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IV Latinamerican Workshop of PGPR
26TH to 28TH August | Foz do Iguaçu/PR

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Embrapa Agrobiologia
Ministério da Agricultura, Pecuária e Abastecimento

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Verônica Massena Reis
Jerri Edson Zilli
Editores Técnicos

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Editores Técnicos

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Presentention

The XVI Symposium on Biological Nitrogen Fixation in Non-Legumes, together with the IV PGPR Latin American Workshop, brought together over 300 participants from 14 countries. The event was held in Foz do Iguaçu from the 26th to the 29th, 2019. It covered a wide range of disciplines including evolution, ecology, genomics, biochemistry, physiology, production, crop engineering and new applications of plant growth promoting microorganisms. There were lectures by international experts invited from Brazil and other countries including Argentina, Australia, Germany, Spain, United States, United Kingdom, Uruguay, besides others. There was posters and oral presentations selected from the submitted abstracts, included in this document. The main objective of this event was to discuss the aspects of microbial use and their interaction with agricultural crops, in order to combine basic and applied research focusing on biological products to increase crop yield. Two national scientists were honored, Dr, Robert Boddey of Embrapa and Dr. Fabio Pedrosa of the Universidade Federal do Paraná, for their lives dedicated to research on this subject. For the Latin American Workshop the honoree was Dr. Anton Hatmann, partner of several research projects in the area of interaction between diazotrophic bacteria and cereals such as wheat, corn and sugarcane. Embrapa was the organizer of this event that had the partnership of CNPq, Capes and different partner companies in the development of technologies for the field. A success story of our country and an example for the world.

Gustavo Ribeiro Xavier
Director-General
Embrapa Agrobiologia

Summary

Introductory Welcome

On behalf of the Local Organizing Committee, we are honoured to announce the organization of the XVI Symposium on Biological Nitrogen Fixation with Non-Legumes, together with the IV Latin-American workshop on PGPR and the XIX RELARE, in Foz do Iguaçu (Iguassu Falls, Brazil) from 26-31th August 2018. The event will cover a wide range of disciplines including evolution, ecology, genomics, biochemistry, physiology, crop production, crop engineering and new applications of growth-promoting microorganisms. The Symposia will be comprised of plenary talks by international experts as well as posters and oral presentations chosen from submitted abstracts. We welcome participants from academia, industry as well as from the public and farming sector and look forward to fruitful discussions on the latest developments in this field.

Hosts and Site

Hosts

Empresa Brasileira de Pesquisa Agropecuária

Our mission is to provide research, development, and innovation solutions for the sustainability ofagriculture and for the benefit of Brazilian society.

Embrapa Agrobiologia

Basic theme research unit which is a reference for studies focused on knowledge advances in the area of Biological Nitrogen Fixation (BNF), and a pioneer in the development of research on organic agriculture and recovery of degraded areas.

Site

Hotel Golden Park International Foz

Rua Almirante Barroso, 2006. Centro. CEP 85851-010. Foz do Iguaçu, Paraná State of Brazil.

Scientific Committee

Brazil

- Veronica Massena Reis - Embrapa
- Jerri Édson Zilli - Embrapa
- Bruno José Rodrigues Alves - Embrapa
- José Ivo Baldani - Embrapa
- Mariangela Hungria da Cunha - Embrapa
- Fabio Bueno dos Reis Junior - Embrapa
- Adriana Silva Hemerly - Universidade Federal do Rio de Janeiro
- Fabio Lopes Olivares - Universidade Estadual do Norte Fluminense
- Emanuel Malpempi de Souza - Universidade Federal do Paraná
- Luciane Maria Pereira Passaglia - Universidade Federal do Rio Grande do Sul

International

1) Dr. Anton Hartmann - Alemanha
2) Dr. Fabricio Dario Casan - Universidad Nacional de Río Cuarto, Argentina
3) Dr. Ray Dixon - John Innes Centre - Reino
4) Dr. Frederico José Battistoni Urrutia - Instituto de Investigaciones Biológicas “Clemente Estable, Uruguai
5) Dra. Maribel Eugenia Parada Ibañez - Universidade de La Frontera, Temuco - Chile
6) Professor Yoav Bashan - Auburn University, USA

Organization

FB EVENTOS - Congress website: http://www.mpcp2018.com.br/index.php/

Local Organizing Committee

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History of Previous Events

BNF Non-legume Symposia (BNF-NL)

The BNF Non-legume Symposia (BNF-NL) have a long tradition since the 1980s. This symposium was separated from the group of bacteria that produce nodules in plants and has as an example the legume family. This research was born in Brazil with the work of the researcher Johanna Döbereiner in the description of species and genera of nitrogen fixing bacteria present in plants of agricultural interest, especially cereals and energetic grasses such as sugarcane.

In the years 2008 and 2012, the 11th and 13th BNF-NL-symposia were already organized successfully as satellite meetings of the ENFCs in Ghent and Munich, respectively. The 14th BNF-NL-Symposium was organized by our Chinese colleagues as satellite to the Asian Congress of Plant-Microbe Interaction in Cheng-Du in October 2014. The last meeting was organized as a satellite meeting of the European BNF Conference, in Budapest - Hungary.

Latin-American Workshop of PGPR

The LATIN-AMERICAN WORKSHOP OF PGPR is a recent meeting, based on the initiative of research and application of Plant Growth-Promoting Rhizobacteria (PGPR). This topic has grown worldwide to the point that the 1st regional meeting was initiated in 2009 by the Asian PGPR Workshop. The 1st meeting in America was organized in Colombia / Medellín by Dr. Camilo Ramírez and was a joint venture with the 9th International PGPR conference with the International Committee leadership by Dr. Joseph W. Kloepper and Dr. David M. Weller. In 2014, the 2nd workshop was organized in Argentina / Los Falda by Dr. Fabricio Cassan and Dr. Claudio Valverde.

After this previous organizations, it was clear that PGPR is an important theme to be discussed in Latin America and new groups were formed in this important topic for agriculture, especially in the tropics and desert areas of this continent. In 2016 Chile organized the 3rd Workshop in Pucon by Dra. Maribel Parada Ibañez and Dr. Gonzalo Tortella Fuentes.

Why a join event?

