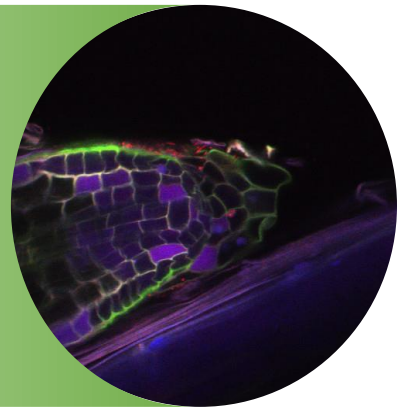




ABSTRACTS BOOKLET



PGPR
workshop

12th PGPR Workshop

Toulouse, France

May 29th – June 2nd, 2023

Presentation

The International PGPR Workshop is the most comprehensive conference on the field of PGPR/PGPB (Plant Growth Promoting Rhizobacteria/Bacteria), with a long tradition that started in Canada in 1987. Subsequently, the Workshop has been held in Switzerland (1990), Australia (1994), Japan (1997), Argentina (2000), India (2003), The Netherlands (2006), Oregon (2009), Colombia (2012), Belgium (2015) and Canada (2018). Unfortunately, the Workshop was not held in 2021 due to COVID-19; thus, the relaunch of the periodic meeting is a good opportunity to gather leading scientists and industry in an environment of scholarly sessions and cooperation opportunities.

This 12th International PGPR Workshop has been planned on the tradition of the previous meetings, where we expect to have lead researchers, students, and commercial companies presenting and discussing issues, such as, new visions and approaches to the already studied growth promotion mechanisms, the use of PGPR and the effect on microbial populations, the industrial usage of the PGPR including methods of fermentation and formulation, and the novel utilization of PGPR, in interaction with other organisms different from higher plants. In addition, given planet-wide adverse environmental phenomena, climate changes, and disasters, an increasing challenge arises to preserve, rehabilitate, and restore damaged soils, where the usage of PGPR/PGPB is a leading research field addressing these challenges and is expected to boom in the coming years.

Organizing Committee

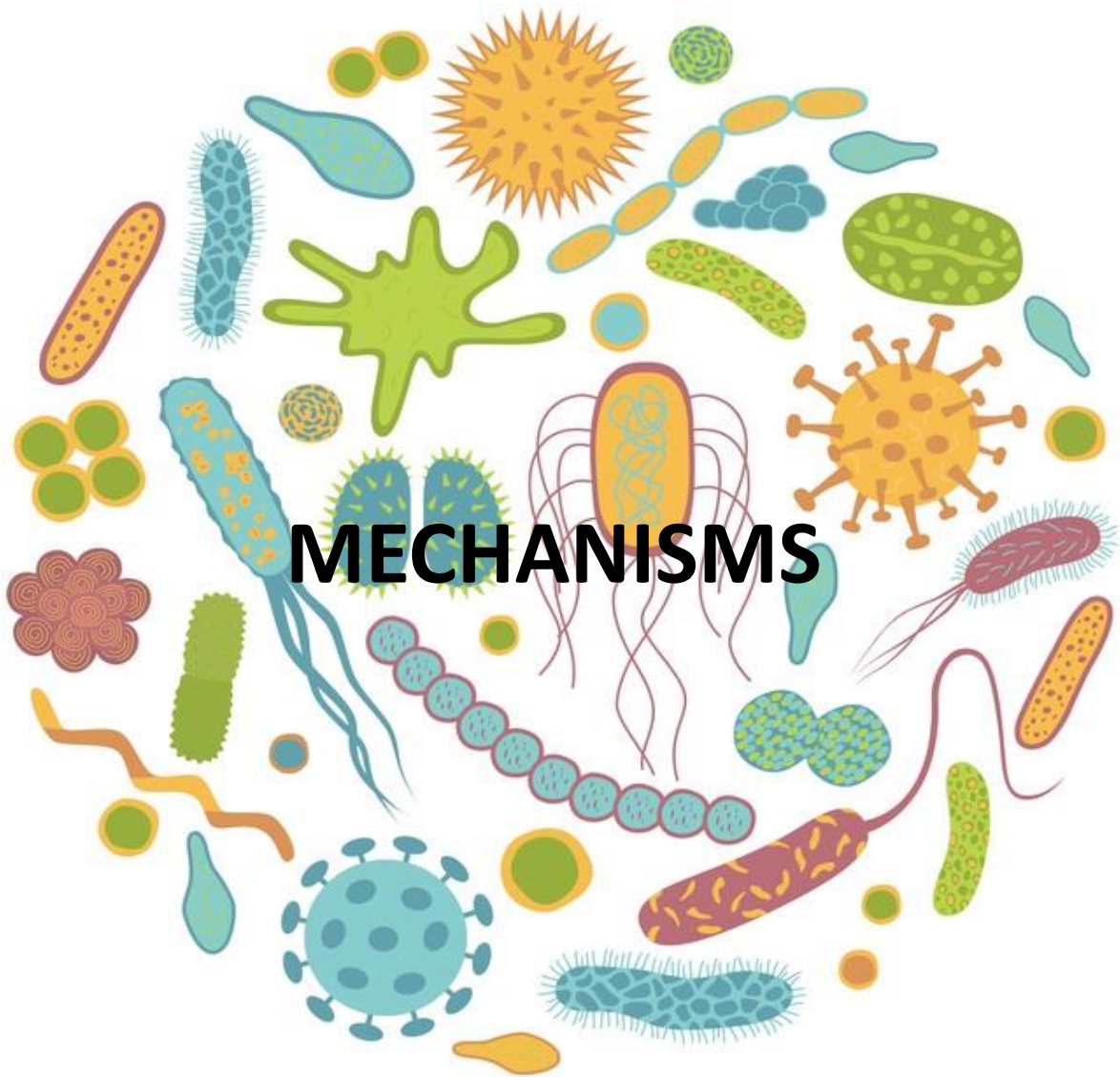
- Tony Adesemoye. USDA ARS National Soil Dynamics Lab, Alabama, USA.
- Marleny Burkett. Mosaic, Plant Response, Inc. Florida USA.
- Luz Bashan. Bashan Institute of Science, Alabama, USA.
- David Held. Auburn University, Alabama, USA.
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- Camilo Ramirez. University of Antioquia, Medellin Colombia.
- Choong-Min Ryu. Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon, Korea.

On behalf of the Organizing Committee, we welcome you to this 12th International PGPR Workshop.

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We offer a research topic in Frontiers in Plant Science: [Highlights from the 12th Plant Growth-Promoting Rhizobacteria Workshop | Frontiers Research Topic \(frontiersin.org\)](https://www.frontiersin.org/research-topics/12th-plant-growth-promoting-rhizobacteria-workshop).



SOMETHING NEW IN THE PGPR TOOLBOX

By Choong-Min Ryu

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For more than four decades, a group of rhizosphere bacteria known as plant growth-promoting rhizobacteria (PGPR) has been intensively studied for possible use in improving plant health and growth. Here, I provide an overview of the new finding, upcoming concepts, and current knowledge on the modern and emerging tools in PGPR research, their signatures, roles in plant growth promotion, biological control against plant pathogens, and modifier for phyto-microbiome. Lately, we are facing a new era to step forward in understanding new findings such as holobiome complexity, soil memory, and microbial chemical ecology during plant interaction with PGPR. The tools include cutting-edge X-omics technologies like genomics, metabolomics, and dual RNA-sequences to understand the mechanism of interaction between PGPR and plant/microbe, PGPR adjuvants to improve the capacity, new inoculants to re-engineer root microbiota community, and fusion PGPR-new technology. In such a PGPR toolbox, we must choose appropriate tools to solve current problems, such as new and re-emerging diseases and climate change. The modern and emerging tools deliver us a better job on successful PGPR applications in the field.

BI-DIRECTIONAL COMMUNICATION ALONG THE MICROBIOME-ROOT-SHOOT AXIS

By Corné M.J. Pieterse¹ and Plant-Microbe Interactions Team Utrecht

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Plants nurture a large community of root-associated microbiota, which in turn provide them with essential services, such as enhanced nutrient uptake, growth promotion, and protection against pathogens. Our research is focused on understanding plant-beneficial functions encoded by the root microbiome and the role of plant genes and traits that recruit these functions. We demonstrated that upon foliar pathogen infection, plant roots recruit a consortium of synergistic microbes to their rhizosphere that in turn trigger induced systemic resistance (ISR). Using the *Arabidopsis-Pseudomonas simiae* WCS417 model, we identified the root-specific transcription factor MYB72 as a central regulator in the onset of ISR, which is effective against a broad spectrum of pathogens. Metabolomics of root exudates revealed that MYB72 controls the biosynthesis of iron-mobilizing coumarins, such as scopoletin, which are secreted in the rhizosphere where they aid in iron uptake. Scopoletin also has antimicrobial activity that inhibits growth of soil-borne fungal pathogens, but to which beneficial rhizobacteria such as WCS417 are insensitive. Microbiome analysis of coumarin-deficient *Arabidopsis* mutants revealed that coumarins function in rhizosphere community assembly, possibly to promote recruitment of ISR-stimulating rhizobacteria to the roots. Whole genome expression profiling of WCS417 exposed to root exudates of wild-type and coumarin-deficient mutants revealed that coumarins have strong effects on bacterial functions such as motility, which may steer the transition of the free-living phase to growth in biofilm on the root surface. Recent data show that rhizobacteria-induced coumarins can also be transported from the roots to the shoots, where they may serve as players in the onset of ISR in the leaves, highlighting coumarins as central players in bi-directional communication along the microbiome-root-shoot axis¹. Besides coumarins, also other chemical and structural root defense barriers emerged as players in shaping plant-microbiome interactions. Understanding their mechanistic basis will provide a firm knowledge basis for the development of future crops that maximize profitable functions from the root microbiome.

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<https://doi.org/10.1016/j.tplants.2020.09.008>



ORAL PRESENTATIONS

LYSINIBACILLUS SPP.: AN IAA-PRODUCING ENDOSPORE FORMING-BACTERIA THAT PROMOTES PLANT GROWTH

By Manuel Pantoja-Guerra^{1,2}, Marleny Burkett-Cadena³, Johanna Cadena³, Christopher A. Dunlap⁴ & Camilo A. Ramírez⁵

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Lysinibacillus is a bacterial genus that has generated recent interest for its biotechnological potential in agriculture. Strains belonging to this group are recognized for their mosquitocidal and bioremediation activity. However, in recent years some reports indicate its importance as PGPR. This research sought to provide evidence of the PGP activity of *Lysinibacillus* spp. and the role of the indole-3-acetic acid (IAA) production associated with this activity. Twelve *Lysinibacillus* spp. strains were evaluated under greenhouse conditions, six of which increased the biomass and root architecture of corn plants. In most cases, growth stimulation was evident at 108 CFU/mL inoculum concentration. All strains produced IAA with high variation between them (20–70 µg/mL). The bioinformatic identification of predicted genes associated with IAA production allowed the detection of the indole pyruvic acid pathway to synthesize IAA in all strains; additionally, genes for a tryptamine pathway were detected in two strains. Extracellular filtrates from all strain's cultures increased the corn coleoptile length in an IAA-similar concentration pattern. Five of the six strains that previously showed PGPR activity in corn also promoted the growth of *Arabidopsis thaliana* (col 0). These strains induced changes in root architecture of *Arabidopsis* mutant plants (*aux1-7/axr4-2*), the partial reversion of mutant phenotype indicated the role of IAA on plant growth. This work provided solid evidence of the association of *Lysinibacillus* spp. IAA production with their PGP activity, which constitutes a new approach for this genus. These elements contribute to the biotechnological exploration of this bacterial genus for agricultural biotechnology.

ACTINOMYCETES-BASED INOCULANT FOR HORTICULTURAL CROPS: CONSORTIUM FORMULATION AND BEST APPLICATION STRATEGY IDENTIFICATION

by Rihab Djebaili | Beatrice Farda | Enrico Sabbi | Claudia Ercole | Maddalena Del Gallo | Marika Pellegrini

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The aims of the work were to select actinomycete strains to develop a microbial-based biostimulant useful for horticultural crops and identify the best application strategy for it. The strains selected for testing were selected based on plant growth-promoting activities among those belonging to the library of plant growth-promoting bacteria (PGPB) strains of University of L'Aquila (Italy). *Streptomyces thinghirensis* K23 and *Nocardiopsis alba* J21 strains were chosen for *in planta* trials based on their biostimulant traits. Associations abilities were investigated through microscope and culturable approaches, on plantlets grown under gnotobiotic conditions. Subsequently, *in planta* trials were conducted on different tomato and lettuce varieties, applying inoculum through seed tanning and irrigation and under greenhouse conditions. Findings unveiled a good colonization rate, with the development of biofilms and associative structures on the root system of tomato and lettuce. *In planta* results showed positive outcomes for both seed tanning and irrigation. Seed tanning promoted better germination of tomato and lettuce plants, leading to the development of longer plants that were richer in pigments and bioactive compounds ($p < 0.01$). Irrigation also achieved positive results but with smaller percentage increases than seed dressing ($p < 0.05$). The activities were carried out by the University of L'Aquila as part of the POFACS project (Title: *Conservabilità, qualità, sicurezza dei prodotti ortofrutticoli ad alto contenuto di servizio* - ARS01_00640, PON 2014-2020). The same conditions will also be tested in open field experiments and on other crops to identify the range of effectiveness and the most efficient application strategy.

AUXIN-MEDIATED GROWTH PROMOTION AND DEFENSE INDUCTION IN ARABIDOPSIS BY TWO AVOCADO RHIZOBACTERIAL STRAINS

by Alfonso Mendez Bravo | Elvis M. Cortazar-Murillo | Edgar Guevara-Avenidaño | Alejandro MéndezBravo |
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Escuela Nacional de Estudios Superiores Unidad Morelia, Laboratorio Nacional de Análisis y Síntesis Ecológica,
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Avocado (*Persea americana* Mill.) is a tree crop of great social and ecological importance for Mexico and several producing countries around the world. The crop productivity is hampered by spreading phytopathogens, often controlled through the indiscriminate application of harmful agrochemicals, and by soil degradation, which calls for new management alternatives to promote the use of biocontrol agents and biofertilizers. Two *Bacillus* strains from the avocado rhizosphere with broad spectrum antifungal activity, were assessed for their plant growth promoting effect and defense induction in *Arabidopsis thaliana*. *In vitro* assays and pot experiments showed that diffusible and volatile (VOCs) bacteria-produced compounds modified root development and increased fresh weight of Arabidopsis plants. These compounds differentially activated several hormonal signaling pathways involved in development and defense responses, such as auxin, jasmonic acid and salicylic acid; mutant screenings suggested that developmental stimulation of the root system architecture by bacteria requires an intact auxin signaling pathway. Furthermore, both strains were able to enhance plant growth and decreased the symptoms of *Fusarium* wilt when soil inoculated. Our results evidence the potential of these two rhizobacterial strains and their metabolites as biocontrol agents of avocado phytopathogens and as biofertilizers in non-natural plant hosts.

