii*, which are strong competitors for the foliar pathogens of many crops. Further testing under field conditions is needed, taking into account different application strategies and potato varieties.

References

Battaglia, G., Domina, M. A., Lo Brutto, R., Lopez Rodriguez, J., Fernandez de Labastida, M., Cortina, J. L., Pettignano, A., Cipollina, A., Tamburini, A. and Micale, G. 2023. Evaluation of the purity of magnesium hydroxide recovered from saltwork bitterns. Water 15: 29. https://doi.org/10.3390/w15010029

Kowalska, J. 2024 a. Strategies for yield losses limitation caused by main potato pathogens in organic farming. IX Congress on Plant Protection, Zlatibor, Serbia, 25-28.11.2024.

Kowalska, J. 2024 b. Protection of organic potato: strategies for disease management with basic substances. P.21. In: Book of abstracts IV International Organic Fruit Symposium and II International Organic Vegetable Symposium 17-20. September 2024, p. 92. 17-20 September 2024, Warsaw, Poland.

Kowalska, J., and Krzymińska, J. 2024. Basic substances as usefulness substances to limit major pathogens of potatoes in organic farming. In : Book of Abstracts XX International Plant Protection Congress, Ateny, Grecja 1-5.07.2024, p. 328. https://www.ippcathens2024.gr/abstracts

Kowalska, J., Krzymińska, J., Matysiak, K., and Jakubowska, M. 2022. Screening for antagonistic yeasts to manage *Alternaria* spp. in organic farming. Agriculture-Basel 12(10): 1693. https://doi.org/10.3390/agriculture12101693

Macaigne, N. and Duclos, J. 2020. https://patents.google.com/patent/WO2020188225A1/en

Marchand, P. A. 2017. Basic Substances under EU Pesticide Regulation: An Opportunity for Organic Production? Organic farming 3(1):16-19.

Romanazzi, G., Orçonneau, Y., Moumni, M., Davillerd, Y., and Marchand, P. A. 2022. Basic substances, a sustainable tool to complement and eventually replace synthetic pesticides in the management of pre and postharvest diseases: Reviewed instructions for users. Molecules 28;27(11): 3484.

Toffolatti, S., Davillerd, Y., D'Isita, I., Facchinelli, Ch., et al. 2023. Are Basic Substances a Key to Sustainable Pest and Disease Management in Agriculture? An Open Field Perspective. Plants 12(17): 3152. https://doi.org/10.3390/plants12173152

Wang, Z. L., Zhang, X., Fan, G. J., Que, Y., Xue, F. and Liu, Y. H. 2022. Toxicity effects and mechanisms of MgO nanoparticles on the oomycete pathogen *Phytophthora infestans* and its host *Solanum tuberosum*. Toxics 10(10): 553. doi: 10.3390/toxics10100553.

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Antibacterial activity *in vitro* and possible mechanism of soulatrolide and derivates against *Ralstonia solanacearum* in banana crops

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Abstract: The most important losses in banana crops have been by phytopathogenic agent. One of these phytopathogenic bacteria is *Ralstonia solanacearum.* It is identified as the second most important phytopathogenic bacteria from a scientific and economic point of view after *Pseudomonas syringae* (Abdoussalami et al., 2023). There is a need for a transition to new control products to replace chemical synthesis products. Currently, global contributions in "One Health" models are highlighted in a joint search for integrated control solutions for plant pathogens included unexplored sources soil and plant biodiversity (Dey and Sen, 2023). The *Calophyllum* genus is a source of metabolites with antimicrobial potential, with reports in the medical field and few in the control of phytopathogenic. One of the major components of species *Calophyllum brasiliense*, is a primranocoumarina denominated soulatrolide. In this study, the biological activity and possible action mechanism of soulatrolide and compounds type 4-phenylcoumarins were evaluated on a phytopathogenic microbial isolate of *R. solanacearum* (Mesa et al., 2029).

The metabolites were characterized using spectroscopic techniques of ^{1}H -RMN on Bruker Ascend II HD 600 MHz and mechanisms of action were established by microscopy SEM. *In vitro* biological assays were carried out using the disk diffusion method with the extract and compounds on *R. solanacearum* with range concentration of (1000-312.5ppm).

A promising activity on *R. solanacearum* has now been found with inhibition zones > 25.0 ± 0.1 and via transmission electron microscopy indicated destruction of the cell structure. This is the first report of promising activity of *C. brasiliense* on *R. solanacearum*. The results of this work will have a comprehensive understanding of the activity of the compounds tested. This information motivate to continue with the studies of *C. brasiliense* and its metabolites in order to contribute alternatives for problems which interfere with control of phytopathogenic bacteria through research aiming to uncover biocontrollers from the biodiversity with application in foods of great global importance.

Key words: biocontrol, phytopathogenic bacteria, phenylcoumarins, bioinputs, metabolites

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Universidad de Antioquia – CODI – for the financing of the code project 2023-62870.

References

Abdoussalami, A., Hu, Z., Islam, A. R. M. T., and Wu, Z. 2023. Climate change and its impacts on banana production: a systematic analysis. Environ. Dev. Sustain. 25: 12217-12246. https://doi.org/10.1007/s10668-023-03168-2

Dey, P., and Sen, S. K. 2023. A review on solanaceous plant diseases caused by *Ralstonia solanacearum* having serious economic impact. Plant Arch. 23(2). https://doi.org/10.51470/PLANTARCHIVES.2023.v23.no2.072

Mesa, A., Blair, S., Peláez, C., and Carda, M. 2019. Antiplasmodials soulattrolide derivatives from *Calophyllum brasiliense* and its mechanism of activity. J. King Saud Univ. Sci. 31(4): 1208-1214. https://doi.org/10.1016/j.jksus.2019.03.005

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Comparative metagenomic analysis of plant pathogenic microorganisms in drainage water from greenhouse of paprika, tomato and strawberry in South Korea

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Abstract: Greenhouse cultivation is rapidly expanding worldwide as an optimized environment for maximizing crop productivity. However, once plant diseases occur in greenhouses, they spread more rapidly than in open field cultivation, leading to greater economic losses. Additionally, the management of drainage water after cultivation poses a significant challenge. In this study, we analysed the microbial communities present in greenhouse drainage water to develop an effective plant disease management strategy and promote sustainable recirculating agriculture. Drainage samples were collected in spring and autumn from tomato, paprika, and strawberry greenhouses, and microbiome analysis was conducted to examine fungi, bacteria, and viruses. In tomato greenhouses, the spring samples contained 92 fungal species, 98 bacterial species, and 45 viral reads, whereas the autumn samples contained 86 fungal species, 98 bacterial species, and 95 viral reads. In paprika greenhouses, the spring samples contained 97 fungal species, 100 bacterial species, and 53 viral reads, while the autumn samples contained 90 fungal species, 98 bacterial species, and 88 viral reads. In strawberry greenhouses, both spring and autumn samples contained 100 fungal, bacterial, and viral reads each. When analyzing the top 10 microorganisms from each sample, the majority were commonly found in soil, wastewater, or freshwater, regardless of crop type or sampling season. This result suggests that the controlled environment of greenhouse cultivation influences the microbial community composition.

Among the identified microorganisms, major fungal pathogens were detected: *Fusarium oxysporum* in tomatoes; *F. oxysporum, F. solani, Rhizoctonia solani*, and *Botrytis cinerea* in paprika; and *F. oxysporum, F. solani, R solani*, and *Sclerotinia sclerotiorum* in strawberries. Seasonal comparisons revealed that the diversity and read count of plant pathogenic fungi were higher in spring than in autumn. Furthermore, pathogens not infecting the cultivated crops but capable of causing diseases in other plants and animals were also identified. These findings indicate that plant diseases detected in the greenhouse microbiome analysis likely occurred during the cultivation period and suggest that soilborne fungal pathogens may spread through drainage water. This preliminary study provides fundamental data for plant disease management in greenhouse systems. Further research is required to establish efficient drainage water treatment strategies to support sustainable agriculture.

Key words: microbiome analysis, plant pathogen, paprika, tomato, strawberry

Session VIII
Industry and academia: a winning partnership for biocontrol product development

A web-based platform for biologicals in coffee production: a dynamic tool to offer updated content and connection between stakeholders

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Abstract: The development of biocontrol relies on the connection between the different players of crop production from industry, sales representative, academia, consultants and farmers with updated knowledge on the problem characterization and fine-tuned recommendation of a microbial-based product that meets each need. However, the difficulty in connecting those different players reduces the conversion rate of knowledge generated by one side to the others and is converted in valuable information to crop production. We have proposed an innovative model that connects them through a web-based platform with live updates about biologicals in coffee production. The platform has been designed to bring about different aspects of coffee issues regarding the crop production (growth regulation, nutrition, pest, disease, weed and post-harvest) and how microbial(-based/derived) solutions are proposed to cope with each issue. Each content has been written by specialists in the subject. The platform is open access and users are able to also propose content updates. The review process follows a scientific journal format with updates sent to the editor-in-chief, if it passes in the flag as a relevant content update, it is transferred by them to the associate editor specialist on the subject. If the update is evaluated as pertinent, the content is incorporated to the original text and the contributor receives the deserved credit. Each user, editor, reader or contributor needs to register to the system to have access to the platform. Therefore, the initiative also connects the different players of coffee production. The success of the project relies on the adoption by as many players of the biocontrol community as possible. The content will be initially proposed in Portuguese but the translation to other languages is a plausible possibility in follow up steps of the project. We present preliminary data on the advances in content update and gains in efficacy from the adoption of the platform.

Key words: biocontrol, biostimulant, inoculant, crowdsourcing, private-public partnership

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Abiotic stressors and their influence on *Lysobacter enzymogenes* efficacy against grapevine downy mildew

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Abstract: *Plasmopara viticola*, the causal agent of downy mildew on grapevines, is one of the most destructive diseases in viticulture. In conventional grapevine production, chemical control remains the most efficient strategy, while organic production primarily relies on copper-based fungicides. In line with the European Green Deal's Farm to Fork strategy, the need for sustainable and environmentally friendly plant protection strategies remains crucial. *OptiLyso* is a joint project that aims to develop a microbial plant protection product based on the bacterium *Lysobacter enzymogenes* isolate JKI-BI-6432 against fungi and oomycetes. The use of biological control with beneficial microorganisms, such as *L. enzymogenes*, represents a promising alternative. In previous studies this bacterium demonstrated a broad antagonistic activity against plant pathogens in *in vitro* and greenhouse experiments (Drenker et al., 2023).

For successful field application, the biocontrol efficacy of *L. enzymogenes* must be maintained under diverse environmental conditions. Factors such as solar radiation, rainfall, heat stress, and temperature variation may influence its effectiveness. Assessing these abiotic factors during product development is essential for optimizing performance and identifying suitable additives or formulations to address potential challenges. In this study, potted grapevine plants were exposed individually to key abiotic factors after spray treatment with *L. enzymogenes* formulations and potential additives. Solar radiation was simulated using sunlight-like lamps, while climate chambers simulated different humidity and temperature levels. Rainfastness was tested using a technical set up with different precipitation levels. One day after treatment, plants were inoculated with *P. viticola*, and disease severity was assessed after seven days. The findings of the study showed that perfomance can be improved through adjustments in production and formulation.

L. enzymogenes shows great potential as a sustainable microbial plant protection product. Understanding the impact of abiotic factors on its biocontrol performance is vital for optimizing efficacy and ensuring its practical integration into field applications.

Key words: microbial antagonist, biocontrol, viticulture

Reference

Drenker, C., El Mazouar, D., Bücker, G., Weißhaupt, S., Wienke, E., Koch, E., Kunz, S., Reineke, A., Rondot, Y. and Linkies, A. 2023. Characterization of a disease-suppressive isolate of *Lysobacter enzymogenes* with broad antagonistic activity against bacterial, oomycetal and fungal pathogens in different crops. Plants 12: 682.

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Aureobasidium pullulans and its multiple potentials in biological control: pullulan production for postharvest fungal diseases management

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Extended Abstract: *Aureobasidium pullans* is a black yeast mainly known for its antagonistic properties against plant fungal pathogens. In fact, this microorganism represents a sustainable solution to limit fungal diseases during storage through multiple antagonistic modes of action, as reported by many studies (Di Francesco et al., 2023; Ippolito et al., 2000). Nevertheless, *A. pullulans* is known to be an excellent producer of pullulan, a polysaccharide known to be tasteless, odourless, non-toxic, non-hygroscopic, and safe for human consumption. Due to its edible nature, it is recognized as a GRAS (Generally Recognized as safe) substance, making it ideal in various food-related sectors.

In recent years, plastic-based materials have been widely used for fruit packaging and their production results in the emission of harmful substances for the environment and human health. In this way, pullulan could represent a sustainable alternative as fruit coating polymer to limit the development of fungal diseases, also combined with biocontrol agents (BCAs) characterized by effective activity against one or more postharvest fungal pathogens.

In this study, ten different *A. pullulans* strains were tested for their polysaccharide production attitude. The expression level of *pgm1* and *ugp* genes, known to be a key factor in the biosynthesis pathway of pullulan, as evaluated and showed a significant difference between the strains. The pullulan products were also biochemically characterized to verify the differences between them and with the commercial one. The biopolymers were formulated as apple and grape coating, used to control respectively *Colletotrichum acutatum* and *Botrytis cinerea* by *in vitro* and *in vivo* assays, with or without the addition of live cells of the tested yeast strains.

All the yeast strains demonstrated to be able to produce pullulan. The AP1, UOR18, M13 strains were chosen for the highest polysaccharide production, and in particular *A. pullulans* AP1 strain. The analysis of *pgm1* and *ugp* genes confirmed the obtained polysaccharide production by the strain AP1. FTIR analysis was used to verify the chemical properties of the obtained pullulans. All the polymers were quite similar to each other and to the standard. However, pullulan produced by AP1 stain was the most similar to the standard and also reported a higher water content. The AP1 pullulan was probably more able to entrap moisture This fact could increase moisture content in internal fruit atmosphere composition if pullulan is applied as coating, so extending the shelf-life period.

In vitro and *in vivo* results showed that by incorporating the cells of each pullulan-producer strain into the film/coating formulation, the polymer's efficacy was higher as a potential treatment to prolong fruit shelf life. Encouraging results were achieved by incorporating *A. pullulans* AP1 cells in the film/coating as preventative application against *C. acutatum* and *B. cinerea* of apple and grape, respectively. Integrating a coating matrix with an active BCA, this can increase the microorganism's survival rate (Lieu et al., 2024) and enhance its uniform spread, providing a successful fruit colonization by the BCA (Marin et al., 2016).

Our results showed how, by the metabolism of *A. pullulans,* it is possible to obtain a product that can be used for the same purposes as the microorganism itself. Nevertheless, there are many factors to consider for developing an active coating/film, such as the viability and stability of the BCA and the total absence of potential food safety hazards.

Key words: yeast strains, polysaccharide, coating, postharvest fungal pathogens, fruit

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References

Di Francesco, A., Zajc, J., and Stenberg, J. A. 2023. *Aureobasidium* spp.: Diversity, Versatility, and Agricultural Utility. Horticulturae 9: 59.

Ippolito, A., El Ghaouth, A., Wilson, C. L., and Wisniewski, M. 2000. Control of postharvest decay of apple fruit by *Aureobasidium pullulans* and induction of defence responses. Postharvest Biol. Technol. 19: 265-272.

Lieu, M. D., Phuong, T. V., Nguyen, T. T. B., Dang, T. K. T., and Nguyen, T. H. 2024. A review of preservation approaches for extending avocado fruit shelf-life. J. Agr. Food Res. 16: 101102.

Marín, A., Cháfer, M., Atarés, L., Chiralt, A., Torres, R., Usall, J., and Teixidó, N. 2016. Effect of different coating-forming agents on the efficacy of the biocontrol agent *Candida sake* CPA-1 for control of *Botrytis cinerea* on grapes. Biol. Control 96: 108-119.

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Microbiome communities exhibit type-specific function in key Champagne grape varieties from the Champagne region in France

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Abstract: Under the increase of biotic and abiotic challenges in viticulture, it is essential to understand the intricate relationships between grapevine microbiota, varietal distinctions, and developmental stages. This study addresses existing knowledge gaps by applying shotgun metagenomics to explore microbial functions in key Champagne grape varieties – Pinot Noir, Meunier, and Chardonnay. Samples of leaves and inflorescence at full flowering stage were collected from three distinct Moët & Chandon vineyard locations in France to ensure a representative functional analysis of microbial communities. We focused on untreated vineyard samples, from which DNA was extracted for Illumina sequencing. Functional annotation revealed significant functional variations in metabolic potential across grape varieties and sample types ($p < 0.05$) including amino acid, carbohydrate metabolism, energy production, and secondary metabolite biosynthesis. Functional CAZy annotations revealed distinct patterns in Chardonnay, Pinot Noir, and Pinot Meunier, with variation in sample type influencing the distributions of Glycoside Hydrolases and Glycosyl Transferases, including specific enzyme classes like zeatin O-beta-glycosyltransferase. The Principal Component Analysis and Non- Metric Multidimensional positioning confirmed distinct microbial community patterns between leaves and inflorescences. Differential abundance analysis using LEfSe identified key biomarkers involved in plant-microbe interactions, including ABC transporters, pyruvate metabolism, and pathways related to replication, recombination, and repair. KEGG pathway Analysis highlighted three major microbial function, including 2-oxocarboxylic acid metabolism, biosynthesis of amino acids, and secondary metabolites. These findings provide novel insight into variety and plant part-specific grapevine microbiomes, potentially guiding microbiome management strategies.

Key words: champagne grape, functional annotation, grapevine, microbiome, metabolic pathway

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Bio-source substances against postharvest diseases of fruits: Mechanisms, applications and perspectives

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Abstract: The safety and quality of fruit after harvest have always been an issue of people's livelihood. Due to increasing concerns about food safety and environmental pollution caused by excessive application of fungicides, there is a growing interest in the exploitation of antifungal bio-source substances. Therefore, it has been the focus of postharvest pathology to reveal the mode of the action and the effect on disease prevention of these antifungal bio-source substances. In recent years, some new bio-source substances have been explored that have enormous potential in postharvest disease control in various fruits, and their mechanisms against fungal pathogens have also become understood. We will demonstrate the recent advances in the mechanisms and applications of these bio-source substances for suppressing fungal pathogens and maintaining the intrinsic quality of fruits. Moreover, further research hotspots and development directions are also proposed.

Key words: antifungal bio-source substances, bio-source, disease, fruit, quality

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Harnessing *Streptomyces* for sustainable biocontrol in agriculture: The discovery of *Streptomyces* sp. GanoSA1

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Abstract: Phytopathogens significantly threaten global agriculture, causing devastating losses in crop yield and quality. These plant pathogens, including fungi, bacteria, viruses, and nematodes, can trigger diseases that weaken plant health, reduce productivity, and threaten food security. The economic impact is immense, as farmers suffer financial losses due to reduced harvests and increased costs for disease management. Moreover, phytopathogen outbreaks can disrupt supply chains and increase food prices. Climate change and global trade further exacerbate the spread of these pathogens, necessitating sustainable disease management strategies such as biocontrol, resistant crop varieties, and integrated pest management, enhanced the growth of oil palm seedlings and reduced the effect and severity of BSR on test seedlings, suggesting that it may have the potential to be used as a biopesticide. This paper describes the discovery of *Streptomyces* GanoSA1 from *in vitro*, greenhouse and field, proving its potential for biocontrol activity that is environmentally friendly and sustainable.

Key words: *Streptomyces*, biocontrol, phytopathogens, sustainable agriculture, green technology

Introduction

Sustainability is a crucial aspect of modern agriculture, emphasizing the need for environmentally friendly and long-lasting solutions to plant disease management. Many crops face significant economic and environmental challenges due to soil-borne fungal pathogens. Conventional control strategies heavily depend on chemical fungicides, which contribute to environmental degradation and have limited long-term efficacy. Biological control has emerged as an essential approach in plant disease management to pursue sustainable agricultural practices and environmentally friendly strategies. Amongst all microbes with potential biological activities, actinomycetes, a group of versatile and prolific bacteria, have garnered significant attention for their potential in pest management and crop protection. Awareness rising over the environmental impact of synthetic pesticides and the evolution of resistance in target organisms have underscored the urgency of exploring alternative methods. Biological control, which relies on the ecological relationships between organisms, offers a promising impact for sustainable agriculture. Actinomycetes, characterized by their filamentous structure and ability to produce many bioactive compounds, are valuable contributors to this approach. This paper highlights the scientific journey to the discovery of *Streptomyces* GanoSA1, which started with the isolation, screening, identification, and evaluation of bioactivity. Through a comprehensive exploration of recent research findings, we seek to provide a comprehensive overview of the current state of knowledge regarding the application of actinomycetes in the biological control of *Ganoderma* in oil palm.

Methods

Actinomycetes were isolated from the oil palm rhizosphere (*Elaeis guineensis* Jacq.) and screened against several phytopathogens with a focus towards *Ganoderma boninense* PER71 through a modified dual plate assay. The potential isolates' identities were determined using 16S rRNA gene sequence analysis. The antifungal mechanism by the strain was determined *in vitro*, and the biocontrol effect was determined *in plantae*. Disease incidence and plant growth parameters were monitored under a controlled greenhouse experiments and long-term field trials. Enzymatic assays were conducted to assess induced systemic resistance (ISR) mechanisms.

Results and discussion

Our findings demonstrated that *S. nigrogriseolus* GanoSA1 exhibited antifungal activity against several fungal pathogens through multiple mechanisms, including bioactive compound production, competition for nutrients, and secretion of hydrolytic enzymes such as chitinase and glucanase. In nursery trials, seedlings treated with *S. nigrogriseolus* GanoSA1 showed a significant reduction in disease incidence and a decrease in disease severity index compared to untreated controls. Furthermore, field studies revealed that long-term biocontrol formulation application improved plant health and reduced fungal infection. The application of *S. nigrogriseolus* GanoSA1 also enhanced plant growth, as evidenced by increased seedling height, stem diameter, and chlorophyll content. The upregulation of pathogenesis-related proteins, such as peroxidase (POX) and polyphenol oxidase (PPO), further suggests the induction of ISR in treated plants.

Conclusion

The application of *S. nigrogriseolus* GanoSA1 for biocontrol activities offers an effective and sustainable alternative to chemical fungicides for managing fungal diseases in crops. This approach aligns with green technology initiatives and sustainable agricultural practices by leveraging its dual benefits of disease suppression and plant growth promotion. Integrating *Streptomyces*-based biocontrol into crop management strategies could significantly mitigate disease losses while promoting environmentally friendly crop protection.

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Orange you glad it's not chemicals? Biocontrol of *Citrus* soilborne diseases

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Abstract: *Citrus* fruits are some of the most widely cultivated and consumed fruits across the globe. However, they are vulnerable to stresses, such as the root rot complex, which can result in extensive economic losses. The use of resistant rootstocks may mitigate this disorder. Unfortunately, the impact of such cultural practices is often limited, resulting in a heavy reliance on agrochemicals despite their known negative environmental and consumer health implications. The need for sustainable and safer alternatives has become imperative, with microbial biocontrol emerging as a possible candidate. However, the variability and inconsistency in control efficacy have severely limited its industrial applications. In this study, three possible biological control agents (BCA), *Bacillus subtilis* N19 (N19), *Pseudomonas fluorescens* N83(N83) and *Trichoderma hamatum* P4 (P4), were selected to develop more effective and consistent management programs. These agents were screened curatively and preventatively against the common soilborne pathogens *Phytophthora nicotianae* (PN) and *Pythium irregulare* (PI), *in planta*, under greenhouse conditions. The effect of the BCAs was based on seedling growth parameters, including height change percentage, whole seedling mass, root mass, and root volume. Three curative applications (3C) of N19 improved seedling height (54 %), whole mass (39 %), root mass (33 %), and root volume (18 %) of the host. A 3C application of P4 also increased all four growth parameters measured. Seedlings that received a 3C application of P4 also showed a 58 % to 69 % reduction in the percentage of pathogens reisolated compared to the untreated, diseased controls. Three applications of N83 only increased seedling height (6 3%) and total mass (154 %). Preventative applications of N19, two weeks prior to infection (pti) resulted in increased seedling height (87 % to 427 %). Additionally, the lowest percentages of pathogens were reisolated at this time, ranging between 18 % and 19 %, compared to the relevant controls (73 % and 74 %). Preventative N83 treatments applied two and four weeks pti improved total seedling mass (90 % to 198 %) and root mass (76 % to 193 %). Preventative P4 treatments increased only the seedling height (222 % to 295 %) when applied six weeks pti but showed the highest reduction (35 % to 79 %) in the reisolated pathogen levels compared to the untreated, diseased controls. The preventive and curative effects of the three possible BCA were demonstrated successfully. Curatively, N19 and P4 were the most effective, with three applications resulting in the highest plant growth parameters and the lowest pathogen reisolation levels. Preventative use of P4 had the longest lasting preventative effect followed by N83 and then N19. The results obtained can contribute to the development of more effective biological control management practices against *Citrus* soilborne pathogens.

Key words: growth promotion, curative, preventative, greenhouse, biological control

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Boosting the performance of *Pythium oligandrum* for biocontrol and biostimulation in potato

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Abstract: Potato (*Solanum tuberosum*) ranks as the world's third most important food crop, following rice and wheat, with an annual production of 330 million tons, making it crucial for food security globally. However, pathogens like *Phytophthora infestans*, the causal agent of late blight, and *Alternaria solani* that causes early blight, pose significant threats to potato cultivation globally. These pathogens cause substantial annual losses and have developed highly aggressive strains that undermine current control strategies. The situation is exacerbated by climate change, which is expected to further reduce yields. The oomycete *Pythium oligandrum* is a mycoparasite with potential as a biocontrol agent and biostimulator in potato. Although commercial preparations of *P. oligandrum* are approved for use in potato and other crops within the EU, adoption of *P. oligandrum* remains limited due to challenges such as inconsistent standardization and limited shelf life, which deter farmers from opting for it over chemical alternatives. Potato crops are typically cultivated in sandy soils; however, much of the research on *P. oligandrum* has been conducted either *in vitro,* in compost or in nutrient-rich soils, leading to uncertainties when applied to field conditions. In this study, we examine the differences in root colonization by *P. oligandrum* across soils with varying sand concentrations. We are also investigating the effectiveness of *P. oligandrum* biocontrol when it is faced with both *P. infestans* and *A. solani,* as may be the case within the potato cropping system. Finally, we evaluate the growth-promoting properties of *P. oligandrum* across 96 potato cultivars and investigate correlations with specific genetic markers, as a prelude to breeding for potato cultivars that better host with biocontrol agent. Our findings aim to improve our understanding of *P. oligandrum* efficacy under crop production systems, and to enhance its effectiveness as a biocontrol and growth-promoting agent.

Key words: growth promotion, pathogen co-inoculation, *Solanum tuberosum*, potato disease

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Standardised methods to screen the efficacy of a novel biological control fungicide against fungal pathogens *in vivo* and *in vitro*

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Extended Abstract: Plant pathogens cause great losses to agricultural production with important economic and environmental impact. Chemical fungicides have been largely used in crop protection to combat and reduce the damage caused by pathogens. However, concerns raised towards the reduction of the use of these conventional crop protection methods due to long-term risks on human and animal health and their impact on the environment (Lahlali et al., 2022). As a result, biological control is a promising alternative to conventional disease management; a wide range of biocontrol agents and products derived from natural plant extracts have been developed and studied by researchers to contain fungal and bacterial diseases (Bonaterra et al., 2022). In this context, effective, fast, accurate, repeatable and cheap screening methods of biological control products have become an important tool in plant pathology.

In this study, we present standardised *in vitro* and *in vivo* tests for screening a potential biological plant protection product against multiple fungal pathogens. These standardised tests will help expedite the development of the biological product and help understanding its mode of action. Secondly these methods can be used as a benchmark in potentially monitoring resistance for biological fungicides.

The product evaluated in this study is a plant extract that has been tested for its potential as a plant protection product against multiple fungal pathogens including *Puccinia hordei, Blumeria graminis* f. sp. *tritici and Puccinia recondita* f. sp. *tritici* for barley and wheat, *Erysiphe necator* and *Plasmopara viticola* for grape *Podosphaera leucotricha* on apple, *Phytophtora infestans* on potato *in vivo*, while *Botrytis cinerea* and *Sclerotinia sclerotium in vitro.* The spores of the respective fungal pathogens were collected within a sampling program throughout different fields and regions across Germany and Europe. The sampling of the plant pathogens was performed by EpiLogic GmbH, Freising (Germany) through field samples and/or air sampling. Depending on the pathogen, *in vitro* and/or *in vivo* tests have been used. *In vitro* tests consisted of the direct contact of the fungal pathogen with the potential bio-fungicide. The potential bio-fungicide is dissolved in either liquid or in agar culture medium. After incubation the mycelial growth was assessed. *In vivo* tests consisted of treating infected leaf segments/discs with different plant extract concentrations. After incubation, the percentage of infection is scored by comparing the growth of the fungal pathogen with the untreated control. The advantage of the *in vivo* test is that it allows for the determination of fungicide efficacy on living tissues, which is crucial for the testing of obligate fungal pathogens, such as *Puccinia* spp. and *Erysiphe necator*. Therefore, the biological product was tested under three different conditions *in vivo*. Plants were treated one day before infection to assess its preventive activity, during growth and one day before the infection to highlight the capacity of enhancing plant defences, and one day after infection to evaluate the curative effect of the candidate. Combining the results obtained from different treatments and different assays the biological product could be determined for its efficacy as a biological fungicide. By testing multiple fungal isolates in the assay, possible adaptation and efficacy spectrum against field fungal populations could also be tested.