Overall, the thematic areas defined for the workshop were, on the one hand, defined in terms of microbial taxa (*Bacillales*, *Azospirillum*, *Pseudomonas*, *Burkholderia*, actinomycetes, and other PGPR microorganisms).

Overall subjects were probiotic mechanisms of plant growth promotion by other taxa as well.

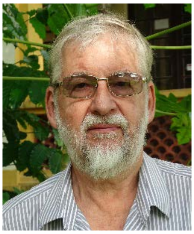
This year we included yeasts, fungi and combination of symbiotic bacteria with associative ones.

Themes of biocontrol, rhizoremediation, biotic and abiotic interactions, molecular ecology of rhizosphere, ecological impact of inoculants, and industrial aspects of inoculant production.

This meeting is an effort of scientific communication between different taxa, plants and environmental conditions. There are no borders. We need to put people together, like these microorganisms do! So welcome to Foz do Iguaçu, Brazil.

Honoured Scientists: Life's dedicated to Science

Robert Michael Boddey



Born in Birmingham, England, UK, 1948. Graduated (BSc) in metallurgy, Imperial College, London) in 1969. Volunteer (VSO) chemistry and maths teacher at a Secondary School, Makeni, Northern Province, Sierra Leone, West Africa from 1969 to 1972. Studied for a further BSc at Leeds University (UK) in Agricultural Chemistry (1972-1975). Moved to University of the West Indies, St Augustine, Trinidad and Tobago for a PhD, thesis entitled "Biological nitrogen fixation associated with the wetland rice crop". Left Trinidad in 1979 to work for 18 months with Dra Johanna Döbereiner at the Embrapa Soil Biology unit (now known as Embrapa Agrobiologia), Seropédica, Rio de Janeiro State. Remained there until today being contracted permanently by Embrapa in 1986. Married with a Brazilian wife and one son, became a Brazilian citizen in 2007. His work originally was concentrated on using and improving different methods (both with the stable isotope 15N and also non-isotopic methods) to quantify biological nitrogen fixation associated with forage grasses, sugarcane and rice as well as grain, tree and forage legumes. More recently the work has expanded to evaluation of the sustainability of different agroecosystems (e.g. grazed pastures, soybean-based crop rotations under direct drill or conventional tillage, sugar cane) using long term experiments, stable isotopes (^{13}C and ^{15}N) and studies on nutrient cycling. This has recently led the team to work on carbon "sequestration" and N_2O emissions from various agroecosystems and the importance, or otherwise, of biofuels as a strategy to mitigate CO_2 emissions. He has published over 170 papers in refereed journals, and a further 90 or so full-length articles in Conference proceedings or as Book Chapters. Citations Web of Science 5670, Factor H 43. Was awarded "Embrapa Researcher of the Year" in 2010.

Fabio de Oliveira Pedrosa

Professor Fábio de Oliveira Pedrosa of the Biochemistry department of Universidade Federal do Paraná. Started his carrier as an Agronomist at the Universidade Federal Rural do Rio de Janeiro (UFRRJ), Seropédica, in 1966, where he had his first contact with Science. He was guided by the Scientific Initiation of Professor Johana Doberëiner from 1968. After completing the Agronomy course, he completed his master's degree in Biochemistry at Univeridade Federal do Paraná (UFPR) under the guidance of Professor Glaci Terezinha Zacan, when he discovered the metabolism of arabinose in *Bradyrhizobium japonicum* (1970-1973). Soon after, he went to New York to obtain a PhD in Biochemistry from the Graduate School of Medical Sciences at Cornell University in New York (1974-1977) under the supervision of Prof. Dr. Bernard Leonard Horecker. In his postdoctoral degree in Physiology of Nitrogen Biological Fixation (1982-3) he worked with Geoff Yates at the Nitrogen Fixation Unit at the University of Sussex, UK. The friendship and collaboration with Geoff Yates, who had met in 1978, was very close and lasted until Geoff's death in 2013. Fabio published more than 200 scientific works and guided more than 50 masters, doctors and postdoctors and published more that 250 papers and books during his carrier. He received a price in 2001 for Science and Technology and is a Member of the Brazilian Academy of Sciences. During his career Fábio has accumulated a variety of functions, as Coordinator of the Paraná Genome Program since 2000; Coordinator of the Nitrogen Fixation Nucleus of the Biochemistry Course, supported by PRONEX (1996-2004); National Director of Binational of the Brazil / Argentina Biotechnology Center since 2007; Head of the Department of Biochemistry and Molecular Biology and General Coordinator of the National Institute of Science and Technology of Biological Fixation of Nitrogen.

Scientific Program

Monday 27TH of August

HORARIO	ATIVIDADE
08:00 - 09:00	Late registration
08:30 - 10:40	**SESSION 1 | Nitrogen, Environment and Global Change** (Chairman: Bruno José Rodrigues Alves) 08:30 - 09:10 | Global vision of use of biological inputs in agriculture on the theme: Nitrogen, Environment and Global Changes (Grahan O'Hara | Murdoch University, Australia) 09:10 - 09:40 | Microorganisms associated with plants – overview of Brazilian agriculture and N_2O emissions (Bruno Alves | Embrapa, Brazil) 09:40 - 10:10 | Plant growth-promoting bacteria from extreme environments to restore fertility of desert lands (Yoav Bashan | Bashan Foundation, USA) 10:10 - 10:40 | Nitrogen cycling at the plant-soil interface (Thomas Hurek | Germany)
10:40 - 11:10	Coffee break
11:10 - 12:10	**SESSION 2 | Evolution, Diversity and Ecology of PGP microorganisms (Chairman: Luc Rouws)** 11:10 - 11:40 | Maize Cultivar Induced Selection of Plant-Growth--Promoting Rhizobacteria (Eduardo Balsanelli - 30 min) 11:40 - 12:10 | Whole genome based analysis of evolution and adaptive divergence in Indian and Brazilian strains of *Azospirillum brasilense* (Chhaya Singh - 30 min)
12:30 - 14:00	Lunch
14:00 - 16:00	**SESSION 2 | Evolution, Diversity and Ecology of PGP microorganisms (cont.)** 14:00 - 14:30 | *Paenibacillus riograndensis*, a Gram Positive diazotroph PGPR with different mechanisms of action (Luciane Passaglia | UFRS, Brazil) 14:30 - 15:00 | Deciphering the molecular bases of the interaction between the diazotrophic endophyte *Kosakonia* sp. UYSO10 and sugarcane plants (Federico Batistoni | Clemente Stable, Uruguay) 15:00 - 15:30 | *Azoarcus* genome: new insights in the genus level (Euan K. James | The Hutton Institute, UK) 15:30 - 16:00 | Structural and functional genomic characterization of *Nitrospirillum amazonense* strain CBAmC, a nitrogen-fixing bacterium isolated from surface-sterilized sugarcane stems (Stefan Schwab | Embrapa Agrobiologia, Brazil)
16:00 - 16:30	Coffee break

Monday 27TH of August (cont.)