BACILLUS G7 IMPROVES ADAPTATION TO WATER STRESS IN OLEA EUROPAEA PLANTLETS TRIGGERING MULTIPLE TARGETS

by Galicia-Campos, E | Ramos-Solano, B | Montero-Palmero, B | Gutierrez-Mañero, FJ | Garcia-Villaraco, A

Universidad San Pablo-CEU Universities. Faculty of Pharmacy

Despite the innate adaptive mechanisms of plants to survive to different environmental stresses, these can further be improved by beneficial bacteria. Effective PGPR strains are able to trigger multiple targets in the plant resulting in a tailored adjustment to adverse conditions. In this work, the capacity of a *Bacillus* strain to stimulate metabolism of one year-old olive seedlings subjected to water stress (salinity and water scarcity) was studied after 10 root inoculations. In October (month 12), after the high stress summer period, photosynthesis, photosynthetic pigments, osmolytes (proline, soluble sugars), antioxidants (enzymes and non-enzyme) malondialdehyde, and a transcriptomic analysis, followed by RTqPCR analysis of specific genes related to salinity stress was performed. The strain was able to modulate several targets decreasing oxidative stress. A huge increase in energy dissipation (NPQ) was detected associated to increases in photosynthetic pigments, suggesting an activation of antennas associated to NPQ. Consistent with the lower oxidative stress are increase in osmolytes and antioxidant molecules, while the antioxidant enzymes are not activated. As the effective PSII photosynthetic yield decreased while CO₂ fixation was enhanced, a better use of the absorbed energy seems to be taking place. Interestingly, G7 appears to be activating the ABA-signaling pathway at different points: i) enhancing ABA synthesis, ii) repressing negative regulators (phosphatases 2C family) and iii) activating the AP2/ERF network related to adaptation to abiotic stress. In summary, *Bacillus* G7 is capable of improving plant adaptation to water stress, activating multiple targets of plant's innate mechanisms of adaptation.

GENETIC TRAITS ALLOWING BACILLUS SUBTILIS TO COLONIZE THE ROOTS IN PRESENCE OF A COMPETITOR

by Maude Pomerleau | Vincent Charron-Lamoureux | Pascale B. Beauregard

Université de Sherbrooke

Because of their multiple beneficial activities on plants, consortiums of two or more PGPR such as *Bacillus subtilis* and *Pseudomonas fluorescens* are a promising avenue to efficient biofertilizers formulation. However, we observed that if we inoculate *B. subtilis* on tomato plants concomitantly to *P. fluorescens* or *P. protegens*, its capacity to establish itself on roots significantly decreased. To determine what traits could mitigate this lack of colonization, directed evolution was performed in presence of *P. fluorescens* for 21 cycles. Colonization capacity of *B. subtilis* was followed during the experiment, and six evolved strains from the six lineages were frozen every seven cycles. Whole genome analysis and colonization assay of the evolved strains revealed that stronger colonization, even in presence of *P. fluorescens*, is most often linked to single nucleotide polymorphisms (SNPs) in genes encoding for regulators of biofilm formation. The phenotype appeared to stem from a loss-of-function only in the case of the mild biofilm repressor *ywcC*, while a strain deleted for the strong biofilm repressor *sinR* could not recapitulate the evolved strains with a SNPs in *sinR*. Thus, we suggest that a moderate increase in biofilm formation favors stronger root colonization. More importantly, we observed that our evolved mutants also displayed stronger colonization of the model plant *Arabidopsis thaliana*, and in presence of various strains of *P. protegens* and *fluorescens*, indicating that the observed adaptations could promote colonization in many scenarios.

METABOLOMICS OF PLANT-BACTERIA INTERACTION (PGPB) ISOLATED FROM AIR UNDER DROUGHT STRESS CONDITIONS

by Beatriz Genoveva Guardado-fierros | Jacobo Rodriguez-Campos | Miguel Angel Lorenzo-Santiago | silvia Maribel Contreras-Ramos

CIATEJ

Nine airborne bacteria were isolated from two sites in the Guadalajara metropolitan area; the first one was characterized by its high influx of people, and the second one for being an industrial zone. These bacteria were morphologically characterized and molecularly identified by 16S gene sequencing. The genera identified to belong to *Exiguobacterium*, *Rhodococcus*, *Kocuria*, and *Staphylococcus*, and their PGP activity was determined in planta in beans (*Phaseolus vulgaris*). After that, they were evaluated in chili (*Capsicum annuum*) planta to improve their growth and resistance to drought conditions. Their survival, height, and chlorophyll ratio a/b were analyzed. The best bacteria strains were selected for determined the metabolomic profile of plant-bacteria interaction when bacteria were inoculated under drought stress in a climatic chamber. Acetate ethyl and n-butanol extracts were obtained from plant substrate and roots to analyze by Mass spectrometer with a time of fly (QTOF-UPLC). Spectrums were analyzed with MaxenÒ software to assign a metabolomic profile. They were compared with extracts from plants without bacteria inoculated. Some compounds were determined as a marker of drought stress and as cross talking between plant bacteria. To our knowledge, this is the first report about the metabolomic profile of airborne PGPB bacteria.

PHYSIOLOGICAL AND GENETIC MODIFICATIONS IN TOMATO PLANTS SUBJECTED TO MODERATE WATER STRESS AND ELICITED WITH A PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR)

by Lucas, JA | Garcia-Villaraco, A | Montalbán, B | Montero-Palmero, B | Gutierrez-Mañero, FJ

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Physiological and genetic changes produced by a plant growth promoting rhizobacteria (PGPR) *Pseudomonas* sp. (N 5.12) after two inoculations in tomato plants subjected to moderate water stress challenge were studied. To cause moderate water stress, the plants were irrigated with 20 ml of PEG6000 at 10%. Photosynthesis efficiency, photosynthetic pigments, compatible osmolytes, enzyme activities related to oxidative stress, stress oxidative level (malondialdehyde-MDA) and gene expression related with Absciscic acid (ABA; *NCDE1* gene) synthesis, proline synthesis (*P5CS*) and plasma membrane ATPase (*PMATPase*) were analyzed in the post-challenge state. The PEG treatment decreased the photosynthetic efficiency, but the plants inoculated with PGPR maintained it at the same level as the non-stressed controls. Strain N 5.12 caused an increase in photosynthetic pigments, proline, glycine-betaine and phenolic compounds, decreasing the level of oxidative stress in the plants. N 5.12 activated the expression of *NCDE1* and Plasma Membrane ATP-ase genes, suggesting that ABA signaling-pathway is involved in protection to moderate water stress according to *NCDE1* expression; activation of ATP-ase genes suggest an active absorption of ions/nutrients to lower osmotic potential forcing water absorption. This PGPR strain had a very beneficial effect on tomato plants adaptation under moderate water stress, improving their physiological state, which could have a very positive effect in real crop conditions in a framework in which the scarcity of water in the future may be one of the great obstacles in increasing agricultural production.

PLANT GROWTH PROMOTING TRAITS OF AVOCADO RHIZOSPHERIC AND ENDOPHYTIC BACTERIA AND THEIR COMBINED EFFECT ON NON-HOST DEVELOPMENT

by Frédérique Reverchon | Alejandro Méndez-Bravo | Lorena C. Herrera-Cornelio | Daniel F. GarcíaToscano | Karla C. Magaña-Calderón | Alfonso Méndez-Bravo

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Plant growth promoting rhizobacteria (PGPR) have been traditionally inoculated as single strains, although recent evidence shows that combining rhizospheric and endophytic bacteria could enhance their beneficial effect on plant development. We thus aimed at producing consortia from avocado rhizospheric and endophytic bacteria with plant growth promoting and antifungal properties and assess their effects on the growth of *Arabidopsis thaliana*, *in vitro*, and on *Physalis ixocarpa*, a native Mexican horticultural crop, under greenhouse conditions. We first evaluated 31 bacterial strains in dual cultures for their antifungal activity against *Fusarium* spp. Strains that exhibited significant antifungal properties were subsequently tested for their ability to solubilize phosphate on NBRIP medium. Seven strains were then selected and evaluated for their capacity to promote the growth of *A. thaliana*. All stains enhanced *A. thaliana* biomass and stimulated the formation of lateral roots and root hairs, most likely through auxin production. Consortia were then elaborated from rhizospheric and root endophytic bacteria, and their effects were tested on the growth and development of *A. thaliana* and *P. ixocarpa*. All consortia were able to promote *A. thaliana* growth *in vitro*. Under greenhouse conditions, two consortia formed by *Bacillus* spp. induced a 40%-increase in plant height and a floral onset initiating 30 days earlier than uninoculated plants. Collectively, these findings confirm the potential of combining PGPR with multiple beneficial traits for plant development and productivity.

PLANT GROWTH-PROMOTING RHIZOBACTERIA (PGPR) INDUCE RESISTANCE IN COTTON PLANTS AGAINST BEET ARMYWORM (*SPODOPTERA EXIGUA*)

by Simon Zebelo | Esther Ngumbi | Kate Nangle | Yuanyuan Song | Joseph Kloepper | Henry Fadamiro

Department of Agriculture, Food and Resource Sciences University of Maryland

Plant growth-promoting rhizobacteria (PGPR) are root-colonizing bacteria that increase plant growth and often elicit biological control of plant diseases. However, very little is known about the effect of PGPR on plant-insect and multitrophic interactions and the potential biochemical and molecular mechanisms by which PGPR enhances plant defense against insect herbivores. Here we report that PGPR treatment of cotton (*Gossypium hirsutum*) plants induces increased emission of volatile organic compounds (VOCs) and enhances the plant's direct and indirect defense mechanisms against insect herbivory. In a series of greenhouse and laboratory studies, we demonstrated that the beet armyworm (*Spodoptera exigua*) exhibited reduced oviposition, larval feeding, and development on cotton plants treated with some PGPR (*Bacillus* spp.) strains compared to untreated plants. In contrast, the parasitoid, *Microplitis croceipes* were preferentially attracted to PGPR-treated plants. The chemical analyses of PGPR-treated cotton plants showed significantly enhanced emission of biologically-relevant VOCs, which may explain the reduced herbivory and increased parasitoid attraction. Furthermore, significantly higher amounts of the plant defense compound, gossypol, and the phytohormone jasmonic acid (JA), were detected in PGPR-treated plants compared to untreated plants. Similarly, transcript levels of the genes involved in gossypol biosynthesis and JA-regulated genes were significantly higher in PGPR-treated plants. These findings demonstrate that the treatment of plants with rhizobacteria may induce significant biochemical and molecular changes with potential ramifications for plant-insect and multitrophic interactions.

USE OF GROWTH-PROMOTING BACTERIA FOR THE OPEN FIELD CULTIVATION OF INDUSTRIAL HEMP

by Giancarlo Pagnani | Beatrice Farda | Rihab Djebaili | Claudia Ercole | Daniela M. Spera | Maddalena Del Gallo | Marika Pellegrini

University of Teramo | University of L'Aquila

Over the past few decades, hemp cultivation has returned to the interests of the public and the industrial sector, which have shown an ever-increasing co-involvement with the many uses of this crop, such as construction, textile, food-re and physiotherapeutic. Industrial hemp is a crop with a low environmental impact. However, in the case of crops that require high harvesting standards, like dioecious inflorescence varieties, the cultivation operations need increases. In addition, hemp can be subject to attacks by various organisms that can result in a decrease in product quality. Any routine farm activities aimed at improving, containing, or eliminating these needs and threats are usually associated with the use of chemically synthesized substances, which can be a potential source of pollution of soils and surface or sub-surface waters. It is therefore necessary to find cultural management that allows for good crop yield and quality while protecting the environment and soil fertility. Among sustainable-use lawns in recent years, treatment by growth-promoting bacteria is undoubtedly an innovation that leads to better and faster growth than untreated crops, improving the sustainability of agricultural production. Pursuing experiments on other hemp varieties, the consortium formed by *Azospirillum brasilense*, *Burkholderia ambifaria*, *Gluconacetobacter diazotrophicus*, and *Herbaspirillum seropedicae* was tested on Kompolti and Carmagnola varieties under a two-years open field experiment. In the presence of bacterial treatment, both varieties showed improved plant growth and development. Inflorescences quality was also improved. The results suggest a promising application potential of the selected consortium in sustainable hemp farming.



POSTER PRESENTATIONS

HARNESSING PLANT-MICROBE INTERACTIONS FOR SUSTAINABLE AGRICULTURE: COMPLEMENTARY APPROACHES FOR IDENTIFYING NOVEL PLANT GROWTH-PROMOTING BACTERIA

by Alex Evenko-Greenapple

Agricultural Research organization (ARO). The Hebrew University of Jerusalem (HUJI)

Plant growth-promoting rhizobacteria have emerged as an environmentally friendly alternative to chemical fertilizers as they can enhance plant growth and productivity while alleviating abiotic stress. However, translating laboratory findings to real-world scenarios is challenging. Thus, the objective of this study was to isolate and identify novel PGPR bacteria from the rhizosphere soil of various tomatoes species, including local relatives and commercial varieties. We employed both culture-dependent and independent techniques, isolating bacteria from root samples as well as extracting bacterial DNA and performing community analysis from rhizosphere. By using these complementary approaches, we were able to identify several effective PGPR, which have the potential to enhance plant growth and resistance to abiotic stresses. In this study, we estimated the performance of PGPR by measuring their effect on shoot dry weight of plants grown under both abiotic stress and ambient conditions. We have identified 21 isolates that promote growth in comparison to *E. coli* control. The best isolate was able to achieve a 30% and 60% improvement under abiotic stress and ambient conditions, respectively. Additionally, we utilized Host mediated microbiome enrichment (HMME) to filter from microbial population in the rhizosphere of tomato plants. This involved transferring rhizosphere for several cycles. Bacteria were then isolated and screened in planta as reported above. Transcriptomics is now being used to investigate the MOA of the bacteria and plant response. This study emphasizes the importance of using complementary techniques to identify new PGPR bacteria and their potential for sustainable agriculture in the face of climate instability.