The chosen biological product has shown a disease severity reduction (efficacy, %) of up to 99.7 % against rust in wheat (*Puccinia recondita* f. sp. *tritici*) and 98.6 % against rust in barley (*Puccinia hordei*) when treated after infection. Interestingly, efficacy was retained when plants were treated one day before infection with up to 77.5 % efficacy for *Puccinia recondita* f. sp. *tritici* and 32.8 % efficacy for *Puccinia hordei.* The biological product also showed efficacy of 83.4 % against *Blumeria graminis* f. sp. *tritici*, 78.4 % against *Erysiphe necator* and 74.7 % against *Podosphaera leucotricha* when treated immediately after infection. The efficacy of the biological product was highly reduced when applied on plants one day before infection, with efficacy ranging from 18.6 % to 27.5 % in powdery mildew of wheat and apple. The biological product also showed efficacy in *in vitro* tests against *Botrytis cinerea* and *Sclerotinia sclerotium* with geometric mean EC50 values of 8.6 ml/l and 2.2 ml/l, respectively. The *in vitro* assay shows that in contact, the biological product reduced mycelial growth of both pathogens. The biological product was also tested against *Pyrenophora teres* both *in vitro* and *in vivo*, which showed efficacy *in vitro* with a geometric mean EC50 value of 7.7 ml/l, but no efficacy *in vivo* on leaf segments against *Pyrenophora teres*.

However, the biological product showed no efficacy against the plant pathogenic oomycetes *Plasmopara viticola* and *Phytophtora infestans* when tested *in vivo* on leaf segments. This study suggests that the biological product can be proposed as a potential broad-spectrum biofungicide.

Further on, *in vivo* tests allow to provide further insights on the mode of action of the biological product, such as its preventive or curative effect and its capacity to prime plant defences response after repeated applications. In conclusion, through standardised small scale *in vivo* and *in vitro* testing, we could determine in a short period of time the potential of the test product as an effective biological fungicide.

References

Bonaterra, A., Badosa, E., Daranas, N., Francés, J., Roselló, G., and Montesinos, E. 2022. Bacteria as Biological Control Agents of Plant Diseases. Microorganisms 10(9): 1759. https://doi.org/10.3390/ microorganisms10091759

Lahlali, R., Ezrari, S., Radouane, N., Kenfaoui, J., Esmaeel, Q., El Hamss, H., Belabess, Z., and Barka, E. A. 2022. Biological Control of Plant Pathogens: A Global Perspective. Microorganisms 10(3): 596. https://doi.org/10.3390/microorganisms10030596

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Contribution of crop residues of grasses and exhausted substrate from mushroom production in the establishment of *Trichoderma asperellum* (BV 10)

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Abstract: The crops stubble represents a niche for necrotrophic pathogen survival and a plausible strategy is to displace them from this niche. However, the high lignin content does not always offer a suitable environment for the establishment of antagonist. Through field observations in a sugarcane straw, mushrooms of the order Agaricales were associated to *Trichoderma* spp. The simultaneous presence of these fungi in the soil can provide synergistic benefits for agriculture, such as the decomposition of organic residues, the cycling and availability of nutrients, formation of soil structure, suppression of pathogens and promotion of growth. In this context, the objective of this work was to evaluate whether the fungus *T. asperellum* (BV 10) establishes itself better when associated with grass straw or exhausted substrate from Shimeji (*Pleurotus ostreatus*) production, after application of products based on the fungus. The experiment was developed in the Laboratory of Biocontrol of Plant Diseases, Department of Plant Pathology, at the Federal University of Lavras, and consisted of six treatments arranged in four blocks, for sterilized and non-sterilized stubble and substrate, and with the amendment or not of a straw decomposition accelerator product. Firstly, exhausted substrate was obtained from a mushroom producing company, samples of maize and sugarcane stubble, with part of the samples being sterilized in the laboratory. The treatments were arranged in a 9-centimeter Petri dish, with a solution of nutrient agar and water, without the use of antibiotics, in a randomized block design. The plates with the treatments were placed in cabinets, at an average temperature of 25 °C and 24 hours of darkness. After applying *T. asperellum* (BV 10) to the stubble, the sporulation of the fungus was monitored and 7 days later, quantified the number of conidia per ml, using a Neubauer Chamber. Statistical analyses performed in the RStudio Program. According to the results, the autoclaved sugarcane stubble provided largest colonization rate with the *T. asperellum* (BV 10) treatment. In both autoclaved and non-autoclaved maize stubble, the treatment with only *T. asperellum* (BV 10), obtained largest colonization average. In relation to the non-autoclaved exhausted substrate, the *T. asperellum* (BV 10) + Stubble decomposition accelerator treatment, was the most effective, compared to the autoclaved exhausted substrate, which resulted in lower colonization averages for all treatments. The management of grass straw with products based on *T. asperellum* can be adopted with the aim of not only controlling phytopathogens but also acting on the decomposition of crop residues. While the exhaust substrate can be subjected to future studies to better understand the association with *T. asperellum*, and a possible adoption of this interaction in the production system.

Key words: biological control, stubble, plant disease management

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Optimizing resistance inducers application: a model for simulating their protection dynamic over time

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Extended Abstract: Restrictions in the use of pesticides (Directive 128/2009/EC and EU Green Deal) encourage EU Member States to promote low pesticide-input pest control by the implementation of IPM (Integrated Pest Management) tools, and by giving priority to non-chemical products. Innovative systems and products have been therefore progressively introduced for sustainable agriculture, including weather-driven models and decision support systems (DSSs), resistant plant genotypes, intelligent spraying, biological control agents, as well as Plant Resistance Inducers (PRIs). PRIs are molecules that prime a defense reaction in plants under pathogens' attack. Two different kinds of defense reactions are known: pattern-triggered immunity (PTI, based on molecular expression patterns activated upon the detection of microbial molecules or plant endogenous molecules) and effector-triggered immunity (ETI, highly specific reaction resulting from direct and indirect interactions between effectors produced by the pathogen and the products of plant resistance genes during a strong and local defense response).

Recent studies demonstrated that PRIs are reliable alternatives to reduce the amount of copper and sulphur in the control of *Plasmopara viticola* and *Erysiphe necator*, the two main grapevine fungal diseases (Taibi et al., 2023 a). Some of these products have a natural origin, such as laminarin (a beta-glucan extracted from the brown alga *Laminaria digitata*) and its sulfate derivatives, chitosan (derived by chitin obtained from insects, the exoskeleton of crustaceans, and fungal cell walls), Cos-Oga (mixture of chitosan fragments and pectin fragments from plant cell walls), cerevisane (extracted from cell walls of *Saccharomyces cerevisiae*), or living microorganisms like *Pythium oligandrum*. Others PRIs are chemicals, such as benzothiadiazole, acibenzolar-S-methyl, fosetyl-Al, or potassium phosphonates, the latter also showing a direct effect on the pathogen.

To better exploit their effectivness and avoid unjustified lossess of resources by the plants through defence allocation costs, PRIs application should be performed when there is a risk of pathogens' infection. The combination of plant disease epidemiological models able to predict infection events with these alternative products is therefore essential (Taibi et al., 2023 b).

To support the application of sustainable viticulture, Horta srl (www.horta-srl.com), a former spin-off company of Università Cattolica del Sacro Cuore (Piacenza, Italy), and now part of BASF Digital Farming, developed a DSS called vite.net® (Rossi et al., 2014). The DSS – which is currently used in Italy and in other European countries such as Spain, France, Portugal, and Greece – collects information in real-time about different vineyard components (air, soil, plants, pests, and diseases) and analyzes these data by using advanced modelling techniques. The DSS provides up-to-date information for the management of the vineyard at plot level, about the grapevine phenology and canopy development, the risk of infection by main fungal pathogens (i. e., downy and powdery mildews, *Botrytis* bunch rot, and black-rot) and the population dynamics of pests (berry moth, American leafhopper, mealybug), as well as the protection level provided by the last application of plant protection products of chemical origin.

To optimize the timming of PRIs, a new model, which simulates the protection dynamic provided by different resistance inducers, was developed. The model for PRIs consists of a generic algorithm, which must be parameterized for each product and target pathogen, based on experimental data collected through *ad hoc* experiments or expert elicitation. In this work, the model was parameterized for six PRIs: two chemicals (FosetylAluminium and K-phosphonate), three natural (COS-OGA, Cerevisane and Laminarin) and one microbiological (the oomycete *Pythium oligandrum*) and for the target pathogen *Plasmopara viticola*. The experiments described in detail in in Taibi et al. (2023 b) and Rossi et al (2024) were used to parameterize the model for leaves and clusters. Briefly, grapevine plants were treated with PRIs at label doses; after 1, 3, 6, 12 and 19 days, leaves and clusters were artificially inoculated with a suspension of *P. viticola* sporangia. The severity of downy mildew symptoms was then evaluated and the efficacy of the treatment was calculated compared to an inoculated and untreated control.

The model consists of two equation: the first equation defines the PRI efficacy increase pattern in the first days after treatment while the second equation determines the PRI efficacy decline pattern after the maximum efficacy has reached. Specific equation parameters are: 1st day in which the PRI shows efficacy (DAYstart); level of PRI efficacy at start (EFFstart); maximal efficacy of the PRI (EFFmax); day when the PRI has EFFmax (DAYmax); lag phase for PRI efficacy decline (lag); and rate of PRI efficacy decline after DAYmax (rate). The model was parameterized for the above mentioned PRIs using the maximum likelihood method. The coefficient of determination (R^2) of the model output compared to the field data ranged between 0.97 for cerevisane and 0.99 for *Pythium oligandrum*; other goodness-of-fit indicators were Mean Absolute Error (MAE) 0.036, Model Efficiency (EF) 0.870, Root Mean Square Error (RMSE) 0.097, Coefficient of Residual Mass (CRM) 0.032 and Concordance Correlation Coefficient (CCC) 0.993.

The implementation of this new model in the DSS will emphasize the multi-modelling approach of vite.net to optimize disease management in viticulture. DSS users can optimize, and therefore improve, the use of PRIs by appling them only when necessary, based on the outputs of both plant disease models and the new model for PRIs protection dynamic; this approach will likely reduce the cost by avoiding unjustifed sprays and will enable to increase the substitution of chemical fungicides with alternatives when possible.

Key words: Plant Resistance Inducers, model-based applications, Decision Support System

References

Rossi, V., Salinari, F., Poni, S., Caffi, T., and Bettati, T. 2014. Addressing the implementation problem in agricultural decision support systems: the example of vite.net®. Computers and Electronics in Agriculture 100: 88-99.

Rossi, V., Taibi, O., Furiosi, M., and Caffi, T. 2024. Do plant resistance inducers reduce *Plasmopara viticola* infection on grapevine berry clusters at different growth stages? Plant Disease https://doi.org/10.1094/PDIS-07-24-1575-RE

Taibi, O., Salotti, I., and Rossi, V. 2023 a. Plant resistance inducers affect multiple epidemiological components of *Plasmopara viticola* on grapevine leaves. Plants 12(16): 2938.

Taibi, O., Fedele, G., Salotti, I., and Rossi, V. 2023 b. Infection Risk-Based Application of Plant Resistance Inducers for the Control of Downy and Powdery Mildews in Vineyards. Agronomy 13: 2959. https://doi.org/10.3390/agronomy13122959.

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Hop-associated bacteria with growth-promoting traits and antifungal activities: a sustainable solution for Italian hop farms

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Abstract: In Italy, hop cultivation and pest management are still challenging. Nevertheless, the exploitation of the hop-associated microbiome offers a promising solution to identify possible beneficial microorganisms. In 2023 and 2024, root-associated bacteria were isolated from both hop wild accessions and commercial cv. *Cascade* orchards in Modena and Perugia provinces (Italy), respectively. Among a set of 263 isolates, the seven best performing bacterial isolates were selected, based on their antagonistic activities against *Verticillium dahliae* and *Botrytis cinerea* and plant growth promotion traits. Our findings propose a set of promising biocontrol bacterial isolates, belonging to the genera *Pseudomonas, Pantoea* and *Burkholderia,* for the development of alternative and sustainable strategies to control hop fungal diseases.

Key words: hop microbiota, beneficial bacteria, fungal diseases, biological control

Introduction

Female cones of hop (*Humulus lupulus* L.) are a key ingredient in the brewing industry. To meet the increasing domestic demand for hops production, many Italian farmers began cultivating hops across various regions, with a predominant focus on organic farming practices (Barbagallo et al., 2024). The quality of hop is significantly influenced by fungal infections during its cultivation, resulting in a range of diseases, such as wilts (*Verticillium* spp.) and grey mold (*Botrytis cinerea*), whose management depends on the possible choice of resistant cultivars and extensive use of fungicides (Gargani et al., 2018).

Various plant niches, such as rhizosphere, phyllosphere and endosphere, are colonized by a set of microorganisms, collectively known as plant “microbiome”. These microorganisms have long been recognized as crucial for plant growth and health (Turner et al., 2013). Research on the hop microbiome is a largely underexplored field that may offer promising ways for developing biocontrol strategies.

Our study aimed to investigate the microbial community associated with two hop-plant compartments (rhizosphere and rhizome-endosphere) for identifying hop-associated bacteria as potential biostimulant and biocontrol agents to enhance hop quality and productivity.

Materials and methods

Sampling and microbial isolation

During the growing seasons 2023 and 2024, hop samples were collected in the provinces of Modena and Perugia from wild type and cultivated hop plants (cv. *Cascade*). A set of 263 bacteria were isolated from the rhizosphere and rhizome-endosphere and cultured on both LB and ISP-2 agar media supplemented with cycloheximide (100 mg/ml), according to Anzalone et al. (2022) with modifications. All bacterial isolates were further assessed for their ability to induce Hypersensitive Reaction (HR) on tobacco and tomato plants (Klement and Goodman, 1967). Four isolates tested positive to HR assays and they were not used in further experiments.

Antagonistic activity

In dual culture assay, HR-negative isolates were screened on PDA for their antifungal activity against *B. cinerea* and *V. dahliae* (Xhemali et al., 2023). Fungal growth inhibition was measured after 12 days of incubation at 26 ± 2 °C.

Plant growth-promoting traits

A selection of bacterial isolates showing consistent antifungal activity against both *B. cinerea* and *V. dahliae* were tested for their tolerance to saline stress (Anzalone et al., 2022), ability to solubilize phosphate and produce siderophores, ammonia, and hydrogen cyanide (Vurukonda et al., 2017).

Molecular identification

The best performing bacterial isolates were molecularly identified through the 16s rRNA gene sequence analysis (Weisburg et al., 1991) by using the BLAST platform on NCBI.

Results and discussion

Bacteria associated with various plant parts, including the rhizosphere and endosphere, play crucial roles in plant health and productivity (Turner et al., 2013). In this study, microbial communities associated with hop rhizome were investigated through a cultivation-based approach. A total of 263 bacterial isolates were obtained from different hop compartments.

The major aim of this study was to isolate and identify new potential candidates for the biological control of fungal hop pathogens; therefore, antagonistic assays against *B. cinerea* and *V. dahliae* were carried out (Figure 1). The isolated bacteria exhibited varying degrees of fungal growth inhibition, with the highest reduction being 63 % (strain HB138) and 44 % (strain HB107) against *V. dahliae* and *B. cinerea*, respectively (Table 1). Most isolates were more effective against *V. dahliae*, likely because both *V. dahliae* and the isolates originate from the same niche, the soil rhizosphere, as previously reported by Berg et al. (2006). However, several isolates showed a significant inhibition against both pathogens (Figure 1), indicating their potential use as biocontrol agents.

The molecular identification of 41 bacteria, selected on the base of their pronounced antagonistic activity, revealed a strong representation of three genera: *Pantoea*, *Pseudomonas*, and *Burkholderia*. Similar studies demonstrated that rhizosphere and root-associated bacterial communities are frequently dominated by these three genera, which are known for their potential for developing microbial-based biopesticides (Bulgarelli et al., 2012; Coutinho et al., 2009).

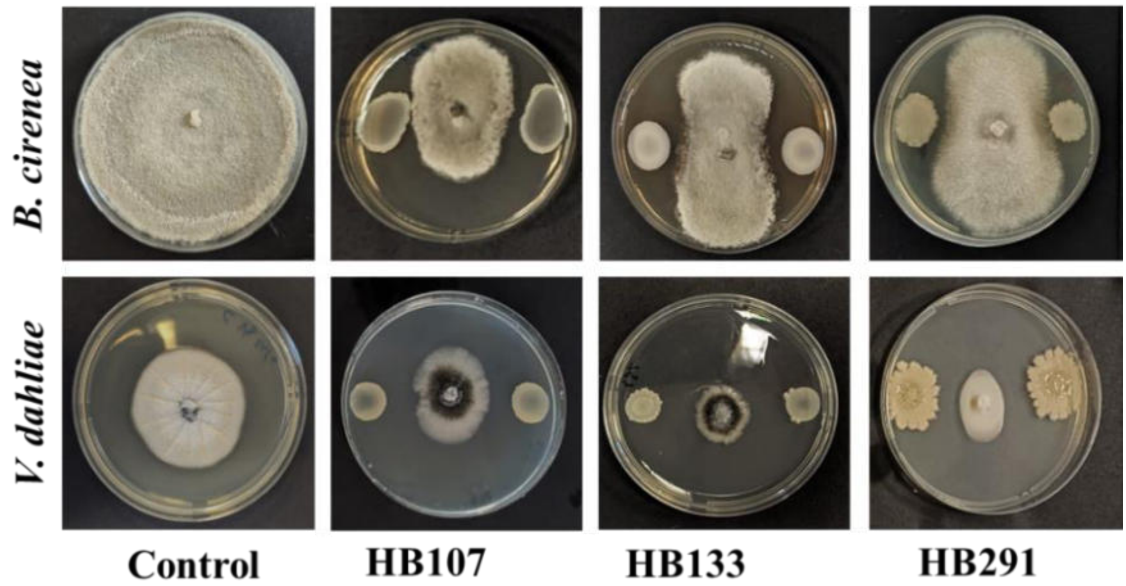

Figure 1. *In vitro* antagonistic activity of three representative bacterial isolates against two phytopathogenic fungi affecting hop in dual-culture assays on PDA medium; the bacterial inhibitory activity was assessed 6 days after *B. cinerea* and 12 days after *V. dahliae* inoculation.

Plant growth-promoting (PGP) traits shown by the identified isolates highlight their multifunctionality in enhancing plant health and suppressing pathogens. Notably, isolates HB050 and HB197 showed both marked antagonistic properties and multiple PGP traits, such as salt tolerance, phosphate solubilization and the production of ammonia, siderophores and hydrogen cyanide (Table 1). These traits are widely recognized as key mechanisms by which bacteria improve plant growth and suppress pathogens (Glick, 2012).

Table 1. Best performing bacterial isolates for plant growth promoting and antagonistic activity against phytopathogenic fungi affecting hop.

Isolates		**Source**			**Antagonistic activity (%)**		**Growth promotion traits**				
Code	**Identification**	**Locality**	**Plant**	**Niche**	**VD**	**BC**	**NaCl**	**PS**	**Sid.**	**HCN**	**NH_3**
HB050	*Pantoea* spp.	MO	Cas.	R	58	29	+	+	+	+	+
HB107	*Pseudomonas* spp.	MO	Cas.	RE	53	44	-	+	+	+	+
HB133	*Pseudomonas* spp.	MO	Cas.	RE	37	33	-	-	+	+	+
HB138	*Pantoea* spp.	MO	WT	RE	63	21	+	-	+	+	+
HB150	*Pantoea* spp.	MO	WT	RE	33	24	+	-	+	+	+
HB197	*Burkholderia* spp.	MO	WT	RE	37	17	+	+	+	+	+
HB291	*Pseudomonas* spp.	PG	Cas.	R	44	26	-	+	+	+	+

MO: Modena; PG: Perugia; WT: Wild type; Cas.: Cv. *Cascade*; R: Rhizosphere; RE: Rhizome-endosphere; VD: *V. dahlia*; BC: *B. cinerea*; PS: Phosphate solubilization; Sid.: Siderophore.

Overall, these findings highlight the potential of these candidates as effective biocontrol and biostimulant agents. Future experiments will focus on *in planta* validation to study the colonization ability and pattern of such beneficial microbes, and to confirm their effectiveness in promoting hop growth and health under field conditions.

Acknowledgements

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References

Anzalone, A., Di Guardo, M., Bella, P., Ghadamgahi, F., Dimaria, G., Zago, R., Cirvilleri, G., and Catara, V. 2021. Bioprospecting of beneficial bacteria traits associated with tomato root in greenhouse environment reveals that sampling sites impact more than the root compartment. Front. Plant Sci. 12: 637582. https://doi.org/doi:10.3389/fpls.2021.637582

Barbagallo, R. N., Rutigliano, C. A., Rizzo, V., and Muratore, G. 2024. Innovative trends and strategies for the integral valorization of products in the beer supply chain. Ital. J. Food Sci. 36(3): 20. https://doi.org/10.15586/ijfs.v36i3.2542

Berg, G., Opelt, K., Zachow, C., Lottmann, J., Götz, M., Costa, R., and Smalla, K. 2006. The rhizosphere effect on bacteria antagonistic towards the pathogenic fungus *Verticillium* differs depending on plant species and site. FEMS Microbiol. Ecol. 56(2): 250-261.

Bulgarelli, D., Rott, M., Schlaeppi, K., Ver Loren van Themaat, E., Ahmadinejad, N., Assenza, F., and Schulze-Lefert, P. 2012. Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. Nature 488(7409): 91-95.

Coutinho, T. A. and Venter, S. N. 2009. *Pantoea ananatis*: an unconventional plant pathogen. Mol. Plant Pathol. 10(3): 325-335.

Gargani, E., Ferretti, L., Faggioli, F., Haegi, A., Luigi, M., Landi, S., Simoni, S., Benvenuti, C., Guidi, S., Simoncini, S., D'Errico, G., Amoriello, T., Ciccoritti, R., Roversi, P. F., and Carbone, K. 2017. A survey on pests and diseases of Italian Hop crops. Italus Hortus 24(2): 1-17.

Glick, B. R. 2012. Plant growth-promoting bacteria: mechanisms and applications. Scientifica 1: 963401.

Klement, Z., and Goodman, R. N. 1967. The hypersensitive reaction to infection by bacterial plant pathogens. Ann. Rev. Phytopathol. 5(1): 17-44.

Turner, T. R., James, E. K. and Poole, P. S. 2013. The plant microbiome. Genome Biol. 14: 1-10.

Vurukonda, S. S., Giovanardi, D., and Stefani, E. 2017. Symbiotic agriculture: plant growth promotion and biocontrol activity of beneficial microorganisms. Proceedings of the XXIII National Conference of Italian Society of Plant Pathology. In: J. Plant Pathol. 99 (Supplement), p. S49.

Weisburg, W. G., Barns, S. M., Pelletier, D. A., and Lane, D. J. 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. 173(2): 697-703.

Xhemali, B., Cortiello, M., Gjinovci, G., Bresilla, B., Modica, F., Stefani, E., and Giovanardi, D. 2023. First finding in Europe of *Colletotrichum scovillei*, a new agent of pepper anthracnose and assessment of potential bacterial biocontrol agents. In: Proceeding of the XIV International Scientific Agriculture Symposium "AGROSYM 2023", Jahorina, Bosnia and Herzegovina, October 05-08, 2023, pp. 712-717.

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Microbial volatile organic compound dynamics in solid-state fermentation: evaluation of antifungal activity

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Extended Abstract: Volatile organic compounds (VOCs) are low-molecular-weight compounds biosynthetically produced by plants, animals, humans, fungi, and bacteria, and involved in inter- and intraspecific signalling. Microbial VOCs are metabolic molecules that play a crucial role in microbial communication, including the regulation of *quorum* sensing. In recent years, VOCs have gained interest in research focused on new environmentally friendly molecules active against phytopathogens. This interest aligns with the European Farm to Fork and Biodiversity Strategy under the European Green Deal, which aims to reduce the use of chemical and hazardous pesticides by half by 2030. Numerous VOCs produced by different bacterial genera, including *Bacillus*, *Pseudomonas*, and *Streptomyces* are known for their activity against phytopathogenic bacteria and fungi. Generally, microbial VOCs activity is exerted through growth inhibition of other microorganisms present in the same environment, affecting hyphae morphology or conidiation. Even if the production of VOCs occurs naturally in microbial metabolism, the volatilome of each species can be influenced by the microbial community composition, nutrient availability, and temperature

Streptomycetes, gram-positive and filamentous bacteria belonging to the phylum Actinobacteria, are among the highest producers of natural secondary metabolites with antimicrobial and antibiotic activities. They are widespread in soil, particularly in the rhizosphere. They are also found in symbiosis with plants, mainly in roots. Interest in *Streptomyces* spp. has increased in recent years thanks to genomic and metabolomic analyses, which have revealed a high number of potential secondary metabolites present in cryptic Biosynthetic Gene Clusters (BGCs), i. e., clusters of genes that are expressed under specific conditions, such as the presence of stress or the substrate in which they grow on. For these properties, diverse Streptomycetes strains are being studied as possible biocontrol agents (BCAs) or as sources of specific bioactive molecules that can serve as alternatives to conventional chemicals. Moreover, Streptomycetes produce a wide range of volatiles. The best-known VOC produced by the *Streptomyces* genus is geosmin, a compound with the earthy odour associated with petrichor. Various volatiles produced by *Streptomyces* spp., like 3-methyl-butanol, 2-pentanone, and 2-hexenal, have antimicrobial activity against several phytopathogens, including *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, *Fusarium oxysporum*, *Fusarium culmorum*, and *Botrytis cinerea*. Growing *Streptomyces* spp. on unconventional substrates, such as agrifood waste, through solid-state fermentation could valorise agricultural wastes and trigger cryptic BGCs with antimicrobial activity that can be used in phytopathogen control. Solid-State Fermentation (SSF) is an alternative cultivation process in which microorganisms are grown on a solid substrate without the presence of free water. This method is used for the industrial applications to produce secondary metabolites, spores, and biotransformations.

Streptomyces sp. DEF603 is a strain isolated from *Vitis vinifera*, whose antimicrobial activity has been tested in vitro. SSF of *Streptomyces* sp. DEF603 on defatted wheat bran was previously optimised in our research group. Genomic analysis confirmed the presence of genes encoding for molecules with antimicrobial activity in *Streptomyces* sp. DEF603 genome.

In this work, the volatilome released by *Streptomyces* sp. DEF603 during SSF was analysed together with the evaluation of the antimicrobial activity to find potential active molecules against phytopathogens. An untargeted HS-SPME-GC-MS analysis was carried out at four sampling times (4, 7, 10, and 14 days after inoculation) during SSF to follow the shifts and changes in VOCs composition in both inoculated and non-inoculated (control) wheat bran. The analysis allowed us to putatively annotate 132 compounds, mainly belonging to the classes of terpenoids, alkanes, aldehydes, ketones and alcohols among others. Data were successively analysed through Principal Component Analyses (PCAs) highlighting a clear separation (i) between inoculated and non-inoculated samples and (ii) among samples collected at different time points. Subsequently, the data were analysed using ANOVA, and significantly altered metabolites were visualized with a clustered heat map. This analysis revealed that the volatilomes segregated into two distinct clusters: non-inoculated and inoculated substrates. Subclusters were also observed in both groups, whose disposition in the graph is related to chemical variations induced by the time (days of fermentation). In the volatilome of *Streptomyces*-fermented substrate, common VOCs produced by Streptomycetes, such as geosmin and 2-methyl-pentanone, were also found.

The antimicrobial activity of VOCs produced by *Streptomyces* strain DEF603 during SSF was tested against four phytopathogens: *Fusarium culmorum*, *Fusarium oxysporum* f. sp. *basilici*, *Botrytis cinerea*, and *Alternaria alternata*. We observed that the antimicrobial activity increased with time in SSF, reaching a peak at 14 days with growth inhibition of almost 70 % in all fungi, except for *F. oxysporum* f. sp. *basilici*, which was less sensitive to microbial VOCs. At the same time, VOCs produced by non-inoculated defatted wheat bran appeared to both increase the growth and the development of aerial mycelium of the four fungal pathogens. In addition to these results, VOCs produced on SSF caused a morphological variation in *B. cinerea* aerial mycelium and spore production.

Synthesis of bioactive VOCs by *Streptomyces* sp. strain DEF603 active against phytopathogens is a further confirmation of the potential of this strain to act as a putative BCA to be applied in the field as a complement to chemicals, in line with principles of the Integrated Pest Management (IPM). Further analyses are required to confirm the efficacy of the strain in in systems that try to mimic open field conditions.