HORARIO	ATIVIDADE
16:30 - 18:10	**SESSION 3 | What to seek in PGP** (Chairman: Jose Ivo Baldani) 16:30 - 17:10 | Nitrogen-fixing bacteria do not live alone (Ann M. Hirsch | UCLA, USA) 17:10 - 17:40 | Genome Wide Association Studies uncovers genes associated with plant growth promotion driven by endophytic bacteria (*Azoarcus olearius*) (Fernanda Amaral | USA) 17:40 - 18:10 | Importance of bacterial signals in the communication of PGP-microbes with host plants (Anton Hartmann | Germany)
18:10 - 19:00	**Flash presentations from the poster session** 18:00 - 18:10 | Rhizospheric bacterial diversity in soils cultivated with tomato and bean plants after N-fertilisation (Eulogio J. Bedmar | Universidad de Granada, Spain) 18:10 - 18:20 | Transcriptome analyses of maize roots inoculated with *Herbaspirillum seropedicae* (Luiz Eduardo Souza da Silva Irineu | UENF, Brazil) 18:20 - 18:30 | Genetic, physiological and metabolic characterization of *Pseudomonas* sp. strains exhibiting biofertilizer traits and broad-spectrum biocontrol potential (Samina Mehnaz | Chartered University, Pakistan) 18:30 - 18:40 | Transcriptomic responses of *Burkholderia catarinenses* to a phytopathogenic fungus and wheat (Evelise Bach | UFRS, Brazil) 18:40 - 18:50 | Microbial prospection and combination of N2-fixing and cell-wall degrading properties to increase sugarcane straw decomposition (Rafael Luiz Frinhani Rocha | UENF, Brazil)
19:00 - 20:00	**Poster section**: all numbers

Tuesday, 28TH of August

HORARIO	ATIVIDADE
08:00 - 10:10	**SESSION 4 | The Holobiont Plant: Multitrophi Interactions** (Chairman: Jean Luis Simões-Araújo) 08:00 - 08:40 | Regulatory Cascades in Rice-Endophyte Interactions (Barbara Reinhold-Hurek, Max Planck | Institute, Germany) 08:40 - 09:10 | Communication between plants and beneficial bacteria: what the plant genes can tell us? (Adriana Hermely | UFRJ, Brazil) 09:10 - 09:40 | The sugarcane microbiome profile unravels the structure, diversity and colonization pattern of plant-beneficial microbial communities (Rafael Soares Correa de Souza | Unicamp, Brazil) 09:40 - 10:10 | Roles of copper microRNAs in planta microbe interactions (Paulo Cavalcanti | UFRJ, Brazil)
10:10 - 10:40	Coffee break
10:40 - 12:20	**SESSION 5 | Bacterial and Plant Physiology** (Chairman: Stefan Schwab) 10:40 - 11:20 | Old species, novel bacteria – innovations in PGPR's (Ray Dixon | John Innes Centre, Uk) 11:20 - 11:40 | Metabolic pathways due to loss of Glnb signaling in *Azospirillum brasilense*: a proteomic approach (Diana Alejandra Estigarriba | UFPR, Brazil) 11:40 - 12:00 | The PII interactome in *Azospirillum brasilense* (Edileusa Cristina Marques Gerhardt | UFPR, Brazil) 12:00 - 12:20 | The effect of sugarcane apoplastic fluid on the global gene expression pattern of strain HRC54 of *Herbaspirillum seropedicae* (Daniela Duarte Villarinho Pessoa | Embrapa Agrobiologia, RJ, Brazil)
12:20 - 14:00	Lunch
14:00 - 16:00	**SESSION 6 | Applications of PGPR in Agriculture** (Chairman: Mariangela Hungria) 14:00 - 14:40 | Development of Biofertilizers – A journey from Associative Nitrogen Fixation to PGPR (Kauser A. Malik | Pakistan) 14:40 - 15:10 | Co-Inoculation Using The PGPR Species of *Azospirillum Brasilense* – Brazilian Case (José Roberto Pereira Castro | ANPII, Brazil) 15:10 - 15:40 | A brief history of microbiology. the history of *A. brasilense* Az39 in agriculture (Fabricio Cassan | Rio Quarto - Argentina) 15:40 - 16:00 | Induction of plant-stress tolerance and defense genes by Azospirillum brasilense cells or their metabolites in maize plants (Marco Antonio Nogueira | Embrapa Soja, Brazil)
16:00 - 16:30	Coffee break

Tuesday, 28TH of August

HORARIO	ATIVIDADE
16:30 - 18:00	**Flash presentations from the poster section** 16:30 - 16:40 | Characterization of bacteria associated to bamboo for use in plant micropropagation (Cristina Belincanta | UFSC, Brazil) 16:40 - 16:50 | *Rhizobium* sp. (br 10268) colonizes internal sugarcane tissues, produces phytohormones and accelerates mini-sett germination (Luc F. M. Rouws | Embrapa Agrobiologia, Brazil) 16:50 - 17:00 | Increased plant growth promotion performance of *Herbaspirillum seropedicae* in the presence of the saprophytic fungus *Trichoderma longibrachiatum* (Alice Ferreira Alves | Uenf, Brazil) 17:00 - 17:10 | Physiological keys to elucidate the differential response of *Lotus* spp.- *Fusarium solani* interaction (Oscar A Ruiz | IIB-INTECH/IFRGV, Argentina) 17:10 - 17:20 | Microbiome of maize seed: source of beneficial bacteria for germination and seedling growth? (Lidiane Figueiredo Dos Santos | UENF, Brazil) 17:20 - 17:40 | Co-inoculaiton, macronutrient contents and dry mass production in corn after two co-inoculated seasons (Carolina Fedrigo Coneglian | UEM, Brazil) 17:40 - 17:50 | Genome-guided isolation of a novel derivative of the antifungal metabolite burkholdin from the pgpr *Burkholderia catarinensis* (Evelise Bach | UFRS, Brazil) 17:50 - 18:00 | RNA-seq reveals new functions of ntry/ntrx two-component system of *Herbaspirillum seropedicae* (Paloma Bonato | UFPR, Brazil)
18:00 - 18:30	**Close ceremony of this event and choice for the next host**

SESSION 1: Nitrogen, Environment and Global Change

Key Note I.I

Global vision of use of biological inputs in agriculture on the theme: Nitrogen, Environment and Global changes

O'Hara, G W[1]

[1]Centre for Rhizobium Studies, School of Veterinary and Life Sciences, Murdoch University, Murdoch, 6150 Australia (gohara@murdoch.edu.au).