IMPROVEMENT OF SOIL QUALITY AND IMPLEMENTATION OF THE DURUM WHEAT PRODUCTION CHAIN THROUGH THE SELECTION AND APPLICATION OF MICROBIAL BIOSTIMULANTS

by Gabriele Bellotti | Antinori Maria Elena | Di Rico Francesca | Morcia Caterina | Piazza Isabella | Terzi Valeria | Carnevali Paola | Lucini Luigi | Puglisi Edoardo

Università Cattolica del Sacro Cuore | CREA-GB | Barilla

Including Plant Growth Promoting Rhizobacteria (PGPR) into wheat cultivation has the potential to serve as an environmentally sustainable alternative to conventional fertilization methods and may be integrated into plant nutrition strategies. Furthermore, PGPR may aid farmers in mitigating the detrimental effects of climate change and augment plant resilience in response to severe weather conditions, such as extended periods of drought. This research examined the culturable bacterial diversity of three varieties of *Triticum durum* and identified potential PGPR based on their ability to fix nitrogen, solubilize phosphorus, produce indole acetic acid, siderophores, and 1-aminocyclopropane-1carboxylic acid deaminase. Ten of the best isolates among a total of 300 were combined to form 10 consortia and were applied to seedlings for a preliminary pot experiment. All consortia improved ($P < .005$) at least one of the measured parameters: chlorophyll, flavonoid, fresh and dry weight, height, and number stems, with one consortium composed of *Bacillus* spp. and two *Pseudomonas* spp. performing best. Genomic analysis carried out on the strains showed which bacterial genomic feature could engage plant growth. Rhizosphere metagenomic and metabolomic analysis will be conducted on pot trials to study the consortia effect on plant root exudates and metabolites (ongoing analysis). In conclusion, selected PGPRs have been found to enhance plant growth and reduce stress through genetic features that allow them to colonize plant roots and improve nutrient uptake. This symbiotic relationship has the potential to improve crop yields and promote sustainable agriculture.

INOCULATION OF BENEFICIAL BACTERIA AND ADEQUATE NITROGEN SUPPLY IMPROVE THE NUTRITION AND YIELD OF EMERALD GRASS (ZOYSIA JAPONICA)

by Marcelo Carvalho Minhoto Teixeira Filho | Graziela Franceschini Ferraz | Raíssa Pereira Dinalli | Carlos Eduardo da Silva Oliveira | Rodolfo de Niro Gazola | Castro Alves da Silva | Arshad Jalal

São Paulo State University (UNESP) - Ilha Solteira Campus

The inoculation of plant growth-promoting bacteria (PGPB) can reduce the use of nitrogen (N) fertilizers in Emerald grass cultivation, which is high. In this sense, the objective was to evaluate the effects of PGPB inoculated at the beginning of the Emerald grass sprouting and N doses in the area of production of grass mats. The experiment was carried out in an area of Emerald grass cultivation with a history of 18 years of commercial grass production. The experimental design was randomized blocks with 4 replications, in a 4x4 factorial scheme, the first factor was the inoculation (*Azospirillum brasilense* - strains Ab-V5 and AbV6, *Bacillus subtilis* strain CCTB04, *Pseudomonas fluorescens* strain CCTB03, being all liquid inoculants guaranteed 2×10^8 CFU mL⁻¹, and without inoculation), the second factor was the reduction of the N doses (100, 75, 50 and 0% of recommended dose, equal to 280 kg N ha⁻¹, split in 5 times) applied in coverage as urea. With higher N doses, higher accumulations of N, P, K, Ca, S, Fe, Mn and Zn in the plants shoot were observed with the inoculation of *P. fluorescens*. The same occurred for Ca, Mg, S, Cu, Mn and Zn accumulations with *B. subtilis* inoculation. Inoculation with *A. brasilense* associated with higher N doses reduces P, K, Ca, Mg, S, Fe shoot accumulations. Inoculation with *B. subtilis* and *P. fluorescens* promoted higher shoot dry mass of Emerald grass, being 41 and 47% higher in relation to without inoculation, regardless of the N dose.

LEAF YIELD, PLANT NUTRITION, AND LEAF GAS EXCHANGE OF LETTUCE PLANTS IN A HYDROPONIC SYSTEM IN RESPONSE TO *BACILLUS SUBTILIS*

by Carlos Eduardo da Silva Oliveira | Marcelo Carvalho Minhoto Teixeira Filho | Arshad Jalal | Geovana Cunha Caetano | Karen Vicentini Tamburi | Letícia Schenaide Vitória | Victoria Moraes Giolo | Jailson Vieira Aguilar | Liliane Santos de Camargos | Tiago Zoz

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Inoculation with *Bacillus subtilis* has been promising in increasing plant yield and nutrient acquisition. The study aimed to estimate the dose of *B. subtilis* that increases yield, gas exchange, and nutrition of hydroponic lettuce plants. The research was carried out in a greenhouse in Ilha Solteira, Brazil. The randomized block design with four replications was used. The treatments were doses of inoculant with *B. subtilis* via nutrient solution (0, 7.8, 15.6, 31.2, and 62.4 ml 100 L⁻¹). The inoculation with *B. subtilis* at the dose of 33 and 29 ml provided the highest fresh (22%) and dry (40%) mass of shoot, respectively. There was an increase of 31% in the accumulation of N, 33% of P, 44% of K, 104% of Ca, 43% of Mg, and 53% of S in the shoot at the doses of 25, 26, 20, 15, 24, and 30 ml 100 L⁻¹. There was also an increase in the rate of liquid photosynthesis, intercellular CO₂ concentration, total chlorophyll, stomatal conductance, transpiration, and water use efficiency at doses between 34 and 39 ml 100 L⁻¹. The dose of 35 ml 100 L⁻¹ of *B. subtilis* via nutrient solution is the most suitable for hydroponic lettuce production, as it provides the highest yield of leaves. Inoculation with *B. subtilis* at doses between 18 and 35 ml 100 L⁻¹ provided the highest fresh and dry mass of shoots accumulation of N, P, K, S, Ca, and Mg in lettuce grown in a hydroponic system.

NEUTRAL AND ALKALINE-SALINITY STRESS MANAGEMENT THROUGH AUTOCHTHONOUS PGPB FROM RHIZOSPHERE MICROBIOME OF A SALT-TOLERANT WILD BRASSICACEAE

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Brassica fruticulosa is a wild Brassica species endemic of the Mediterranean coasts and it is cross-compatible with valuable crops such as white mustard (*Sinapis alba* L.). In the Catalonia region we found inland and coastal populations with contrasting phenotypes in salinity and alkalinity tolerance. The rhizosphere microbiome of salt-tolerant *B. fruticulosa* plants was isolated and plant growth promoting bacteria (PGPB) were selected based on their salinity tolerance and PGP mechanisms. Based on this result, the aims of this study were: a) to identify the candidates selected and analyse their PGP activities (phosphate solubilization ability, siderophores production, indolacetic acid like molecules production and ACC deaminase activity) when exposed to neutral-salinity (salt) and alkaline-salinity (alk-salt) stress; b) to determine the effects of their individual inoculation in *Sinapis alba* plants submitted to salt and alk-salt stress. The taxonomic results revealed that R60 and E1 belonged to the *Pseudomonas* genus, R52 and T7 to *Pantoea* genus, and E14 to *Enterobacter* genus. These isolates demonstrated the ability to maintain their PGPB activities under both salinity conditions, being T7 the top. The plant-PGPB interaction assay under salt and alk-salt conditions resulted in an increased biomass and an improvement of salinity tolerance of all the inoculated plants, outstanding the interaction of E1 and T7 with *S. alba*. These findings provide basic knowledge of autochthonous PGPBs, becoming a key factor for a future development of a novel bioinoculant useful for other salt-sensitive Brassicaceae species of high economic interest.

SCREENING POTENTIAL BIOLOGICAL CONTROL AGENTS AGAINST ALLORHIZOBIMUM VITIS, THE CAUSAL AGENT OF GRAPEVINE CROWN GALL DISEASE

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Cold-climate grape-growing regions around the world experience severe economic loss as a result of crown gall disease. The soil-borne bacterial causal agent of grapevine crown gall, *Allorhizobium vitis*, infects plant xylem tissue and spreads via xylem sap. As such, symptoms can appear along the whole length of the grapevine. These symptoms include root necrosis below the soil surface and tumour (gall) growth on aerial plant parts, leading to reductions in both plant performance and crop quality. Partial or complete vine death is common in young vines that become infected soon after planting. There is currently no effective disease management practice nor biological control agent commercially available to prevent or treat grapevine crown gall. This study aimed to screen potential biological control agents (BCAs) against *A. vitis in planta*. Five BCAs (three *Bacillus* spp. and two *Pseudomonas* spp.) were identified through an *in vitro* plate screening assay as having shown consistent inhibitory activity against *A. vitis*. These BCAs were tested in grapevine (*Vitis vinifera*) planting material using a needle inoculation assay, where potential BCAs and *A. vitis* were coinoculated along the grapevine stem at a 1:1 ratio. The BCAs were also tested in tomato (*Solanum lycopersicum*) seedlings using a root soak assay, where roots were soaked in a cell suspension of a potential BCA and *A. vitis* at a 1:1 ratio and planted into *A. vitis*-infected soil. The needle inoculation assay showed no inhibitory effect on the growth nor size of galls. Root soak assays are in progress.

TN-SEQ APPROACHES IDENTIFY COMMONALITIES AND DIFFERENCES IN ROOT COLONIZATION STRATEGIES IN BURKHOLDERIA PGPR

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The bacterial group of burkholderias includes several genera with a large number of species interacting with eukaryotic hosts, ranging from mutualism to pathogenesis. To use these strains in agriculture we need to understand to what extent the bacterial strategies used for the different biotic interactions differ depending on the host and if strains with agricultural potential could also pose a threat towards other plant hosts or humans. One strategy to determine bacterial genetic traits involved in the colonization of rice roots is the highthroughput screening of transposon insertional mutant libraries (Tn-seq). In detecting which genes influence the fitness of the strains we aim at unravelling their commonalities and differences in root colonization strategies. Tn-seq analyzes were firstly carried out on two PGPR models, *Burkholderia vietnamiensis* LMG10929 (Bv) and *Paraburkholderia kururiensis* M130 (Pk). Twice more genes were involved in rice roots colonization for Bv compared to Pk, including genes contributing to the tolerance of plant defenses. Several functions commonly enhancing root colonization were found in both bacterial strains but strain-specific functions were also identified implying different genetic strategies between burkholderia strains to colonize *Oryza sativa*. Tn-seq approaches thus revealed as an efficient tool to better understand the genetic bases that underlie the interactions between burkholderias strains and their host. This approach was then developed on a collection of *Burkholderia* strains from different eukaryotic hosts, to unravel genetic strategies used to colonise each host.

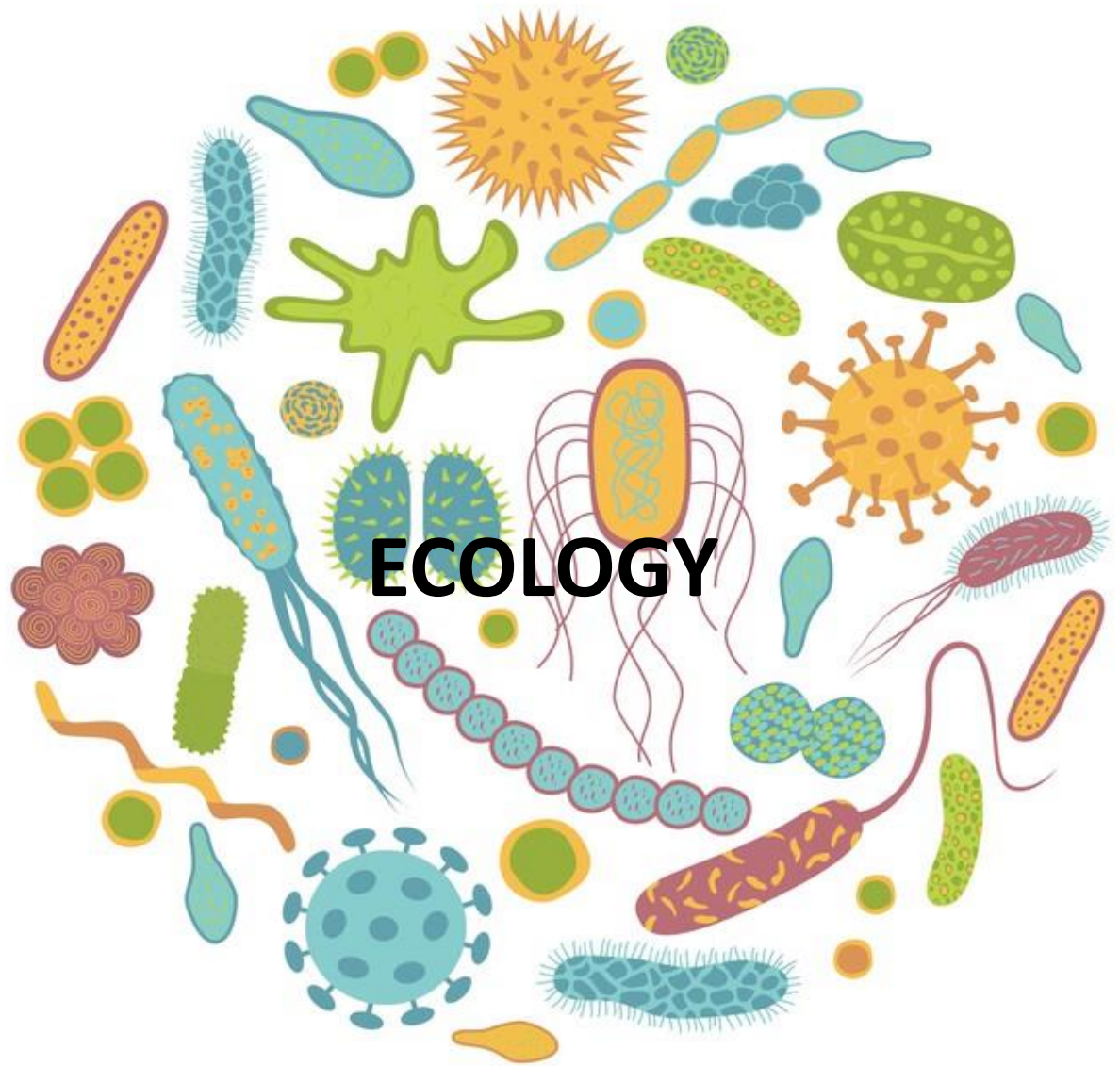
BIOAUGMENTED PHYTOEXTRACTION OF ZINC BY PGPM AND MISCANTHUS PLANTS: INNOVATIVE STRATEGIES INCREASE STRESS RESISTANCE AND PLANT-MICROORGANISMS COOPERATION

by Francesca Di Rico | Gabriele Bellotti | Maria Elena Antinori | Amjad Ali | Angelica Barone | Andrea Ferrarini | Stefano Amaducci | Edoardo Puglisi

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Soil contamination with heavy metals, water scarcity and sustainable energy production are three major challenges in the context of climate change. In this work, we tried to address all of them by evaluating the use of PGPM in bioaugmented phytoextraction of zinc (Zn) by Miscanthus, a sustainable bioenergy crop, in zinc contaminated soils and under drought stress. 70 bacterial and fungal isolates from rhizosphere of Miscanthus plants grown in soil contaminated with Zn were identified and evaluated in vitro for PGP activities (phytormone and siderophore production, nitrogen fixation, phosphorus solubilization), drought stress resistance (growth under osmotic stress and ACC deaminase activity) and zinc bioadsorption. Strains were selected also depending on the possibility of priming their resistance to heavy-metal and drought stress in vitro, before inoculum. Persistence of this priming effect in selected strains up to their delivery was implemented by experimenting and exploiting cooperation among microorganisms (“microbial hitchhiking”). Evaluation of the synergical effect of microorganisms and plant on drought stress resistance and zinc remediation was performed in pot experiments by comparing the yield and status of plants grown in presence or not of PGPM, under the diverse stresses.