Key words: Solid-State Fermentation, *Streptomyces*, volatile organic compounds, biocontrol agent, phytopathogens control

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Enhancing pigment production in bacterial biocontrol agents to improve UV radiation tolerance

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Abstract: Despite success in laboratories and greenhouses, field efficacy of bacterial biocontrol agents remains inconsistent, primarily due to UV radiation's germicidal effects. However, bacteria have evolved molecular mechanisms to protect themselves against UV. For instance, *Lysobacter* spp. produce xanthomonadins, pigments conferring yellow colour to bacterial colonies and tolerance to UV (Wang et al, 2013). Notably, xanthomonadin biosynthesis is positively regulated by the signal molecule 3-hydroxybenzoic acid (3-HBA). Based on this, this study aimed to enhance the survival of the biocontrol agent *Lysobacter capsici* (AZ78) exposed to UV by increasing the production of xanthomonadins and investigating the molecular mechanisms involved in UV tolerance. AZ78 produced colonies with a more intense yellow colour when grown on LBA with 3-HBA. These AZ78 colonies produced more xanthomonadins and were more tolerant to UV radiation. To investigate the role of genes responsible for xanthomonadin production in AZ78, electrocompetent cells were electroporated with the transposon delivery vector pUT/mini-Tn5 Cm (Artiguavane et al., 1997). Some chloramphenicol-resistant AZ78 transformant colonies showed a yellow colour paler than the wild type and a reduced xanthomonadin production. Overall, this work shows that it is possible to produce AZ78 cells that are more tolerant to UV radiation by increasing the output of xanthomonadins. Moreover, the analysis of AZ78 mutants will increase the knowledge of the molecular pathways involved in xanthomonadin production and UV tolerance in AZ78.

Key words: microbial biocontrol agents (mBCAs), *Lysobacter capsici* AZ78, xanthomonadins, 3-hydroxybenzoic acid (3-HBA), transposon mutagenesis

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References

Artiguenave, F., Vilaginès, R., Danglot, C. 1997. High-efficiency transposon mutagenesis by electroporation of a *Pseudomonas fluorescens* strain, FEMS Microbiol. Lett. 153: 363-369.

Wang, Y., Qian, G., Li, Y., Wang, Y., Wang, Y., Wright, S., Li, Y., Shen, Y., Liu, F., Du, L. 2013. Biosynthetic mechanism for sunscreens of the biocontrol agent *Lysobacter enzymogenes*. PLoS One 8: e66633.

Session IX
Mechanisms behind
Plant – microbe – antagonist interactions

Mechanisms behind microbe-microbe and plant-microbe interactions

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Abstract: Microorganisms employed for biological control of plant diseases achieve their beneficial effect through a variety of mechanisms. These mechanisms can include competition with pathogens for nutrients and space, through production of toxic specialized metabolites (antibiosis) and cell wall-degrading enzymes, or direct parasitism. Certain biological control agents also exert their effect by priming and activating immune responses in the plant. However, the exact contribution of individual mechanisms to biocontrol depends on species, strain, plant organ, pathosystem and the environment. By understanding the mechanisms contributing to successful disease control, we can make knowledge-based improvements of efficacy. Variation in biocontrol efficacy between individuals from a single species can be used to identify interaction mechanisms. We evaluated 63 genome-sequenced strains of the biocontrol fungus *Clonostachys rosea* for their ability for biocontrol, *in vitro* antagonism towards fungal plant pathogens and colonization of wheat roots. There were significant differences between *C. rosea* strains to antagonize the fungal and oomycete pathogens *Rhizoctonia solani*, *Botrytis cinerea*, *Fusarium graminearum* and *Aphanomyces euteiches*. Low correlations between these traits indicate adaptations towards host specificity in *C. rosea*. Furthermore, a genome-wide association study (GWAS) identified genomic regions in *C. rosea* that segregated either with antagonism towards *F. graminearum* or *A. euteiches*, suggesting a mechanistically different basis for host specificity. Analyses of transcriptomes and small RNAs (sRNA) further identified induction of distinct gene sets in *C. rosea* following interaction with *F. graminearum* or *B. cinerea*, further suggesting different mechanisms of antagonism towards different pathogens. These analyses indicated that production of, and tolerance towards, specialized metabolites (SM) is important in the antagonistic ability of *C. rosea*. Deletion of specific polyketide synthase and non-ribosomal peptide synthetase genes in *C. rosea* resulted in decreased antagonism and biocontrol. Furthermore, deletion of the dicer 2 (*dcl2*) gene in *C. rosea*, acting in the RNA silencing pathway, resulted in mutants with reduced SM production, reduced antagonism and reduced biocontrol ability. We further identified fungal microRNA-like RNAs (milRNAs) in *C. rosea* and their endogenous gene targets associated with SM biosynthesis and antagonism, supporting the phenotypic result. At the cross-species level, these milRNAs were predicted to target genes involved in fungal virulence, SM production and cell wall biogenesis in *F. graminearum* and *B. cinerea*, and plant defense-related genes in wheat roots. Furthermore, sRNA sequencing identified 18 wheat miRNAs responsive to *C. rosea,* and three were predicted to target the *C. rosea* polyketide synthase gene *pks29*, previously shown for its role in fungal antagonism and biocontrol. Two of these miRNAs were observed to enter *C. rosea* from wheat roots with fluorescence analyses and to downregulate the expression of *pks29*, providing evidence for cross-kingdom RNA silencing of the *C. rosea* gene by wheat miRNAs. This work provides insights into the mechanisms underlying the interaction between biocontrol fungi and fungal and plant hosts and provides a basis for future studies of fungal biocontrol interactions.

Key words: *Clonostachys rosea*, genome analysis, polyketide synthase, RNA silencing

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Effective control of wheat and barley diseases by *Bacillus velezensis* BE2 through direct antagonism and induced systemic resistance

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Abstract: *Septoria tritici* blotch and net blotch, caused by *Zymoseptoria tritici* and *Pyrenophora teres* f. *teres*, respectively, cause significant yield losses up to 50 % in wheat and barley. Biocontrol agents are gaining attention as sustainable alternatives to fungicides, offering an eco-friendly disease management approach. This study aimed to assess the efficacy of *Bacillus velezensis* BE2 in controlling these fungal pathogens. *In vitro* assays demonstrated that strain BE2 significantly inhibits fungal growth through direct antagonism. Microscopic analysis confirmed its ability to suppress spore germination and mycelial development. Cell-free supernatant analysis using UPLC-MS identified seven metabolite families, including key cyclic lipopeptides potentially involved in biocontrol. To explore BE2's biocontrol mechanisms, we analyzed plant gene expression at different time points post-infection. Differential expression analysis revealed that BE2 modulates key defense pathways, including oxidative stress responses, chitinase activity, and phytohormone regulation. The *in planta* efficacy of BE2 was assessed by quantifying fungal DNA at the leaf level following bacterization using foliar and root application methods. Root application proved more effective in reducing pathogen pressure. Overall, these findings demonstrate that *B. velezensis* BE2 protects wheat and barley through direct antifungal activity and by priming plant defenses, making it a promising candidate for sustainable disease management in cereal crops.

Key words: wheat, barley, *Z. tritici, P. teres,* lipopeptides, *B. velezensis* BE2 strain, gene expression, biocontrol

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Effect of a bacterial SynCom on the tomato microbiome and transcriptome under challenge by *Xanthomonas euvesicatoria* pv. *perforans*

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Abstract: Biological control has been extensively explored as an alternative to copper-based compounds for managing tomato bacterial spot (TBS), yet to our knowledge, few studies have investigated how biocontrol agents interact with the host-bacterial pathogen dynamics. This study evaluated a SynCom of six Plant Growth Promoting Rhizobacteria (PGPR) in the genera *Bacillus, Pseudomonas, Glutamicibacter,* and *Leclercia*, on tomato plants leaf-inoculated with *Xanthomonas euvesicatoria* pv. *perforans* (Xep). The SynCom was applied via seed soaking and soil drenching before Xep inoculation. Symptoms appeared as water-soaked lesions at 3 days post inoculation (dpi), progressing to necrotic spots and larger lesions at 6 dpi. MIX2 treatments significantly reduced TBS severity. The effects of Xep and MIX2, both separately and combined (MIX2 + Xep), on the tomato microbiome and transcriptome were assessed at different time points. Xep inoculation reduced the phyllosphere bacterial community α-diversity at 3 and 6 dpi compared to non-inoculated treatments, with *Xanthomonas* dominating at both time points. No significant diversity differences were observed in the rhizosphere, although in MIX2 (with or without Xep), Actinobacteriota decreased while Proteobacteria increased. At 6 dpi, Xep and MIX2 + Xep phyllosphere and Xep, MIX2 + Xep and MIX2 rhizosphere showed an increased fungal α-diversity. RNA-Seq was performed on tomato leaf discs at 12 hours (hpi), 3 dpi, and 6 dpi. Differentially expressed genes (DEGs) were identified for all treatments compared to the untreated control. MIX2 treatment alone resulted in few DEGs at all time points, while Xep inoculation triggered a high number of DEGs in both untreated and MIX2 plants. Although Xep and MIX2+Xep plants shared many DEGs, distinct sets were specifically enriched or depleted in each condition. KEGG analysis revealed significant enrichment of pathways related to plant-pathogen interaction, MAPK signaling, hormone signal transduction, and photosynthesis at all time points, with specific pathways enriched only in Xep or MIX2 + Xep. Our results provide insight into the tripartite interaction between pathogen, host, and biocontrol bacteria, demonstrating how microbial communities are shaped by both the pathogen and our SynCom. Transcriptomic analysis suggests that SynCom MIX2 primes plant responses upon foliar bacterial pathogen attack that are not activated in the absence of the pathogen.

Key words: *Solanum lycopersicum* L., bacterial spot, biocontrol, synthetic community, amplicon-based metagenomics, RNA-Seq

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Elucidate the modes of action of biocontrol agents against *Sclerotinia sclerotiorum* on rapeseed

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Abstract: Rapeseed (*Brassica napus*), one of the most widely grown rapeseed crops in the word, is highly susceptible to white mold caused by the fungal pathogen *Sclerotinia sclerotiorum*. The management of this necrotrophic and polyphagous pathogen primarily relies on chemical treatments. Consequently, the development of novel biocontrol agents offers a promising alternative for promoting the sustainable cultivation of this crop. However, microbial biocontrol products are often perceived by farmers as lacking in efficacy and reliability when used in the field. This issue is generally attributed to a lack of knowledge of the factors that modulate their efficacy, including their mode of action.

In previous work, two bacteria have shown particularly promising activity under controlled conditions and in the field to protect rapeseed against *S. sclerotiorum* and are currently being developed by a company. Antibiosis and resistance induction have been demonstrated as two possible modes of action implemented by these bacteria, with variation depending on the strain. Studies are therefore needed to decipher in detail the mechanisms underlying the protective efficacy of each bacterial strain. In this context, our first objective was to identify, among the various elements present in the by-products of bacterial fermentations, those that play a key role in rapeseed protection. A second objective was to characterize the active metabolites produced by these bacteria that may explain the effects observed.

To address the first objective, we investigated the contribution of washed bacterial cells, killed bacterial cells and cell-free supernatant produced in two culture media to the development of *S. sclerotiorum* on rapeseed, for each antagonistic bacterium. Detached leaves of rapeseed were treated with the various constituent derived from bacterial fermentation either 1, 3 or 7 days prior to pathogen inoculation. Inoculated leaves are then incubated at 21 °C, a temperature conducive to pathogen development, until lesion size measurements were conducted. Preliminary results indicate that cell-free supernatant has a prominent role in reducing the development of *S. sclerotiorum* and, for one of the two strains, in inducing defense-related genes in rapeseed.

To further characterize the active compounds within the cell-free supernatant, a 'candidate metabolite' approach and a non-targeted metabolomics approach have been implemented. The first approach focused on evaluating the effect on *S. sclerotiorum* of combinations of purified metabolites present in the cell-free supernatant and known to be associated with biocontrol activity. The second approach was to fractionate the cell-free supernatant and perform LC-MS analyses for further characterization. The main results of these approaches will be presented.

Key words: *Sclerotinia sclerotiorum*, biocontrol, mode of action, fermentation constituent, metabolomics

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Biocontrol and its regulation of *Bacillus velezensis* against apple ring rot

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Abstract: *Bacillus velezensis* PG12 was isolated from apple fruit. The fungal pathogen of apple ring rot *Botryosphaeria dothidea* was significantly suppressed by *B. velezensis* PG12 *in vitro* and *in vivo*. Lumpy appearance and abnormal structure of the mycelia from the edge of inhibition zone were observed using scanning electron microscopy (SEM) in *in vitro* assays. Furthermore, the lipopeptide crude extracts from cell-free supernatant of PG12 had remarkable antifungal activity against *B. dothidea*, indicating that lipopeptides played a major role in the biological control ability of *B. velezensis* PG12. One iturin-like compound (Rf 0.4) showed inhibitory activity against *B. dothidea* using thin layer chromatography (TLC)-bioautography analysis and were further fractionated by semipreparative high performance liquid chromatography (HPLC). The fraction with a molecular weight of 1043.55 m/z was identified as iturin A by electrospray ionization quadrupole-time-of-flight mass spectrometry/mass spectrometry (ESI-Q-TOF MS). Taken together, *B. velezensis* PG12 was an effective biocontrol agent against apple ring rot caused by *B. dothidea* and iturin A was an important factor in its activity.

Cyclic diguanylate (c-di-GMP) is a nearly universal second messenger in bacteria and modulates various important physiological processes, including motility, biofilm formation, antifungal antibiotic production and host colonization. c-di-GMP is synthesized from 2 GTP molecules by diguanylate cyclases (DGCs) characterized by GGDEF domain and degraded by Phosphodiesterases (PDEs) characterized by EAL or HD-GYP domains into the linear pGpG which can be further degraded into GMP by the oligoribonucleases. Deletion of single gene encoding diguanylate cyclase or phosphodiesterase in *B. velezensis* PG12 did not affect its biocontrol efficacy against apple ring rot. However, artificial modulation of c-di-GMP level in the cells leads to a significant change of its biocontrol efficacy, suggesting that c-di-GMP positively regulates biocontrol efficacy of *B. velezensis* PG12 against apple ring rot disease. More evidences indicate that c-di-GMP does not affect the antagonistic activity of *B. velezensis* PG12 against *B. dothidea in vitro* and *in vivo*, but positively regulates biofilm formation of *B. velezensis* PG12 and its colonization on apple fruits. Importantly, deletion of *ydaK* could rescue the inhibition of biofilm formation, bacterial colonization and biocontrol efficacy caused by low c-di-GMP level, indicating that YdaK is the potential c-di-GMP receptor to regulate biofilm formation, colonization and effective biological control. However, YdaK did not affect the antagonistic activity of *B. velezensis* PG12 against *B. dothidea.* Based on these findings, we propose that c-di-GMP regulates biofilm formation, subsequently the bacterial colonization on apple fruits and thus biocontrol efficacy of *B. velezensis* through its receptor YdaK. This is the first report showing that c-di-GMP plays a role in biocontrol efficacy of beneficial bacteria.

Key words: *Bacillus velezensis*, c-di-GMP, biofilm, colonization, biological control, apple ring rot disease

Decoding the ecological impact of tailocins in soft rot Pectobacteriaceae

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Abstract: Phages, as bacterial viruses, often integrate their genetic material into bacterial genomes during infection, leaving a lasting evolutionary imprint. Interestingly, some bacteria have domesticated phage-derived genes for their own benefit. Among such adaptations there are R-type tailocins, protein complexes structurally similar to the contractile tails of Myoviridae phages. These nanomachines specifically target and kill related bacterial strains by puncturing their membranes. Recently, we described a model R-type tailocin produced by *Dickeya dadantii* 3937, a key representative of the soft rot Pectobacteriaceae (SRP), which includes *Pectobacterium*, *Dickeya*, and *Musicola* species. These plant pathogens cause soft rot in economically significant crops, such as potatoes, resulting in considerable global losses.

In this study, we expanded on our earlier findings to investigate the prevalence and ecological role of tailocins across the soft rot Pectobacteriaceae. Furthermore, we examined their ecological significance, including their impact on naturally occurring SRP strains and other co-occurring bacterial species within shared environments.

We also explored how the production of tailocins affects the metabolism and virulence of the producing strains. This work contributes to a deeper understanding of the role of tailocins in shaping bacterial interactions and their potential influence on the functional traits of producer bacteria.

Key words: bacteriophage, phage tail-like particles, inter-genus, interactions, prophage

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Intraspecific variation in virulence of a mycoparasitic fungus suggests evolution of host specificity

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Abstract: Microbial parasites of plants can have devastating effects on ecosystem stability, agricultural production and food security. However, parasites can in addition themselves be parasitized by other microorganisms, a phenomenon referred to as hyperparasitism. The ability of microbial hyperparasites to decrease plant pathogen populations through top-down cascades is the basis for their use as biological control agents (BCAs) in agriculture. However, different strains of the same species may display significant variation in biocontrol ability. Therefore, a deeper understanding of the evolutionary dynamics affecting hyperparasite populations and their genomic consequences is needed and may contribute to future biocontrol solutions. In this PhD project, important ecological, genomic and mechanistic questions in necrotrophic fungal parasites of plant pathogenic fungi (mycoparasitism) will be addressed. Initially, 63 strains of the BCA fungus *Clonostachys rosea* are assessed for *in vitro* antagonism and mycoparasitism of several host fungi and oomycetes, including *Rhizoctonia solani*, *Botrytis cinerea*, *Fusarium graminearum* and *Aphanomyces euteiches*. The data collected will be used to test the hypotheses that 1. There is variation between *C. rosea* strains in virulence towards different host fungi, and 2. Certain strains display high virulence towards a specific host (specialization) while others display intermediate virulence towards all hosts (generalism). Differences in virulence is further hypothesized to partially depend on different interaction mechanisms towards the intrinsically different host fungi, which include ascomycetes, a basidiomycete and an oomycete. A genome-wide association approach will be used to identify genomic regions, and thereby genes/alleles, contributing to high virulence towards specific plant pathogenic host fungi. Future work includes transcriptomics study of a limited number of *C. rosea* strains selected based on their performance during *in vitro* interaction with plant pathogenic species to further support identification of virulence related genes.

Key words: *Clonostachys rosea*, *in vitro* interaction, virulence

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Microbial interactions in crop protection: multi-omic analyses of the plant perception to biological control agents

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Abstract: The integration of microbial biocontrol agents (BCAs) in the management of sustainable agriculture represents a promising strategy to mitigate pest and pathogen damage while enhancing plant resilience and growth.

The biological control capacities of *Trichoderma afroharzianum* T22 (Ta) and/or *Beauveria bassiana* Bb74040 (Bb) were evaluated against a fungal pathogen and a sucking insect pest. Tomato plants were treated with Ta and Bb, applied individually or in a consortium, in the presence or absence of: (i) the foliar pathogen *Botrytis cinerea*, or (ii) the aphid pest *Macrosiphum euphorbiae*. *In vivo* bioassays demonstrated a significant reduction in the incidence and severity of *B. cinerea* induced symptoms in plants treated with BCAs, both individually and in consortium. Additionally, levels of aphid infestation and fertility were markedly lower in BCA-treated plants compared to controls.

Integrated metabolomic, proteomic, and hormonomic analyses elucidated the biochemical and metabolic mechanisms underlying plant responses to the single or dual BCA applications, with and without biotic stressors. Multi omics profiling revealed distinct metabolic, proteomic and hormonal shifts between stressed and unstressed plants, with further variations depending on the biological treatment applied.

In *B. cinerea*-infected control plants, proteins associated with plant starvation, ROS metabolic processes, and cellular responses to toxic substances were highly represented. Furthermore, among the detected metabolites, Botrydial – a toxic compound linked to *B. cinerea* infection – was abundant, along with several metabolites involved in oxidative stress responses.

Conversely, in infected and BCA treated plants, a metabolic shift was observed, characterized by a higher accumulation of defense related compounds such as tomatin, capsaicin and multiple oxylipins which are precursors of jasmonic acid. Accordingly, the hormonomic analysis showed an enhanced expression of the jasmonate metabolic pathway, indicating a systemically modulated plant response mediated by the microbial interaction, and suggests an alternative defensive strategy in the presence of beneficial microbes.

Furthermore, the responses of the aphid infested plants demonstrated that BCA treatments reduced the accumulation of secondary metabolites in comparison to the untreated infested plants. These results are consistent to those observed in the *in vivo* experiments, where aphid survival and infestation severity were significantly lower on BCA treated plants.

Current efforts are focused on integrating data from the three omics approaches into a comprehensive network to elucidate how plants perceive and respond to beneficial microbes as well as react to the presence of the targeted stressors with and without BCAs. These findings further reinforce the potential of microbial consortia as effective biocontrol and biostimulant tools for sustainable agriculture.

Key words: biocontrol, antagonists, *Trichoderma afroharzianum*, *Beauveria bassiana,* induced systemic resistance (ISR), jasmonic acid pathway, sustainable agriculture

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Application of heat-treated *Lysobacter capsici* AZ78 cells and its polycyclic tetramate lactams effectively controls *Plasmopara viticola* by stimulating grapevine defence mechanisms

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Abstract: The genus *Lysobacter* controls a broad range of plant pathogens through diverse modes of action, including the production of Polycyclic Tetramate Lactams (PTMs), secondary metabolites resistant to high temperatures and exhibiting strong antifungal activity. As PTMs are stored in the cell membranes, this study investigated the efficacy of applying heat-treated cells of *L. capsici* AZ78 in controlling *Plasmopara viticola*, the causal agent of grapevine downy mildew. Moreover, it sheds light on the ability of *L. capsici* AZ78 cells and PTMs to stimulate defence mechanisms in grapevine plants.

Key words: *Lysobacter capsici*, *Plasmopara viticola*, polycyclic tetramate lactams, callose, reactive oxygen species

Introduction

The *Lysobacter* genus includes bacterial species that efficiently control plant pathogenic fungi and oomycetes by releasing antifungal secondary metabolites (Puopolo et al., 2018). Polycyclic Tetramate Lactams (PTMs) are the most studied, given their broad host range and resistance to high temperatures (Yu et al., 2007; Li et al., 2009). Beyond inhibiting hyphal growth, PTMs also trigger apoptotic processes in yeasts (Ding et al., 2016). Notably, Yue et al. (2021) showed that *L. enzymogenes* PTMs are primarily localised within bacterial cell membranes. Recently, Brescia et al. (2021) detected PTMs in cell-free supernatants of *L. capsici* AZ78 (AZ78), a biocontrol agent of *Plasmopara viticola*. However, little is known about their presence in AZ78 cell membranes and if they play a role in controlling *P. viticola*.

Based on this, we evaluated the biocontrol efficacy of heat-treated AZ78 cells against *P. viticola*. In parallel, we assessed the capacity of these treatments to trigger plant defence responses. To further substantiate the involvement of PTMs, we isolated them directly from the AZ78 cell membranes and tested them against *P. viticola*.

Materials and methods

Plant protection efficacy assays

Grapevine leaf discs and two-year-old Pinot Noir plants were used in *in vitro* and *in planta* assays, respectively. Two-year-old *V. vinifera* cv. Pinot Noir grapevine plants, grafted onto

Kober 5BB were used for *in planta* assays. Heat-treated AZ78 cells were prepared by incubating AZ78 cell suspension (1×10^8 CFU/ml) at 90 °C for 20 min, followed by incubation at -20 °C for 5 min. Viable and heat-treated AZ78 cells (1×10^8 CFU/ml) were sprayed 24 h before *P. viticola* inoculation (2.5×10^5 sporangia/ml). For PTM assays, mixtures of PTMs (50 mg/l - 2.5 mg/l) were mixed in equal volumes with the *P. viticola* inoculum and drop-inoculated onto grapevine leaf discs. In all the cases, samples were incubated under greenhouse conditions for six days (Brescia et al., 2021). Then, disease severity was assessed according to the EPPO standard scale (EPPO, 2004). Untreated and copper-treated leaf discs (Coprantol Hi Bio; 2 g/l) were negative and positive controls, respectively. Five leaf discs per Petri dish were used, and each treatment consisted of five replicates (Petri dishes) for *in vitro assays*. For *in planta assays,* each treatment was carried out on five grapevine plants (replicates).

In planta and *in vitro* assays were repeated, and results were statistically analysed using R on R studio version 4.3.0. Data related to disease severity were analysed using One-Way ANOVA, and means were compared with Tukey's test ($\alpha = 0.01$).

Grapevine plant response to treatments

The ability of PTMs and AZ78 cells to stimulate grapevine defence mechanisms was investigated by assessing callose accumulation and reactive oxygen species (ROS) production. Callose was visualised via aniline blue staining using fluorescence microscopy, with callose appearing as turquoise fluorescence (Palmieri et al., 2012). ROS production was detected using 3' 3' diaminobenzidine (DAB) staining (1 mg/ml) (Trouvelot et al., 2008). ROS was visible as a dark brown precipitate under the bright field microscope. The experiments were repeated.

Results and discussion

In the plant protection efficacy assays, the untreated control had the highest severity, ranging between 80 % and 100 % in all cases, whereas the copper-based fungicide showed a plant protection efficacy higher than 90 %. Notably, our results demonstrated that heat-treated AZ78 cells retained strong protective efficacy against *P. viticola*, comparable to AZ78 viable cells and the commercial copper-based fungicide. This indicates that the associated thermostable PTMs could play a role in the control of *P. viticola*.

To confirm the involvement of PTMs in *P. viticola* control, we isolated the PTM mixture from AZ78 cell membranes and applied it to grapevine leaves. The PTM mixture provided significant protection against *P. viticola* even at the lowest concentration (2.5 mg/l), mirroring the effects of AZ78 cells. Furthermore, the PTMs independently induced plant defence responses, validating their role in controlling *P. viticola.*

Together, these results prove that PTMs are associated with AZ78 cell membranes and are involved in biocontrol activity. This study, the first to highlight the ability of PTMs to trigger defence mechanisms in plants, could potentially lead to the development of non-viable AZ78 cells and/or PTMs as novel plant protection products for the control of *P. viticola* on grapevines, offering hope for more effective and sustainable wine production.

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References

Brescia, F., Vlassi, A., Bejarano, A., Seidl, B., Marchetti-Deschmann, M., Schuhmacher, R., Puopolo, G. 2021. Characterisation of the antibiotic profile of *Lysobacter capsici* AZ78, an effective biological control agent of plant pathogenic microorganisms. Microorganisms 9: 1320.

Ding, Y., Li, Z., Li, Y., Lu, C., Wang, H., Shen, Y., Du, L. 2016. HSAF-induced antifungal effects in *Candida albicans* through ROS-mediated apoptosis. RSC Adv. 6: 30895-30904.

Li, S., Calvo, A. M., Yuen, G. Y., Du, L., Harris, S. D. 2009. Induction of cell wall thickening by the antifungal compound dihydromaltophilin disrupts fungal growth and is mediated by sphingolipid biosynthesis. J. Eukaryot. Microbiol. 56: 182-187.

Palmieri, M. C., Perazzolli, M., Matafora, V., Moretto, M., Bachi, A., Pertot, I. 2012. Proteomic analysis of grapevine resistance induced by *Trichoderma harzianum* T39 reveals specific defence pathways activated against downy mildew. J. Exp. Bot. 63: 6237-6251.

Puopolo, G., Giovannini, O., Pertot, I. 2014. *Lysobacter capsici* AZ78 can be combined with copper to effectively control *Plasmopara viticola* on the grapevine. Microbiol. Res. 169: 633-642.

Puopolo, G., Tomada, S., Pertot, I. 2018. The impact of the omics era on the knowledge and use of *Lysobacter* species to control phytopathogenic micro-organisms. J. Appl. Microbiol. 124: 15-27.

Trouvelot, S., Varnier, A. L., Allègre, M., Mercier, L., Baillieul, F., Arnould, C., Gianinazzi-Pearson, V., Klarzynski, O., Joubert, J. M., Pugin, A., Daire, X. 2008. A β-1,3 glucan sulfate induces resistance in grapevine against *Plasmopara viticola* by priming defence responses, including HR-like cell death. Mol. Plant Microbe In. 21: 232-243.

Yu, F., Zaleta-Rivera, K., Zhu, X., Huffman, J., Millet, J. C., Harris, S. D., Yuen, G., Li, X.-C., Du, L. 2007. Structure and biosynthesis of heat-stable antifungal factor (HSAF), a broad-spectrum antimycotic with a novel mode of action. Antimicrob. Agents Ch. 51: 64-72.

Yue, H., Jiang, J., Taylor, A. J., Leite, A. D. L., Dodds, E. D., Du, L. 2021. Outer membrane vesicle-mediated codelivery of the antifungal HSAF metabolites and lytic polysaccharide monooxygenase in the predatory *Lysobacter enzymogenes*. ACS Chem. Biol. 16: 1079-1089.