To achieve the vision of eliminating extreme human hunger globally by 2030 it will be imperative that biological inputs are used in agriculture to obtain sustainable food production. Nitrogen is a key nutrient limiting production of both cereal and legume crops, and pastures for grazing animals, in the majority of farming systems. Increasing plant-based biological inputs of nitrogen will be essential for sustaining and improving productivity in agriculture. Legume symbiotic nitrogen fixation and the use of microbial inoculants to enhance plant productivity are key áreas of historical success that need to be continued and improved. Whilst our understanding of the biology of these systems is increasing, especially with the application of molecular knowledge and tools, many fundamental challenges remain. Environmental stresses from adverse soils (e.g. acidity, alkalinity, poor structure, poor fertility, hydrophobicity) and climate-related shocks (e.g. high/low temperatures, too much/too little rain) limit legumes because of effects on their rhizobia, the symbiosis and the plant. These stresses also limit the beneficial effects of other microbial inoculants. Key areas of challenges and opportunities include enhanced survival of inoculants (especially when applied to dry or drying soils), optimising plant-microbe interactions to maximise productivity and development of appropriate technologies for microbial inoculants that are accessible for small-holder farmers in developing countries. Improving our knowledge and understanding of the plant microbiome, impacts of the environment on soil microorganisms and the consequences of HGT from inoculants to soil bacteria will be essential to ensure maximum benefits are obtained from the use of microbial inoculants and biological inputs in agriculture.

Keywords: legume, rhizobia, plant-growth-promoting bacteria, symbiotic nitrogen fixation.

Acknowledgements: Australian Research Council, GRDC.

Short Note I.I

On the effectiveness of crop inoculation with plant growth promoting bacteria in Brazil for the mitigation of greenhouse gases

Alves, B J R[1*]; Martins, M R[1]; Santos, R[2]; Reis, V M[1]; Urquiaga, S[1]; Jantalia, C P[1]; Boddey, R M[1]

[1]Embrapa Agrobiologia, BR 465, km 7, Seropédica, RJ, 23891-000, Brazil. [2]Universidade Federal Rural do Rio de Janeiro, BR 465, km 7, Seropédica, RJ, 23890-000, Brazil (bruno.alves@embrapa.br).

There is strong evidence that the observed increase in atmospheric mean temperature of Earth is provoked by emission of greenhouse gases (GHG) due to anthropogenic activity. In Brazil, approximately one third of annual emissions of GHG in CO_2 equivalents comes from agriculture, of which 40% is due to nitrous oxide (N_2O) production. As a committed mitigation strategy at the United Nations, Brazil needs to reduce emissions from N fertilizers by expanding crop reliance on biologically fixed N_2. This process is adopted countrywide in soybean crops that cover over 30 million ha. Grasses such as maize and sugarcane that occupy a little less area but consume about 1 million ton of N fertilizers annually, are the challenge for the desired change. *In vitro* studies confirm the potential of plant growth promoting bacteria (PGPB) to supply N from biological nitrogen fixation (BNF) or growth induction by hormone exudation, which may increase N-use efficiency. Some bacteria strains are already part of commercial inoculants for maize, wheat and sugarcane. Data obtained from field studies show that the growth promotion effect can improve N-fertilizer use efficiency or even guarantee significant plant yield without N fertilization. In addition, reduction in N_2O emissions were also reported as an effect of PGPB inoculants. Nonetheless, it is apparent that the effectiveness of the technology is highly dependent on the general nutritional status of the plant-microorganism system, without which limited effects are observed. Apart from the above framework, published information was gathered together to verify to which extent a broader adoption of the available technology would impact on the GHG mitigation and intensity in Brazil.

Keywords:biological nitrogen fixation; nitrogen use efficiency; nitrous oxide.

Financial Support: Faperj, CNPq.

Short Note I.II

Plant growth-promoting bacteria from extreme environments to restore fertility of desert lands

Bashan, Y[1,2,3,*]; de-Bashan, L E[1,2,3]; Lopez, B R[1,2]; Garcia, D E[1]; Galaviz, C[1]; Gonzalez, J[1]; Moreno, M[1]
[1]Environmental Microbiology Group, Northwestern Center for Biological Research (CIBNOR), La Paz, B.C.S., Mexico. [2]Bashan Institute of Science, Auburn, Alabama, USA. [3]Dept. of Entomology and Plant Pathology, Auburn University, Alabama, USA (bashan@ bashanfoundation.org).

Southwest USA and Northwest Mexico are suffering for decades from accelerating desertification. One of the ways to combat desertification is to use strategies that enhanced fertility of degraded desert soil. One of the tools to do so is by plant growth-promoting bacteria from extreme environments and also by agriculturally origin PGPB as inoculants. During the last 20 years, we have isolated numerous strains of PGPB from extreme desert environment. They were both rhizoplane and endophytic bacteria. Many of them were capable of promoting growth of many desert plants in greenhouse conditions. Few were used for field experiments where survival of the plants after over a decade in the field was high for cardon cactus and mesquite trees. The combination of nurse tree and cardon cactus both inoculated with extreme environment PGPB were successful. In addition, we used the agricultural PGPB, *Azospirillum* sp. as inoculant of cardon cactus with similar success in establishing growth and survival of the plant over a long period of time, Establishment of the inoculated native plant in the field was so successful that after a decade of growth the nitrogen fixing capacity of originally degraded desert was equal or superior to that of pristine desert. This strategy showed that using PGPB for environmental restoration is a useful tool in return fertility of soils to their original level thus allowing our plant species to thrive in these restored soils.