Overall, this work shows that the use of PGPM can be an effective strategy for the remediation of Zn contaminated soils using Miscanthus plants, especially under drought stress. Priming the selected strains for resistance to heavy-metal and drought stress and finding efficient delivery systems for the microorganisms to colonize the plant rhizome are important steps in the development of bioaugmented phytoextraction formulas.



LINKING PLANT GROWTH PROMOTING BACTERIA FROM FARM TO FORK WITH HEALTH ISSUES

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Biodiversity in the farm's soil is linked to plant health and performance. The plant microbiome is vertically transmitted by seeds but also horizontally acquired from soil – both sources are crucial for a healthy plant microbiome. While the seed microbiota contains preferentially plant growth promoting bacteria, environmental microbes support adaptation to local conditions. Although all plant tissues harbor a specific microbiome, all of them are connected with each other and show typical patterns along plant's lifecycle. Edible parts of the plants are equipped with a specific microbiome, which will be shown exemplarily for apple, strawberry and lettuce. Post-harvest management is influencing the food microbiome as well. The food microbiome can be an important external factor that influences the gut microbiome. While this is well known for food-borne pathogens, it is less studied for the entire microbiome. To explore this, we have compared metagenome assembled genomes (MAGs) originated from fruit and vegetables and indigenous human gut microbiota. This combined catalogue was used to detect fruit and vegetable associated MAGs including originally plant growth promoting bacteria in human gut, which link the farm to fork concept with the human health. Moreover, this will provide a basic foundation for a potential role of plant associated bacteria and fresh produce consumption on the gut microbiota and linking them with the disease outcomes in frame of the exosome concept.

THE CONTRIBUTION OF RHIZOBACTERIA TO PLANT ADAPTATION DUE TO DROUGHT ENVIRONMENTAL CHANGE

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Adapting to environmental changes enhances plant performance under altered environmental conditions like drought. There is a wide range of adaptability between plant species. Drought stress significantly impacts agricultural productivity because it causes the plants to change in several morphological, biochemical, and physiological ways. For example, it inhibits root and shoots growth, reduces photosynthesis and transpiration rates, produces reactive oxygen species, modifies osmotic conditions, and alters pathways that control leaf senescence and signal stress. As a result, there is a greater need for environmentally sound solutions to guarantee the security of the global food supply. Plant growth-promoting rhizobacteria ensure plants' survival and growth during drought through several mechanisms, including enhanced phytohormone synthesis, antioxidant activity, and osmotic changes. They alter root architecture, maintain high relative water content, increase photosynthesis rate, activate the antioxidant defense system, produce 1-aminocyclopropane-1-carboxylate deaminase, exopolysaccharides, phytohormones, and volatiles, and change the expression of genes responsive to stress. More reliable strain selection and performance in difficult environmental circumstances will continue to be prerequisites for the commercial application of PGPR as bioinoculants or biostimulants. The ability to forecast plants' adaptation to drought depends critically on our ability to understand the variables influencing plant-PGPR interactions in the soil and adaptation to environmental perturbations. Recent discoveries in the field and future perspectives will be presented.

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CHANGES IN GRAIN IONOME COMPOSITION IN PEA-WHEAT INTERCROPPING ARE ASSOCIATED WITH THE ABUNDANCE OF *PSEUDOMONAS* SPP. AND ENTEROBACTERALES

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Increasing stable crop yields and nutrient quality are essential to ensure food security and promote human health. Cereal-legume intercropping (IC) has been proposed to meet these challenges since IC allows to reduce the use of nitrogen fertilizer, thanks to N uptake by the cereal promoted by the symbiosis between the legume and N-fixing bacteria. Other bacterial groups, such as *Pseudomonas* spp., are known to promote pea nutrition in Fe, Mg and P, and Enterobacterales to impact Fe and P dynamic in pea and wheat. The goals of the present study were to (i) characterize the impact of pea-wheat IC on the abundance of *Pseudomonas* spp. and Enterobacterales and (ii) assess a possible relation of their abundance with changes in grain ionome associated with IC. Four pea (*Pisum sativum*) varieties were cultivated in sole- or IC with wheat (*Triticum aestivum*) in clay and sandyloam-clay soil types. Bacterial abundances were assessed by qPCR, and grain ionome by mass spectrometry. *Pseudomonas* spp. were more abundant in pea, and Enterobacterales in wheat when cultivated in IC. Grain ionome was also impacted by IC with an increased content in Cu, Mn, Fe and S in wheat, and in Mn, Mo, and Ni in pea. A positive correlation was found between the *Pseudomonas* spp. abundance and the Mo content in pea grains. These results indicate that pea-wheat IC increases the abundance of bacterial groups and suggest that the increased occurrence of *Pseudomonas* spp. could contribute not only to Fe but also to Mo nutrition.

EXPLORATION AND EXPLOITATION OF THE RICE MICROBIOME FOR PLANT HEALTH IN CAMBODIA

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Rice (*Oryza sativa*) is the staple food for more than half of the world's population. Several diseases including fungi, bacteria, viruses, and nematodes, cause great loss to rice production. To increase rice yield and defend against pest, farmers use high agrochemical inputs which are not sustainable and cause environmental pollutions, loss of soil biodiversity and soil compaction. The plant microbiota can host phytobeneficial microbes that can improve rice health and reduce the use of agrochemical products. The main objective of this study is to identify plant health-related microbial taxa, by exploring the leaf and root microbiome (by 16S and 18S amplicons sequencing, ASV analysis) of healthy and diseased rice in Cambodia. This approach i) identified a range of phytopathogens in leaf and root tissues, allowing to make correlations between disease establishment and overall rice health in belowground and aboveground compartments; ii) led to the identification of specific signature of bacterial taxa correlated with asymptomatic or symptomatic rice, with for example a significant increase of *Methylobacterium* ASV in leaves of healthy rice compared to diseased one. Rice-health correlated taxa were searched in culture collections developed in parallel by culture-dependant approaches for further biocontrol tests against phytopathogens.

FLAVOBACTERIUM DAUAE TCH3-2 MODULATES THE BENEFICIAL INTERACTION BETWEEN PLANTS AND NATIVE SOIL MICROBIOTA

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Rhizosphere microbiota plays an important role in plant fitness such as plant growth and disease resistance. *Flavobacterium dauae* TCH3-2, a novel species isolated from rhizosphere soil of tomato, promotes the growth of tomato plant with indigenous upland soil microbiota, but not alone. In this study, to evaluate the effect of *F. dauae* TCH3-2 on host plant and microbial community, we conducted transcriptome of tomato roots and rhizosphere microbiome analysis. The RNA-seq data revealed that *F. dauae* TCH3-2 activates the expression of genes involved in plant hormone signaling including gibberellic acid and phytosterol in tomato roots. In addition, the microbiome analysis showed that there was significant microbiome shift by *F. dauae* TCH3-2 treatment compared to untreated control group in tomato rhizosphere. Based on the microbiome analysis, we constructed a synthetic community (SynCom), which mimics upland soil microbiota, comprising seven bacterial isolates from tomato rhizosphere soil. Combination of SynCom and *F. dauae* TCH3-2 significantly promoted the growth of tomato plant under both soil and hydroponic cultivation systems. Taken together, these data suggest that *F. dauae* TCH3-2 and the native soil microbiota might collaborate to promote plant growth via the activation of plant hormonal signaling. Further studies are in progress to detect the growth-promoting substances produced by SynCom and *F. dauae* TCH3-2.

IDENTIFICATION OF MICROBIAL CONSORTIA AND MECHANISMS INVOLVED IN SOIL SUPPRESSIVENESS TO *FUSARIUM CULMORUM*

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Soil suppressiveness is a sustainable alternative to controlling fungal diseases. In most cases, disease suppressiveness is microbial by nature. In a previous study, we screened 28 soil samples from different regions and found 4 soils with a high and consistent level of suppressiveness against *Fusarium culmorum*, which is one of the most widespread fungal plant pathogens. Via soil sterilization and soil transplantation, we confirmed that the protective effect observed in these soils is microbiological. Based on the bacterial taxonomic analysis, only limited commonalities among these suppressive soils were found. However, functional amplicon sequencing of NRPS-associated Adenylation domains (A-domains) and 10X metagenomic sequencing results indicated several genes and biosynthetic gene clusters (BGCs) that strongly correlate with a suppressive phenotype against *F. culmorum*. We isolated bacteria from activated suppressive soil and performed whole-genome sequencing of these bacteria to obtain the full genetic information. Based on this information, we are designing bacterial Synthetic Communities (SynComs) to further verify the contribution of specific BGCs to the disease-suppressive phenotype. By integrating metabolomic, metatranscriptomic, and metagenomic data collected from different functional SynComs treatments, we aim to decipher the mechanisms involved in suppressiveness.

LOW NITROGEN FERTILIZATION OVER TWO CONSECUTIVE YEARS ENRICHES POTENTIAL PGPRS IN THE BRASSICA NAPUS SEED MICROBIOME

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A diverse and multifunctional seed microbiome contributes to soil microbial diversity, plasticity, and resilience. Seeds can also transmit PGPRs, which might have a priority effect over environmental microbiota. While recent studies proved adverse impacts of mineral nitrogen (N) fertilization on soil and rhizosphere microbiomes, the consequences for seed microbiomes are still underexplored. Here, we analyzed the impact of different levels of N fertilization over two subsequent growing seasons on the seed microbiome of *Brassica napus* by a combined approach of amplicon sequencing, qPCR, and microscopy. On average, the Brassica seed microbiota consisted of 646 bacterial ASVs, dominated by Proteobacteria, Actinobacteria, Bacteroidetes, and Firmicutes and harbored approx. 10^8 bacteria gene copy number per seed. Two consecutive years of either low or high N fertilization resulted in a significant shift in seed microbiota. Here, PGPRs, such as order Rhizobiales and features assigned to Rhizobia, were identified as biomarkers for low N fertilization regimes. Those results were supported by quantitative measurements, where 16S rRNA and bacterial nifH gene copy numbers were significantly enriched. The alternation of N levels between the first and the second growing season (high to low, or low to high) had, however, no impact on seed microbiota. In addition, high N levels correlated with increased seed-to-seed transmission, whereas low N levels correlated with an increased acquisition of environmental microbiota, suggesting a flexible, soil-influenced seed microbiome assembly. Our data suggest, especially in the long term, innovative microbiome management via biological nitrogen fixation to sustain mineral fertilizer applications in future agriculture.

OPTIMIZING HEAVY METAL EXTRACTION WITH THE AID OF CHEMICAL BIOSTIMULANT AND PLANT GROWTH-PROMOTING RHIZOBACTERIA IN COPPER PHYTOREMEDIATION

by Gabriele Bellotti | Andrea Fiorini | Federico Capra | maria elena antinori | Francesca Di Rico | eren taskin |
Michele Manica | Patrizia Vida | Giacomo de Maio | edoardo puglisi

Università Cattolica del Sacro | Manica S.p.A. | NTI S.r.l.

Copper (Cu) is an essential micronutrient for living organisms, but its accumulation in soil from Cu-based fungicides can possibly lead to toxic effects on biodiversity and soil fertility. Plant-associated rhizobacteria can enhance Cu phytoextraction ability in plants. In this study, bacterial strains were isolated from the rhizosphere of fescue and alfalfa and were *in-vitro* tested for plant nutrition and Cu bioadsorption capacity. The most promising rhizobacteria were re-applied as a consortia on fescue and alfalfa plants in a vineyard to enhance Cu uptake in plants. Genomic analysis on bacterial strains identified potential genes associated with Cu bioadsorption and plant growth promotion. Field experiments over two years were conducted to evaluate the efficacy of biostimulants in improving the phytoremediation potential of the plant species. Metagenomic amplicon sequencing was performed on soil rhizosphere to identify potential microbial interactions. Genomic analysis confirmed that the selected bacterial strains are genetically equipped with PGP characteristics that can support the plant to cope with stress caused by high concentrations of copper in the soil. Results showed that bacterial consortia with algae enhanced Cu uptake in plant biomass. This study provides valuable insights into the potential of plant-associated bacteria for enhancing Cu bioadsorption in crops and highlights the importance of microbial interactions in the rhizosphere soil. The study also indirectly confirms the importance of inter-row vegetative cover in vineyards to limit copper losses to the soil. Further analysis is needed to determine the specific mechanisms underlying the increased biomass production and copper removal in the plants.