Metagenomics insight into rhizosphere: Microbial indicators of tomato health and disease resistance

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Abstract: Powdery mildew disease of tomato caused by *Oidium neolycopersici* constitutes a significant barrier to the cultivation of tomato in South Africa. The rhizosphere microbiome of tomato has not been explicitly studied to understand the dynamics of the community structure and functional diversity in the management of the disease. This study explores the use of shotgun metagenomics approach to investigate the impact of microbial abundance and their functional roles on the tomato plant health. The rhizosphere soil samples were collected from healthy and powdery mildew infected tomato plants, alongside the bulk soil. DNA was extracted from the samples and sequenced using shotgun metagenomics. The result obtained showed a higher microbial abundance in the samples obtained from healthy tomato rhizosphere, followed by the diseased and then the bulk soil samples. A total of 18 bacterial phyla consisting of Actinobacteria, Acidobacteria, Aquificae, and Bacteroidetes were dominant in the healthy samples, while Bacteroidetes, Gemmatimonadetes, and Thermotoga were the most dominant in the diseased samples. However, Ascomycota and Basidiomycota, while the archaeal community was composed mainly of Thaumarchaeota, Crenarchaeota, and Euryarchaeota. Furthermore, the functional analysis conducted using the SEED subsystem reveals that carbohydrate, nitrogen, sulfur, and phosphorus metabolism, as well as secondary metabolism, were more abundant pathways recorded in the healthy samples. No significant differences were observed in the alpha diversity across the study sites, while beta analysis demonstrated significant variation (p = 001). More so, 21 plant growth-promoting (PGP) genes were identified in the samples of healthy rhizosphere, 9 was recorded in the diseased samples, and 4 in bulk soil. In addition, the disease-resistant genes, including nucleotide-binding and antimicrobial genes, were most abundant in heathy samples (15 genes), followed by diseased samples, then the bulk soil (3 genes each). These findings reveals that the rhizosphere microbiome plays important roles in plant health management and also have potential for the sustainable cultivation of tomato.

Key words: plant growth-promoting genes, rhizosphere microbiome, SEED subsystem, Shotgun sequencing

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Enhancing tomato defense mechanisms: modulation of long non-coding RNAs in response to foliar application of the biocontrol agent *Streptomyces* sp. DLS2013

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Abstract: Beneficial bacteria induce a state of alert (priming) in plants that allows them to deploy defensive measures more effectively upon subsequent pathogenic attack. In a previous study, foliar treatments with *Streptomyces* sp. DLS2013 induced transcriptomic changes in protein coding genes related to plant defense and photosynthesis. Here, we have investigated the associated transcriptional response of long non-coding RNAs (lncRNAs) inferring their putative role in plant defense by co-expression analysis with the protein coding genes. This study provided lncRNA candidates as additional benchmarks in tomato resistance to bacterial speck.

Key words: biocontrol bacteria, induced resistance, RNA-seq

Introduction

In tomato plants, *Streptomyces* sp. DLS2013 was found to trigger the activation of the immune system at the transcriptional level through various molecular pathways, after foliar treatments (Cassanelli et al., 2025). Non-coding RNAs (ncRNAs) play key roles in regulating gene expression involved in different biological processes such as abiotic and biotic stresses (Li et al., 2023). To investigate the involvement of tomato's long non-coding RNAs (lncRNAs) in enhancing the immune response, we used RNA-seq data to predict and characterize those that are transcriptionally active in leaves treated with DLS2013. By means of transcriptional co-regulation analysis including lncRNAs and protein-coding genes, a group of relevant co-expressed gene's modules were identified. These data will be further investigated to explore lncRNAs functions as a regulatory factor in biocontrol *Streptomyces* sp. DLS2013 to induce tomato resistance against *Pseudomonas syringae* pv. *tomato* infection.

Materials and methods

Tomato leaf transcript prediction and functional annotation

Raw RNA-seq reads from 30 samples of tomato leaves (cv. H3402) treated with DLS2013 and water (Mock) at three timing points (i. e., T1: 6 hours post treatment; T2: 12 hours post treatment; T3: 24 hours post treatment) were retrieved from NCBI Short Read Archive (SRA) database under the BioProject PRJNA1127845. Reads were cleaned with fastp v0.23.4 and mapped to the tomato genome (assembly SL4.0) with Hisat2 program (v2.1.1). Transcript assemblies were performed using Stringtie v1.3.3. LncRNA identification and classification were performed using Plant-LncPipe (Tian et al., 2024). Functional gene ontology (GO) annotations of mRNAs were retrieved from Riveira et al. (2024). KEGG pathway analysis of SL4.0/ITAG4.0 proteome was done using Blast-KOALA (KEGG Orthology And Links Annotation) server (https://www.kegg.jp/blastkoala). Salmon v1.2.0 was used for alignment and gene expression quantification with default parameters.

Co-expression analysis of differential expressed lncRNA-mRNA pairs

Differential expressed mRNAs (DEG) and lncRNAs (DEL) for Mock-DLS2013 comparisons were identified by using DESeq2 (threshold |log2 fold-change| ≥ 1, q-value < 0.05). To define co-regulated DEL: DEG pairs, the expression values of lncRNAs and mRNAs were evaluated through Spearman's correlation test (cutoff |r| > 0.9 and p-adjust < 0.01 was applied). The topGO package v3.2 (in R) was applied to identify significantly enriched GO terms in DEG (using *elim* algorithm in conjunction with Fisher's exact test).

Results and discussion

Tomato leaf transcript prediction and co-expression analysis

Reconstruction of lncRNA transcripts led to the identification of 2350 putative loci. Differential expression analysis comparing DLS2013-treated versus mock plants and considering both lncRNA and protein-coding genes, showed at T1 the up-regulation of 664 DEG and 25 DEL while down-regulation of 328 DEG and 38 DEL; at T2, 413 DEG and 19 DEL were over-expressed along with down-regulated 312 DEL and 25 DEL; at T3, transcription increased for 372 DEG and 16 DEL, and decreased in 244 and 21 DEL, respectively. These results suggest that, after the DLS2013 challenge, a mitigation in transcriptional response over the time, mainly affected protein-coding genes compared to lncRNA loci. Nevertheless, minimal overlap was observed across the three time points, as single lncRNAs exhibited consistent upregulation (MSTRG.32145) or downregulation (MSTRG.41210) throughout the experiment, indicating a possible time-dependent recruitment for DEL. Indeed, previous investigations have revealed that transcriptional reprogramming of lncRNAs at T1 is responsible for activating both pattern- and effector-trigger immunities (PTI/ETI) against bacterial speck on tomato leaves (Rosli et al., 2021). In this vein, co-expression analysis among DEG and DEL at T1 followed by GO and KEGG functional annotation were performed, to disclose potential lncRNA candidates involved in tomato defense response. Relaying on relative distance between co-regulated lncRNA – mRNA pairs, 24 DEL were predicted to have potential *cis*-acting effect on nearby 37 DEG (laying within 100 Kb up or downstream the lncRNA) in 117 lncRNA-mRNA pairs. At the same time, 53 DEG were predicted to exert potential long distance (*trans*-acting) effects on 514 mRNAs DEG in 1462 lncRNA-mRNA pairs. Given that the number of co-expressed genes associated with a lncRNA may indicate its functional relevance, Table 1 prioritises the top ten DEG ranked based on the total number of co-expressed genes related to plant defense pathways and photosynthesis biological processes, transcriptionally affected by DLS2013 treatment (Cassanelli et al., 2025). As expected, the number of co-expressed mRNAs belonging to defense-related pathways are generally proportional to the lncRNA rank, albeit not in all instances. Furthermore, the diverse directionality in lncRNA-mRNA co-expression patterns, coupled with the uneven distribution of mRNAs within each pathway, suggests that lncRNAs may play roles in regulating plant defense through distinct combined effects acting on signalling (jasmonic and salicylic acids), production of antimicrobial agents (stilbenoid, polyamines and protease inhibitors) and redirection of carbon source metabolism (photosynthesis).

Table 1. Co-expression analysis of DEL – DEG pairs: top 10 lncRNAs according to co-expressed mRNAs mapping on plant defense and photosynthesis biological processes.

DEL lncRNA code	Total Co-exp mRNAs	JA pathways	SA pathways	Pa-Phe pathways	Protease inhibitors	Photosynth.
32145 (↑)	116	13 (↑)	6 (↑)	3 (↑)	15 (↑)	0
5173 (↓)	109	9 (↑)	5 (↑)	4 (↑)	16 (↑)	0
9540 (↓)	163	2 (↑)	1 (↑)	1 (↑); 1 (↑)	10 (↑)	8 (↓)
3331 (↓)	81	3 (↑)	3 (↑)	2 (↑)	9 (↑)	3 (↓)
20679 (↓)	144	1 (↑)	1 (↑)	1(↓); 1 (↑)	0	14 (↓)
37089 (↓)	150	1 (↑)	0	1 (↓)	0	16 (↓)
22871 (↑)	26	1 (↑)	3 (↑)	1 (↑)	8 (↑)	0
9745 (↓)	53	1 (↑)	1 (↑)	0	7 (↑)	2 (↓)
6771 (↓)	26	1 (↑)	4 (↑)	1 (↓); 3 (↑)	0	0
18913 (↑)	16	1 (↑)	1 (↑)	0	6 (↑)	0

(↑) up-regulated gene; (↓): down-regulated gene; JA: Jasmonic acid biosynthesis and signalling; SA: Salicylic acid biosynthesis and signalling; Pa-Phe: polyamine and phenylpropanoid signalling; Photosynth: photosynthesis biological processes.

Functional enrichment of co-expressed DEG

Functional analysis extended to all 551 DEG co-expressed with at least one DEL confirms that amongst the up-regulated DEG, there was an enrichment in Biological Process categories associated with plant defense mechanisms. Conversely, the down-regulated genes exhibited an enrichment in functions related to photosynthesis (Figure 1). The hypothesis that lncRNAs may contribute to the negative modulation of photosynthesis both during PTI and ETI has been previously formulated by Rosly et al. (2021). One of the primary benefits of photosynthesis inhibition is the redistribution of metabolic resources toward the production of defense-related substances (Jiang et al., 2023).

This study highlighted lncRNA candidates as additional benchmarks in tomato resistance to bacterial speck, while also supporting their role in regulatory modules that prime plant defense responses, particularly those elicited by *Streptomyces* sp. DLS2013 treatment.

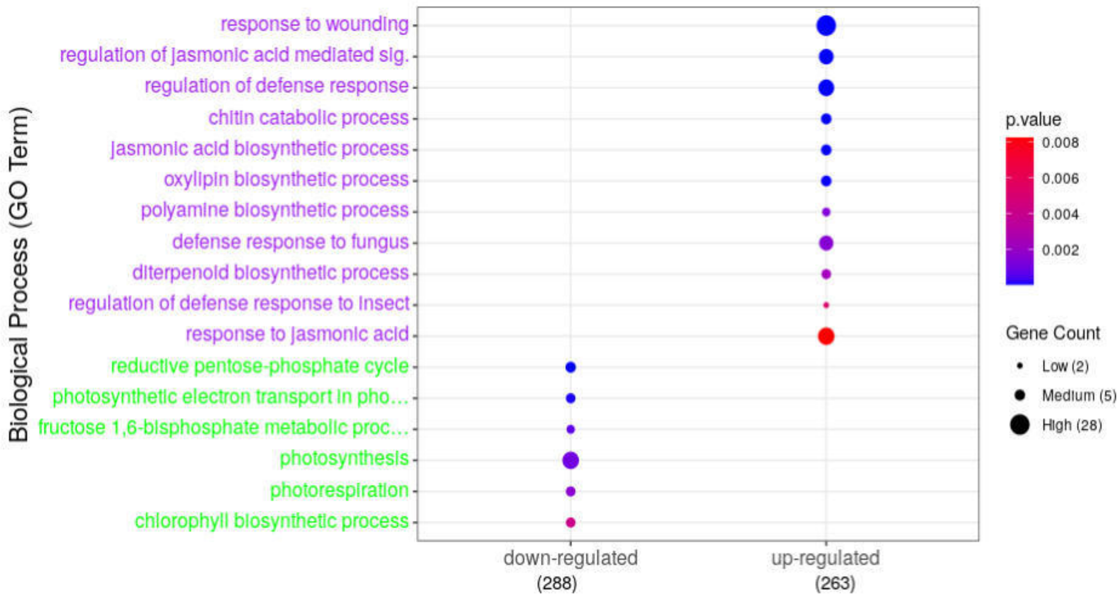

Figure 1. Bubble plot representing functional enriched analysis of co-expressed DEG. Plant defense related GO terms and photosynthesis biological processes were reported in purple and in green, respectively.

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References

Cassanelli, S., Bellameche, F., Caradonia, F., Cortiello, M., Perez Fuentealba, S., and Giovanardi, D. 2025. Foliar application of *Streptomyces* sp. DLS2013 induces transcriptional changes on tomato plants and confers resistance to *Pseudomonas syringae* pv. *tomato*. J. Plant Dis. Prot. 132: 19. https://doi.org/10.1007/s41348-024-01027-4

Jiang, X., Walker, B. J., He, S. Y., and Hu, J. 2023. The role of photorespiration in plant immunity. Front Plant Sci. 14: 1125945.

Li, Q., Shen, H., Yuan, S., Dai, X., and Yang, C. 2023. miRNAs and lncRNAs in tomato: Roles in biotic and abiotic stress responses. Front. Plant Sci. 13: 1094459.

Rivera-Silva, R., Chavez Montes, R. A., and Jaimes-Miranda, F. 2024. Gene ontology functional annotation datasets for the ITAG3.2 and ITAG4.0 tomato (*Solanum lycopersicum*) genome annotations. Data Brief 6(54): 110401.

Rosli, H. G., Sirvent, E., Bekier, F. N. Ramos, R. N., and Pomobo, M. A. 2021. Genome-wide analysis uncovers tomato leaf lncRNAs transcriptionally active upon *Pseudomonas syringae* pv. *tomato* challenge. Sci. Rep. 11: 24523.

Tian, X. C., Chen, Z. Y., Nie, S., Shi, T. L., Yan, X. M., Bao, Y. T., Li, Z. C., Ma, H. Y., Jia, K. H., Zhao, W., and Mao, J. F. 2024. Plant-LncPipe: a computational pipeline providing significant improvement in plant lncRNA identification, Horticulture Research 11: 1-9.

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Synergistic effects of arbuscular mycorrhizal fungi and a rapid root colonizing fungus against *Fusarium* wilt in tomato

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Abstract: Soil-borne pathogens pose a particular challenge to crop protection and agricultural productivity due to the longevity of their propagules in the soil. Arbuscular mycorrhizal fungi (AMF) are known for their bioprotective effects, but their efficacy is limited and depends on a well-established symbiosis prior to pathogen challenge. Therefore, the combination of a beneficial rapid root colonizing fungus with AMF may result in a more successful bioprotective effect, allowing protection even with simultaneous inoculation.

In this study, we investigated the synergistic potential of combining the root endophytic fungus *Serendipita herbamans* (Sh) with a single AMF strain to control *Fusarium oxysporum* f. sp. *lycopersici* (Fol) in tomato plants. Our experiments, conducted both in the greenhouse and in the growth cabinet, evaluated the efficacy of single inoculations versus coinoculations of these beneficial fungi. In addition, we performed targeted metabolomics to reveal the involvement of protective plant metabolites in root and shoot parts at an early stage of the interaction (21 dpi).

Over an eight-week cultivation period, a reduction in disease incidence was observed when Sh was inoculated alone (67 %) or in combination with AMF (53 %). This reduction persisted at 16 weeks, with disease incidence reduced by 25 % and 42 % for single and combined inoculations, respectively. AMF readily colonized the roots in all treatments and improved the phosphorus status of the plants. In addition, the growth reduction induced by Fol in the tomato plants was alleviated. Plant metabolite analysis revealed different metabolite patterns for roots and shoots at 21 dpi. In roots, AMF were the main driver for the increase of defense-related metabolites, while in the shoots, AMF and Sh alone, as in combined inoculation, increased the relative amounts of phenolic acids such as cinnamic acid and ferulic acid.

In conclusion, the bioprotective effect of Sh-AMF co-application is a result of their complementary mechanisms. While Sh rapidly colonizes the rhizoplane and contributes to early protection, AMF not only enhance plant growth and improve phosphorus uptake but also play a role in activating defense-related metabolites within three weeks after inoculation. These findings highlight the potential of combining beneficial fungi with distinct colonization strategies for sustainable disease suppression. Future studies should further characterize these interactions to identify common mechanisms underlying fungal-mediated biocontrol of plant pathogens, in particular the key factors driving their efficacy, and evaluate their potential under field conditions.

Key words: *Serendipita*, plant defense, plant metabolites, root endophytes, *Fusarium* wilt, arbuscular mycorrhiza, soil-borne pathogens

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Foliar application of *Microfighter* activates defense genes in grapevine: a preventive strategy against potential diseases

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Abstract: The increasing use of biopesticides to make viticulture more sustainable has led to questions about their mode of action in the field. In this study, the effect of the biopesticide *Microfighter*, together with a Zinc-based biostimulant, was investigated at the transcriptomic level on grapevine. The dynamic expression of eight defense-related genes was assessed up to 72 hours post-treatment. The expression profile of the tested genes showed distinct responses between both bioproducts in timing and amplitude. A fast response of PR (*PR5* and *PR11*), *PAL* and *SMAT1* genes in both treatments was highlighted, suggesting their possible involvement in priming the plant defense. Moreover, *PAL* and *PR5* genes were identified as potential markers for the biopesticide's activity.

Key words: downy mildew, biopesticide, gene expression, qRT-PCR, PR-genes

Introduction

The control of a major grapevine disease like downy mildew usually requires the massive use of copper-based fungicides with deleterious effects on the environment and its microbial biodiversity (Roviello et al., 2021). Therefore, the implementation and use of biopesticides might represent a promising approach to reducing the use of copper. The EU-financed project "LIFE Microfighter" has the ambition to reduce the input of copper by at least 50 %. *Microfighter* is an innovative biopesticide, where the antagonistic bacterium *Pseudomonas synxantha* strain DLS65 is coupled with a natural zeolite. In previous studies, *Microfighter* was able to reduce the incidence of downy mildew of grapevine in pilot experiments (Bellameche et al., 2024). Notoriously, the efficacy of biopesticides in field applications is influenced by various factors, including environmental conditions, disease pressure, and the timing of their application (Mawcha et al., 2024); therefore, understanding the mode of action is essential for optimizing their use. In the present study, a transcriptomic approach was used, and the expression of a set of genes, known for their role in modulating resistance mechanisms in grapevine, was investigated to describe the possible mechanism involved in protecting the plant upon a biopesticide treatment and to identify potential markers for this protection effect.

Materials and methods

Plant material and biopesticide

Ywo-year-old grapevine plants (cv. Lambrusco Salamino) in excellent phytosanitary health, cultivated under glasshouse conditions, were used for this study. The biopesticide *Microfighter* was prepared and provided by Symbiagro Srl. Italy, the industrial partner of the EU "LIFE Microfighter" project. As a comparison, a Zinc-based biostimulant with secondary antifungal properties was used.

Microfighter application and sampling

Five plants for each foliar treatment were individually sprayed with *Microfighter* at a concentration of 6 g/l or sterile distilled water as control; in the same way, the Zinc-based biostimulant was sprayed on another set of plants. Samples were taken at 0, 2, 4, 8, 24, 48, and 72 hours post-treatment (HPT), and each sample consisting of one leaf per plant. Samples were immediately flash-frozen in liquid nitrogen and stored at -80 °C until RNA extraction.

RNA isolation and qRT-PCR analysis

RNA Extraction and DNAse treatment were performed using the RNeasy Plant Mini Kit (Qiagen), with modifications, according to Landi and Romanazzi (2011). For each sample, 200 ng of RNA was reverse transcribed to cDNA using PrimeScriptTM RT Master Mix (Perfect Real Time, TaKaRa, Japan) according to the manufacturer's instructions. qPCR assays were carried out as described by Cassanelli et al. (2025). A total of eight genes were selected based on their relevance to the plant defense-related pathways, including 1-amino cyclopropane- 1-carboxylic acid (*ACC),* Thaumatin-like protein (*PR5*), Chitinase type 1 (*PR11*), Defensin (*PR12*), Phenylalanine ammonia lyase (*PAL*), Chalcone Synthase (*CHS*), Peroxidase (*PER*) SA Methyl Transferase (*SMAT1*) (Dufour et al., 2016). The PCR reactions were performed in triplicate, and relative gene expression was determined using the $2^{-\Delta\Delta CT}$ method (Taylor et al., 2018), normalized against two reference genes, EF1 and TA, as internal controls.

Results and discussion

Gene expression dynamic

Understanding the mode of action of biopesticides is crucial for developing an innovative biofungicide and implementing reliable field application methods. To achieve this, physiological, biochemical, and molecular approaches can be employed; however, gene expression analysis offers a more detailed insight into their impact on the plant's metabolism (Bodin et al., 2020). This study investigated dynamic changes in the expression of eight defense-related genes in grapevine leaves treated with biopesticides (Figure 1). As expected, all genes were not differentially expressed at the starting point of the experiment, indicating the good phytosanitary health of the plants. As an early response, genes belonging to PR proteins (*PR5* and *PR11*), *PAL* and *SMAT1* were upregulated after 2 to 4 hpt in both treatments. The early upregulation of these genes highlights the rapid response of grapevine plants to the treatment, allowing them to enter a state of alert (priming) and deploy defensive measures more effectively against subsequent pathogenic attacks (Mauch-Mani et al., 2017). Interestingly, at 8 hpt, most of the analyzed genes were upregulated in grapevine leaves treated with biopesticides. This timing likely reflects the period required for the biopesticide, containing beneficial bacteria, to penetrate and establish interaction with the plant tissues (Lugtenberg and Kamilova, 2009). These suggest that the bacteria successfully activated plant defense signaling pathways, likely through recognizing microbe-associated molecular patterns (Jones and Dangl, 2006).

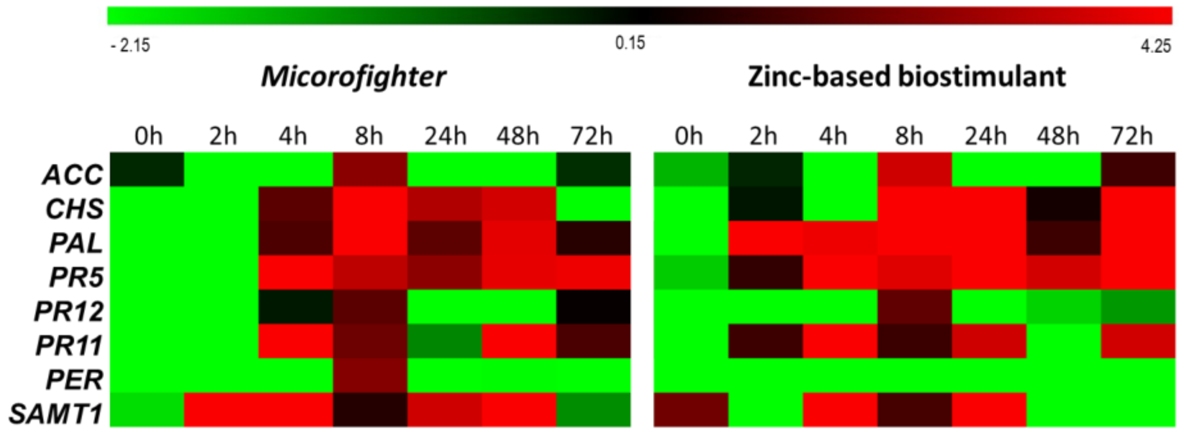

Figure 1. Heatmap showing the expression pattern of eight defense-related genes in grapevine after foliar treatments with *Microfighter* and the Zinc-based biostimulant. Heatmap was generated using the software MeV viewer (http://www.tm4.org). Data presents Log2 fold change induction compared to mock-treated plants with sterile distilled water; green = downregulated genes, red = upregulated genes.

Activation of PR5 and PAL genes

Both foliar treatments on grapevine induced the activation of the analyzed genes, with their up- and down-regulation levels varying depending on the bioproduct and the timing (data not shown). Interestingly, genes like *PR5 and PAL* showed robust overexpression at all sampling times (Figure 2), suggesting their role in improving grapevine resistance. The upregulation of *PAL* is related to the phenylpropanoid pathway, a critical mechanism for producing secondary metabolites, like phytoalexins and lignin, which are essential for structural defense and pathogen resistance (Ramaroson et al., 2022). Same, *PR5* was found to be associated with antifungal activity (Dos Santos et al., 2023).

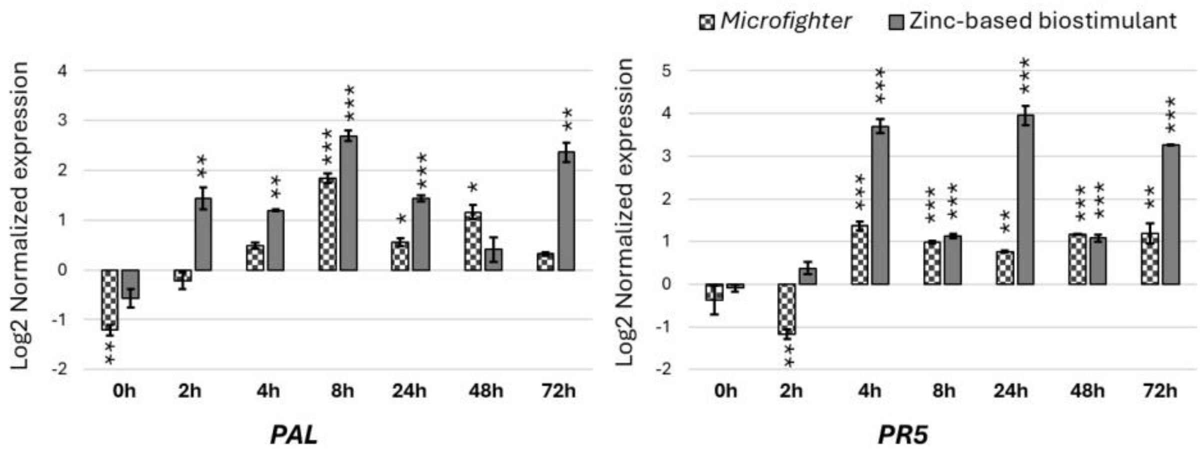

Figure 2. Relative gene expression of a putative markers *PR5* and *PAL* in grapevine leaves treated with bioproducts at 0, 2, 4, 8, 24, 48 and 72 hpt. Data indicates mean values (n = 3) of fold change in response to treatment. Differences compared to intreated control were determined by Student t-test: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

These findings suggest that *Microfighter* can trigger early and late defence mechanisms in grapevines by modulating key genes, providing a basis for their use against downy mildew in sustainable viticulture and, possibly, enhancing plant resistance against other phytopathogens.

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References

Bellameche, F., Modica, F., Fagioli, L., Giovanardi, D., and Stefani, E. 2024. Preliminary characterization and mode action of *Pseudomonas synxantha* DLS65 as the active ingredient of Microfighter, an innovative biopesticide. J. Plant Pathol. doi.org/10.1007/s42161-024-01752-7.

Bodin, E., Bellée, A., Dufour, M. C., André, O., and Corio-Costet, M. F. 2020. Grapevine stimulation: A multidisciplinary approach to investigate the effects of biostimulants and a plant defense stimulator. J. Agric. Food Chem. 68(51): 15085-15096.

Cassanelli, S., Bellameche, F., Caradonia, F., Cortiello, M., Perez Fuentealba, S., and Giovanardi, D. 2025. Foliar application of *Streptomyces* sp. DLS2013 induces transcriptional changes on tomato plants and confers resistance to *Pseudomonas syringae* pv. *tomato*. J. Plant Dis. Prot. 132(1): 1-20.

Dos Santos, C. and Franco, O. L. 2023. Pathogenesis-related proteins (PRs) with enzyme activity activating plant defense responses. Plants 12(11): 2226.

Dufour, M. C., Magnin, N., Dumas, B., Vergnes, S., and Corio-Costet, M. F. 2016. High-throughput gene-expression quantification of grapevine defense responses in the field using microfluidic dynamic arrays. BMC genomics 17: 1-20.

Jones, J. D., and Dangl, J. L. 2006. The plant immune system. Nature 444(7117): 323-329.

Landi, L., and Romanazzi, G. 2011. Seasonal variation of defense-related gene expression in leaves from Bois noir affected and recovered grapevines. J. Agric. Food Chem. 59(12): 6628-6637.

Lugtenberg, B., and Kamilova, F. 2009. Plant-growth-promoting rhizobacteria. Annu. Rev. Microbiol. 63(1): 541-556.

Mauch-Mani, B., Baccelli, I., Luna, E., and Flors, V. 2017. Defence priming: An adaptive part of induced resistance. Annu. Rev. Plant Biol. 68: 485-512.

Mawcha, K. T., Malinga, L., Muir, D., Ge, J., and Ndolo, D. 2024. Recent advances in biopesticide research and development with a focus on microbials. F1000Research 13: 1071. doi.org/10.12688/f1000research.154392.3.

Ramaroson, M. L., Koutouan, C., Helesbeux, J. J., Le Clerc, V., Hamama, L., Geoffriau, E. and Briard, M. 2022. Role of phenylpropanoids and flavonoids in plant resistance to pests and diseases. Molecules 27(23): 8371.

Roviello, V., Caruso, U., Dal Poggetto, G. and Naviglio, D. 2021. Assessment of copper and heavy metals in family-run vineyard soils and wines of Campania region, south Italy. Int. J. Environ. Res. Public Health 18(16): 8465.
Taylor, S. C., Nadeau, K., Abbasi, M., Lachance, C., Nguyen, M., and Fenrich, J. 2019. The ultimate qPCR experiment: producing publication quality, reproducible data the first time. Trends Biotechnol. 37(7): 761-774.