Keywords: desertification; cactus; native plants.

Short Note I.III

Nitrogen cycling at the plant-soil interface

Große, J[1]; Burbano, C S[1]; Oskiera, M[1]; Reinhold-Hurek, B[1]; **Hurek, T**[*1]

[1]Department of Microbe-Plant Interactions, Faculty of Biology, University of Bremen, Bremen, Germany (thurek@uni-bremen.de).

Soil quality and fertility are an essential component for bio-economic productivity. Nitrogen losses due to microbial N-cycling and wash out are a major concern. Catch crops can be an option to preserve or even improve soil productivity because of their effect on soil fertility and health. A long-term field experiment of the CATCHY project (Catch-cropping as an agrarian tool for continuing soil health and yield-increase) with two contrasting crop rotations was established in two different locations in Northern and Southern Germany. Single catch crops (white mustard, Egyptian clover, phacelia and bristle oat), catch crop mixtures (a mixture of the above and a commercial mixture) and main crops (wheat and maize) have been grown. To investigate how catch crops can affect the microbial diversity and particularly the microbial nitrogen cycling communities, we are studying first the short-term effect of different catch crop mixtures on the microbiomes associated with soils and roots. We compared these microbiomes with wheat plants, representing the microbial community before a catch crop treatment. Roots, rhizosphere and bulk soils were collected from representative samples of wheat plants, fallow treatment (without roots), and three catch crop variants. Quantitative analyses were carried out by qPCR based on key functional marker genes for mineralization (ureC), nitrification (amoA), dissimilatory nitrate and nitrite reduction to ammonium –DNRA-(nrfA), denitrification (nirK, nirS, nosZ), and nitrogen fixation (*nif*H). We detected the presence and activity of microbial populations capable of performing the main processes of the N-cycle except DNRA. The microbial population structure was studied using rRNA and *nif*H amplicon sequencing of DNA extracts obtained from root, rhizosphere and bulk soils of wheat plants and catch crops. DNAs were sequenced using the Illumina platform (MiSeq platform). The OTU-based analysis showed that the main driver for community composition appeared to be the location, likely due to soil differences. However, microbial communities differed with plant species and could thus lead to different functional traits after catch crop cultivation. Plants enriched a specific microbiome in rhizosphere soil and roots, selecting for particular bacteria and excluding others, leading to specific communities at the soil-root interface in contrast to bulk soil. For maize as the following main crop in crop rotation, microbial communities were also analyzed. Our data indicate that different catch cropping techniques may take effect on the main crop already after one cropping cycle.

Keywords: soil fertility; nitrogen cycle; microbial population.

Poster I.1

Response of diazotrophic community to nitrogen fertilization in no-tillage system

Urrea-Valencia, S[1*]; Etto, R M[1]; Bini, A R[1]; Caires, E F[1]; Potma, D[1]; Parize, E[2]; Ayub, R[1]; Galvão, C W[1]
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Nitrogen (N) is an indispensable nutrient for organisms; thus, in agro-ecosystems it is a limiting factor for the crops growth. Biological nitrogen fixation is performed by a microbial group, called diazotrophics, which contain the nitrogenase metalloenzyme and can converts dinitrogen into ammonia. This process is mediated by specific genes, such as the marker gene *nif*H. The diazotrophic group is diverse, and contains non-cultivated microrganisms, therefore, molecular studies are a good alternative for monitoring *nif*H gene in the soil. Agricultural practices affect physical and chemical characteristics of the soils and as result, the composition of the microbial communities. For this reason, in the present study, the responses of the nitrogen fixation group to N enrichment and *Azospirillum brasilense* AbV5/AbV6 inoculation were monitored by qPCR, using the *nifH* gene as a target. The experiment was conducted in a no-tillage system and in a split-plot design, using either *A. brasilense* inoculation or no inoculation as a principal factor, and N doses as a secondary factor. For maize, 0, 80, 240 kg N ha^{-1} was applied at V4 physiological state, and for black oat, 15 kg N ha^{-1} was applied at sowing, because it is a cover crop that come right after soybean. DNA extractions were conducted using samples of 0-10 cm of soil collected at flowering of the two successive cultures. The number of *nifH* copies of each sample was determined by qPCR. The *nifH* copy number on maize varied from 1.2 to 7.3 x 10^6 copies/g of soil, being the smallest number observed in 240 kg N ha^{-1}. The black oat exhibited higher numbers than maize, from 29 to 340 x 10^6 copies/g of soil. No statistical differences were detected between *A. brasilense* inoculated and non-inoculated treatments. These results corroborate that N fertilization directly affects the soil diazotrophic community and demonstrate the importance of the black oat as a cover crop in notilling system to increase the diazotrophic population.

Keywords: biological nitrogen fixation; q*PCR;* nifH*.*

Financial Support: Capes, INCT Fixação Biológica de Nitrogênio, CNPq, Fundação Araucária.

Poster I.2

Rhizospheric bacterial diversity in soils cultivated with tomato and bean plants after N fertilisation

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The effect of N-fertilisation on bacterial diversity in the rizosphere of tomato and bean plants has been analysed. An agricultural sandy-loam soil (pH = 7.0) was amended independently with 260 kg N/ha and used to fill 20 kg containers which were kept under greenhouse conditions (22º/16ºC day/night temperature; 16h/8h light/dark cycle). Unfertilised soil was used as a control. Soil moisture content was adjusted weekly to 80% WFPS. N_2O production by soils was measured by gas chromatography twelve hours after watering. Four consecutive crops of about 4 months each were made. For each crop, plants were removed from the soil at 10% flowering, and then soils fertilised again up to the initial fertilisation rate. Soil samples for DNA extraction were taken from the rhizosphere at the beginning and at the end of the fourth crop. Bacterial diversity was analysed by pyrosequencing. Application of any N-fertiliser decreased bacterial diversity. The number of OTUs found in the rizosphere of tomato plants grown in control and rhizospheric urea-, ammonium- and nitratetreated soils, was 560, 181, 193 and 160 for tomato, and 591, 158, 172 and 133 for common beans, respectively. Beta diversity analysis revealed that the type of N-fertilisation affected the relative abundance of both dominant (>1%) and rare (<1%) taxons. A multiple correlation analysis including the soil properties pH, [NH_4+], [NO_3-], total organic carbon, total carbon, total nitrogen and the relative abundance of bacterial OTUs indicated that variations in bacterial biodiversity were due mainly to nitrate, which explained 58% of the variance, and ammonium, which was responsible for the 32% of the variance. Determination of nitrous oxide revealed clear differences due to the N form used for fertilisation whose release was also affected by the plant, being 1.5-fold higher in common beans cultivated soils.