P-CYCLING AND N FIXING BACTERIA OF RICE ROOT COMPARTMENTS ARE AFFECTED BY REDUCED P FERTILIZATION

by Camilla Valli | Sarah Zecchin | Lucia Cavalca

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The demand for rice (*Oryza sativa*, L.) production is continuously increasing, following the world population size (FAO 2021). Since phosphorus (P) is not a limiting factor for plants in rice paddy soils, environmental and economic issues ask for tailoring mineral phosphate inputs. Due to the role of soil microbial communities in element cycling, this study was aimed to assess the impact of P fertilization on microbial communities in rhizosphere compartments (soil, rhizoplane, endosphere) of rice plants cultivated under continuous flooding in the presence (50 kg ha⁻¹ P₂O₅) and in the absence of P fertilization. IlluminaMiSeq sequencing of bacterial and archaeal 16S rRNA genes and fungal ITS, demonstrated that different levels of P fertilization caused a significant (PERMANOVA, $p < 0.01$) differentiation of bacterial and archaeal communities of root compartments, particularly of the endosphere. P fertilization increased the microbial functionality related to phosphate solubilization/mineralization and nitrogen fixation as assessed by Real-Time quantitative PCR and inferred functional profiling. The reduction of P fertilization needs to be further investigated, since the initial response of microbial community to the absence of a boost of nutrient was a decrease of P cycling and nitrogen-fixing bacteria in the proximity of rice roots. *Acknowledgement: Regione Lombardia (P-RICE, n. 23, d.d.s. 4403-28/03/2018).*

VARIABLE AND CORE FUNCTIONAL RHIZOSPHERE MICROBIOTA ASSOCIATED WITH SEVEN *MEDICAGO TRUNCATULA* GENOTYPES

by Liya Zhu | Olivier Rué | Florence Deau | Julie Cremaschi | Valentin Loux | Samuel Mondy | Jingjing Peng | Sylvie Mazurier | Philippe Lemanceau | Barbara Pivato

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The rhizosphere microbiota emerges as a novel component that expands plant adaptation to its environment, and thus paves the way for future breeding approaches. Knowledge regarding the "functional core microbiota", which refers to the microbial taxa bearing essential functions for holobiont fitness, is required to move forward in this area. The aim of the present study was to compare the functional microbiota recruited by different plant genotypes in order to identify its core and variable components. Seven genotypes of *Medicago truncatula* (DZA315-16, DZA315-26, DZA045-6, F83005-5, SA028064, Jemalong A17 and J6), representatives of a core collection of 184 genotypes, were studied. Functional genes have been described using shotgun metagenomics followed by Prodigal. Core and variable functional microbiota have been identified based on shared genes and on genes explaining differences among the genotypes, respectively. Functional assignments have been obtained based on proteins annotation and Gene Ontology using BlastX and EggNOG. 76,264 genes were shared, whilst 3547 genes explained the differences between the three DZA genotypes and SA, 4662 among the three DZA and the F, A17, J6 genotypes, and 32 among SA and the three genotypes F, A17, J6. 60.5% of these genes have been assigned to biological processes, 22.6% to cellular components and 16.9% to molecular functions. Particularly, inorganic ion, amino-acid, and carbohydrate transport were the most represented in biological processes. This global analysis shows that different genotypes modulate rhizosphere microbial functional genes and provides new insights into the variable and core microbiota among different plant genotypes.

ESTABLISHING A GENOMIC MAP OF NATURAL VARIATION OF VEGETATIVE GROWTH RESPONSE TO BENEFICIAL LEAF BACTERIA IN *ARABIDOPSIS THALIANA*

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Microbiota can largely contribute to plant health by mobilizing and supplying nutrients and by providing protection from abiotic stresses and pathogens. However, the effectiveness of microbiota on plant health is highly influenced by climatic and soil conditions, thereby deeply affecting their use in a wide range of agricultural conditions. In addition, the effects of microbiota on plant health can highly depend on the genotype of the host plant. There is therefore a growing interest in exploring natural genetic variation in plants to exploit the beneficial effects of individual members of the microbiota.

By studying native plant-microbiota interactions and considering genetic variation within the main bacterial species of the microbiota, we established a genomic map of local adaptation in *Arabidopsis thaliana* in response to the main non-pathogenic bacterial members of its leaf microbiota. We highlighted extensive intra-specific genomic diversity within some of the most abundant and prevalent bacterial species of the leaf compartment of 168 natural populations of *A. thaliana* located southwest of France. Genome-wide association studies revealed strong 'host genotype × bacterial genotype' interactions for diverse vegetative growth-related traits. We observed that the genetic architecture was polygenic and dependent on the identity of the bacterial strain, the growth conditions of the plants (in vitro vs field), and the developmental stage at which plants were inoculated. This highly flexible genetic architecture nonetheless revealed an enrichment of genes involved in (i) development, in particular root development, in in vitro conditions, and (ii) in plant innate immunity in field conditions.

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POSTER PRESENTATIONS

EXPLORING THE PLANT GROWTH-PROMOTING AND BACTERIAL WILT CONTROL POTENTIAL OF NOVEL VERRUCOMICROBIOTA ISOLATES IN TOMATO RHIZOSPHERE

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Bacteria belonging to the phylum Verrucomicrobiota have been commonly found in rhizosphere soil. However, bacterial strains within this phylum are largely uncharacterized due to limited cultivation. In this study, we aimed to isolate Verrucomicrobiota strains from tomato rhizosphere soil and evaluate the beneficial effects on tomato plants. To isolate Verrucomicrobiota strains from tomato rhizosphere, rhizosphere soil microbial fraction (MF) was cultivated in a mineral medium supplemented with various carbon sources and antibiotics. Microbiome analysis revealed that bacteria within the phylum Verrucomicrobiota enriched in the medium supplemented with plant root tissue. Through enrichment cultivation of MF, we obtained two novel Verrucomicrobiota species V1 and V2. To assess plant growth-promoting and disease control activity of these isolates, bacterial suspensions of V1 and V2 were treated into tomato cultivars Hawaii 7996 and Zuiko. Compared to control, the treatment of V2 enhanced plant growth in tomato plant. Interestingly, V2 strain intensively colonized tomato roots and stimulated root hair formation and elongation under a hydroponic cultivation system. In addition, the treatment of V1 significantly reduced the severity of bacterial wilt disease in tomato plant compared to control. Taken together, our results revealed that two species of Verrucomicrobiota enhance plant growth and disease resistance in tomato plants.

FLAVOBACTERIUM DAUAE TCH3-2 MUTANTS DEFECTIVE IN TOMATO GROWTH PROMOTION AND STIMULATION OF MICROBIAL COMMUNITY

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Plant-rhizobacteria interactions play an important role in providing benefits to the host plant such as promoting plant growth, activating disease resistance, and enhancing tolerance to abiotic stress. A novel rhizobacteria species *Flavobacterium dauae* TCH3-2 promotes the growth of tomato plants by syntrophic interaction with native soil-mimicking synthetic community (SynCom). However, the underlying mechanism of this syntrophic interaction is unknown. In this study, using the transposon mutagenesis of TCH3-2, we screened Tn-inserted mutants of TCH3-2 defective in plant growth-promotion (PGP) by the combination with SynCom. We obtained 3,500 transposon-inserted TCH3-2 mutants and assessed the syntrophic traits with *Ketobacter* sp., a keystone taxon of SynCom, using softagar overlay techniques. Compared to TCH3-2 wildtype, 53 mutant strains showed the altered syntrophic traits with *Ketobacter* sp. To determine whether the selected mutants are defective in stimulating microbial community for tomato growth promotion, those mutants were co-treated with microbial fraction (MF) of native upland soil in tomato seedlings using hydroponic and soil cultivation system. The treatment of specific TCH3-2 mutants including TM31 and TM60 with soil MF showed reduced PGP activity, compared to TCH3-2 wildtype. Further characterization of the selected mutants is under progress to identify the mutated genes in TCH3-2.

INOCULATION OF A BACTERIAL BIOCONTROL AGENT MODIFIES THE RHIZOSPHERE MICROBIOME AND REDUCES SYMPTOMS CAUSED BY PHYTOPHTHORA CINNAMOMI IN AVOCADO

by Edgar Guevara Avendaño | Mariel García Meléndez | Itzel Anayansi Solís García | Violeta Patiño Conde | Fernando Pineda-García | Guillermo Ángeles | Alfonso Méndez Bravo | Eria Rebollar | Frédérique Reverchon

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The rhizosphere microbiome is an important determinant of plant fitness. However, the incidence of soil-borne pathogens and/or the inoculation of PGPR can alter rhizosphere microbial communities, thereby affecting plant health. Our objective was to elucidate the effect of inoculating *Bacillus* sp. A8a, a PGPR strain with antagonistic activity against *Phytophthora cinnamomi*, on the rhizosphere microbiome of artificially infected avocado trees. We established a greenhouse assay consisting of four treatments: (1) control trees, (2) trees infected by *P. cinnamomi*, (3) trees inoculated with strain A8a and (4) infected trees inoculated with strain A8a. We examined rhizosphere bacterial communities during the infection process through 16S rDNA amplicon sequencing and evaluated plant physiological and anatomical responses to the infection and PGPR inoculation. We found that strain A8a reduced disease incidence by 50% and decreased tannin accumulation in infected avocado stems. Moreover, PGPR inoculation in healthy trees increased the proportion of Magnetospirillaceae, Micropepsaceae and Comamonadaceae in the rhizosphere bacterial community. When inoculated in infected trees, strain A8a enriched the rhizosphere in Chitinophagaceae and Bacillaceae at the early stages of the disease. At the final stage of the disease, we detected a decrease in Rhodocyclaceae in PGPR-inoculated trees, compared with non-inoculated infected trees. Collectively, our findings provide a better understanding of the rhizosphere microbiome response to the inoculation of a biocontrol agent and could help design microbial consortia for the mitigation of Phytophthora root rot in avocado.

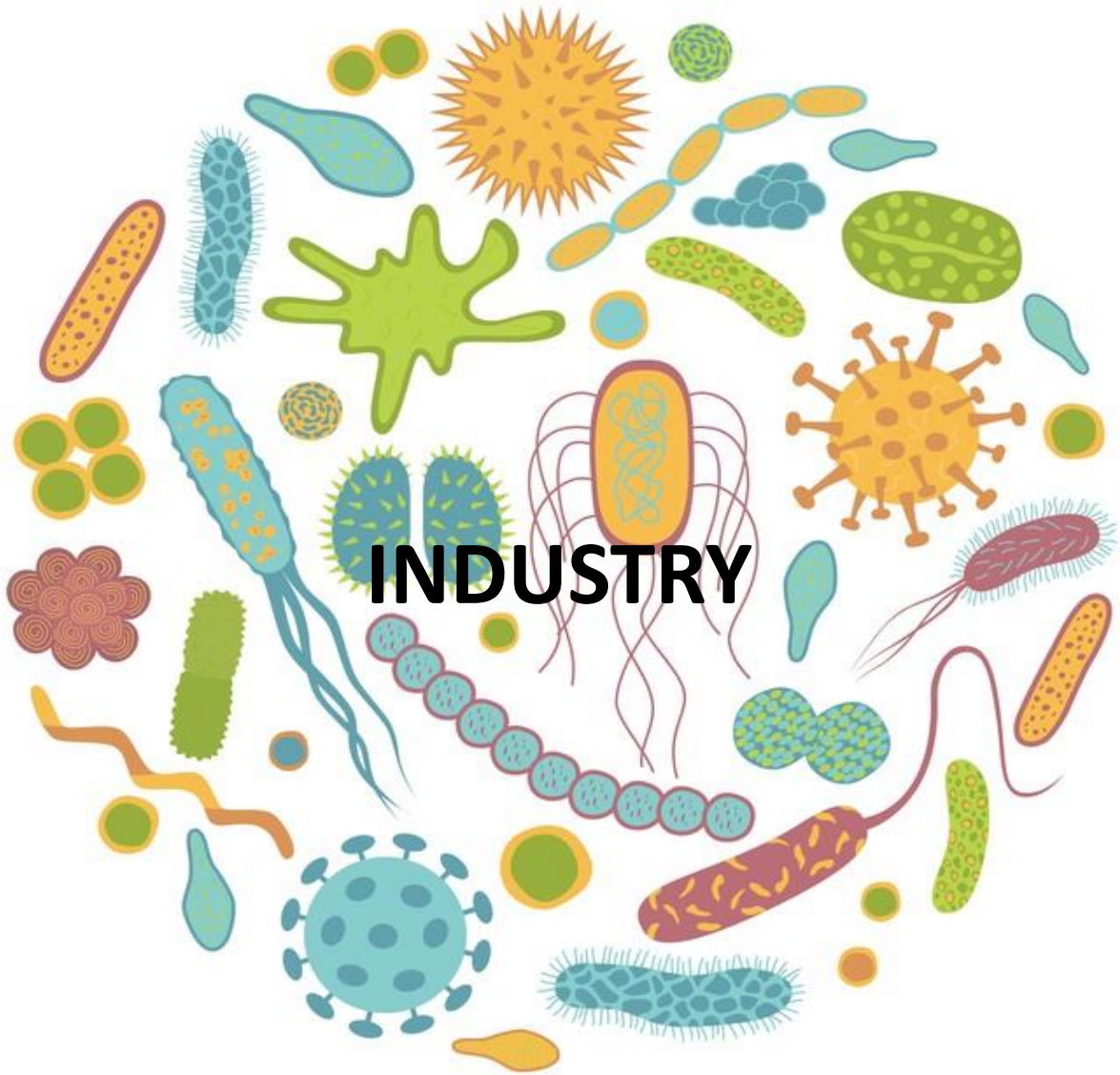
RICE PLANT GROWTH-PROMOTING RHIZOBACTERIA: A FOCUS ON PHOSPHATE SOLUBILIZATION

by Camilla Valli | Alice Melzi | Sarah Zecchin | Lucia Cavalca

Università degli studi di Milano

The application of microbial inoculants is an innovative biotechnology to preserve the productivity and sustainability of rice cultivation. Plant growth-promoting rhizobacteria (PGPR) can be applied directly to the soil by seed inoculation, plant tissue treatments or soil applications, to increase the crop biomass growth and to control several plant pathogens, thus avoiding or reducing chemical control. In particular, PGPR are able to release soluble phosphorous (P) ions from insoluble mineral or organic forms is drawing attention, also in consideration of severe depletion of raw materials for phosphate fertilizer manufacturing. In the present work, 299 bacterial strains isolated from rhizosphere compartments of rice were characterized for different PGP activities. ACC deaminase activity, phytate mineralization and N₂ fixation were the most represented PGP traits. Twenty-one strains possessing most of the tested PGP activities were inoculated ingrowth pouches experiments to determine *in vivo* the ability to promote seed germination. Four PGPR identified as *Pseudomonas koreensis*, *Priestia aryabhatai* and *Bacillus zanthoxyli* showed a major significant promotion of biomass growth. Pot experiments with Green Fluorescent Protein-transformed *Pseudomonas koreensis* strain are ongoing to evaluate plant colonization and rice growth promotion under different levels of bioavailable phosphate.