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Root exudates from stressed plants influence rhizosphere inter-kingdom interactions

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Abstract: Plants live in close association with microbes that populate the soil. The presence of this complex soil-plant-associated microbiome plays a key role in regulating plant growth and health. Recent findings have clearly demonstrated that plant-microbe interactions are important for providing an equilibrium in the soil, for supplying many nutrients and participating in numerous biological processes, particularly in the rhizosphere. The microbial composition in the root zone may be altered by both abiotic and biotic stress, resulting in the modulation of the microbial community which may influence the plant responses to the adversity and possibly aid in overcoming the consequences of the constraint. Root exudates (REs) released by plants are pivotal in mediating multitrophic interactions within the rhizosphere, acting as primary signaling molecules that affect microbial community composition and function. Although such effectors are of great importance, the mechanisms by which stressed plants alter REs chemical profiles, regulate production and release of compounds that subsequently affect rhizospheric microbial dynamics remain poorly understood.

In this study, tomato plants were exposed for 24 h to different stress by an abiotic factor of inflicted wounding, and biotic conditions caused by a foliar pathogen *Botrytis cinerea*, chewing insect pest *Spodoptera littoralis,* and sucking insect pest *Macrosiphum euphorbiae*. The REs were collected, then used in chemotropic assays by microscopic observation, to evaluate the properties of the diverse REs to attract the fungal biological control agents (BCAs) *Trichoderma afroharzianum* and/or *Beauveria bassiana*, and the plant pathogen *Fusarium oxysporum*.

All REs demonstrated inhibitory and repellent effects, respectively, on the conidia and germ tubes of *Fusarium oxysporum*, while inducing attraction of BCA germ tubes. Notably, REs from insect-stressed plants exhibited the highest chemotropic activity with the highest attractivity to the BCAs and the strongest repellency to the fungal pathogen. This suggested that REs, whose chemical composition may be reasonably modulated by the different stressors to which the plant has been exposed, produced a differential response in pathogenic versus non-pathogenic microbes.

To elucidate the molecular underpinnings of this inter-kingdom signaling, we analyzed the root-secreted metabolome and performed a fractionation of the REs. Then, an activity-guided chemotropic assay was used to determine the attractiveness to *F. oxysporum*. Findings indicated that low molecular weight compounds are involved in the repellent activity against pathogen. Investigations on BCAs responses to fractionated REs are in progress. Results will be useful to enhance understanding if shared and/or diverse molecular signatures are associated with stress responses in plants and may influence their interactions with soil microbial communities. These insights will advance our knowledge on plant-

microbe interactions and highlight the potential for developing stress-resilient crops with enhanced pathogen resistance.

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Building microbial consortia towards saprophytic stubble colonization and reduction of *Rhizoctonia solani* severity in common bean

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Abstract: *Trichoderma* spp. is widely used worldwide to reduce losses caused by plant diseases, however its effectiveness is not guaranteed when exposed to UV radiation. In tropical conditions, particularly in crop rotation systems, stubble maintenance can hinder the survival and colonization of *Trichoderma* spp. In this context, the efficacy of this fungus can be enhanced when associated with melanized fungi, such as *Epicoccum nigrum*. This fungus is pigmented which provides higher UV tolerance compared to non-pigmented ones. Within a maize-bean and potato-bean rotation, *Rhizoctonia solani* AG3 may build up in population, therefore competition for substrate by saprophytic fungi is a plausible option to reduce the pathogen overwintering. Both *in vitro* and *in vivo* tests were conducted to evaluate the potential of *Trichoderma asperellum* and *Epicoccum nigrum* solo or combined on the displacement of *R. solani* from the stubble and damping-off severity. *In vitro* the carbon and nitrogen source requirements were evaluated to determine the Niche Overlap Index (NOI) and both the compatibility and inhibition of the pathogen were determined by dual culture. For the greenhouse test, maize or potato stubble was sprayed with *R. solani*, and subsequently *T. asperellum* either alone or in combination with *E. nigrum* were applied before planting beans. The *in vitro* tests demonstrated that both microorganisms can colonize stubble and reduce *R. solani* inoculum. Additionally, the biocontrol agents were compatible with each other. In successive inoculation tests (first applying the antagonist followed by the pathogen), treatments with *T. harzianum* alone and *T. asperellum* + *E. nigrum*, reduced the severity of the disease in potato stubble compared to the control ($p < 0.05$). While in maize stubble, *E. nigrum* and the combination of *T. asperellum* + *E. nigrum* reduced the disease rate. Conversely, in simultaneous inoculation, this same pattern was maintained only in potato stubble, while maize stubble could not sustain the beneficial microbial consortium. In conclusion, the consortium of *E. nigrum* and *T. asperellum* can displace *R. solani* but the origin of the stubble must be taken into consideration.

Key words: antagonistic, consortia, crop rotation, damping-off, severity of disease

Session X
Commercial use of microbials for integrated and organic disease management

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Selection of new microbial plant protection strains for EU markets: regulatory and manufacturing challenges

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The growing emphasis on sustainable agricultural practices has positioned biocontrol Plant Protection Products (PPPs) as critical alternatives to chemical pesticides. Microbial-based biocontrol agents, in particular, offer precise and environmentally sustainable pest and disease management solutions. Selecting microbial strains that comply with strict regulatory frameworks and meet industrial production standards is an inherently complex process. This paper addresses these challenges, focusing on the regulatory safety requirements under EU legislation and the industrial criteria critical for the successful commercialization of microbial biocontrol PPP.

The EU regulatory framework, primarily governed by Regulation (EC) No 1107/2009, is designed to ensure the safety of microbial PPPs for human health, animal welfare, and environmental protection. Stringent regulatory requirements make early-stage screening for regulatory compliance imperative (EC, 2020 a). During the selection of biocontrol strains, developers must strictly consider that explicit prohibitions and cautionary criteria are established. Authorization will not be granted, or the assessment will be subject to further scrutiny with a high likelihood of rejection, under the following conditions:

- **Pathogenicity to humans and animals:** Microorganisms pathogenic to humans or animals are excluded from regulatory approval. Strains classified under risk groups 3 or 4 in international biosafety classifications (BAUA, 2010; SBB, 2024) are not considered suitable for PPP development. Risk group 2 microorganisms should be considered with caution, approval is unlikely.
- **Pathogenicity to non-target organisms:** Microbial strains with demonstrated pathogenicity to bees, soil fauna, or aquatic invertebrates are unlikely to receive regulatory approval, unless it is clearly established by a thorough risk assessment that under field conditions no unacceptable impact on populations of these organisms is expected to occur.
- **Genetic instability in viruses**: Microorganisms that are non-virulent variants of plant pathogenic viruses must demonstrate genetic stability. Strains at higher risk of regaining virulence are typically excluded from approval. Clarifications provided in the 'Explanatory Notes' (EC, 2023) indicate that genetic stability is critical, particularly when differences in virulence depend on single-point mutations.
- **Production of metabolites of concern**: Metabolites of concern are those with potential (eco)toxicological effects on human health, non-target organisms, or the environment. Regulatory evaluations require a stepwise approach to identify and assess these metabolites (EC, 2024). If the primary mode of action of the microorganism depends entirely on these metabolites, the product may be regulated as a chemical, according to Part A of Regulation (EU) No 283/2013. Additionally, if a microorganism produces medically important antimicrobials (MIA) as defined by the World Health Organization (WHO, 2018), the interference with such antimicrobials would prevent an approval of this microorganism (EC, 2020 b).

- **Antimicrobial resistance:** The detection of transferable antimicrobial resistance (AMR) genes, particularly those associated with medically important antimicrobials, is a critical regulatory concern. Whole genome sequencing is necessary to identify and evaluate AMR genes, especially if they are located on mobile genetic elements (EC, 2020 b; WHO, 2018).
- **Industrial considerations:** The industrial production of biocontrol strains necessitates careful alignment of biological efficacy with economic scalability. Four core criteria are identified as critical to industrial viability:
- **Ease of cultivation and scalability:** Microbial strains must exhibit compatibility with large-scale production systems, whether through solid-state or liquid fermentation. Essential factors include rapid, synchronized and robust growth rates of the target structures, under standard fermentation conditions. Strains sensitive to minor changes in culture parameters or prone to erratic behaviors, such as inconsistent metabolite production or the formation of undesirable by-products, may undermine scalability (Burges, 1998; Crater and Lievense, 2018).
- **Stability and shelf-life:** The long-term viability of microbial biocontrol products is heavily dependent on their stability during storage and transportation. Key attributes include tolerance to desiccation, resistance to temperature fluctuations, and oxidative stress. Such traits reduce logistical challenges and enhance the marketability of the products (Teixidó et al., 2022).
- **Compatibility with agricultural practices:** Biocontrol agents must integrate seamlessly into existing agricultural practices. This includes compatibility when co-applied with fertilizers, pesticides, and other biological agents, as well as adaptability to various application methods (Ravensberg, 2011).
- **Cost-effectiveness:** Economic viability is critical for the widespread adoption of biocontrol products. Strains with simple cultivation requirements, and minimal processing demands are more cost-effective. Processes that minimize waste and reduce energy consumption further enhance cost efficiency. A well-defined regulatory strategy, incorporating strategic data collection and adherence to safety standards, significantly mitigates development costs and facilitates smoother market access (Khan and Ahmad, 2024).

The selection of microbial strains for biocontrol demands a harmonized approach that addresses both regulatory and industrial considerations. By prioritizing safety, efficacy, scalability, and economic feasibility, developers can ensure that biocontrol products meet the EU rigorous standards while achieving commercial success. This integrated strategy not only streamlines market entry but also promotes sustainable agricultural practices, aligning with broader environmental and food security objectives.

Key words: microbial biocontrol products, EU regulatory framework, safety requirements, industrial production criteria

References

BAUA 2010. Federal Institute for Occupational Safety and Health. TRBA 466 – Classification of prokaryotes (bacteria and archaea) into risk groups. Technical Rule for Biological Agents. Gemeinsames Ministerialblatt No. 68-80 of 06.12.2010, pp. 1428-1667 Available at: https://www.baua.de/EN/Service/Technical-rules/TRBA/TRBA-466 [Accessed 28 Oct. 2024].

BAUA 2024. Federal Institute for Occupational Safety and Health. Technical Rules for Biological Agents (TRBA). Selected TRBA. Available at: https://www.baua.de/EN/Service/Technical-rules/TRBA/TRBA [Accessed 28 Oct. 2024].

Burges, H. D. 1998. Formulation of microbial pesticides: beneficial microorganisms, nematodes and seed treatments. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Crater, J. S., and Lievense, J. C. 2018. Scale-up of industrial microbial processes. FEMS Microbiology Letters 365(13): fny138.

EC 2000. European Commission Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32000L0054 [Accessed 28 Oct. 2024].

EC 2009. European Commission Regulation (EC) No 1107/2009 concerning the placing of plant protection products on the market. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32009R1107 [Accessed 28 Oct. 2024].

EC 2020 a. European Commission Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions A Farm to Fork Strategy for a fair, healthy and environmentally friendly food system. Available at: https://eur-lex.europa.eu/resource.html?uri=cellar:ea0f9f73-9ab2-11ea-9d2d-01aa75ed71a1.0001.02/DOC_1&format=PDF. [Accessed 28 Oct. 2024].

EC 2020 b. SANTE/2020/12260. European Commission Guidance on the approval and low-risk criteria linked to "antimicrobial resistance" applicable to microorganisms used for plant protection in accordance with Regulation (EC) No 1107/2009. Available at: https://food.ec.europa.eu/system/files/2020-11/pesticides_ppp_app-proc_guide_180652_microorganism-amr_202011.pdf [Accessed 28 Oct. 2024].

EC 2022 a. European Commission Regulation (EU) 2022/1439 amending Regulation (EU) No 283/2013 as regards the information to be submitted for active substances and the specific data requirements for micro-organisms. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32022R1439 [Accessed 28 Oct. 2024].

EC 2022 b. European Commission Regulation (EU) 2022/1440 amending Regulation (EU) No 284/2013 as regards the information to be submitted for plant protection products and the specific data requirements for plant protection products containing micro-organisms. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32022R1440 [Accessed 28 Oct. 2024].

EC 2022 c. European Commission Regulation (EU) 2022/1441 amending Regulation (EU) No 546/2011 as regards specific uniform principles for evaluation and authorisation of plant protection products containing micro-organisms. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32022R1441 [Accessed 28 Oct. 2024].

EC 2023. PAFF-PPL-October 2023-Doc.A.07.01. European Commission Explanatory notes for the implementation of the data requirements on micro-organisms and plant protection products containing them in the framework of Regulation (EC) No 1107/2009. Available at: https://food.ec.europa.eu/system/files/2023-10/pesticides_ppp_app-proc_guide_imp-data-req_micro-organisms-ppp_imp-reg-11072009.pdf [Accessed 28 Oct. 2024].

EC 2024. European Commission Guidance on the risk assessment of metabolites produced by micro-organisms used as plant protection active substances (SANCO/2020/12258). Available at: https://food.ec.europa.eu/document/download/ffdf09b5-77a5-45b5-95f9-b16272f7535a_en [Accessed 28 Oct. 2024].

IBMA 2023. International Biocontrol Manufacturers Association. What are biocontrol technologies? Available at: https://ibma-global.org/what-is-biocontrol [Accessed 28 Oct. 2024].

Khan, E., and Ahmad, I. Z. 2024. Production and commercialization of biocontrol products. In: Kumar, A., Santoyo, G., Singh, J. (eds.): Plant and Soil Microbiome, Biocontrol Agents for Improved Agriculture, pp. 165-180. Academic Press.

Ravensberg, W. J. 2011. A Roadmap to the Successful Development and Commercialization of Microbial Pest Control Products for Control of Arthropods. Springer, Dordrecht, The Netherlands.

SBB 2024. Belgian Biosafety Server. Contained use: International classifications schemes for micro-organisms based on their biological risk. Available at: https://www.biosafety.be/content/contained-use-international-classifications-schemes-micro-organisms-based-their-biological [Accessed 28 Oct. 2024].

Teixidó, N., Usall, J., and Torres, R. 2022. Insight into a Successful Development of Biocontrol Agents: Production, Formulation, Packaging, and Shelf Life as Key Aspects. Horticulturae 8(4): 305.

WHO 2018. Worl Health Organization. Critically Important Antimicrobials for human medicine, 6th Revision. Available at: https://iris.who.int/bitstream/handle/10665/312266/9789241515528-eng.pdf?sequence=1 [Accessed 28 Oct. 2024].

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Trichoderma-formulated products alternated or not impact in *Sclerotinia sclerotiorum* management considering the origins: laboratory and field borne sclerotia

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Abstract: White mold, caused by *Sclerotinia sclerotiorum*, has the sclerotia as initial inoculum. The sclerotium parasitism reduces the apothecium formation, ascospore release, and disease incidence. In this regard, *Trichoderma*-based products are deployed on large scale wherever this disease is important; but little is known about the contribution of alternating *Trichoderma*-based products on the sclerotia parasitism, disease incidence, and contribution to plant yield. Furthermore, little is known about the contribution of the sclerotia origin on the performance of the biocontrol agent. In the present work, *Trichoderma*-based products were sprayed solo and alternately at the V2 and V4 phenological stages to evaluate their effectiveness in controlling white mold in soybean. Two field trials were conducted in different locations. The evaluations were: yield-related variables, grain yield (kg/ha), and white mold-related variables (disease incidence, sclerotia parasitism, germination, and viability, and the number of apothecia) on sclerotia originated from the field and produced in laboratory. Regarding the disease parameters, all BCA treatments were different from the control regarding incidence of white mold, while germination of sclerotia was decreased with *Trichoderma* spray. The sclerotia origin (lab or field) affected its capability to produce apothecia and cause disease. The application of these BCAs regardless of the sequence they were applied reduced sclerotia viability, disease incidence and sustained higher yield.

Key words: biological control agents, *Trichoderma harzianum*, white mold, yield increase, carpogenic germination.

Introduction

Brazil is currently the leading global producer of soybeans (USDA, 2023). However, the expansion of the cultivation area has increased the need for integrated management methods to control diseases, weeds, and insects (Bortolotto et al., 2015; Bueno et al., 2021). Although higher yields can lead to increased input costs, they can also cause quantitative and qualitative losses due to damage from biotic agents, such as plant diseases (Bennett et al., 2012).

One significant fungal pathogen affecting soybean is *Sclerotinia sclerotiorum* Lib de Bary (Willbur et al., 2019; Macena et al., 2020). This fungus infects plants both through carpogenic or myceliogenic germination, leading to considerable losses in Brazil, particularly at elevations higher than 600 meters (Boland and Hall, 1994; Meyer et al., 2016). Without control measures, yield losses can reach 70 % (De Faria et al., 2022).

Managing white mold requires both chemical and biological control methods, as no soybean cultivars are completely resistant to this fungus, although susceptibility may vary (Kandel et al., 2018; Sumida et al., 2015). The main active ingredients commercially available in Brazil include fluazinam, methyl thiophanate, procymidone, and carbendazim (Meyer et al., 2020). These fungicides are typically applied during the flowering stages (R1 and R2) with a 10-day interval between sprays (Lehner et al., 2017). However, these chemical controls primarily protect the flowers and do not effectively reduce the initial inoculum, which consists of the sclerotia already present in the area, and which multiplies from season to season (Garg et al., 2010; Angelique et al., 2012; Young and Werner, 2012). From our obtained results, the control treatment that received the recommend chemical fungicide treatment was not sufficient to achieve the highest yield under conditions favorable to the disease epidemics. Therefore, it is imperative to improve the management techniques to reduce the inoculum and standardize the possible modes of action that could inhibit germination in the field using the biological control as we have demonstrated for both field trials.

Several models using biological control agents (BCAs) that exhibit mycoparasitic activity on sclerotia have been developed (Rembinski et al., 2022; Haddad et al., 2017; Silva et al., 2022). These models range from field observations of BCA activity and their colonization of sclerotia to controlled laboratory tests that assess the effects on germination and viability (da Silva et al., 2022; Sumida et al., 2018). Another important consideration concerns the conditions that lead to sclerotium germination. They are related to the source of the initial inoculum and the pathogen population, as it can significantly influence the dynamics of the mycoparasitic processes associated with BCAs, potentially varying from strong to weak sources of inoculum (Foley et al., 2016; Taylor et al., 2018). The sclerotia can be produced in the laboratory or collected from the field, both have two different conditions. Understanding these processes – both the epidemiology of the initial inoculum source and its interactions with the mycoparasitic activity of BCAs is crucial for validating more efficient and promising methods to reduce the severity of white mold in the field.

Various BCAs can reduce the losses caused by white mold in soybean. They achieve this by producing cell wall-degrading enzymes, parasitizing sclerotia, inducing systemic defense responses, promoting plant growth, and recruiting other beneficial organisms. Numerous studies have highlighted these benefits of *Trichoderma* spp. (Macena et al., 2020; Singh et al., 2021; de Azevedo Silva et al., 2021; Sumida et al., 2018), although there is limited knowledge regarding the use of different biological control agents and their synergistic effects. Additionally, using different types of beneficial microorganisms, even if they belong to the same species, may help to improve agricultural production and reduce the effects of diseases caused by the initial inoculum. These microorganisms might work together in ways that enhance plant health and increase productivity.

To reach the sclerotia, the BCAs are commonly recommended to be sprayed during the V2 and V4 phenological stages of the plant to characterize sclerotia viability (Meyer et al., 2016) or following soybean desiccation (Conte et al., 2022). At these plant phenological stages, the canopy is not fully developed, allowing the mycoparasite to reach the sclerotia on the soil (O'Sullivan et al., 2021). This application timing coincides with that of post-emergent herbicides (Canuto da Silva et al., 2024), thus reducing production costs while employing a user-friendly approach by incorporating *Trichoderma*-based products into the agroecosystem (Adetunji and Varma, 2020; Dutta et al., 2022).

This study aims to evaluate: (i) the effects of applying *Trichoderma harzianum* from two different formulations applied solo or alternating on the white mold disease and crop yield parameters; (ii) the contribution of the sclerotia origin on the products performance (field and laboratory).

Materials and methods

The *in vivo* experiment with *Trichoderma* sp. to control *S. sclerotiorum* was conducted in two different locations. These fields were selected based on the prevalence of sclerotia confirmed for virulence before planting, according to (De Faria et al., 2022). The first area (Area 1) was at Nepomuceno (21°19'08.9"S 45°06'41.1"W) 964 meters high, and the second (Area 2) at Três Corações (21°34'41.6"S 45°08'29.9"W) 972 meters high, both in Minas Gerais, Brazil. The cultivar used was Brasmax Zeus IPRO. Sowing at Area 1 occurred on 11/10/2022 and Area 2 on 15/10/2022, respectively both with soil cover with grass stubble. Planting was done with 14 seeds per meter, with a row spacing of 0.5 meters. The plots consisted of five treatments and four blocks, with each plot having an effective area of 18 m² (6 lines spaced by 0.5 meters by 6-meter length). The treatments were: Control (water); BCA 1 (*Trichoderma harzianum* IBLF 006, Ecotrich; dose 250 g/ha); BCA 2 (*Trichoderma harzianum* BK-Th001, Natucontrol; dose 800 g/ha); BCA 1_BCA 2 (Ecotrich followed by Natucontrol); and BCA 2_BCA 1 (Natucontrol followed by Ecotrich), each product was applied according to the label of the product for the biological target *S. sclerotiorum*. The applications of the treatments were at the phenological stages of V2 and V4 only with the help of a CO_2-pressurized pump aiming the middle of the lines and, consequently the sclerotia present in the soil and the stubble. To isolate the effect of only *Trichoderma* in the system, Procimidona (ParrudoBR) was applied at a dose of 1 l/ha to control *S. sclerotioru*m ascospore infection starting at R1 and the application was repeated three more times with 14 days of interval to all treatments. To tackle foliar diseases, the products Carbendazim (Carbendazim STK 500 SC-B) 500 ml/ha and Tebuconazole (Tebuconazole CCAB 200 EC) 0.75 l/ha were also sprayed for all treatments at the same phenological stages as the white mold chemical fungicides. Glyphosate (Roundup WG), at the dose of 2 kg/ha, was applied at V2, and thiamethoxam + lambda-cyhalothrin (Engeo Pleno S) at the dose of 200 ml/ha to control insects when necessary.

To assess the experiments, the incidence of white mold was quantified by evaluating the percentage of eighty plants of the main planting lines (the four lines of the middle) at R6. The parameters: yield (kg/ha), was measured by the weight of the grains collected from the main four lines of the center of the plot with the discount of the moisture; thousand-grain weight, measured by weighting one hundred grains eight times and transforming it into one thousand grains weight; number of pods, measured by counting the number of pods of the main stem; and number of grains per pod, measured by dividing the number of grains by the number of pods of the main stem.

A test of parasitism capacity of the fungus *T. harzianum* IBLF 006 and BK-Th001 over *S. sclerotiorum* was conducted. The experiment initially took place in the field, where sclerotia were collected from the area as well as produced in the laboratory to assess parasitism, under both conditions. The sclerotia were then placed inside bags made of aphid-proof mesh, with only one layer covering the sclerotia. A tray containing B horizon soil was placed in the middle of the soybean planting rows, and the sclerotia were placed on the tray. The tray was covered with the stubble of the last crop, concealing the sclerotia. Ten days after the second application, the sclerotia were removed from the field and taken to the laboratory. In the laboratory, the sclerotia from each treatment were individually laid over a acrylic box (20 × 20 × 5 cm) with a lid, filled with autoclaved sand, and incubated in a Growth chamber at 17 °C to stimulate the carpogenic germination. Weekly, the number of apothecia on each sclerotia was counted until 60 days after the experiment was set up to estimate the germination and number of apothecia per sclerotia (Meyer et al., 2016). The experiment was conducted in a randomized block design (n = 4).

The data were tested according to the Shapiro-Wilk normality test (p-value > 0.05), Bartlett's homogeneity test (p-value > 0.05), and Dixon's test (p-value ≤ 0.05). For the mean comparison, Tukey's test was used (p-value ≤ 0.05). The software used was the R program 2023.06.1 Build 524.

Results and discussion

There was a significant effect for the treatments regarding all considered variables. Results obtained for disease parameters areas, a lower disease incidence (p = 0.001) was obtained for the *Trichoderma* treatments (Figure 1) with reductions from 48.9 to 94 % according to the considered treatment combination, but there was no difference between the different *Trichoderma* treatments.

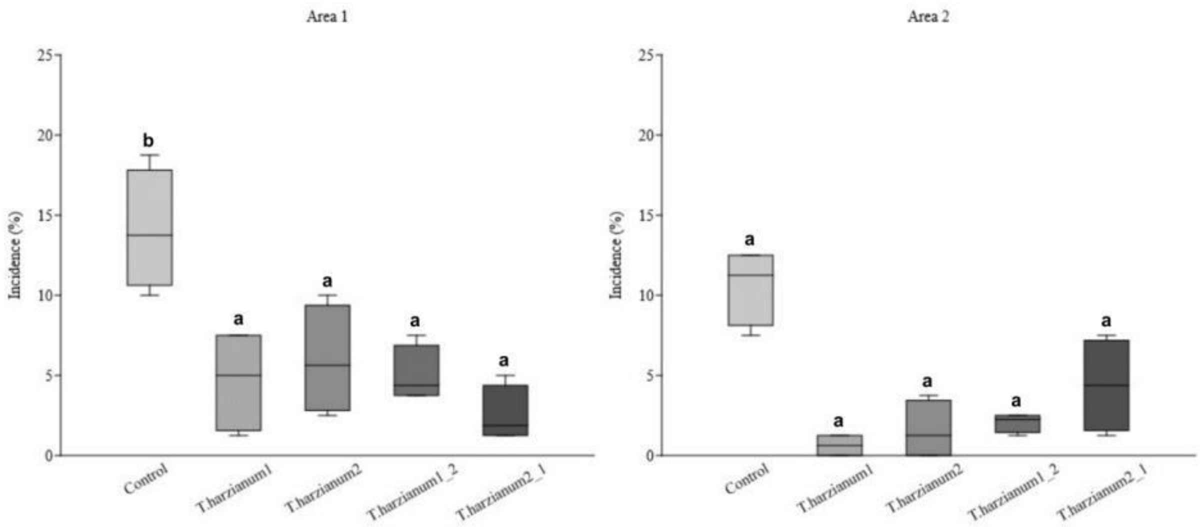

Figure 1. White mold incidence (%), considering the presence or absence of white mold symptoms regardless of its severity (n = 4 and 80 plants per plot), in two different field trials (area 1 and area 2), according to different treatments: Control (Water); *T. harzianum* 1: *Trichoderma harzianum* IBLF 006 (Ecotrich, Ballagro); *T. harzianum* 2: *Trichoderma harzianum* (Natucontrol, Biotrop); *T. harzianum* 1_2: Ecotrich_ Natucontrol; and *T. harzianum* 2_1: Natucontrol_ Ecotrich. *Means followed by the same letter are not statistically different by the Tukey test (p <= 0.05).

The incidence levels of *S. sclerotiorum* are determined by multiple factors, primarily influenced by moisture and temperature. Optimal conditions for carpogenic germination occur under high moisture levels and temperatures ranging from 10 to 20 °C (Wu and Subbarao, 2008). This stage is crucial for causing epidemics in crops (Purdy, 1979). According to data from INMET (2024), the average minimum temperature recorded at the Lavras weather station from October 2022 to February 2023 was 17 °C. This temperature is conducive to apothecia germination, which increases the incidence of white mold, especially in the absence of BCAs, whether in crop rotation or not.

About the sclerotia that received treatments with biocontrol agents (BCAs) from both the field and laboratory in the two evaluated areas (Area 1 and Area 2), sclerotia germination (Table 1) was analyzed. In both areas, sclerotia collected from the field and laboratory exhibited high germination rates in the control group and the application of the BCAs significantly reduced germination ($p < 0.001$). Parasitism (Table 1) showed no significance in Area 1 for both field ($p = 0.247$) and laboratory ($p = 0.7212$) samples. In Area 2, only the treatments with *T. harzianum* 1 and a combination of *T. harzianum* 1 and 2 were able to demonstrate parasitism to sclerotia collected from the field. In contrast, the laboratory samples did not show significant parasitism ($p = 0.1905$).

Table 1. Rates of germination, and parasitism of the sclerotia after 60 days.

	Treatments	Germination (%)		Parasitism (%)	
		Field	Lab	Field	Lab
Area 1	Control	85.27 a	22.42 a	8.10 a	0.00 a
	T. harzianum 1	5.075 b	5.62 b	2.85 a	2.50 a
	T. harzianum 2	5.32 b	2.50 b	0.00 a	0.00 a
	T. harzianum 1_2	10.58 b	0.00 b	0.00 a	3.12 a
	T. harzianum 2_1	15.65 b	0.00 b	0.00 a	2.27 a
	P valor	< 0.001	< 0.001	0.2471	0.7212
Area 2	Control	100 a	10.55 a	0.00 b	0.00 a
	T. harzianum 1	13.33 b	0.00 b	22.91 a	12.50 a
	T. harzianum 2	5.00 b	0.00 b	0.00 b	0.00 a
	T. harzianum 1_2	24.79 b	2.50 b	25.41 a	6.81 a
	T. harzianum 2_1	16.66 b	0.00 b	0.00 b	2.77 a
	P valor	< 0.001	< 0.001	0.0032	0.1905

*Control (Water); *T. harzianum* 1: *Trichoderma harzianum* IBLF 006 (Ecotrich, Ballagro); *T. harzianum* 2: *Trichoderma harzianum* (Natucontrol, Biotrop); *T. harzianum* 1_2: Ecotrich_ Natucontrol; and *T. harzianum* 2_1: Natucontrol_ Ecotrich. Means followed by the same letter are not statistically different by the Tukey test ($p <= 0.05$). Germination, considering all the sclerotia that produced apothecium; Parasitism, considering all the parasitized sclerotia. *Means followed by the same letter are not statistically different by the Tukey test ($p <= 0.05$).