Keywords: N-fertilisers; biodiversity; GHG.

Financial Support: ERDF-cofinanced grant PEAGR2012-1968 from Junta de Andalucía, Spain. ACH is recipient of a grant from MECD (FPU 2014/01633).

Poster I.3

Effect of three inputs combination in maize crop rhizosphere: evaluation of potential production and greenhouse gases fluxes

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Inputs combination could lead divergent responses for monitoring soil microbial behavior in greenhouse gases (GHGs) emissions. In addition, few studies have quantified GHGs-related genes at rhizosphere level under three inputs combinations that are mostly applied in maize cultivated in Brazil. In this study, we evaluated the influence of three common inputs and their combinations on maize plants development in macrocosm experiment (100L pots): *(a)* S - maize stover coverage at experiment setup, *(b)* F - topdressing urea fertilization at V5 stage, and *(c)* I - microbial inoculant (*Azospirillum braziliense*) at seeds sowing (totalizing in 8 treatments: F, I, S, F+I, F+S, I+S, F+I+S and C, the control group); observing microbial responses through genes related to nitrous oxide (N_2O) and methane (CH_4) emissions at V5 (the last period stage of potential production determination) and V15 stage (the start of the most crucial period for determining grain yield) rhizosphere sampling. Gas fluxes were collected from round-shaped chambers (20 cm inner diameter) installed in the center of the pots, from every 72h after seeds sowing until V15 stage and measured by gas chromatography (GC). Sampling of rhizosphere soil was performed at V5 and V15 stages; as well as dry weight of shoots was measured to obtain potential production of plants and genes for N_2O mitigation (*nosZ*), and for CH_4 emission (*mcrA*) and consumption (*pmoA*) were evaluated through quantitative PCR (qPCR) to correlate gases fluxes. As main results, inputs combinations merged their individual effects on gases fluxes, or presented synergistic effect with special attention to inoculant + maize stover treatment (I+S) that showed similar dry weight gains at V15 stage (51.92±1.80 g/per plant) to F+I+S (52.64±2.45 g/per plant) with the advantage of 12.1% less emissions in carbon equivalent from total gases emitted during the experiment (CO_2, N_2O and CH_4; 4897.08±153.40 kg/ha) in comparison to F+I+S (5574.12±62.88 kg/ha).

Keywords: Azospirillum braziliense*; maize stover coverage; topdressing urea fertilization.*

Financial Support: FAPESP (2015/08564-6, 2016/11268-2), CNPq (149662/2014-9).

Poster I.4

Quantification of BNF in two subsequent ratoons of commercial sugarcane varieties using ^{15}N isotopic dilution technique

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Biological nitrogen fixation (BNF) naturally associated with grasses has been highlighted as a strategy for the sustainable sugarcane (*Saccharum* spp.) production, becoming a potential alternative to the use of synthetic nitrogen fertilizers and, consequently, contributing to reduction in costs and environmental impacts associated to the fertilization and mining of N from soil organic matter. The objective of this study was to measure the stalk yield and the contribution of BNF in commercial varieties of sugarcane by the ^{15}N isotopic dilution technique. The long-term experiment has been carried out in Seropédica, Rio de Janeiro State, Southwest Brazil, since 1989 without any N-fertilizer application. The treatments were three commercial varieties RB867515, RB92579 and SP813250 of sugarcane. The experiment has been conducted in a 120 m^2 concrete tank filled with soil with low N content. Liming and fertilization with P, K and micronutrients followed the soil analysis and requirements of the crop. No significant effect between varieties were detected by Tukey test ($\alpha = 0.05$) for any parameter measured in the ratoon harvested in 2017. In this year the mean yield of sugarcane stalks was 105 Mg ha^{-1} and the mean total N accumulated in stalks, dry leaves and green tops was 84 kg ha^{-1}. On the other hand, a significant varietal effect ($\alpha = 0.05$) was observed for total N accumulated by the sugarcane ratoon harvested in 2018, ranging from 48 kg ha^{-1} (var. RB92579) to 98 kg ha^{-1} (var. SP81-3250), even with no significant differences detected in yield of stalks. The mean contribution of BNF, expressed as fraction of nitrogen derived from atmosphere, was 45% in 2017 and 48% in 2018. This result shows that BNF supplies a significant fraction of N required by commercial varieties of sugarcane usually grown for Brazilian sugar-alcohol industry, representing a significant reduction in cost of production and reducing the negative environmental impact of applying synthetic N-fertilizer.

Keywords: Saccharum *spp.; bioenergy; tropical soil.*

Financial Support: CNPq e Faperj.

Poster I.5

Biomass production and contribution of BNF to five genotypes of elephant grass grown in naturally poor soils

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Elephant grass (*Pennisetum purpureum* Schum.) is one of the grasses with the highest potential for biomass production, which is a highly desirable characteristic for the energy sector. The contribution of the biological nitrogen fixation (BNF) to elephant grass decreases the need for nitrogen fertilization. This minimizes dependence on fossil energy sources and contributes to a positive energetic balance of the crop. The objective of the present study was to measure the biomass production and the contribution of BNF in genotypes of elephant grass grown in soils naturally poor in available N. The experiment was conducted in Seropédica, Southwest Brazil, in two soil types (Acrisol and Planosol). The genotypes used in the study were: Cameroon, Bag 02, Gramafante, Roxo and CNPGL F 06-3. Harvests of each experiment were made every six months, from January 2016 to January 2017. The experimental results show that the cultivars present yields of biomass varying according to the type of cultivated soil, and according to the season of the year. The annual yield of biomass in the Acrisol ranged from 20.7 Mg ha^{-1} to 27.5 Mg ha^{-1}, the best results being with the varieties Bag 02 and Gramafante. In the Planosol, the results ranged from 14.6 Mg ha^{-1} to 19.0 Mg ha^{-1}, and Bag 02 and Cameroon were the highest yielding varieties, with Roxo being the least productive in both soils. The contribution of BNF in crop nutrition was observed in the range of 38 to 72% in the Acrisol and 58 to 97% in the Planosol, a soil that is poorer in N available. BNF contributed with 44 to 70 kg ha^{-1} year^{-1} of N in the Acrisol, and with 46 to 64 kg ha^{-1} year^{-1} of N in the Planosol. The accumulation of N by the plant and the contribution of the BNF behaved asindependent factors.