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PGPR-BASED FORMULATIONS: LAB TO FIELD OPTIMIZATION FOR BETTER EFFICACY

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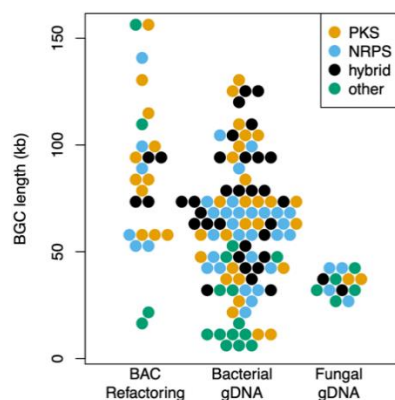
Agricultural products based on PGPR have grown rapidly in the last decade because of stakeholders' interests in foods that are free of chemical residues, in reducing chemical inputs and their negative environmental impacts, and in improving sustainability of soil resources. Acceptance of these products is increasing, but adoption has been comparatively slower with farmers expressing concerns about non-consistent efficacy of most of the products. We evaluated multiple factors that may affect variability of product efficacy from lab to field under different conditions and cropping systems. Many questions, which may help in product development, including strategies that may improve PGPR collection and screening processes were explored. Our results showed trial-to-trial variability in growth promotion by *Bacillus* strains and biocontrol activities during *in vitro* and *in planta* assays did not always correlate. Responses during physiological trait assays such as pathogen growth inhibition, phosphorus solubilization, nitrogen fixation, and protease enzyme, siderophore, chitinase, and biosurfactant production expressed in the laboratory did not reliably predict efficacy in the plant. Thus, *in vitro* and *in planta* tests should be considered independently in selecting effective PGPR candidates for commercialization. Adequate understanding of the active ingredients (organisms), especially the conditions that promote vegetative growth or sporulation, was very important. Careful determination of the appropriate application/delivery methods and formulation types is crucial and may be unique. To successfully reduce and supplement the use of chemical inputs, PGPR-based formulations must be developed to be compatible with widely used agrochemicals and farming practices.

NEW TOOLS FOR TARGETED CLONING AND OVER EXPRESSION OF BIOSYNTHETIC GENE CLUSTERS

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Over 130+ biosynthetic gene clusters (BGCs) ranging from 12 to 150 kb from over 100 diverse bacterial and fungal strains were successfully captured and cloned using CRISPR-based enzymes to precisely excise pathways of interest. To improve BGC heterologous expression, we developed a new Actinobacteria BGC expression vector (pDualP) and a companion vector for *Bacillus* expression that uniquely includes two inducible promoter elements that flank the cloning site. BGCs cloned and conjugally transferred to Actinobacteria or *Bacillus* heterologous hosts include ACT, RED, nystatin, erythromycin, rapamycin, vancomycin, diffidin, bacillusin A, and many dozens of novel clusters. We de-orphaned the stravidin BGC from *Streptomyces* sp. NRRL S-98 in two months using the same inducible approach¹. Second, we observed a substantial enhancement of the antimicrobial activity of heterologously-expressed, soil-derived metagenomic BGCs through induction with pDualP and demonstrate the ability to reconstitute complete BGCs from fragments of metagenomic libraries. Finally, we applied these directed BGC cloning tools to known and novel fungal BGCs for successful heterologous expression in *Aspergillus*, including imizoquin and pestalamide. These results indicate that sequenced BGCs can be cloned intact from complex (meta)genomes, and that direct cloning into a dual-inducible expression vector can greatly accelerate downstream small molecule expression and characterization.



Over 130 diverse BGCs refactored or captured directly from bacterial and fungal gDNA into heterologous expression vectors.

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ORAL PRESENTATIONS

CONSORTIUM BIO INOCULANT TO SUSTAINABLE AGRICULTURE: FROM LABORATORY TO MARKET

by Silvia Maribel Contreras-Ramos | Erika Nahomy Marino-Marmolejo | Flor Yohana Flores Hernandez | Beatriz Genoveva Guardado Fierros | Diego Antonio Tuesta Popolizio

Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco A.C.

PGPR was isolated from cucumber roots, soil, and organic waste; they were characterized and selected with better PGP characteristics to develop a formulation of a consortium bioinoculant for agriculture. This work showed the academic history, pilot system scaling, concept tests, transfer technology, pre-commercial field test, and diverse results. It passed from laboratory to market with different challenges to make the differences and advance in Technology Readiness Levels (TRL) to sure the maturity of the technology. Technology partnerships were built in order to have more support in the development. The formulation showed an exciting increase in yield results on different crops such as blueberries (13% and 10% bigger caliber), onions (24%), cucumber (43%), tomato (44%), potato (26%), chili bell (36%), lemon (20%), wheat (13%), papaya (40%), maize (7%), etc. It has been used in different soil, weather, temperatures, and agroecology management with favorable results for sustainable agriculture. In addition, the farmers observed higher size fruit, homogenization in growing plants and fruits, survival transplantation, and higher resistance to abiotic stress such as extreme temperatures (hot or cold) and drought. Also, users reported better quality fruit for exportation parameters such as size.

DEVELOPMENT OF AN EFFECTIVE AND STABLE PGPB-BASED INOCULANT AS A POTENTIAL TOOL FOR SUSTAINABLE AGRICULTURE

by Beatrice Farda | Fabrizia Pasquarelli | Rihab Djebaili | Enrico Sabbi | Maddalena Del Gallo | Loretta Pace | Marika Pellegrini

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Plant growth-promoting bacteria (PGPB) are microbes with biostimulant and biocontrol capabilities that effectively substitute toxic agrochemicals in sustainable agriculture. Their effect is linked to the application of the necessary number of viable microbial cells (10^7 - 10^9 CFU/ml or CFU/g). Therefore, formulation and cell viability evaluations are crucial in developing PGPB-based inoculants. The present study aimed to develop an effective and stable liquid biostimulant obtained by a simple and less expensive production process. A PGPB consortium composed of four beneficial bacteria (*Azospirillum brasilense*, *Burkholderia ambifaria*, *Gluconacetobacter diazotrophicus*, and *Herbaspirillum seropediceae*) was selected and investigated in cooperation with a biostimulant manufacturer (SPAA Srl). The research performed so far has enabled us to: i) optimize the bioreaction's parameters in a combined bio-fermentation at a laboratory scale, ii) evaluate several protective agents and stabilization techniques to enhance cell viability, and iii) assess the formulation's stability under accelerated shelf-life conditions. The optimal cell density was obtained by stirring a bioreaction at 28 °C for four days and differential addition of the strains. The treatment using glycerol + xanthan gum and a pH shock was the most effective approach to maintain good cell viability after three months of accelerated shelf-life tests. Findings revealed the capability of cost-effective polymers and polyols as protective agents to extend cell viability during storage. These results paved the base for formulating a bioproduct on a larger scale.

DEVELOPMENT OF INNOVATIVE BIOSTIMULANTS USING PANTOEA AGGLOMERANS C1 METABOLITES TO PRODUCE PLANT POSTBIOTICS

by Luziatelli Francesca | Ficca Anna Grazia | Bonini Paolo | Ruzzi Maurizio

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Strains belonging to *Pantoea agglomerans* species are frequently found in association with plant hosts. These bacteria are agronomically relevant for their plant-growth-promoting (PGP) features, biocontrol activity, and involvement in plant disease management. Using comparative genomics, we revealed that, in this species, PGP traits are determined either by highly conserved genes (auxin/indole 3-acetate [IAA] synthesis) or strain-specific genes (nitrate bioremediation). These observations emphasize the need to focus on single strains rather than on the species for developing novel plant biostimulants based on PGP bacteria and their metabolites. By integrating metabolomics and fermentation technologies, this study aimed to define the optimal culture conditions to obtain auxin/IAA-enriched plant postbiotics using *P. agglomerans* strain C1. Metabolomics analysis allowed us to demonstrate that the production of a selected array of compounds with plant growthpromoting- (IAA and hypoxanthine) and biocontrol activity (NS-5, cyclohexanone, homo-Larginine, methyl hexadecenoic acid, and indole-3-carbinol) can be stimulated by cultivating this strain on minimal saline medium amended with sucrose as a carbon source. Targeted metabolomics demonstrated that the aeration efficiency significantly affected the IAA product selectivity and the IAA precursor indole-3-pyruvic acid accumulation in liquid culture. Our findings indicate that multi-omics approaches are essential for clarifying single microbes' PGP potential and using their biochemical machinery to develop bio-based biostimulants with a well-defined composition and well-characterized activities.

METHODOLOGICAL AND INTERPRETATIONAL PROBLEMS IN PLANT GROWTH-PROMOTING BACTERIA INOCULATION STUDIES

by Luz de-Bashan | Paolo Nannipieri

Bashan Institute of Science | University of Florence, Italy

The positive effects of inoculating different plants with plant growth-promoting bacteria/rhizobacteria (PGPB/PGPR) have been widely reported. However, in most cases, the data presented are the results from very limited sets of experiments or were gathered only under greenhouse conditions. Furthermore, reports of using different strains of bacteria are usually full of methodological deficiencies. In the literature on inoculants, it is common to find reports that use fresh weight, lack of proper controls, and incorrect techniques to determine bacterial traits, such as the ability to produce the phytohormone indoleacetic acid or the capacity to solubilize inorganic phosphorus (P) effectively. In addition, in many cases, the reports lack a proper description of inoculation procedures, such as fermentation processes, incorrectly identify strains, show a lack of clear design when producing a consortium, and fail to properly trace the inoculated bacteria on plant roots or in rhizosphere soil. Generally, the fate of the inoculum is not traced, but its detection with the expression of plant-beneficial activities is important to support any direct action on the plant. Based on our experience as journal editors, we present evidence of the pitfalls researchers fall into when reporting on the inoculation of plants with growth-promoting (rhizo)bacteria.

REGENERATIVE AGRICULTURE STRATEGIES AND PGPM APPLICATION COOPERATIVELY FOSTER MAIZE SUSTAINABLE PRODUCTION IN THE MEDITERRANEAN AREA

by Maria Elena Antinori | Gabriele Bellotti | Francesca Di Rico | Federico Ardenti | Giacomo Mortella | Andrea Fiorini | Luigi Lucini | Edoardo Puglisi

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Climate change affects agriculture by two main challenges, i.e. the necessity of reducing GHGs emissions and the contingency of facing drought, temperature increase, reduction of biodiversity. Regenerative agriculture and the application of PGPM both answer to these challenges, but seldom their effects are simultaneously evaluated in field. This work, realized and financed in the framework of PRIMA project SIRAM (Sustainable Innovations for Regenerative Agriculture in the Mediterranean Area), focuses on the application of no tillage plus cover crops, manure/vermicompost fertilization and PGPM application as strategies to foster maize sustainable cultivation in Emilia-Romagna region (Italy).

Nine out of 90 bacterial isolates and three out of 40 fungal isolates from maize rhizosphere were selected on the basis of PGP activities in vitro and in pot experiments. In particular, IAA and siderophores production, ACC deaminase activity, phosphate solubilization, nitrogen fixation and drought resistance were evaluated in vitro, also in terms of effect on seed germination and seedlings development. Different formulations (seed coating and vermicompost inoculum) for selected PGPM were tested both in vitro and in pot in terms of efficacy and shelf-life to find the optimal formula to apply in field. Moreover, the combined action of PGPM and vermicompost fertilization was measured in pot in comparison with standard chemical fertilization.

Simultaneous application of regenerative agriculture strategies and PGPM not only enhances plant yield and nutritional status, thus allowing maize sustainable production in a context of climate change, but also supplies innovative formulas for the application of microbial biofertilizers.



POSTER PRESENTATIONS

MICROBIAL COLLECTION OF PLANT GROWTH PROMOTING RHIZOBACTERIA AT AUBURN UNIVERSITY

by Kathy Lawrence | Auburn University

Dr. Joseph Kloepper began the plant growth-promoting rhizobacterial (PGPR) collection in 1989 looking for strains that promoted plant health, and nutrient uptake. The emphasis was species that could produce spores. Spore-forming rhizobacteria in agricultural practices are versatile, primarily in their capacity to withstand dry treatments, and in their long shelf-life. These qualities, increased their appeal and the practicality of being integrated into agricultural practices and products. Bacterial strains were isolated, organized into collections, and subsequently tested for an array of benefits that they might be able to offer plants. Modes of action were studied, and effects on bacterial, fungal, and nematode plant pathogens as well as plant-damaging insects were evaluated in collaborations with other faculty at Auburn University and elsewhere. Currently, the collection is growing with the addition of microbes from the rhizospheres of plants growing in more diverse habitats. Gram-Negative bacteria are now being studied, as collecting spore-forming rhizobacteria is no longer a restriction. New sub-collections of bacteria have been added from the rhizospheres of native plants growing in; high-saline coastal soils along the Gulf of Mexico, the Chihuahuan desert of the American southwest, and the frigid soils of south-central Alaska. The near 10,000 strains are identified to species using 16S rRNA gene sequencing. The results include individual strain testing for growth promotion. Auburn University's IP Exchange is partnering with agribusiness to further the advancement of biological natural improvement of plant health and meeting the world food supply demands by overcoming plant-limitations caused by drought, cold, and salty soils.