The variation in germination rates observed between the origins (field and laboratory) suggested that sclerotia from the field had a higher germination rate, while laboratory-sourced sclerotia did not. This higher germination rate may be attributed to the accumulation of sclerotia formed in the field, which were subject to optimal temperature conditions and adapted to the local environment (Huang and Kozub, 1991). Although all biocontrol treatments led to a reduction in carpogenic germination, *Trichoderma* strain was seldom recovered from the sclerotia, while other microbes were frequently observed. This indicates that microbial succession may have occurred – initially, *Trichoderma* may have reduced pathogen viability, but it did not persist on the sclerotia, allowing saprophytic communities to take over the sclerotia debris, or a microbial succession may have taken place and a microbial community with higher mycoparasitic relationship would have taken over (Sharma et al., 2020). Additionally, an alternative mechanism that might be present in this situation is microbial selection, which alters the soil community (Umadevi et al., 2018). Such microbial succession may have contributed to soil suppressiveness against diseases (Wang et al., 2019) and led to a reduction in disease incidence among the biological treatments compared to the control group (Figure 1).

The number of apothecia (Figure 2) observed in both areas, regardless of the origin of the sclerotia reduced with treatments with BCAs compared to the control. Specifically, in area 1 ($p < 0.001$) and area 2 ($p < 0.001$) for sclerotia from the field, and similarly in area 1 ($p < 0.001$) and area 2 ($p < 0.001$) for sclerotia from the laboratory. Additionally, the number of apothecia produced by sclerotia collected from the field was 73 % higher than that produced by from the ones from the laboratory. This supports the earlier discussion that field-collected sclerotia demonstrated a greater ability to develop the disease compared to those originating from the laboratory.

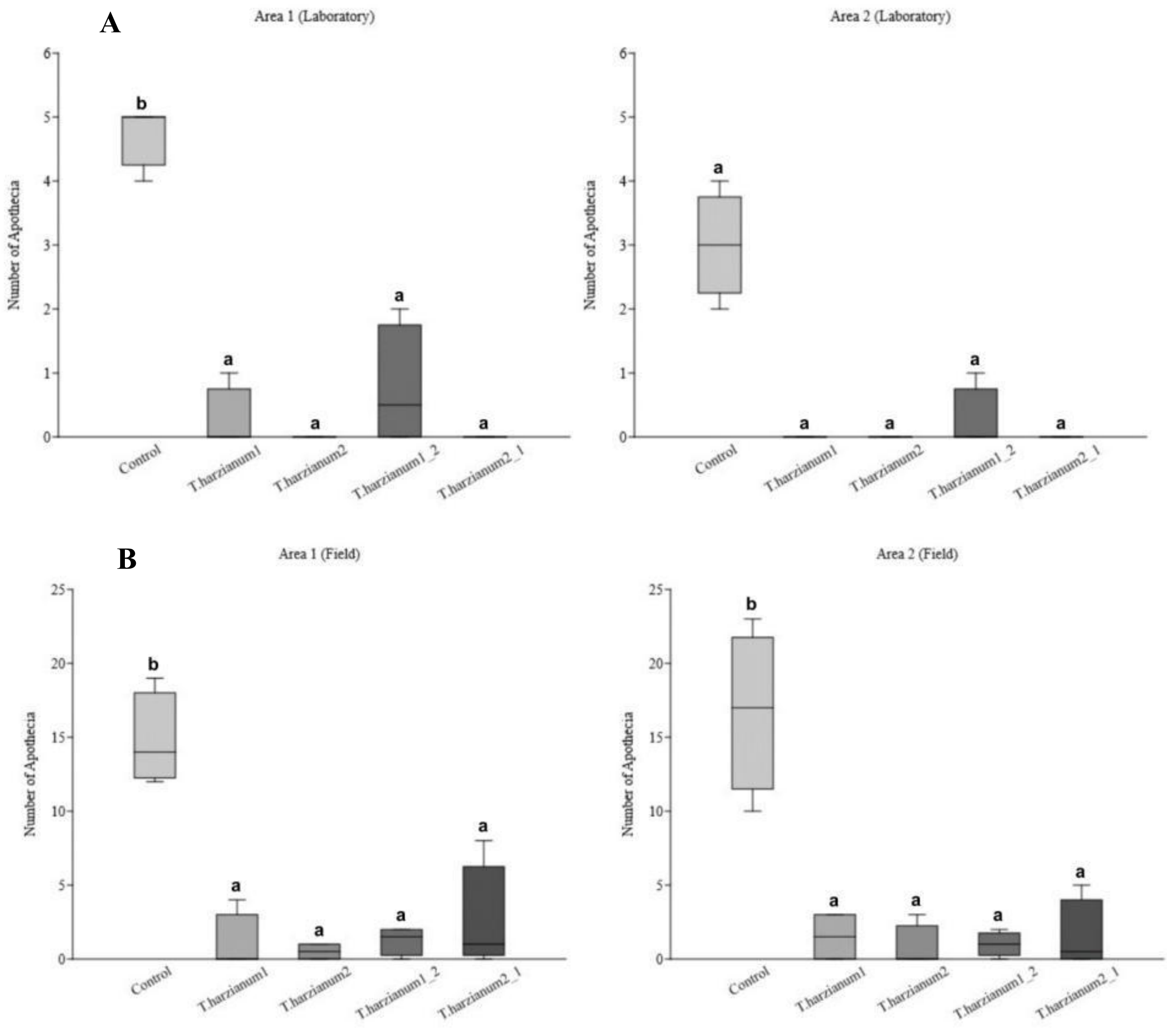

Figure 2. Number of apothecia produced from sclerotia originated from sclerotia population obtained from the (A) field or produced in the laboratory (B) in field trials carried out in two locations (area 1 and area 2). Where: Control (Water); *T. harzianum* 1: *Trichoderma harzianum* IBLF 006 (Ecotrich, Ballagro); *T. harzianum* 2: *Trichoderma* harzianum (Natucontrol, Biotrop); *T. harzianum* 1_2: Ecotrich_ Natucontrol; and *T. harzianum* 2_1: Natucontrol_ Ecotrich. *Means (n = 4) followed by the same letter are not statistically different according to Tukey's test ($p <= 0.05$).

The yield data (Figure 3) indicate that the treatments *T. harzianum* 1 and *T. harzianum* 2 resulted in an increase compared to the control group. For area 1 ($p < 0.001$), an increase in yield ranged from 19.11 to 81.6 %, with the highest for *T. harzianum* 2 followed by *T. harzianum* 1 (81.6 %), an increase of up to 21 60 kg-bags per hectare compared to the control. In area 2, there was also a significant effect ($p < 0.001$), between the treatments and the control from 20.81 to 34.1 %, with *T. harzianum* 1 (34.1 %) as promoting the highest yield, an increase of 27 more 60 kg-bags than the control.

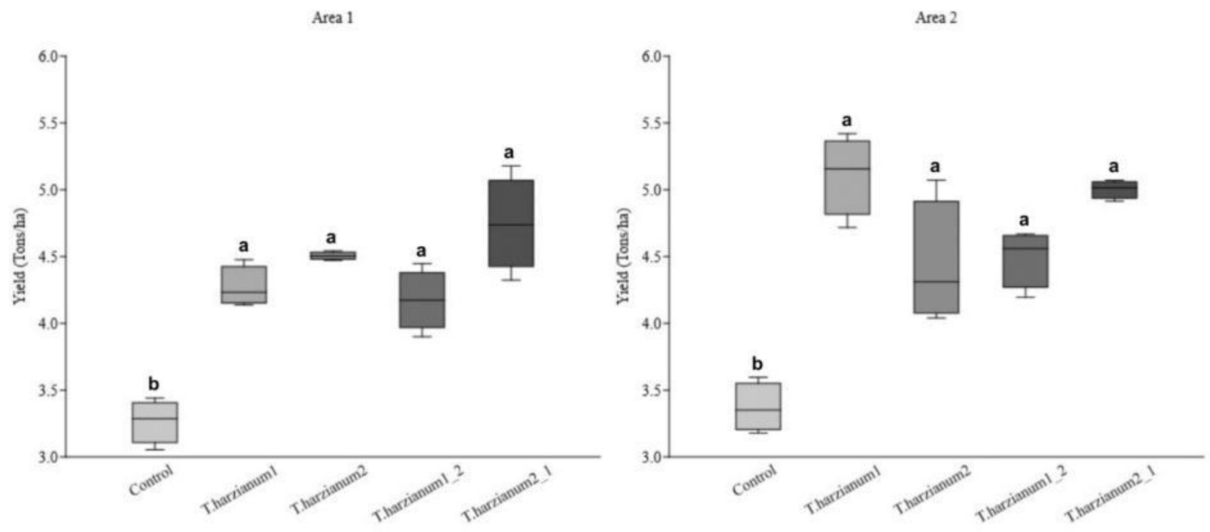

Figure 3. Soybean yield in two different field trials (area 1 and area 2) according to different *Trichoderma*-based sequential treatments: Control (Water); *T. harzianum* 1: *Trichoderma harzianum* IBLF 006 (Ecotrich, Ballagro); *T. harzianum* 2: *Trichoderma harzianum* (Natucontrol, Biotrop); *T. harzianum* 1_2: Ecotrich_ Natucontrol; and *T. harzianum* 2_1: Natucontrol_ Ecotrich. *Means ($n = 4$) followed by the same letter are not statistically different according to Tukey's test ($p <= 0.05$).

While the fungicide has the exclusive role of protecting the plants against the *S. sclerotiorum* ascospore infection, the biocontrol agent can have a dual role and act as a biostimulant as well (Nieto-Jacobo et al., 2017). Notably, *T. harzianum* possesses growth-promoting properties that enhance plant growth through both direct and indirect mechanisms, such as microbial recruitment and improved productivity (Hang et al., 2022). Therefore, the increased yield can be a combination of both its disease biocontrol and growth promoting.

Finally, from the obtained results, we postulate that alternating the application of *T. harzianum*-based products did not affect the incidence of white mold or plant yield and these findings may have a direct implication on practical adoption of biocontrol since the grower will have more freedom of choice and according to local availability or price constraints the grower can opt to alternate the used biocontrol product in each application without detrimental effect on the biocontrol performance and implications in plant yield. Furthermore, it is imperative to use local sclerotia population to infer on the contribution of biocontrol agents on disease management and laboratory population may not explain such benefit due to its higher susceptibility to natural parasitism.

Conclusions

Treatments with *T. harzian*um species reduced disease incidence in the two areas, with a rate ranging in area 1 from 48.9 % to 82.3 % and in area 2, from 58.5 % to 94 % and the order of application of each product did not interfere with its performance.

The germination of the sclerotia from the field was higher than the germination from the Laboratory ones.

References

Adetunji, C. O., and Varma, A. 2020. Biotechnological application of *Trichoderma*: A powerful fungal isolate with diverse potentials for the attainment of food safety, management of pest and diseases, healthy planet, and sustainable agriculture. *Trichoderma*: Agricultural Applications and Beyond: 257-285.

AGROFIT – Sistema de agrotóxicos fitossanitários 2024. https://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons (accessed February 13, 2024).

Angelique, J. P., Carl, A. B., Martin, I. C., Dean, K. M., Daren, S. M., Kiersten, A. W., and Paul, D. E. 2012. Biology, yield loss and control of *Sclerotinia* stem rot of soybean. Journal of Integrated Pest Management 3(2): B1-B7.

Athmanathan, A. et al. 2015. Impact of temperature, moisture, and storage duration on the chemical composition of switchgrass, corn stover, and sweet sorghum bagasse. BioEnergy Research 8: 843-856.

Bennett, A. J., Bending, G. D., Chandler, D., Hilton, S., and Mills, P. 2012. Meeting the demand for crop production: the challenge of yield decline in crops grown in short rotations. Biological Reviews 87(1): 52-71.

Boland, G. J., and Hall, R. 1994. Index of plant hosts of *Sclerotinia sclerotiorum*. Canadian Journal of Plant Pathology 16(2): 93-108.

Bonato, E. R., and Bonato, A. L. V. 1987. A soja no Brasil: história e estatística.

Bortolotto, O. C., Pomari-Fernandes, A., de Freitas Bueno, R. C. O., de Freitas Bueno, A., da Cruz, Y. K., Sanzovo, A., and Ferreira, R. B. 2015. The use of soybean integrated pest management in Brazil: a review. Agronomy Science and Biotechnology 1(1): 25-25.

Bueno, A. D. F., Panizzi, A. R., Hunt, T. E., Dourado, P. M., Pitta, R. M., and Gonçalves, J. 2021. Challenges for adoption of integrated pest management (IPM): the soybean example. Neotropical Entomology 50: 5-20.

Canuto da Silva, L., de Faria, A. F., Guimarães, R. A., Afridi, M. S., de Medeiros, F. H. V., and de Medeiros, F. C. L. 2024. How can an in vitro incompatibility of *Trichoderma*-based products and herbicides impact the parasitism and control of white mold (*Sclerotinia sclerotiorum* (Lib.) De Bary)? Crop Health 2(1): 1-16.

Cardoso, M. L., Oliveira, H. S. S., Uetanabaro, A. P. T., and Kamida, H. M. 2009. Biodegradação de celulose e lignina por fungos: uma breve revisão. SITIENTIBUS série Ciências Biológicas 9(1): 35-40.

Conte, E. D., Dal Magro, T., Dal Bem, L. C., Dalmina, J. C., Matté, J. A., Schenkel, V. O., and Schwambach, J. 2022. Use of *Trichoderma* spp. in no-tillage system: effect on soil and soybean crop. Biological Control 171: 104941.

da Silva, L. R. et al. 2022. Transcriptional responses and reduction in carpogenic germination of *Sclerotinia sclerotiorum* exposed to volatile organic compounds of *Trichoderma azevedoi*. Biological Control 169: 104897.

de Azevedo Silva, F., de Oliveira Vieira, V., da Silva, R. C., Pinheiro, D. G., and Soares, M. A. 2021. Introduction of *Trichoderma* spp. biocontrol strains against *Sclerotinia sclerotiorum* (Lib.) de Bary change soil microbial community composition in common bean (*Phaseolus vulgaris* L.) cultivation. Biological Control 163: 104755.

de Faria, A. F. et al. 2022. Seven Years of White Mold Biocontrol Product's Performance Efficacy on *Sclerotinia sclerotiorum* Carpogenic Germination in Brazil: A Meta-Analysis.

Dutta, P., Deb, L., and Pandey, A. K. 2022. *Trichoderma* – from lab bench to field application: Looking back over 50 years. Frontiers in Agronomy 4: 932839.

Foley, M. E., Doğramaci, M., West, M., and Underwood, W. R. 2016. Environmental factors for germination of *Sclerotinia sclerotiorum* sclerotia. Journal of Plant Pathology & Microbiology 7: 379-383.

Garg, H., Li, H., Sivasithamparam, K., Kuo, J., and Barbetti, M. J. 2010. The infection processes of *Sclerotinia sclerotiorum* in cotyledon tissue of a resistant and a susceptible genotype of Brassica napus. Annals of Botany 106(6): 897-908.

Guilger-Casagrande, M., Germano-Costa, T., Pasquoto-Stigliani, T., Fraceto, L. F., and Lima, R. D. 2019. Biosynthesis of silver nanoparticles employing *Trichoderma harzianum* with enzymatic stimulation for the control of *Sclerotinia sclerotiorum*. Scientific Reports 9(1): 14351.

Hang, X. et al. 2022. *Trichoderma*-amended biofertilizer stimulates soil resident *Aspergillus* population for joint plant growth promotion. npj Biofilms and Microbiomes 8(1): 57.

Haddad, P. E., Leite, L. G., Lucon, C. M. M., and Harakava, R. 2017. Seleção de estirpes de *Trichoderma* spp. para o controle de *Sclerotinia sclerotiorum* em soja. Pesquisa Agropecuária Brasileira 52(12): 1140-1148.

Huang, H., and Kozub, G. 1991. Temperature requirements for carpogenic germination of sclerotia. Botanical Bulletin of Academia Sinica 32: 279-286.

INMET – Instituto Nacional de Meteorologia 2024. Available from: https://portal.inmet.gov.br/. Accessed February 14, 2024.

Juliatti, F. C., Rezende, A. A., Juliatti, B. C. M., and Morais, T. P. 2019. *Trichoderma* as a biocontrol agent against *Sclerotinia* stem rot or white mold on soybeans in Brazil: usage and technology. *Trichoderma* – The Most Widely Used Fungicide 39.

Kandel, R., Chen, C. Y., Grau, C. R., Dorrance, A. E., Liu, J. Q., Wang, Y., and Wang, D. 2018. Soybean resistance to white mold: evaluation of soybean germplasm under different conditions and validation of QTL. Frontiers in Plant Science 9: 505.

Lehner, M. S., Del Ponte, E. M., Gugino, B. K., Kikkert, J. R., and Pethybridge, S. J. 2017. Sensitivity and efficacy of boscalid, fluazinam, and thiophanate-methyl for white mold control in snap bean in New York. Plant Disease 101(7): 1253-1258.

Macena, A. M., Kobori, N. N., Mascarin, G. M., Vida, J. B., and Hartman, G. L. 2020. Antagonism of *Trichoderma*-based biofungicides against Brazilian and North American isolates of *Sclerotinia sclerotiorum* and growth promotion of soybean. BioControl 65: 235-246.

Meyer, M. C., Campos, H. D., Godoy, C. V., and Utiamada, C. M. 2016. Ensaios cooperativos de controle biológico de mofo-branco na cultura da soja-safras 2012 a 2015. Londrina: Embrapa Soja: 49.

Meyer, M. C. et al. 2021. Eficiência de fungicidas para controle de mofo-branco (*Sclerotinia sclerotiorum*) em soja, na safra 2020/2021: resultados sumarizados dos experimentos cooperativos. Embrapa, Circular Técnica: 173.

Montalvão, S. C. L., Marques, E., Martins, I., da Silva, J. P., and de Mello, S. C. M. 2023 Suppression of the phytopathogens *Sclerotinia sclerotiorum* and *Sclerotium rolfsii* by *Trichoderma* spp. Biologia 78(10): 2941-2952.

Neethu, Kannan et al. 2012. A novel strain of *Trichoderma viride* shows complete lignocellulolytic activities. Advances in Bioscience and Biotechnology 03(08): 1160-1166..

Nieto-Jacobo, Maria F. et al. 2017. Environmental growth conditions of *Trichoderma* spp. affect indole acetic acid derivatives, volatile organic compounds, and plant growth promotion. Frontiers in Plant Science 8: 102.

O'Sullivan, C. A., Belt, K., and Thatcher, L. F. 2021. Tackling control of a cosmopolitan phytopathogen: *Sclerotinia.* Frontiers in Plant Science 12: 707509.

Pethybridge, S. J., Gugino, B. K., and Kikkert, J. R. 2019. Optimizing fungicide timing for the management of white mold in processing snap bean in New York. Crop Protection 125: 104883.

Purdy, L. H. 1979. *Sclerotinia sclerotiorum*: history, diseases and symptomatology, host range, geographic distribution, and impact. Phytopathology 69(8): 875-880.

Rahman, Md. M. E., et al. 2016. Suppressive effects of *Bacillus* spp. on mycelia, apothecia and sclerotia formation of *Sclerotinia sclerotiorum* and potential as biological control of white mold on mustard. Australasian Plant Pathology 45: 103-117.

Rembinski, J., et al. 2022. Using *Trichoderma* to Manage Sclerotia-Producing Phytopathogenic Fungi. *Trichoderma* - Technology and Uses. IntechOpen.

Sharma, P., Jambhulkar, P. P., Raja, M., Sain, S. K., and Javeria, S. 2020. *Trichoderma* spp. in consortium and their rhizospheric interactions. *Trichoderma*: Host Pathogen Interactions and Applications: 267-292.

Silva, L. G., Camargo, R. C., Mascarin, G. M., Nunes, P. S. D. O., Dunlap, C., and Bettiol, W. 2022. Dual functionality of *Trichoderma*: Biocontrol of *Sclerotinia sclerotiorum* and biostimulant of cotton plants. Frontiers in Plant Science 13: 983127.

Singh, S. P., Keswani, C., Singh, S. P., Sansinenea, E., and Hoat, T. X. 2021. *Trichoderma* spp. mediated induction of systemic defense response in brinjal against *Sclerotinia sclerotiorum*. Current Research in Microbial Sciences 2: 100051.

Sumida, Ciro Hideki et al. 2015. Chemical and biological control of *Sclerotinia* stem rot in the soybean crop. Ciência Rural 45(5): 760-766.

Sumida, C. H., Daniel, J. F., Araujo, A. P. C., Peitl, D. C., Abreu, L. M., Dekker, R. F., and Canteri, M. G. 2018. *Trichoderma asperelloides* antagonism to nine *Sclerotinia sclerotiorum* strains and biological control of white mold disease in soybean plants. Biocontrol Science and Technology 28(2): 142-156.

Taylor, A., Coventry, E., Handy, C., West, J. S., Young, C. S., and Clarkson, J. P. 2018. Inoculum potential of *Sclerotinia sclerotiorum* sclerotia depends on isolate and host plant. Plant Pathology 67(6): 1286-1295.

Umadevi, P., Anandaraj, M., Srivastav, V., and Benjamin, S. 2018. *Trichoderma harzianum* MTCC 5179 impacts the population and functional dynamics of microbial community in the rhizosphere of black pepper (*Piper nigrum* L.). Brazilian Journal of Microbiology 49: 463-470.

USDA – United States Department of Agriculture Foreign Agricultural Service 2024. Available from: https://public.govdelivery.com/accounts/USDAFAS/subscriber/new. Last accessed on 15 May 2024.

Wang, Z., et al. 2019. A rhizosphere-derived consortium of *Bacillus subtilis* and *Trichoderma harzianum* suppresses common scab of potato and increases yield. Computational and structural biotechnology journal 17: 645-653.

Willbur, J., Mccaghey, M., Kabbage, M., and Smith, D. L. 2019. An overview of the *Sclerotinia sclerotiorum* pathosystem in soybean: impact, fungal biology, and current management strategies. Tropical Plant Pathology 44: 3-11.

Wu, B. M., and Subbarao, K. V. 2008. Effects of soil temperature, moisture, and burial depths on carpogenic germination of *Sclerotinia sclerotiorum* and *S. minor*. Phytopathology 98(19): 1144-1152.

Young, C. S., and Werner, C. P. 2012. Infection routes for *Sclerotinia sclerotiorum* in apetalous and fully petalled winter oilseed rape. Plant Pathology 61(4): 730-738.

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Advanced multifunctional biopolymer product for delayed ripening, enhanced postharvest disease control, and fruit quality preservation

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Abstract: Food loss and waste represent a critical global challenge across the entire supply chain, with a significant portion of these losses attributed to postharvest pathogens that impact fruit quality and marketability. Extensive research has been dedicated to developing biocontrol agents' (BCA) formulations as sustainable alternatives to synthetic chemical treatments. However, regulatory limitations and difficulties in real-world application have restricted their widespread commercial adoption. Edible coatings (EC) represent another promising complementary strategy that creates a semi-permeable barrier on the fruit surface. This barrier modulates gas exchange and water loss, alters the fruit microenvironment, delays senescence, and may enhance overall postharvest performance. This technology also serves as a carrier for functional additives, such as antioxidants, antimicrobial substances, UV protectants, and others, expanding its potential applications. Furthermore, incorporating BCAs into EC matrices may improve antifungal efficacy while also enhancing microorganism stability during storage. Considering these benefits, this research focused on developing a multifunctional edible biopolymer-based product (MP) that incorporates biocontrol agents in its coatings. The main objective was to develop an edible product that effectively controls fruit diseases, slows down ripening, and maintains overall quality, thereby prolonging shelf life. Pears were selected as fruit model, and MP-coated and uncoated pears were monitored throughout their commercial shelf life. Key quality parameters – including firmness, respiration rate, sensory attributes, and disease incidence – were evaluated. The results demonstrated that MP application effectively slowed ripening and color development, reduced respiration rates, and enhanced fruit firmness retention when compared to uncoated fruits. Sensory analysis further indicated that the acceptance of MP-coated fruits was greater than uncoated fruits. Additionally, the multifunctional product reduced disease incidence, reinforcing its role as an effective postharvest disease management strategy. These findings highlight the potential of MP as an innovative, eco-friendly solution to improve fruit storability while minimizing postharvest losses in the fresh produce sector. This work has been financed by the project PID2023-149464OR-I00 of the Government of Spain and by 'Generalitat de Catalunya' (SGR:2021SGR01477, Programa CERCA).

Key words: biocontrol, food loss reduction, edible coating, respiration, gas exchange, fungal control

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Meyerozyma guilliermondii strain 2H13 – a strain without efficacy can have a great effect

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Abstract: *Meyerozyma guilliermondii* strain 2H13 was isolated in the laboratory of Bio-Protect in Konstanz, Germany, ten years ago during a research project to develop microbial plant protection products against Oomycetes. *Meyerozyma guilliermondii* is a yeast-like Ascomycete, ubiquitous in soil, seawater, the human microflora, and the plant surface (grapes, pome fruit, citrus). It has only one problem – it does not work as biocontrol agent, or at least, it does not work as stand-alone plant protection product. During another project to reduce the application of Copper to control late blight on potatoes (*Phytophthora infestans*), it turned out that Copper can be reduced by half, having the same effect as the full dosage, if 2H13 is added to the spray suspension. The addition of 2H13 to copper fungicides increased the yield of tubers compared to the untreated control and to the copper stand-alone treatment in several field trials. Furthermore, using 2H13 at a rate of 0.05 % in the spray suspension is shown to enhance the effect of other single-site fungicides against *Botrytis cinerea*, *Monilia fructigena*, *Neofabraea alba*. The addition of 2H13 to single-site inhibitors like fludioxonil, fluopyram, isofetamid, or strobilurines reduced the development of multiresistant *Botrytis* spp. strains in strawberry fields. That way the first microbial additive was developed. It is currently registered in Germany and Austria, with further countries to follow.

Key words: *Botrytis* spp., biocontrol

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Microbial biocontrol agents: Opportunities and barriers

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Abstract: *Armillaria* species are fungal plant pathogens found worldwide affecting a range of woody trees and shrubs in gardens, horticulture, and forestry. The root disease is primarily spread through an underground network of rhizomorphs and by root-to-root contact. Chemical controls have been banned or are ineffective, making the only available control options expensive and labour intensive. A study was conducted to determine the potential of native *Trichoderma* spp. endophytes to antagonise *Armillaria mellea* and offer protection to host plants. Dual culture assays showed significant reductions in *A. mellea* colony size. Host-associated isolates of *Trichoderma* spp. were endophytically inoculated into strawberry and privet roots. Much variation in *Armillaria* root rot infection was noted, but several isolates reduced disease severity.

The growing number of pesticide withdrawals raises concerns over effective plant disease control. Biocontrol plays a major role in integrated pest management (IPM) in mitigating the effects of withdrawals. A workshop was held in the UK with stakeholders representing major plant health sectors. The opportunities for increasing use of biocontrol and barriers to uptake were discussed. Although commercial products are available, uptake is low. Significant barriers exist and key recommendations for improved uptake include better knowledge exchange and guidance. Many of the opportunities and barriers are applicable worldwide.

Key words: *Trichoderma*, *Armillaria* root rot, biological control, opportunities, barriers

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Integrated biological-chemical interface for eco-friendly control of maize late wilt and cotton charcoal rot diseases

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Abstract: The *Magnaporthiopsis maydis* late wilt disease (LWD) of corn is a major threat in highly affected Mediterranean areas and an emerging concern in other countries. Likewise, *Macrophomina phaseolina* charcoal rot (CRD) is a destructive global disease of cotton plants. Both pathogens are considered the most severe diseases in maize and cotton rotated in Israeli fields, with limited control strategies. This research aims to develop biological control based on selected *Trichoderma* species combined with low-dosage Azoxystrobin that provides the biopesticides strength and stability. First, selected *Trichoderma* species were proved minorly affected by Azoxystrobin (0.005 mg/l). Then, *T. asperellum* and *T. asperelloides* provided high LWD protection to sprouts in the growth room and to open-enclosure potted plants throughout a full season. At harvest, these species' bio-shielding excels in plant growth enhancement, yield increase, and late wilt shield (up to 29 % health recovery and 94 % pathogen suppression). Azoxystrobin alone or in addition had minor influences. In CRD-stressed growth room cotton sprouts, the *T. longibrachiatum* treatment enhanced plant survival and development and reduced pathogen root infection to near-zero levels. In a full-season potted experiment, the eco-friendly treatments with *T. asperellum* and *T. longibrachiatum* alone enhanced cotton plants' growth and health. At harvest, the combined treatments of *T. longibrachiatum* + Azoxystrobin reached a high efficacy level of 86-91 % pathogen repression. In the commercial field, *Trichoderma* species blend seed treatment was equal to the Azoxystrobin treatment in yield improvement (up to 17 %) and *M. phaseolina* infection reduction (up to 37 %). Bio-chemo integrated management has significant benefits compared to chemical interventions. These results establish the potential of the integrated biological-chemical interface to control severe LWD and CRD cases, reduce chemical use, and the development of fungicide resistance.