Keywords: Agroenergy; N_2 fixation; Pennisetum purpureum*.*
Financial Support: CNPq; Faperj.

Poster I.6

Inoculation of *Brachiaria* híbrida Mavuno with the plant growth-promoting bacterium

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Estimates are that in Brazil there are about 180 million hectares of pasturelands, with 70% with some degree of degradation. Recovery of degraded areas requires restoration of soil fertility, for plant growth and forage production, and the use of microbial inoculants with plant growth promoting bacteria may help in these processes. Thus, the work aims to evaluate the productive characteristics and mass of roots, of *Brachiaria* hibrida Mavuno inoculated in sowing with associative diazotrophic bacteria in Argissolo and nitrogen (N) fertilization. The experiment was carried out in pots, in a greenhouse, consisting of eleven treatments and five replicates in a randomized block design. The composition of the AB-V5 and AB-V6 strains of *Azospirillum brasilense*, *Pseudomonas fluorescens* and the composition of AB-V6 and *Rhizobium tropici*, associated or not with N fertilization and co-inoculation of the plants after cutting, were tested. The evaluation was performed through three cuts every 4 weeks for the determination of forage dry mass (DM) production, tillers and roots. For the accumulated DM of forage and roots, DM of leaf, DM stem plus sheath, DM tiller, number of tillers and chlorophyll content the highest averages were presented by treatments AB-V6 and *R. tropici*, *P. fluorescens*, AB-V5 and AB-V6 of *A. brasilense* associated with N. AB-V6 and *R. tropici* produced 6% more forage DM and 26% more DM of roots than the positive control (with N and without inoculation). The treatments submitted to co-inoculation of the aerial part after the cut did not present significant difference (P>0.05) in relation to the other inoculated for all variables analyzed. We concluded that co-inoculation was not effective to express a greater response in terms of forage dry mass production, and growth-promoting bacteria did not show significant differences among them when associated with N. Diazotrophic bacteria applied without N application were significantly lower in all evaluated parameters.

Keywords: Azospirillum brasilense*;* Pseudomonas fluorescens*;* Rhizobium tropici*.*

Financial Support: grant #2017/17573-4, São Paulo Research Foundation (FAPESP).

Poster I.7

Nitrous oxide emission in sorghum inoculated with *Azospirillum brasilense*

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Nitrous oxide (N_2O) is a highly reactive greenhouse gas mainly produced by agricultural activity and use of N fertilizers is one of the key sources. The root growth promotion induced by some diazotrophic bacteria can be strategic to improve fertilizer N use by plants and concomitantly mitigate soil N_2O emissions after N application. This possibility was investigated in this study by setting up a greenhouse experiment. Sorghum (*Sorghum bicolor*) was seeded into 10 L pots containing topsoil (upper 20 cm of an Ultisol). The treatments were (T1) control; (T2) 80 kg N ha^{-1}; (T3) inoculation with *Azospirillum brasilense* strain Sp245+80 kg N ha^{-1}; (T4) 80 kg N ha^{-1}+nitrification inhibitor (Nitrapyrin); (T5) inoculation with *Azospirillum brasilense* strain Sp245+80 kg N ha^{-1}+nitrification inhibitor. The N source was urea that was applied after 25 days of plant emergence. The pot was used as the bottom part of a closed static chamber was set up every day for 1h by hermetically connecting the top part made of a similar pot that was inverted on the basis. The top part received an external insulation cover, vent and connector to air withdrawal from headspace for gas sampling. The highest N_2O fluxes were detected in T2. The inoculation with *Azospirillum* significantly reduced N_2O emissions. The most efficient gas flux reduction was observed in the treatments with nitrification inhibitor, irrespective of bacteria inoculation.

Keywords: Greenhouse gas; mitigation; Sorghum bicolor*.*

Financial Support: Capes, CNPq, FUNARBE.

Poster I.8

Bacterial inoculation of grain-sorghum fertilized with urea coated with NH_3 volatilization inhibitor for yield improvement

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The use of an ammonia volatilization inhibitor has proven to be successful in reducing N losses after urea application to crops, which in combination with seed inoculation with plant-root growth promoting bacteria (PGPB) would enhance the fertilization efficiency. This hypothesis was tested in this study. An experiment was carried out at the experimental station of Embrapa Agrobiologia located in Seropédica, Rio de Janeiro. A randomized block design with 5 treatments and 5 replications was adopted. The treatments were different N fertilization strategies as follows: no N addition (T1); urea (60 kg N ha^{-1}) (T2); seed inoculation with *Azospirillum brasilense* ABV4 and ABV5 strains+urea (60 kg N ha^{-1}) (T3); coated urea (60 kg N ha^{-1}) with NBPT (T4); and coated urea (60 kg N ha^{-1}) with NBPT+seed inoculation with *A. brasilense* ABV4 and ABV5 (T5). Each plot was 4 m long by 4 m wide, with 0.50 m interrow space. At seeding, plants were fertilized with 80 kg P_2O_5 ha^{-1} as single superphosphate, 40 kg K_2O ha^{-1} as potassium chloride, and 3 kg micronutrients ha^{-1} as Cana Micros Plus®. Urea fertilization was split in two, with 20 kg N ha^{-1} at sowing and 40 kg N ha^{-1} as side-dressing. Grain yield and straw were quantified at harvesting. Results were submitted to ANOVA and significantly different means were separated by l.s.d. test at $p<0.05$. Ammonia volatilization in the absence of inhibitor was about 10% of the added N, irrespective of application rate, but when present, the inhibitor halved the N losses. There was no effect of seed inoculation on volatilization losses. The agronomic efficiency of the fertilization with 60 kg N ha^{-1} was about 5 to 10% higher under inoculation and/or inhibitor use. There is no synergy on fertilizer efficiency when combining the technologies of volatilization inhibitor and seed inoculation with PGPB.