CYANOBACTERIA FROM A CARBON DIOXIDE-RICH SULFUR POND: POLYHYDROXYALKANOATES PRODUCTION AND PLANT-GROWTH PROMOTING TRAITS

by Enrico Sabbi | Rihab Djebaili | Anna Maria D'Alessandro | Daniela Maria Spera | Maddalena Del Gallo | Beatrice Farda | Claudia Ercole | Marika Pellegrini

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The study aimed to characterize the microbiota and culturable cyanobacterial microflora of a carbon dioxide-rich sulfur pond. A metagenomic approach of the 16S rRNA gene was performed on DNA extracted from the samples. A culturable approach, using BG11 media, was used to target the cyanobacterial group. Isolates were screened for their *in vitro* abilities to produce polyhydroxyalkanoates (PHAs) and for their plant-growth promoting (PGP) properties (biostimulant and bioconsolidation properties). FT-IR characterization was performed on PHAs. To test the PGP abilities, useful strains were also pooled in a consortium. Biostimulant properties were assessed for *Solanum lycopersicum*. Bioconsolidation abilities were screened on a sandy soil using a multidisciplinary approach (i.e., functional and microscopic methods). 16S rRNA gene analysis revealed a prokaryotic community rich in uncultured (30.8%) and unknown (33.2%) bacterial genera. Known taxa belonged to the genera *Aeromicrobium* (8.6%), *Haliangium* (4.7%), *Iamia* (4.3%), *Nitrospira* (3.9%), and *Lysobacter* (2.9%). Isolation and characterization of the culturable microflora yielded three pure strains with PHA-producing abilities and PGP traits. Polyhydroxybutyrate (PHB) was the main PHA produced by all the isolates. Trials on *S. lycopersicum* showed improvement in germination and plant growth. Scanning electron microscopy (SEM) analysis of the soil treated by the consortium revealed the presence of bacterial footprints, bacterial cells, and firm interconnection bridges between the sand particles. The results obtained from these experiments represent a good start for further studies directed toward bioremediation, biocontrol of pathogens, construction, and artwork restoration.

DEVELOPMENT OF INNOVATIVE PROCESSES FOR PRODUCTIVITY AND SUSTAINABILITY IN AGRICULTURE

by Marika Pellegrini | Rihab Djebaili | Daniela M. Spera | Maddalena del Gallo | Mahmoud Kitouni | Vittorio Di Giammatteo | Mario Nucci

University of L'Aquila | Université F.Mentouri Constantine | CO.VAL.PA

As part of the PRO.S.A. project PEI (EUROPEAN INNOVATION PARTNERSHIP), an innovative cultivation method based on microorganisms in harmony with the soil resource has been developed for the horticulture and potato sector, guaranteeing safe, high-quality, and ecologically sustainable products. To increase agricultural productivity per unit area, many agricultural practices involve the use of chemical fertilizers, which in the long term negatively affect the physical-chemical properties and permeability of the soil, compromising the expansion of plant roots. Ultimately, this leads to a reduction in production yields, a decrease in the quality of crops and the onset of environmental problems and groundwater pollution. The innovation introduced within the project is the use of PGPBs that can improve the efficiency of nutrient use by improving the quality of production and optimizing the metabolic processes of plants. The experiments conducted so far have shown: (1) Improvement of soil nutrient release; (2) Improvement of the amount of phosphorous in tubers. The results obtained have been valorized through a patent with international extension.

POTENTIAL OF COMMERCIAL BIOPRODUCTS TO CONTROL FUSARIUM OXYSPORUM F. SP. CUBENSE RACE 1 (FOC R1) AND TROPICAL RACE 4 (FOC TR4) IN COLOMBIA

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Fusarium wilt of banana (Panama disease) is the most limiting disease of this crop. The detection of the disease on the resistant cultivar Cavendish in 2019 in Colombia caused the declaration of phytosanitary emergency. Public and private research institutions and growers have joined efforts to adapt known control methods and develop new ones. Considering the biological control promissory to face this problem, this study wanted to determine the potential of available bioproducts (T1 to T9) to control Foc R1 and Foc TR4. Chosen treatments had a permit for use against *Fusarium* spp. in other crops. *In vitro* experimental set on Foc R1 showed that T3, T9 and T4 all based on *Trichoderma* spp. and T8 (*Bacillus* spp.) significantly reduced the growth of Foc. Viable population of Foc under *in vitro* incubated soil was temporary reduced by T3, T1, T4 and T9 (*Trichoderma* spp.) However, *in vivo* bioassay showed to T7 based on *Bacillus amyloliquefaciens*, *Bacillus pumilus* and *Agrobacterium radiobacter* K84, and to T2 based on *T. harzianum* as the most effective reducing the disease incidence (61% and 44%, respectively). Similar results were observed on Foc TR4, however, only T7 showed consistency between replications of the entire experiment, and prevented the entry of Foc to inner plant tissue. T7, T2 and T9 mitigated plant physiological damages caused by Foc. Differential display of biocontrol traits between *in vitro* and *in vivo* conditions were observed. We found bioproducts in the Colombian market with high potential to control Foc R1 and Foc R4T.

STUDIES OF VETERINARY AND BIOMEDICAL APPLICATIONS OF THE PGPR STRAIN BACILLUS VELEZENSIS AP183 THAT PRODUCES THE POTENT ANTIBIOTIC BACILLUSIN A

by Mary J. Sandage | Auburn University

PGPR strains produce bioactive secondary metabolites that inhibit plant and animal pathogens. We investigated a collection of PGPR strains for their *S. aureus*-inhibitory capacity in biofilm, mouse and bovine models. *B. velezensis* AP183 provided the most potent inhibition of *S. aureus* proliferation and bioluminescence in a mouse cutaneous wound ($P = 0.02$). Histology revealed abundant Gram-positive cocci in control wounds that were reduced in *B. velezensis* AP183-treated tissues. Experiments were conducted to evaluate *B. velezensis* AP183 prevention of *S. aureus* biofilm formation on a tracheostomy tube substrate. *B. velezensis* AP183 formed a biofilm on a tracheostomy tube inner cannula substrate, and this biofilm was antagonistic to *S. aureus* colonization. *B. velezensis* AP183 was also observed to inhibit the growth of *S. aureus* isolates originated from bovine mastitis cases. All mammary quarters in four cows were induced to have grade 1 clinical mastitis by intramammary inoculation of a *S. aureus* mastitis isolate; following mastitis induction, eight quarters were treated with *B. velezensis* AP183 and milk samples were collected from pretreatment and post-treatment samples for 9 days. In groups treated with *B. velezensis* AP183, SCC and abundance of *S. aureus* decreased with significant reductions in *S. aureus* after 3 days post-inoculation with AP183 ($P = 0.04$). A milk microbiome analysis revealed significant reductions in *S. aureus* relative abundance in the AP183-treated group by 8 days post-inoculation ($P = 0.02$). *B. velezensis* AP183 can inhibit *S. aureus* biofilm formation and its proliferation in murine and bovine disease models.

THE POTENTIAL OF PGPR ACTIVITY IN PHYTOREMEDIATION_CASE STUDIES OF CONTAMINATED SOILS

by Elisabetta Franchi | Danilo Fusini | Ilaria Pietrini | Francesca Pedron | Meri Barbafieri | Irene Rosellini | Marco Vociante | Giannantonio Petruzzelli

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Phytoremediation is the most sustainable and low-cost technology to recover soil and water contaminated by hydrocarbons and heavy metals. However, excessive abiotic stress severely limits plants' growth and survival. Plant root exudates engage suitable bacteria (PGPR) to support their growth in these critical conditions. PGPR can alleviate the soil phytotoxicity of metals by altering their bioavailability and increasing the translocation of the metals within the plant or excluding their uptake. Lead (Pb), mercury (Hg) and arsenic (As) are among the most toxic metals (metalloids). In this work, we report some results of phytoextraction with three different soils contaminated by i) As, ii) Pb and iii) As, Hg. The first case study relates to a Pb contamination deriving from a decommissioned fuel depot. The inoculation of PGPR (endophytes isolated from native plants: *Lotus corniculatus*, *Sonchus tenerrimus* and *Bromus sterilis*) in microcosms with *Brassica juncea* led to an increase of up to 25 times phytoextraction of Pb in the shoots. The second trial addressed persistent arsenic contamination in a brownfield site and tested the performance of *Brassica juncea*, *Helianthus annuus* and *Zea mays*. Adding the As mobilizing agent K₂HPO₄ combined with a microbial consortium of indigenous PGPR led to an increase of up to 140% of total As uptake in *Brassica juncea*. In the third case (mixed contamination of As and Hg), considering total accumulation in *Brassica juncea* treated with an autochthonous PGPR consortium, uptake increases of up to 85 % for As and up to 45 % for Hg were observed.

THE ROLE OF SECONDARY METABOLITES IN THE DIVERSITY OF BACILLUS SPP. AN IMPORTANT GROUP OF PGPR MICROBES

by Christopher Dunlap | Crop BioProtection Research Unit, USDA, ARS Peoria, IL

Lipopeptides constitute an important family of compounds that are produced by species in the *Bacillus subtilis* group. There are three main groups of lipopeptides, surfactins, iturins and fengycins. The compounds that comprise the iturin family are: iturin, bacillomycin D, bacillomycin F, bacillomycin L, mycosubtilin and mojavensin. The compounds that comprise the surfactin family are: surfactin, lichenysin and pumilacidin. While the fengycin family is comprised of several structural variants of fengycin. These lipopeptides are prominent in many *Bacillus* strains that have been commercialized as biological control agents against fungal plant pathogens and as plant growth promoters. The compounds are cyclic peptides with a variable length alkyl sidechain, which confers surface activity properties resulting in an affinity for fungal membranes. This study examines the diversity of these compounds in the thousands of genomes available for this group of species. The distribution of these compounds among the *B. subtilis* group species suggests that they play an important role in their speciation and evolution.

USE OF AVAILABLE TECHNOLOGIES (BIOLOGICAL AND GENETIC CONTROL) TO REDUCE LOSSES CAUSED BY *PLASMODIOPHORA BRASSICAE* IN BROCCOLI

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Clubroot caused by *Plasmodiophora brassicae* is the main constraint of cruciferous crops worldwide. This disease is difficult to control due to the complex life cycle of the pathogen, the high concentration of resting spores released into the soil, and its survival over time, causing serious losses in broccoli, cauliflower, and cabbage crops in all vegetable-producing areas in Colombia. Cultural methods such as the use of limestone to increase soil pH, crop rotation, the removal of symptomatic roots from the field are not widely practiced. Disinfection protocols of agricultural tools, and footwear have not been implemented to limit pathogen's dispersal. Resistant cultivars are not available or not fully meet the requirements of all markets and there are not chemical options registered against this pathogen. The objective of this research was to implement available technologies to reduce this disease. To this end, we tested two commercial bioproducts, one based on three rhizobacteria, and the other one based on *Trichoderma koningiopsis* Th003 (Tricotec® WG). Additionally, we tested the resistant broccoli cv. Monclano® (Syngenta seeds®) against two *P. brassicae* isolates from farms in Pasto, Nariño. The evaluations were done both under greenhouse and field conditions. Our results showed that the rhizobacteria-based bioproduct significantly reduced the disease incidence and severity both in the greenhouse and field. Also, the Monclano® cultivar is resistant against the *P. brassicae* strains from horticultural soils in Nariño. These results suggest overall high potential of local available commercial tools to reduce losses caused by *P. brassicae* in cruciferous crops.

DIRECTED BGC CLONING COMBINED WITH BACTERIAL AND FUNGAL EXPRESSION TOOLS UNCOVER NOVEL BIOSYNTHETIC MECHANISMS AND VALIDATE METABOLOGENOMICS

by David Mead | Terra Bioforge

Over 130+ biosynthetic gene clusters (BGCs) ranging from 12 to 150 kb from over 110 diverse bacterial and fungal strains were successfully captured and cloned using CRISPR enzymes to precisely excise pathways of interest. To improve heterologous expression, we developed a BGC expression vector (pDualP) for Actinomycetota and a companion vector for Bacillus that uniquely includes two inducible promoter elements that flank the cloning site. BGCs cloned and conjugally transferred to heterologous hosts include ACT, RED, nystatin, erythromycin, vancomycin, difficidin, bacillusin A, and many dozens of novel clusters. We rapidly de-orphaned the stravidin BGC from Streptomyces sp. NRRL S-98 using this inducible approach. Second, we observed a substantial enhancement of the antimicrobial activity of heterologously-expressed, soil-derived metagenomic BGCs through induction with pDualP and demonstrate the ability to reconstitute complete BGCs from metagenomic library fragments. Finally, we applied these directed cloning tools to known and novel fungal BGCs for successful heterologous expression in Aspergillus, including imizoquin and pestalamide. These results indicate that sequenced BGCs can be cloned intact from complex (meta)genomes, and that direct cloning into a dual-inducible expression vector can greatly accelerate downstream small molecule expression and characterization.