Key words: diseases, fungicide, integrated control, pests, *Macrophomina phaseolina*; *Magnaporthiopsis maydis*, Real-time PCR, *Trichoderma*

Introduction

The fungus *Magnaporthiopsis maydis* severely affect sensitive maize plants at the maturity stage. The resulting late wilt disease (LWD) is reported in eight countries and is considered the major threat to maize fields in Egypt, Israel, Spain, Portugal, and India and is an emerging concern in other countries (Degani, 2021). While some prevention methods can restrict disease outbreaks, using resistant maize cultivars remains almost the sole method for minimizing yield losses. Yet, virulent lines of the pathogen have been found that can overcome host resistance (Degani, 2021). Late wilt is often associated with secondary plant pathogenic fungi contamination, enhancing the stem symptoms. Indeed, fungi such as *Fusarium verticillioides*, causing stalk rot, *Macrophomina phaseolina*, causing charcoal rot, and *M. maydis* are grouped in a post-flowering stalk rot complex, which was identified as being one of the most widespread and destructive groups of diseases in maize (Khokhar et al., 2014).

Macrophomina phaseolina, the CRD agent, is a common phytopathogen affecting many species, including maize and cotton. This pathogen is a significant threat to Israel's cotton field, with no effective means to restrain its impact, leading to death at the late stages of growth (Degani et al., 2020). While many control strategies were tested over the years to reduce CRD impact, reaching that goal remains a significant challenge (Degani et al., 2024). Both *M. maydis* and *M. phaseolina* are soil-borne and seed-borne pathogens with similar action modes and visible symptoms (Degani et al., 2020). These pathogens are challenging to control because of their long-lasting soil survival. The fungi penetrate the plants' roots, causing root necrosis and disrupting the sprout's development. The main symptoms, water supply disruption, dehydration, and yield reduction, develop later as plants flower and are enhanced under drought conditions (Degani et al., 2020). The two pathogens, *M. maydis* and *M. phaseolina,* can be found abundantly in Israeli commercial fields that share the two summer crop hosts, cotton, and maize, cultivated alternately in two-year rotations. The current study underscores the environmental benefits of integrated approaches, which reduce chemical usage, stabilize biological treatments, maintain high efficacy even in severe disease scenarios, and mitigate the risk of fungicide resistance in pathogens.

Materials and methods

Fungal source and growth conditions

Magnaporthiopsis maydis (isolate Hm-2, CBS 133165) and *M. phaseolina* (isolate Mp-1) were recovered from diseased maize and cotton plants (respectively), identified through various methods, and maintained on a PDA medium at 28 ± 1 °C for 4-7 days. For liquid mycelium growth, five colony discs were incubated in 150 ml of potato dextrose broth (PDB) for 10 days and shaken at 150 rpm under the same conditions.

Growth room seedling assays

Seeds of LWD or CRD-susceptible cultivars were treated with biological and/or chemical pesticides, with 7-10 repeats (Gordani et al., 2023; Degani et al., 2024). Pots' soil (2-2.5 l) was pre-inoculated by incorporating 8-10 g/pot of infected sterilized wheat or millet seeds. Complimentary soil infections were conducted with three 6-mm diameter colony agar discs/plant at sowing or the beginning of sprouting. Plants were cultivated at 28 ± 2 °C with 80 % relative humidity and a 16-hour photoperiod. At ca. 20 and 40 days post-sowing, seedling development and plant roots real-time qPCR pathogen's DNA tracking were assessed.

Full-growth season pot experiments under field conditions

The experiments (comprising biological and/or chemical pesticide treatments with 6-10 repeats) were conducted at the North R & D's plantation farm (Hula Valley, Upper Galilee, northern Israel) (Degani et al., 2023; Gordani et al., 2023). Each pot (10 l) was inoculated with 150 g/pot of sterilized wheat or millet seeds, followed by complimentary soil infections with sowing and/or peeking as described above. The plant's phenological development and qPCR analysis of fungal DNA within plants were assessed at mid-season and harvest.

Hulda commercial field experiment spring-summer 2022

The kibbutz Hulda field trial (Israel Shephelah region with a CRD infestation history) was performed with the Pima V-70 cotton cultivar. The treatments (with 12 repeats) involved chemical sprinkling in the sowing strip with the seeding: Azoxystrobin (Mirador 250 SC, 250 g/l active ingredient) at 200 and 400 ccs per 0.1 ha. Additionally, there were four biological treatments: *T. longibrachiatum* (T7407) or a mix of T7407, *T. asperellum* (P1), and *Trichoderma* sp. O.Y. T7107 seed dressing and two similar bio-control treatments sprinkled the *Trichoderma* species secreted metabolites in the sowing strip with the seeding. The control group received left untreated. There were 168 plots covering an area of about 2.1 ha.

Hulda commercial field experiment spring-summer 2023
The field experiment repetition was performed using the Goliath V-6 cotton cultivar. Twelve treatments (with eight replications) include chemical (thiram, captan, and metalaxyl-M)) and biological (a mix of T7107, T7407, and P1) seed coating. Also, Azoxystrobin (Mirador 250 SC) was used at 100 ml per 0.1 ha sprinkling in the sowing strip with the seeding. Additionally, two chemical treatments with this fungicide at 200 and 400 ml per 0.1 ha were employed through a drip irrigation line 44 and 78 days from sowing. The total area included 96 plots and was about 1.44 ha.

Results and discussion

The LWD protection results consistently demonstrated the efficacy of the integrated approach, with the combination of P1 and T203 alone or with Azoxystrobin showing remarkable outcomes in plant growth promotion, yield enhancement, and infection suppression (Figure 1). Notably, Azoxystrobin alone provided only minimal protection.

For cotton CRD integrated control, we examined the effectiveness of eight *Trichoderma* isolates against the *M. phaseolina* pathogen. Among these, *T. asperellum* (P1) and *T. longibrachiatum* (T7407) were identified as promising treatments for CRD control in seedlings grown in pots. The T7407 treatment led to significant enhancements in plant wet weight (45 %), height (32 %), phenological development (56 %), survival (34 %), and root infection reduction to near-zero levels after 42 days compared to the control. A full-season open encloser potted trail (Figure 2) revealed that on day 68, the Azoxystrobin sole treatment successfully suppressed the pathogen, followed by the T14707 bio-treatment (93 % and 87 % pathogen inhibition). Combining AS with either T7407 or P1 notably improved their effectiveness (from 31 % and 44 % to 85 %). In a commercial field trial (2022), the highest yield was achieved by treating seeds with a *Trichoderma* species blend (P1, T7407, *Trichoderma* sp. O.Y. 7107 isolate). This bio-shielding outperformed Azoxystrobin treatment in yield improvement and was equal to the conventional management in reducing *M. phaseolina* infection. In a follow-up field study (2023), similar achievements to both methods were reached, increasing the cotton plants' yield (up to 17 %) and health (up to 27 %) and reducing *M. phaseolina* DNA root infection (up to 37 %). Moreover, the integrated control significantly outperformed the chemical one.

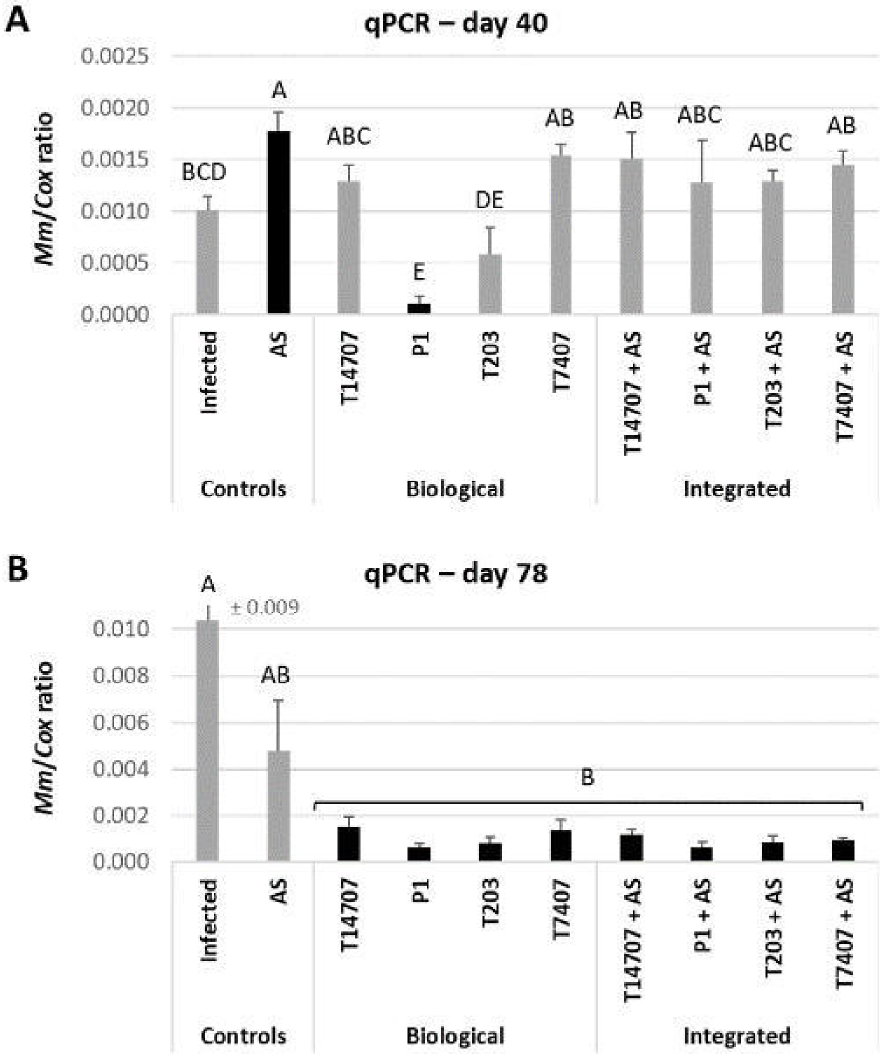

Figure 1. Real-time PCR-based monitoring of the proportionate amount of *M. maydis* DNA inside the plants' roots. This evaluation was made in a semi-field open-enclosure trial at mid-season (**A**, day 40) and harvest (**B**, day 78). The relative of *M. maydis* DNA (*Mm*) amount normalized to the plants' roots cytochrome C oxidase DNA (Cox) is shown in the Y axis. Each value is a mean of 9-10 plants/treatment. Error bars signify a standard error. Different letters (A-E) above the chart's bars represent an ANOVA test significant difference ($p < 0.05$).

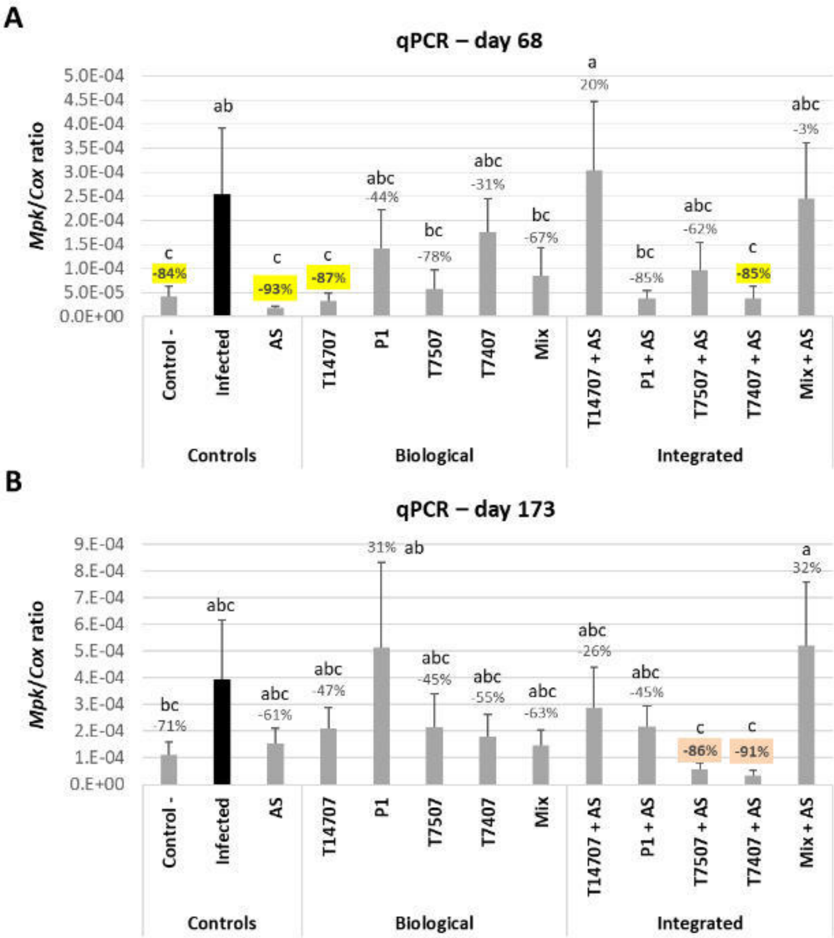

Figure 2. A semi-field trial's qPCR-based monitoring of *M. phaseolina*' DNA inside the plants' roots. This evaluation was made at mid-season (**A**; day 68) and harvest (**B**; day 173). The mock control pots (control -) were kept pathogen-free. Y-axis description as in Figure 1. Values are the mean of six to eight repetitions and Error bars indicate standard error. Different letters (a-c) signify an ANOVA test observable variation ($p < 0.05$).

Over the years, experiments separately tested chemo-agents and bioagents against *M. maydis*. Thus, this work pioneers the exploration of maize LWD integrated control. In cotton CRD, such interface potential has already started to be revealed. For example, in field conditions, plants treated with *Bacillus megaterium* ZMR-4 and Benzothiadiazole exhibited the highest biocontrol efficiency, ranging from 72.8 % to 78.9 % (Adrees et al., 2019). A recent review (Ons et al., 2020) emphasizes the benefits of integrative interface shifting towards greener alternatives to mitigate chemical control strategies' environmental and health risks. It may also help combat multiple soil fungal phytopathogens and is necessary to prevent the development of fungal resistance to fungicides. The bio-friendly seed coating demonstrated here provides a relatively economically feasible solution that can fit any cultivation method and be integrated with various agricultural practices. With some adjustments, the same protocol can include different eco-friendly beneficial bacterial or fungal species and can be combined with varying chemical fungicides.

References

Adrees, H., Haider, M. S., Anjum, T., and Akram, W. 2019. Inducing systemic resistance in cotton plants against charcoal root rot pathogen using indigenous rhizospheric bacterial strains and chemical elicitors. Crop Prot. 115: 75-83.

Degani, O. 2021. A review: late wilt of maize – the pathogen, the disease, current status and future perspective. J. Fungi 7: 989.

Degani, O., Dor, S., Abraham, D., and Cohen, R. 2020. Interactions between *Magnaporthiopsis maydis* and *Macrophomina phaseolina*, the causes of wilt diseases in maize and cotton. Microorganisms 8: 249.

Degani, O., Gordani, A., Dimant, E., Chen, A., and Rabinovitz, O. 2023. The cotton charcoal rot causal agent, *Macrophomina phaseolina*, biological and chemical control. Front. Plant Sci. 14: 1272335.

Degani, O., Chen, A., Dimant, E., Gordani, A., Malul, T., and Rabinovitz, O. 2024. Integrated management of the cotton charcoal rot disease using biological agents and chemical pesticides. J. Fungi 10: 250.

Gordani, A., Hijazi, B., Dimant, E., and Degani, O. 2023. Integrated biological and chemical control against the maize late wilt agent *Magnaporthiopsis maydis*. Soil Syst. 7: 1.

Khokhar, M. K., Hooda, K. S., Sharma, S. S., and Singh, V. 2014. Post flowering stalk rot complex of maize-Present status and future prospects. Maydica 59: 226-242.

Ons, L., Bylemans, D., Thevissen, K., and Cammue, B. P. A. 2020. Combining biocontrol agents with chemical fungicides for integrated plant fungal disease control. Microorganisms 8: 1930.

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Streptomyces spp. biocontrol activities emphasize the bioprospection significance on the development of new products

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Abstract: The *Streptomyces* biotechnological potential has been the subject of huge publication numbers, thus leading interest to be bioprospected as source of different compounds including, pesticides, antibiotics, plant growth promoters, antitumor and others. In this study the biocontrol activity of 15 *Streptomyces* spp. strains isolated from three Brazilian biomes and its transitions were evaluated *in vitro* and at greenhouse experiments. The nematicidal activity against *Meloidogyne incognita* was observed in all strains, which inhibited eggs from hatching. Also, strains HTS2817A and HTS2818A presented biocontrol activities against symptoms caused by *C. gossypii* CML2324 at greenhouse, reducing the incidence of the disease and increasing the shoots dry mass, while 60 % of the isolates increased seed germination and 100 % decreased plants damping-off. Other experiments are still being conduct, like metabolites production to elucidate the biocontrol efficiency and fingerprint genetic profiles to clarify the genetic diversity of strains in study.

Key words: bioprospection, nematicidal activity, fungicide, biocontrol metabolites

Introduction

According to LPSN (Parte et al., 2020), the genus *Streptomyces* have more than 700 species validly published with correct names standing in nomenclature, being the largest genus from the Streptomycetaceae family. In the last decades many publications have reported the wide range of biotechnological potential from *Streptomyces* spp., including activities of agricultural interest, like plant growth promotion and pathogenic microorganisms' biocontrol.

Those characteristics provides advantages for *Streptomyces* members, assisting its adaptation to different ecosystems like soil, rivers, lakes, marine habitats, desert soils and other variable stress conditions (Jensen et al., 2005; Pacios-Michelena et al., 2021), which have encouraged many research groups to explore those characteristics aiming bioprospection of *Streptomyces* genus.

Materials and methods

Soil samples collection and microorganisms' isolation and identification

Seven soil samples (5 kg) were composed by subsamples from same native region of three Brazilian biomes and its transitions, being mostly dry and hot environments. The subsamples were collected at seven different sites from Minas Gerais (MG), Pernambuco (PE), and Bahia

(BA) states (Table 1). Each composite soil sample was sieved and used as substrate to sow *Glycine max* cv. BMX Potencia RR seeds, which were used as trap plants (Zachow et al., 2013). At the flowering stage, plants were harvested and the rhizosphere soil collected for microorganisms' isolation without preheated (Wahyudi et al., 2019) in ACA (Williams and Davies, 1965) and NBRIP (Nautiyal, 1999) media. A total of fifteen strains were isolated, purified and cryopreserved at -20 °C and -80 °C (Balagurunathan et al., 2020) and selected for this study.

Table 1. *Streptomyces* spp. strains used in this study and the biomes from each isolation site.

Strain	Biome	Municipality	State	Site	Isolation medium
HTS0178A	Cerrado/Mata Atlântica	Itaúna	MG	1	ACA
HTS0182A	Cerrado/Mata Atlântica	Itaúna	MG	1	ACA
HTS0188A	Cerrado/Mata Atlântica	Itaúna	MG	1	ACA
HTS0192A	Cerrado/Mata Atlântica	Itaúna	MG	1	ACA
HTS0193A	Cerrado/Mata Atlântica	Itaúna	MG	1	ACA
HTS0200A	Cerrado	Capitólio	MG	2	ACA
HTS0204A	Cerrado	Capitólio	MG	2	ACA
HTS2813A	Mata Atlântica	Juazeiro	BA	3	NBRIP
HTS2815A	Caatinga	Abaré	BA	4	NBRIP
HTS2816A	Caatinga	Abaré	BA	4	NBRIP
HTS2817A	Caatinga	Capoeiras	PE	5	NBRIP
HTS2818A	Caatinga	Juazeiro	BA	3	NBRIP
HTS2819A	Caatinga	Juremal	BA	6	NBRIP
HTS2821A	Caatinga	Uauá	BA	7	NBRIP
HTS2822A	Caatinga	Uauá	BA	7	NBRIP

For strains molecular identification, DNA extraction was conducted using DNeasy UltraClean Microbial Kit following manufacturer instructions, 16S rRNA sequences were amplified using the pair of primers 27f and 1492r (Matsuo et al., 2021) and sequenced by Oxford Nanopore Technologies following manufacturer protocol for Native Barcoding Sequencing Kit (v.14). Sequences were compared with NCBI database for strains identification at a genus level.

In vitro screening: nematicidal activity on Meloidogyne incognita

Nematicidal activity from strains in study was tested *in vitro* on *Meloidogyne incognita* in a completely randomized design experiment. Nematodes were extracted from tomato roots (cv. Santa Cruz) using maceration and centrifugal flotation method as described by Coolen and D'Herde (after Hooper et al., 2005). The nematodes inoculum was adjusted to 1800 ± 18 eggs/ml, with 125 µl used per treatment (approximately 200 eggs) added into 125 µl of *Streptomyces* spp. inoculum grown in potato dextrose broth medium for 10 days at 28 °C and 200 rpm. The experiment was conduct as described by Fan et al. (2020) without stopping hatching process. Statistical analyses were conduct as described below at Data Analysis section.

Biocontrol of Colletotrichum gossypii CML2324 symptoms in cotton seedlings
Anthracnose biocontrol trials in cotton seedlings (*Gossypium hirsutum* cv. IMA 2106 GL) were conducted using *Colletotrichum gossypii* CML2324 strain, obtained from the Lavras Mycological Collection (CML). Cotton seeds were previously disinfested (Brasil, 2009) and then microbiolized with the sporulated *C. gossypii* CML2324 strain cultivated in Petri dishes with potato dextrose agar (PDA) supplemented with 50 g/l of mannitol ($C_6H_{14}O_6$) (Coutinho et al., 2001). Microbiolized seeds were inoculated with *Streptomyces* spp. cultures grown for 10 days at 200 rpm and 28 °C (Ferro et al., 2020). Tests were conducted for 14 days in 50-cell trays with sand and soil (1:1) as substrate and kept in greenhouse with controlled conditions of 30 °C and relative humidity of 85 % between April and October 2023.

Treatments were distributed in a randomized block design (RBD) using absolute negative control (non-microbiolized disinfested seed inoculated with sterile distilled water), chemical control based on carboxin and thiram (Vitavax Thiram 200 SC, Arysta-UPL, Mumbai, India; 800 ml p.c./100 kg of seeds) and treatments with *Streptomyces* isolates inoculum. All treatments were conducted with four replicates of 25 seeds each. The number of germinated seedlings were evaluated at the 7th day after planting, the number of final seedlings and seedlings with incidence were evaluated at harvesting 14 days after sowing (DAS). Plants shoots were collected and oven-dried with forced air circulation at 70 °C for subsequent shoot dry mass (SDM) evaluation. To validate the experiment, all tests were conducted three times.

Data analysis
Statistical analyses were conduct using R Studio software (v.4.4.1, 2024) and “readxl” and “easyanova” packages. Data were submitted by analysis of variance (ANOVA) and Scott-Knott test ($p < 0{,}05$).

Results and discussion

Biological control activities
The fifteen *Streptomyces* strains from this study have shown nematicidal activity *in vitro*, with 100 % inhibiting egg hatching when compared to the negative control, which can be observed on the number of second stage nematodes (Figure 1). Furthermore, 53 % of the strains reduced the number of viable eggs of *M. incognita*. Similar results were observed by Ruanpanun and Chamswarng (2015) with *Streptomyces* spp. isolated from humus and by Azlay et al. (2024) with *S. violascens* on a study for biological control of *M. incognita in vitro*.

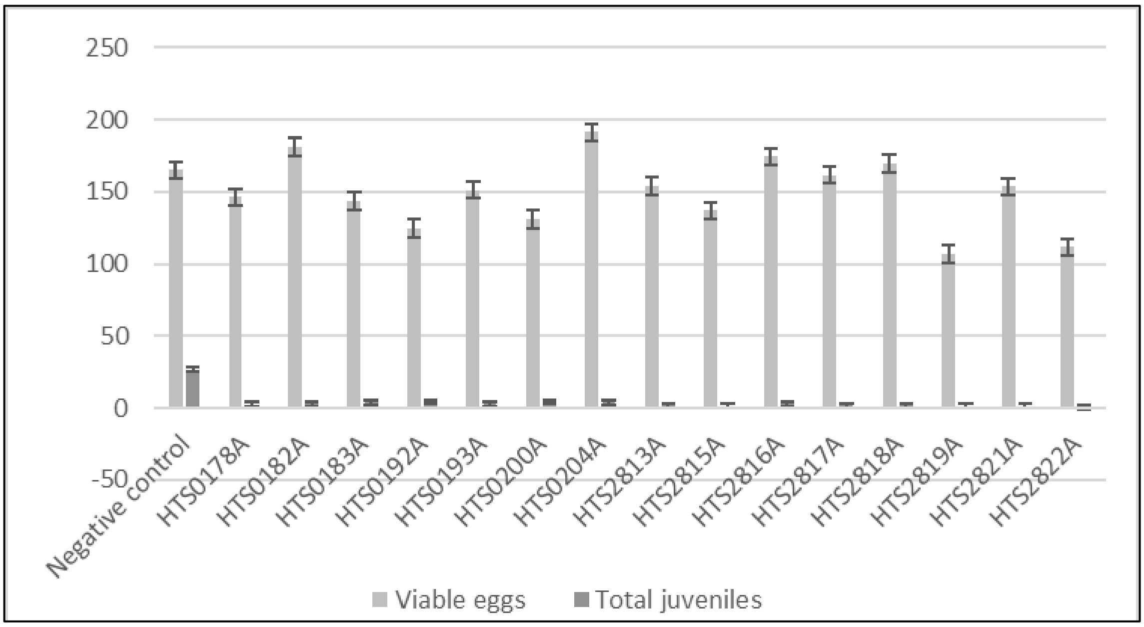

Figure 1. Nematicidal activities of *Streptomyces* strains in study in relation of the number of viable eggs (light gray) and total second stage nematodes of *M. incognita* (dark gray). Means followed by different letters differ from each other by Scott-Knott test ($p < 0.05$).

Streptomyces specific metabolites with strong nematicidal effect have been widely reported, such as actinomycin (Sharma et al., 2019), fervenulin (Ruanpanun et al., 2011) and abamectin (El-Nadgi and Youssef, 2004). Regarding the toxicity of those compounds, they may be environmentally safe and even registered for medicinal use, as is the case of actinomycin (Martens and Demain, 2017) and abamectin in agriculture, with registrations for pesticides (Fent, 2014). Although there was no metabolic compound investigation of the specific nematicidal action, this study reports the potential of many *Streptomyces* spp. strains for the control of *M. incognita*.

Concerning *Streptomyces* strains biocontrol activities against *C. gossypii* CML2324 symptoms at greenhouse, 60 % of the isolates increased seed germination when compared to the pathogen, being statistically similar to the absolute and chemical control. All *Streptomyces* strains decreased plants damping-off and 2 isolates (HTS2817A and HTS2818A) reduced the incidence of the disease and increased the shoots dry mass, also statistically similar to the absolute and chemical control (Table 2).

Table 2. *Streptomyces* strains biocontrol activities against *C. gossypii* CML2324 symptoms at greenhouse experiment. Means followed by different letters differ from each other by Scott-Knott test ($p < 0.05$).

Strain	**Germination**	**Damping off**	**Disease incidence**	**SDM**
CML2324	36 b	81 a	98 a	0.261 b
Absolut control	**68 a**	30 d	74 b	1.398 a
Chemical control	**78 a**	22 d	66 b	1.453 a
HTS0178A	53 b	58 c	89 a	0.661 b
HTS0182A	48 b	68 b	96 a	0.428 b
HTS0188A	**61 a**	50 c	90 a	0.783 b
HTS0192A	**60 a**	49 c	92 a	0.766 b
HTS0193A	**58 a**	51 c	90 a	0.715 b
HTS0200A	55 b	57 c	89 a	0.644 b
HTS0204A	**64 a**	44 c	86 a	1.004 b
HTS2813A	47 b	63 b	93 a	0.551 b
HTS2815A	**58 a**	56 c	88 a	0.714 b
HTS2816A	53 b	51 c	82 a	0.837 b
HTS2817A	**73 a**	**31 d**	**71 b**	**1.367 a**
HTS2818A	**65 a**	**35 d**	**72 b**	**1.361 a**
HTS2819A	**60 a**	46 c	86 a	0.916 b
HTS2821A	45 b	62 b	92 a	0.651 b
HTS2822A	**59 a**	56 c	91 a	0.647 b
CV (%)	15.57	17.31	8.29	25.75

Final considerations

These partial results indicate the potential of *Streptomyces* spp. as biocontrol agents. Both, vegetative cells and/or their metabolites can be used to produce biological pesticides against root-knot nematodes (*M. incognita*) infestations and symptoms caused by phytopathogens such as *C. gossypii*. Consequently, in addition to disease control, strains are able of reducing harmful effects on crops. Other experiments are still being conducted to provide better insights about *Streptomyces* strains in study, such like the ability to produce metabolites like ammonia and hydrogen cyanide (HCN). Also, a genomic profile will be build aiming to reach the genetic diversity of the Brazilian *Streptomyces* strains.