DEVELOPMENT OF METHODS TO EFFICIENTLY CHARACTERIZE BIOCONTROL PRODUCTS

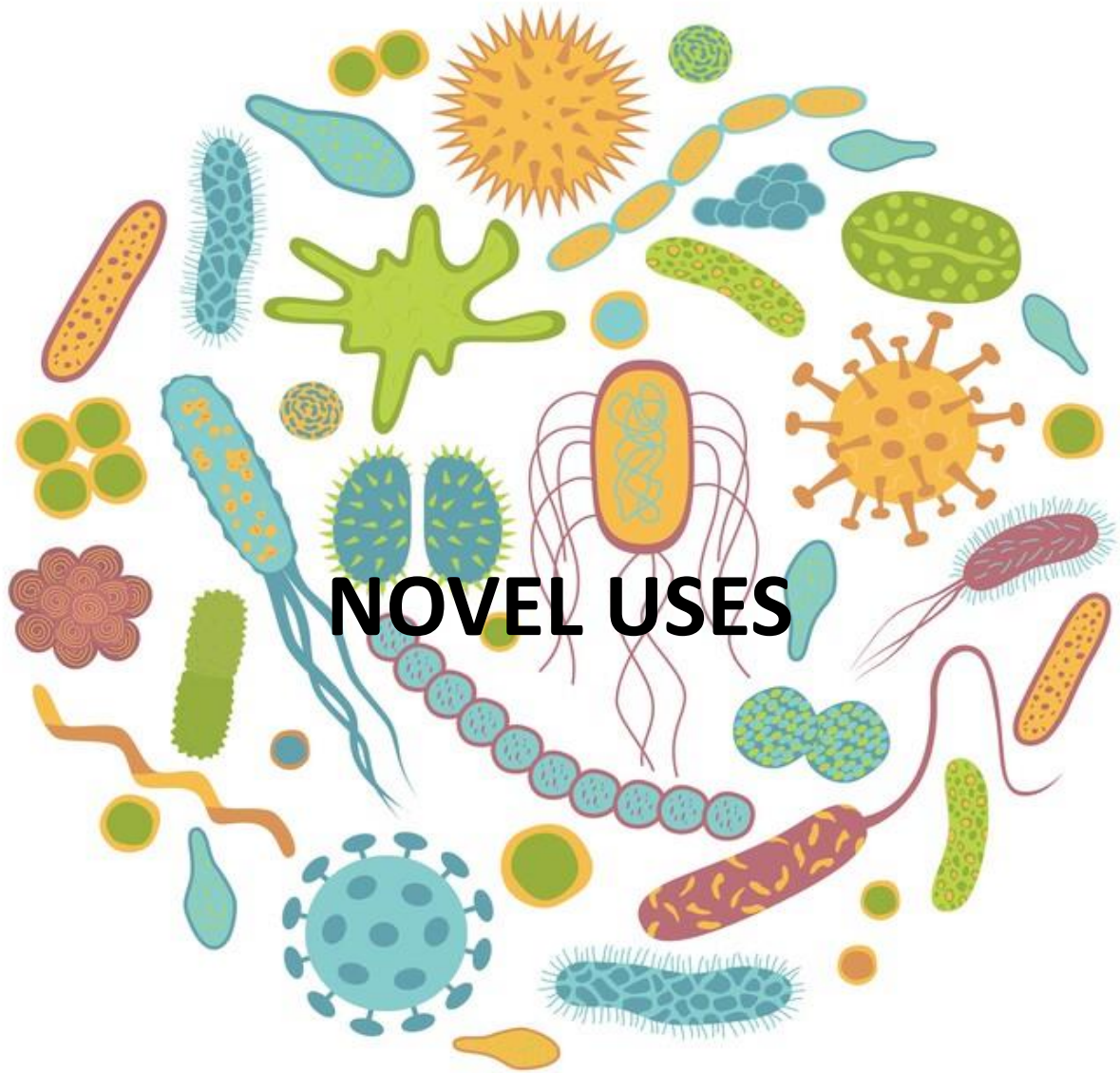
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The use biocontrol and biostimulant PGPR strains is a promising strategy to lessen chemical fertilizers and pesticides application in a context of agroecology (1). One challenge to develop reliable bioproducts is to assess the efficient plant or soil colonization of those PGPR strains, while they are in competition with the soil microflora. To tackle this challenge, IAGE has developed a digital PCR quantification method to detect specifically a PGPR strain developed by Lallemand Plant Care. Pots of bulk and planted soil (raygrass) were inoculated with the strain at known concentration (CFU/g soil) and soil DNA extraction were then performed, followed by dPCR assay to evaluate the specificity and sensitivity of IAGE method. Results have shown that the dPCR assay (i) allow quantification at the strain level, and (ii) with a good correlation with expected concentration (allowing quantification as low as 10 CFU/g soil). Results have also shown dPCR is an efficient and reliable tool to monitor PGPR in fields and assess their efficiency in rhizosphere/root colonization. IAGE is currently developing methods to quantify endophyte and epiphyte strain, allowing PGPR monitoring on various samples and future developments will allow to address only viable cells.

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EXPLORING THE MUTUALISTIC INTERACTION BETWEEN PGPB AND MICROALGAE AND ITS BIOTECHNOLOGICAL POTENTIAL

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Microalgae are promising organisms for the sustainable production of metabolites of commercial interest. Several studies have revealed that microalgae growth and cellular storage of these metabolites can be significantly enhanced by co-cultivation with PGPB. The mutualistic interaction between *Azospirillum brasilense* and the green microalgae *Chlorella* spp. significantly increased microalgal biomass and metabolites such as pigments, lipids, and carbohydrates. In addition, it has been found that bacteria affect the activity of enzymes related to the metabolism of ammonium, phosphorus, fatty acids, and starch. The interaction's mechanism is an exchange of molecules between the two microorganisms, as reported by nanoSIMS, HPLC, and Nuclear Magnetic Resonance (NMR). The microalgae produce tryptophan and thiamine, which the bacteria use to synthesize indoleacetic acid (IAA). In return, IAA and vitamins synthesized by the bacteria affect the growth and metabolism of the microalgae. Glycerol and uracil are molecules that also participate in the mutualistic association. The positive effects of bacteria on microalgae growth and metabolism can be observed without attachment, even by the bacterial production of volatile organic compounds. All these significant metabolic changes during the co-culture are interlinked and offer several biotechnological applications, including wastewater treatment and the production of carbohydrates, lipids, and photosynthetic pigments. The latest application involves using *A. brasilense* to increase CO₂ fixation by microalgae from biogas. Transcriptomic analysis of *A. brasilense* grown under a biogas atmosphere shows a series of genes regulating processes that could be essential for adaptation to the stressful condition generated by biogas.

IMPROVING PGPR EFFICACY VIA A SYMBIOTIC SEED TREATMENT

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Although PGPR can enhance crop productivity, field studies have historically shown lower yields and reduced PGPR efficacy compared to greenhouse studies. A comparative genomic analysis of *Bacillus velezensis* PGPR strains found that all strains contained genes for pectin utilization, and could use pectin or a pectin-rich amendment such as orange peel (OP) powder as a carbon source.¹ Inoculation of soybean seeds with 10⁶ CFU *B. velezensis* AP193 and 10 mg OP per seed resulted in soybean cultivar-specific responses, with some soybean cultivars having up to a 60% increase in growth parameters, whereas for other cultivars no statistically significant growth enhancement was observed.² The lack of response to the symbiotic seed treatment in some cultivars may be influenced by the degree to which OP supported *B. velezensis* growth. We therefore identified PGPR strains that had rapid growth on OP, thereby selecting PGPR strains that could quickly utilize pectin and produce plant growth-mediating factors. Among six *B. velezensis* strains tested in a greenhouse experiment, strains AP191, AP215, and AP216 showed the best results in plant growth promotion when supplemented with OP, and *B. velezensis* AP191 with OP significantly enhanced pod dry weight by 15.8%. A one-time soybean seed inoculation with AP191 and OP showed the greatest yield under field conditions, being the only treatment significantly different from the control treatment ($P < 0.05$; + 0.5 ton/ha), a 9.4% yield increase. Current studies are evaluating formulations of a symbiotic seed treatment to simultaneously provide growth promotion and disease control activities.

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ORAL PRESENTATIONS

BACTERIAL CONSORTIUM INDUCES SYSTEMIC RESISTANCE AND PROMOTES PLANT GROWTH VIA BACTERIAL MOVEMENT AND ENDOPHYTIC COLONIZATION

by Hangyu Dong | Utrecht University

Hyaloperonospora arabidopsidis (*Hpa*) activated *Arabidopsis thaliana* foliar defense and promoted the enrichment of *Xanthomonas* sp WCS2014-23 (X), *Stenotrophomonas* sp WCS2014-113 (S) and *Microbacterium* sp WCS2014-259 in the rhizosphere, which as a bacterial consortium induced systemic resistance and promote the growth of plant. Here, we found that combining X and S significantly increased the fresh weight of *Arabidopsis thaliana* plants than single bacterial and mock treatments in the absence of *Hpa*. To visualize bacterial colonization and movement, a luminescent strain of X (*XLum*) was created, and a Luminometer was used to quantify the colonization. *XLum* traveled along the roots to the leaves during 14 days after inoculation and moved faster when the leaf was inoculated with *Hpa*. After plant surface disinfection, the luminescent signals were detected in both roots and leaves, proving *XLum* as an endophyte that enters the root system and may move from inside the root to the leaf. The luminescence value of the leaf washing-off from *XLum* and S treatment was significantly higher than that of *XLum* alone, indicating S may contribute to the colonization of X on leaves. Together, *Xanthomonas* sp WCS2014-23, as an endophyte, promoted plant growth with *Stenotrophomonas* sp WCS2014-113, moved along the root to the phyllosphere, and colonized, which may be an effective way to participate in the interaction with *Hpa* and induce systemic resistance.

MICROALGA–AZOSPIRILLUM CONSORTIUM FOR CO₂ FIXATION FROM BIOGAS

by Azul Valeria Chacón Hernández | Oskar Alejandro Palacios López | Francisco Javier Choix Ley

Universidad Autónoma de Chihuahua, Facultad de Ciencias Químicas | CONACYT

The capability of microalgae to fix CO₂ from biogas through their photosynthetic process and convert it into high-value metabolites as carbohydrates, lipids, and proteins is considered an environmentally sustainable biotechnology strategy. Today, numerous studies have demonstrated the direct effect of IAA produced by *Azospirillum*, a plant growthpromoting bacterium, on photosynthetic activity and cell compound accumulations of microalgae. Biogas is a by-product from the anaerobic digestion of organic waste and considered an important energy source since it is composed of CH₄ (75%) and CO₂ (25%); although, this composition could be detrimental for several microalgae and bacteria. Besides, IAA biosynthesis by *Azospirillum* is negatively or positively regulated by environmental factors and biotic or abiotic stress. In this study, the interaction of *Azospirillum brasilense* was assessed with two microalgae genera, *Scenedesmus* and *Chlorella*, in order to evaluate the capability of this bacterium to maintain its mutualistic association with both microalgae under the stressful composition of biogas as well as its effect on the CO₂ capture from this gaseous effluent in both microalgae. The results demonstrate that *A. brasilense* maintained a chemical and physical affinity with both microalgae cultured under biogas, mainly supported by the metabolic exchange between both microorganisms, as IAA and Tryptophan (Trp), respectively; the physiological activity improved and CO₂ fixation increased, as well as growth and cell compounds accumulation in each microalga evaluated. Overall, these results propose the mutualism interaction of *A. brasilense* with microalgae for improving biotechnological processes based on microalgae as CO₂ capture and their bio-refinery capacity.

NEW PLATFORM ENABLING PLANT-MICROBES CO-CULTIVATION AND THE STUDY OF THE RESULTING PLANT-PROTECTION MECHANISMS

by Wendalina Tigani | Leiden University

Endophytes communicate with their host through a complex exchange of signals. The reciprocal stimulation to release helpful compounds, such as antimicrobials, is likely contributing to this partnership's resilience. Nevertheless, an increase in endophytic antimicrobials release upon plant signal has never been reported. Due to technical limitations, most plant-associated microbes are studied *in vitro*, missing the eliciting role of the plant, and biasing the selection toward *in vitro* production of bioactive compounds, which may not explain *in situ* antagonism. We created a platform to explore plant's ability to enhance the production of bioactive metabolites by its microbial partners. We grow liquid cultures of *Arabidopsis* with or without endophytic *Streptomyces* isolates and use the spent media for bioactivity assays and metabolites analysis. We identified a streptomycete producing a bioactive phenylalanine homolog in response to co-cultivation with *Arabidopsis*. This resulted in plant protection from the pathogen *Rhizoctonia solani*, and provided the first report of endophytic antimicrobials production enhanced by plant cues. Importantly, this stimulation was also triggered by other two plant species, suggesting the conservation of the plant cue(s) and the possible translation of these results in crop protection measures. Moreover, we were also able to observe the stimulation of plant immunity, an indirect plant-protection mechanism exerted by endophytic *Streptomyces*. Eventually, we show that pathogenic microbes can also take advantage of the colonized plant to increase their antimicrobial production. Few plant-associated *Pseudomonas* strains produce the same antifungal when co-cultured with *Arabidopsis*, underlining the importance of plant cues both in beneficial and pathogenic interactions.

PLANT GROWTH-PROMOTING RHIZOBACTERIA (PGPR) MODULATES SWEET CORN-CORN EARWORM INTERACTIONS

by Tigist Tolosa | Jocelyn Elliott

University of Maryland Eastern Shore | University of Maryland Eastern Shore

Plant Growth-Promoting Rhizobacteria (PGPR) induces systemic resistance against plant diseases and insect herbivores in plants. The impact of single and blends of PGPR strains on plant-insect interactions with a focus on feeding and oviposition behavior of insect herbivores was studied using sweet corn (*Zea mays*) and corn earworm (CEW) (*Helicoverpa zea*) as a model system. Sweet corn plants were treated with single and blends of PGPR strains that include Blend 5 consists of PGPR strains AP-209 and AP-136, whereas Blend 8 consists of three strains, AP-136, AP-218, and AP-219, and a single strain, AP-209. The control sweet corn plants were untreated with PGPR. Adult female CEWs laid a significantly low number of eggs on PGPR-treated plants compared to the number of eggs laid on untreated sweet corn plants. Moreover, the leaf area damage inflicted by the larvae was higher in untreated corn plants when compared with the PGPR-treated plants. Interestingly, plants treated with the single strain blended with other strains were less preferred by the adult and larvae. These findings reveal that treating plants with rhizobacteria may induce significant physiological changes with potential ramifications for plant-insect interactions.

POTENTIAL USE OF PGPR FOR MORE SUSTAINABLE PERENNIAL FORAGE PRODUCTION

by Leanne Dillard | David Held | Russ Muntifering | Megan Griffin | Madison Cole | Kayla Sullins

Auburn University

Recent research at Auburn University has focused on the use of PGPR in perennial forage systems, such as bermudagrass (*Cynodon dactylon*). Forage production of bermudagrass is consistently similar with the use of PGPR and reduced N fertility in bermudagrass, even though bermudagrass is a high N-removal crop. Additionally, nutritive value (crude protein, neutral detergent fiber, and acid detergent fiber) is not negatively affected when PGPR is applied with half the recommended rate of N fertilizer. These results have been seen in several management systems in the southeastern US including fall stockpiling, hay production, and grazing with beef cattle. These results could be the result of increased N use efficiency, which was observed in bermudagrass treated with PGPR and varying levels of N fertility. While other research indicates that tank mixing commonly-used herbicides and multiple PGPR strains did not reduce the efficacy of the herbicide or the viability of the PGPR strains. Our latest work indicates that soil health parameters may improve over time when PGPR is used compared to conventional fertilizer inputs. Specifically, greater soil respiration and soil mesofauna were observed with PGPR compared with conventional fertility management. Due to the large number of forage crops currently used, these results may not apply to other species due to the variety of plant physiological pathways and growth habitats. A need to expand research into other perennial and annual forage crops is necessary to fully evaluate PGPR use in forage systems.