References

Azlay, L., Oubassou, W. Z., Berr, A., Mayad, E. H., and Barakate, M. 2024. In vitro assessment of the nematicidal potential of *Streptomyces violascens* strain AS2 against root-knot nematodes (*Meloidogyne* sp.). Arch. Biol. Sci. 76(3): 345-358.

Balagurunathan, R., Radhakrishnan, M., Shanmugasundararam, T., Gopikrishnan, V., and Jerrine, J. 2020. Protocols in Actinobacteria Research, Springer Protocols Handbooks.

Brasil 2009. Manual de Análise Sanitária de Sementes. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária – Brasília: MAPA/ACS.

Coutinho, W. M., Machado, J. C., Vieira, M. G. G. C., Guimarães, R. M., and Ferreira, D. F. 2001. Uso da restrição hídrica na inibição ou retardamento da germinação de sementes de arroz ou feijão submetidas ao teste de sanidade em meio ágar-água. Revista Brasileira de Sementes 23(2): 127-135.

El-Nagdi, W. M. A., and Youssef, M. M. A. 2004. Soaking faba bean seed in some bio-agents as prophylactic treatment for controlling *Meloidogyne incognita* root-knot nematode infection. Journal of Pest Science 77: 75-78.

Fan, H., Yao, M., Wang, H., Zhao, D., Zhu, X., Wang, Y., Liu, X., Duan, Y., and Chen, L. 2020. Isolation and effect of *Trichoderma citrinoviride* Snef1910 for the biological control of root-knot nematode, *Meloidogyne incognita*. BMC Microbiology 20: 299.

Fent, G. M. 2014 Avermectin, In: Wexler, P. (ed.): Encyclopedia of Toxicology, 3rd ed., pp. 342-344.

Ferro, H. M., Souza, R. M., Lelis, F. M. V., Silva, J. C. P., and Medeiros, F. H. V. 2020. Bacteria for cotton plant protection: disease control, crop yield and fiber quality. Revista Caatinga, Mossoró 33(1): 43-53.

Hooper, D. J., Hallmann, J. and Subbotin, S. A. 2005. Methods for extraction, processing and detection of plant and soil nematodes. In: Luc, M., Sikora, R. A. and Bridge, J. (eds.): Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. 2nd ed., pp. 53-86.

Jensen, P. R., Mincer, T. J., Williams, P. G., and William, F. 2005. Marine actinomycete diversity and natural product discovery. Antonie van Leeuwenhoek 87: 43-48.

Martens, E. and Demain, A. L. 2017. An overview of the industrial aspects of antibiotic discovery. In: Kurtboke, I. (ed.): Microbial Resources, pp. 149-168.

Matsuo, Y., Komiya, S., Yasumizu, Y., Yasuoka, Y., Mizushima, K., Takagi, T., et al. 2021. Full-Length 16S rRNA Gene Amplicon Analysis of Human Gut Microbiota Using MinION TM Nanopore Sequencing Confers Species-Level Resolution. BMC Microbiol. 21: 35.

Nautiyal, C. S. 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiology Letters 170(1): 265-270.

Pacios-Michelena, S., Aguilar, G. C. N., Alvarez-Perez, O. B., Rodriguez-Herrera, R., Chávez-González M., Arredondo, V. R., Ascacio V. J. A., Govea, S. M., and Ilyina, A. 2021. Application of *Streptomyces* Antimicrobial Compounds for the Control of Phytopathogens. Front. Sustain. Food Syst. 5. doi: 10.3389/fsufs.2021.696518.

Parte, A. C., Sardà Carbasse, J., Meier-Kolthoff, J. P., Reimer, L. C., and Göker, M. 2020. List of Prokaryotic names with Standing in Nomenclature (LPSN) moves to the DSMZ. International Journal of Systematic and Evolutionary Microbiology. 70: 5607-5612.

Ruanpanun, P., and Chamswarng, C. 2015. Potential of actinomycetes isolated from earthworm castings in controlling root-knot nematode *Meloidogyne incognita*. Journal of General Plant Pathology 82: 43-50.

Ruanpanun, P., Laatsch, H., Tangchitsomkid, N., and Lumyong, S. 2011. Nematicidal activity of fervenulin isolated from a nematicidal actinomycete, *Streptomyces* sp. CMU-MH021, on *Meloidogyne incognita*. World Journal of Microbiology and Biotechnology 27: 1373-1380.

Sharma, M., Jasrotia, S., Ohri, P., and Manhas, R. K. 2019. Nematicidal potential of *Streptomyces antibioticus* strain M7 against *Meloidogyne incognita*. AMB Express 9: 168.
Wahyudi, A. T., Priyanto, J. A., Afrista, R., Kurniati, D., Astuti, R. I. A., and Akhdiya, A. 2019. Plant growth promoting activity of Actinomycetes isolated from soybean rhizosphere. Online Journal of Biological Sciences 19(1): 1-8.
Williams, S. T., and Davies, F. L. 1965. Use of antibiotics for selective isolation and enumeration of Actinomycetes in soil. Journal of General Microbiology 38: 251-261.
Zachow, C., Muller, H., Tilcher, R., Donat, C., and Berg, G. 2013. Catch the best: novel screening strategy to select stress protecting agents for crops plants. Agronomy 3: 794-815.

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Efficacy and evolution of *Bacillus velezensis* BV02-based biofungicide in integrated coffee disease management

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Abstract: Coffee is one of the most important export crops in Brazilian agribusiness. The phytopatogen *Hemileia vastatrix* is the main disease responsible for significant economic damage and occurs in all coffee-producing regions. Among the management measures, biological control agents such as *Bacillus* sp. have a number of mechanisms of action with effect against phytopathogens. We evaluate the microbiological fungicide application Bio-Imune® (*Bacillus velezensis* BV02) in the integrated management of foliar diseases in coffee crops in two different fields in 2022 season. Our objective was to compare its performance with a standard commercial application program containing fluazinam, azoxystrobin + cyproconazole and copper hydroxide, as well as to document its impact on coffee crop productivity. The efficiency of the treatments in reducing Area Under the Disease Progress Curve of cercosporiosis severity reached 81 %. Likewise, the efficiency of these treatments to rust disease is up to 89 %. In addition, all treatments increased the yield of the coffee crop, with the best results observed in treatments 7 and 8, wich recieved only Bio-Imune® in the final spray. Therefore, the use of Bio-Imune® for the management of coffee foliar diseases can be a more sustainable alternative within the practices of integrated management of diseases in coffee.

Key words: *Hemilea vastatrix*, Bio-Imune, *Cercospora coffeicola*

Introduction

Coffee (*Coffea arabica* L.) is a widely spread crop in Brazil with great socioeconomic importance, since it is the world's largest producer, exporter and has the second largest consumer per capita. Currently, there are 2.25 million hectares in Brazil occupied with coffee plantations, with a production in the 2024 harvest of 58,08 million processed bags (EMBRAPA, 2024). As it is an agricultural crop, coffee is subject to problems caused by the climate, the lack of nutrients in the soil and the attack of phytopathogens, such as the fungi that cause rust and cercosporiosis, for example (Waller et al., 2007).

In Brazil, coffee leaf rust (*Hemileia vastatrix*) is one of the main diseases of the coffee crops, being responsible for causing great economic damage and occurring in all regions where the crop is produced (Pereira et al., 2019). Cercosporiosis or brown eye spot (*Cercospora coffeicola*), cause damage to the nursery plants in early stage of growth and later, on cherries, reaching losses of 50 % in plants that did not receive adequate fertilization and phytosanitary management in the field (Sera et al., 2022).

As an aggravating factor, the control of these phytopathogens is limited, requiring the joint use of different management techniques, from cultural to chemical control (Caixeta et al., 2024). One of the agents explored in biological control is the genus *Bacillus*, which demonstrates satisfactory results in the control of numerous pathogens in different agricultural crops, including coffee (Thanh et al., 2023).

Thus, we evaluated the efficiency of Bio-Imune® (*Bacillus velezensis* BV02) in integrated management with chemical fungicides for controlling foliar diseases in coffee across two fields.

Materials and methods

Data setting and treatments

The trials during the 2022 season were conducted in two different cities in the state of Minas Gerais, Brazil: Indianópolis and Nepomuceno. The experimental design was in randomized blocks, with four replications and each plot of one row of 7 m in length and an area 26.6 m^2. Eight treatments and four replications were evaluated at four different application times (Table 1). The CO_2 pressurized equipment was used for the applications, with a spray volume of 400 l/ha.

Table 1. Treatments and time of application of Bio-Imune® and chemical products.

Nº	October/November	December	Junuary	March
T1	CONTROL	CONTROL	CONTROL	CONTROL
T2	Fluazinam (0,75 l)	Copper hydroxide	Azox + Cypro[1]	Copper hydroxide
T3	Bio-Imune® (1 l) + Azox + Cypro[1]	Copper hydroxide	Bio-Imune® (1 l) + Azox + Cypro[1]	Copper hydroxide
T4	Bio-Imune® (1 l) + Azox + Cypro[1]	Copper hydroxide	Bio-Imune® (1 l) + Azox + Cypro[1]	Bio-Imune® (1 l) + Copper hydroxide
T5	Bio-Imune® (1 l) + Azox + Cypro[1]	Bio-Imune® (1 l) + Copper hydroxide	Bio-Imune® (1 l) + Azox + Cypro[1]	Bio-Imune® (1 l) + Copper hydroxide
T6	Bio-Imune® (1 l) + Azox + Cypro[1]	Copper hydroxide	Bio-Imune® (1 l) + Azox + Cypro[1]	Bio-Imune® (1 l) + Copper hydroxide
T7	Bio-Imune® (1 l) + Azox + Cypro[1]	Copper hydroxide	Bio-Imune® (1 l) + Azox + Cypro[1]	Bio-Imune® (2 l)
T8	Bio-Imune® (1,0 l) + Azox + Cypro[1]	Bio-Imune® (1 l) + Copper hydroxide	Bio-Imune® (1 l) + Azox + Cypro[1]	Bio-Imune® (2 l)

[1]Azox + Cypro: commercial fungicide based on azoxystrobin and cyproconazole, registered for culture use. Dose of 0,75 l/ha. [2] Copper hydroxide: commercial fungicide based on copper hydroxide, registered for culture use. Dose of 1,5 l/ha.

Severity of foliar diseases

The plants were monitored before the first disease symptoms. A total of seven severity assessments of *Cercospora coffeicola* and *Hemileia vastatrix*, was evaluated. Disease severity data were transformed into the area under the disease severity curve according to (Shaner and Finney, 1977). The values presented in the control efficiency are according to the equation proposed by (Abbott, 1925).

Yield

The yield was estimated in each experimental plots, in two central plants and later transformed into bags of processed coffee per hectare, with the equation: Productivity = [(kg/plant × 0.2) × number of plants/ha] / 60 kg, considering that 10 kg of cherry coffee is equivalent to 2 kg of processed coffee, meaning a weight yield of 20 % (Carvalho et al., 2006).

Statistical analysis

The analyzes were carried out according to Tukey test, with 5 % of significance.

Results and discussion

Severity of foliar diseases

In assessing the severity of cercosporiosis and coffee rust diseases, all treatments reduced the severity of both diseases. Regarding control efficiency, the most effective treatments for rust diseases were 5, 6, 7 and 8, with rates of 78 % to 89 %. Regarding cercosporiosis incidence, treatments 6, 7 and 8 showed an efficiency of 43 % to 81 % (Table 2).

Table 2. Area under the disease progress curve (AUDPC) for *Hemilea vastratrix* severity and AUPDC for *Cercospora coffeicola* incidence along with their respective control efficacy in Nepomuceno (MG) and Indianópolis (MG) fields, 2022.

Treatments	AAUPD - *Hemilea vastatrix*						AAUPD - *Cercospora coffeicola*					
	Nepomucemo			Indianópolis			Nepomucemo			Indianópolis		
	m[1]		E (%)	m[1]		E (%)	m[1]		E (%)	m[1]		E (%)
1	356.0	d	--	651.0	a	-	2707.5	c	--	798.0	a	-
2	140.0	c	60.68	115.5	b	82.3	1867.5	b	31.0	154.9	b	**80.6**
3	96.3	b	72.96	129.5	b	80.1	1717.5	b	36.6	207.4	b	74.0
4	102.5	b	71.21	128.6	b	80.2	1710.0	b	36.8	207.4	b	74.0
5	77.3	a	**78.28**	74.4	b	**88.6**	1740.0	b	35.7	151.4	b	**81.0**
6	74.1	a	**79.19**	133.0	b	79.6	1492.5	a	**44.9**	172.4	b	**78.4**
7	68.0	a	**80.89**	106.8	b	83.6	1447.5	a	**46.5**	161.0	b	**79.8**
8	57.6	a	**83.83**	96.3	b	**85.2**	1545.0	a	**42.9**	166.3	b	**79.2**
CV(%)	14.34			28.54			13.64			29.68		

[1]Means with the same letter in each column are not different (Tukey test, α = 0.05).

Yield

For yield, in Nepomuceno (MG), there wasn't a statistical difference between the treatments. Besides, treatments 7 and 8 increased yield by 6.5 and 5.3 bags of processed coffee per hectare compared to the control treatment (1). In Indianópolis (MG), all the treatments, excepted the treatment 2, increased the number of bags to the untreated control. The treatments most productive were 6, 7 and 8, which increased by 11.1, 9.2 and 10.6 bags per hectare, respectively (Table 3).

Table 3. Coffee crop yield and increments (I) in bags of processed coffee per hectare in Nepomuceno (MG) and Indianópolis (MG) fields, 2022.

Yield (Bags of processed coffee per hectare)						
Treatments	**Nepomucemo**			**Indianópolis**		
	m[1]		**I**	**m[1]**		**I**
1	28.1	a	--	31.6	a	-
2	30.1	a	2.0	33.5	a	1.8
3	29.6	a	1.4	36.9	b	5.3
4	28.6	a	0.5	40.4	b	8.8
5	29.2	a	1.1	37.1	b	5.4
6	29.8	a	1.7	42.7	b	**11.1**
7	34.6	a	**6.5**	40.9	b	**9.2**
8	33.5	a	**5.3**	42.2	b	**10.6**
CV(%)	27.52			13.26		

[1]Means with the same letter in each column are not different (Tukey test, $\alpha = 0.05$).

All treatments with biological management control showed efficacy in controlling rust and cercosporiosis in both fields. Treatments 7 and 8 showed the greatest increase compared to the other treatments in both fields. They received Bio-Imune® in the first and third sprays, associated with chemical control, and were the only treatments that received Bio-Imune® (2 l) alone in the last spray (4th). The capacity of competition and the production of many toxic molecules with effect against phytopathogens is already described for *B. velezensis* (Thanh et al., 2023). We could demonstrate that Bio-Imune® (*B. velezensis* BV02) has a high potential to control rust and cercosporiosis and enhance harvest yield of coffee trees.

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References

Caixeta, E. T., Franzin, M. L., Zambolim, L., Venzon, M., Carvalho, C. H. S. D., Oliveira, A. C. B. D., and Resende, M. D. V. D. 2024. Manejo integrado de pragas e doenças do café arábica.

Carvalho, C. H. M., Colombo, A., Scalco, M. S., and Morais, A. R. 2006. Evolução do crescimento do cafeeiro (*Coffea arabica* L.) irrigado e não irrigado em duas densidades de plantio. Ciência e Agrotecnologia 30(2): 243 -250.

Empresa Brasileira De Pesquisa Agropecuária – EMBRAPA 2024. Safra dos cafés do Brasil foi estimada em 58,08 milhões de sacas para este ano de 2024. URL https://www.embrapa.br/busca-de-noticias/-/noticia/86520314/safra-dos-cafes-do-brasil-foi-estimada-em-5808-milhoes-de-sacas-para-este-ano-de-2024?p_auth=9cF0CRlt. Accessed 26 Feb. 2024.

Pereira, I. S., Rosalino, R. C., Neves, F. de O. B. C., Pereira, M. T., and Braga, J. G. de S. 2019. Diferentes fungicidas no controle da ferrugem do cafeeiro. Revista Inova Ciência & Tecnologia 5(2): 25-29.

Sera, G. H., de Carvalho, C. H. S., de Rezende Abrahão, J. C., Pozza, E. A., Matiello, J. B., de Almeida, S. R., ... and dos Santos Botelho, D. M. 2022. Coffee leaf rust in Brazil: Historical events, current situation, and control measures. Agronomy 12(2): 496.

Thanh Tam, L. T., Jähne, J., Luong, P. T., Phuong Thao, L. T., Nhat, L. M., Blumenscheit, C., ... and Borriss, R. 2023. Two plant-associated *Bacillus velezensis* strains selected after genome analysis, metabolite profiling, and with proved biocontrol potential, were enhancing harvest yield of coffee and black pepper in large field trials. Frontiers in plant science 14: 1194887.

Waller, J. M., Bigger, M., and Hillocks, R. J. 2007. Coffee Pests, Diseases and Their Management. CABI Publishing, Wallingford.

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How can the use of biocontrol agents be improved to manage gray mold in vineyards?

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Abstract: Biological control agents (BCAs) offer a promising alternative to synthetic fungicides, ensuring minimal risks for both the environment and human health. Although several studies have explored the use of BCAs to control gray mold in vineyards (caused by *Botrytis cinerea*), there is still a need to optimize their application under field conditions. This work presents an evidence-based strategy, drawing from the results obtained from both laboratory and field experiments, to enhance gray mold control using BCAs. The proposed strategy includes a preliminary context analysis and a consequent reasoned selection of the most suitable BCA. The context analysis involves three key steps: Step 1 – assessing the risk of *B. cinerea* infection and determining whether disease control interventions are needed; Step 2 – considering the pathogen's infection pathway and the plant substrates to be targeted at the time of BCA application; and Step 3 – considering the weather conditions at the time of application and in the following days, as these can influence the survival, growth, and efficacy of BCAs. The approach outlined in this work emphasizes the importance of a reasoned, context-driven selection of BCAs for the control of *B. cinerea* in vineyards.

Key words: biocontrol, disease management, antagonist, integrated control

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The effect of bacterial volatile organic compounds with light-emitting diode lighting on antioxidant capacity in stored broccoli

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Abstract: Bacterial volatile organic compounds have been found to affect the shelf life of fruits and vegetables. This study used BVOCs released by *Lysinibacillus fusiformis* composite with LED white light irradiation treatment (LED-BVOCs treatment), enhanced the synthesis and accumulation of antioxidants during post-harvest storage and delayed senescence in broccoli. Our results show that LED-BVOCs treatment enhances CAT, APX, SOD, and PPO activity and the expression of *BoPOD*, *BoCAT* and *BoAPX* genes in broccoli florets during its storage. Meanwhile, although the level of ROS increased, H_2O_2 and O_2^- levels were lower in LED-BVOCs treatment compared with CK group. According the transcriptomic and metabolic analyses between LED-BVOCs treatment and CK group on the 4 days of storage, 31 differentially expressed genes (DEGs) and 7 differentially abundant metabolites (DAMs) associated with the ascorbic acid-glutathione cycle and polyphenol metabolism were identified in the 4 day LED-BVOCs treatment combination CK group. Specifically, 8 DEGs associated with GSH metabolism, 3 DEGs and 1 DAM associated with ascorbate and aldarate metabolism, 20 DEGs and 6 DAMs associated with phenylalanine metabolism and phenylpropanoid biosynthesis were identified in broccoli florets collected from the day 4 of storage. The ascorbic acid-glutathione cycle and polyphenol are involved in the antioxidation process of plants. These results provide new insights on the potential of BVOCs as biopreservatives to enhance antioxidant capacity and delay senescence in broccoli during storage.

Key words: bacterial volatile organic compounds (BVOCs), antioxidant, broccoli

Chitosan/mandarin essential oil-based films on citrus fruits to prevent the occurrence of grey and blue mould in post-harvest and to control the medfly attack

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Abstract: Citrus fruits, widely consumed around the world, can be negatively affected by pests and fungal infections during their cultivation, handling, transportation, and storage, thus resulting in substantial yield losses and food waste. The use of natural preservatives like chitosan (CHT) and essential oils (EOs) is a promising approach for reducing chemical inputs to preserve food products. This study investigated the effects of CHT, extracted from crab shells and the fungus *Pleorotus ostreatus*, alone and in combination with mandarin (*Citrus* × *reticulata* Blanco, Rutaceae) essential oil (MEO), as growth inhibitor of *Penicillium* spp. (*P. expansum*, *P. digitatum,* and *P. italicum*) spp. fungi, the causal agents of apple and citrus rot and as an oviposition deterrent towards *Ceratitis capitata* (Diptera: Tephritidae), the Mediterranean fruit fly. A solution of 1.0 % CHT of both origins (from crab shells and *P. ostreatus*) added with 1 % MEO resulted as the best combination to significantly reduce mycelial growth and spore germination of *Penicillium* isolates and their pathogenic activity on *Citrus japonica* Thunb. (kumquats) fruits as well as the oviposition percentage of *C. capitata* on the same fruit. According to results here collected, CHT added with MEO represents a valid combination to be used as an edible film and coating as part of an integrated control strategy to improve the shelf-life of fresh citrus fruits. Furthermore, fungal CHT, here used for the first time in combination with MEO, can be an excellent alternative to reply to the eating habits and necessities of the final consumers.

Key words: chitosan, essential oil, kumquat, antifungal activity, oviposition deterrent

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References

Parichanon, P., Farina, P., Vicente, I., Cesarini, M., Hotaj, E., Sarrocco, S., Pellegrini, E., and Conti, B. 2024. Chitosan/mandarin essential oil-based films on citrus fruits for the control of the medfly attack and to prevent the occurrence of grey and blue mould in post-harvest. J. Stored Prod. Res. 108: 102380. https://doi.org/10.1016/j.jspr.2024.102380.

Use of Vacciplant® in the control of grey mold (*Botrytis cinerea*) and its performance in strawberry shelf life

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Abstract: The appropriate use of fungicides is essential to avoid residues on fruits and pathogen resistance. In the search for more sustainable crop management methods that can improve post-harvest fruit quality, the use of biological products becomes a promising alternative. In this context, the objectives of this study were to evaluate the influence of the Vacciplant® product on the productivity, number of flowers, *B. cinerea* control, shelf life and soluble solids content (°BRIX) of strawberries. The experiment was carried out in the field, in the city of Congonhal, state of Minas Gerais, Brazil, at an altitude of 1,250 meters, an average temperature of 22 °C and humidity of 75 %. The experimental design was in randomized blocks, with 8 treatments and 2 weekly applications, for 28 days, of laminarin (Vacciplant®) combined or not with pyrimethanil (Mythos®) and boscalid (Canthus®) in doses of 2 l/ha, 1 l/ha and 0.80 kg/ha, respectively, using a CO_2 pressurized sprayer coupled to a PET bottle. The productivity and incidence of grey mold were evaluated by measuring the weight of healthy and diseased strawberries, harvested at 7, 14, 21 and 28 days after applications (DAA); the number of flowers was assessed at 30 DAA; and the shelf life of fruits from the last harvest was evaluated at the Laboratory of Biocontrol of Plant Diseases, Department of Plant Pathology, at the Federal University of Lavras. Alternating applications of Vacciplant® and Mythos® resulted in higher strawberry productivity; the Mythos®/Cantus®/Vacciplant® treatments and 4 applications of Vacciplant®, respectively, presented the highest number of flowers. Alternating applications of Vacciplant®/Mythos® and Vacciplant®/Canthus® provided longer fruit shelf life. However, Vacciplant® used in 4 or 8 applications increased the soluble solids content, but did not guarantee greater fruit health compared to its combination with chemical fungicides. Therefore, the Vacciplant® product has the potential to be incorporated into a strawberry grey mold management program, as it has proven to be effective in increasing the number of flowers, °BRIX and providing greater post-harvest fruit conservation.

Key words: *Fragaria x ananassa,* biological control, laminarin, plant disease management

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Next-generation antimicrobials: biological synthesis of AgNPs coated with beneficial bacterial metabolites as biocontrol agents

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Abstract: The development of nanotechnology has led to the production of silver nanoparticles (AgNPs) with unique properties due to their small size (< 100 nm) that make them a good candidate for use in agriculture as fertilizers, bactericides and fungicides (Mgadi et al., 2024). Owing to their small size and large surface area, AgNPs have high antibacterial activity and non-drug resistance (Huq et al., 2022). On the other hand, among sustainable agriculture practices is the use of beneficial bacteria (PGPB) to activate plant immunity or as biocontrol elements. The development of a conjugative approach of AgNPs with plant growth promoting bacteria (PGPB) offers enormous potential to improve both plant yield and disease resistance. Accordingly, we reasoned that beneficial bacteria would be an interesting candidate for the synthesis of NPs with unique properties for their application for pathogens control.

Hence, the present work is devoted to the biosynthesis of AgNPs under the rationale that metabolites produced by the PGPB strain *Pseudomonas* Z9.3 are able to reduce Ag, forming AgNP coated with bacterial metabolites. Optimization of physicochemical parameters (temperature, pH, and $AgNO_3$ concentration) for the synthesis of AgNP was carried out. Biosynthesized AgNPs had a spherical shape with an average particle size ranging from 8.24 ± 0.26 to 13.32 ± 0.4 nm, covered with a unique organic matter corona of bacterial metabolites. Antimicrobial activity was tested on bacterial and fungal phytopathogenic strains. Synthesized AgNPs showed antibacterial activity against both types of Gram-positive and Gram-negative bacteria, and phytopathogenic bacteria and fungi. In the case of human bacterial pathogens, antibacterial effect was strain-dependent and dose-dependent. The Gram-positive *S. epidermidis* was the most sensitive strain, showing growth inhibition in the range of 7 to 44 % for S4/pH7 AgNPs, and from 58 to 94 % for S1/pH9 AgNPs and, among the Gram-negative, *Salmonella* sp. with 40 % average growth inhibition The order of the highest antibacterial activity among plant pathogenic bacteria is *X. campestris* pv oryzae > *X. campestris* pv tomato > *P. syringae* DC3000, demonstrating an improved inhibition effect from 22 to 69 % depending on the concentration and type of NPs. The results of antifungal activity showed that *Alternaria* and *Stemphylium* sp. were the most sensitive (74.97 % and 66.3 %, respectively), while *Fusarium* and *Rhizopus* sp. were less sensitive (45.62 % and 32.68 %, respectively). Based on the obtained results of antimicrobial activity, the potential use of these AgNPs as a means of control against human and plant pathogens is closer to field application within sustainable agricultural practices.

Key words: PGPB, AgNP, biological synthesis, antibacterial and antifungal activity

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References

Huq, M. A., Ashrafudoulla, M., Rahman, M. M., Balusamy, S. R., and Akter, S. 2022. Green synthesis and potential antibacterial applications of bioactive silver nanoparticles: a review. Polymers 14(4):742. doi:10.3390/polym14040742

Mgadi, K., Ndaba, B., Roopnarain, A., Rama, H., and Adeleke, R. 2024. Nanoparticle applications in agriculture: overview and response of plant-associated microorganisms. Front Microbial. 15: 1354440. doi:10.3389/fmicb.2024.1354440

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Upscaling efficient alternatives for contentious inputs in organic farming – the recently funded EU-project "SCALE-it"

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Abstract: Contentious inputs in plant protection such as copper, mineral oil or Spinosad, are still widely used in organic farming due to limitations of alternatives in terms of (i) availability (ii) costs (iii) information on methodology of use, efficacy and benefits to the environment and (iv) acceptance by farmers and consumers. On this background, tools, strategies and techniques are applied in the recently funded EU-project SCALE-it, which starts in May 2025 and runs over four years. The key objective of SCALE-it is to increase the availability, accessibility and adoption by farmers of cost-effective alternatives and thus to support the objective of the EU Farm to Fork strategy through the development, testing and promoting of alternative solutions to contentious inputs in organic production. In SCALE-it contentious inputs for plant and animal production are addressed, including plant protection products, fertilisers/manures from problematic sources, antibiotics, anthelmintics, and vitamins from synthetic or GM origin.

Uptake of large-scale production and use of alternatives is currently hampered by (i) slow, complex and expensive registration procedures, (ii) lack of confidence by users and investors, (iii) the expectation of 1:1 replacement of contentious inputs, and (iv) partial lack of information on the mechanisms of action and side effects on the environment. SCALE-it addresses all aspects.

Core of the project is the upscaling of alternatives and the demonstration of strategies that reduce/replace the respective contentious inputs in around 70 on farm demonstration trials across Europe. Wherever possible, system approaches, including e. g., variety selection, agronomic techniques, improved application techniques and alternative products are applied. SCALE-it builds on collaboration with "local ambassadors", i. e., renowned regional advisory professionals with profound experience in their disciplines providing direct access to farmer groups.

With respect to crop protection and reduction of copper, mineral oil and Spinosad, 29 demonstration trials, each running for at least two years, will take place. Field crops addressed in the project are grapevine, table grape, apple, olive, citrus, potato, carrot, tomato and in addition basil, cucumber and tomato under protected cultivation. The alternatives in SCALE-it are based on solutions, developed in former projects, such as the H2020 projects Organic-PLUS and RELACS. Plant extracts and other natural substances, mating disruption by vibrational devices and mechanical devices for beetle collection will be tested here. These alternatives have already reached high technology readiness levels (TRL) and will be further developed in the project.

Key words: copper, mineral oil, Spinosad, pests, diseases