Keywords: Azospirillum*; nitrogen agronomic efficiency;* Sorghum bicolor.

Financial Support: Capes, CNPq, FUNARBE.

Poster I.9

Microbial activity as a parameter for soil quality to Curitibanos region – SC

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The most commonly used farming techniques are being inefficient for soil conservation and itis necessary to adopt new methods to produce food. Soil Microbial Biomass (SMB) is very sensitive to changes in the environment and has potential as an effective indicator of SQ (Soil Quality). The aim was to evaluate the soil microbial activity at different cultivation systems, at a local farm, as standard, the native forest (NF). The cultivation systems were no-tillage (NTi), soybean/oat succession and soybean conventional tillage (CTi). Soil samples were taken after sowing: 15 days, 74 days and 132 days of plant development. The parameters evaluated were soil metabolic quotient (qCO_2), basal respiration (SBR) and microbial biomass Carbon (SMB-C) taken by fumigation-extraction method. The C average amount to the NTi system was 78.10 mg kg^{-1} of dry soil (d.s.), to CTi was 142.49 mg kg^{-1} C d.s. and to native forest, 251,72 mg C Kg^{-1} de d.s. It was observed that the carbon content to the NTi was lower than to the CTi, unlike expected, indicating SMB potential, as SQ marker, since the system was improperly conducted in the farm. The qCO_2 values to NTi and CTi were higher than those obtained to MN during the three evaluation periods, which was expected, because at NF, the respiratory rate is lower, since there is no soil disturbance. However, there were increasing values, each time, to NTi and CTi and even higher to NTi: NTi (236.41; 534.12; 1249.31 mg $C\text{-}CO_2.g^{-1}$ $SMB\text{-}C.h^{-1}$) and CTi 115; 275,38; 530,40 mg $C\text{-}CO_2.Kg^{-1}$ $SMB\text{-}C.h^{-1}$). The results of qCO_2 suggested again that the NTi was improperly handled since values were higher compared to the CTi. It was concluded that SMB activity can detect the inadequate management at conservationist systems, such as NTI, as well as at conventional ones. It is necessary to deepen the studies in the region.

Keywords: conservationism; soil management; microbial biomass.

SESSION 2: Evolution, Diversity and Ecology of PGP microorganisms

Short Note II.I

Maize Cultivar Induced Selection of Plant-Growth-Promoting Rhizobacteria

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Manipulation of the rhizospheric microbial communities of maize is a promising strategy for crop breeding and depends on understanding the ecological interactions influencing growth and productivity. The Brazilian Atlantic Forest soil is a biodiversity hotspot. In this work, we used such soil as bacterial inoculum for 2 maize cultivars, with contrasting growth responses to inoculation with *Azospirillum brasilense*. Plants were grown in pots using as substrate sterile vermiculite and soil (25% w/w) without added nitrogen input in a greenhouse. After 30 days, rhizosphere soil and roots were collected for bacterial isolation and DNA extraction. The remaining substrate and plant roots were homogenized and used as inoculum for a second cycle of maize growth. This process was repeated for five cycles. Bacteria biodiversity was determined by *16S rRNA* V4 deep-sequencing of metagenomic DNA. Isolates were identified by 1*6S rRNA* sequencing and tested for plantgrowth promotion. The rhizospheric microbiome shifted over the cycles on both cultivars, with abundances of Acidobacteria decreasing, and Actinobacteria, Bacteroidetes, and Archaea increasing. Microbiome compositions of the maize cultivars were not different at the phylum level, but differed at genera level. About 1700 bacteria were isolated from each cultivar. We selected the most effective root colonizers from pools of those isolated bacteria, using an in-house designed system. Twelve bacteria with different colony morphology were isolated from each cultivar using this system, and identified as species of *Bacillus*, *Enterobacter*, *Pseudomonas* and *Paenibacillus*. Most of the isolates solubilized phosphate, produced siderophores, showed fungal biocontrol activity, and fixed nitrogen. When re-inoculated in maize plants, these isolates reached as high as 10^9 cells per gram of root, some increased lateral root numbers in the cultivars they were isolated, and a few were able to induce growth of both cultivars. Our data suggest that successive growth cycles of maize are an effective method to select PGPRs.

Keywords: maize; plant-growth-promoting bacteria; nitrogen fixation.

Financial Support: Capes, INCT-FBN/CNPq, Newton Fund Grant BB/N013476/1.

Short Note II.II

Whole genome based analysis of evolution and adaptive divergence in Indian and Brazilian strains of *Azospirillum brasilense*

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Bacteria of the Genus *Azospirillum* are one of the best characterized plant growth promoting rhizobacteria. Out of its 20 species reported so far whole genome sequence of *A. lipoferum*, *A. Brasilense*, *A. amazonense*, *A. thiophilum*, *A. oryzae*, *A. halopreferens* and *Azospirillum* B510 has been determined. Since the first report of genome sequence of *A. brasilense* Sp245 (Wisniewski-Dye et al. 2011) it is becoming increasingly evident that *Azospirillum* genomes harbor extraordinarily large number of genes which seem to be acquired via horizontal gene transfer (HGT) force in its evolutionary journey from aquatic to diverse terrestrialenvironments and plant hosts. In order to understand the evolutionary adaptation in Indian strains of *A. Brasilense*, we have sequenced whole genomes of 4 strains of *A. brasilense* which were isolated from the rhizosphere of Indian cereals and grasses (Chaudhary et al, 2007). Here we present the result of comprehensive analysis of the genomes of 4 Indian (MTCC 4035, 4036, 4038 and 4039) and 3 Brazilian (Sp245, Sp7 and Az39). Whole genome comparison was done starting from chromosomal organization to gene level. Their genome size varies from 7.0 Mb to 8.1 Mb. The number of replicons in Indian strains varies from 6 to 9. Average nucleotide identity among Indian and Brazilian strains varies upto 6%. About 75% of the total gene families constitute the core genome which is apportioned in Chr, p1, p2 and p4 replicons. By comparing the coreand dispensable genomes of Indian- and Brazilian strains with the core genome of all the *A. brasilense* strains, we were able to find unique genes and pathways which were acquired in Indian and Brazilian strains via HGT. To demonstrate adaptive evolution evidenced by phenotypic versatility in *A. brasilense* strains, here we try to explain the genetic relatedness and genome dynamics in the pan genome of *A. brasilense* strains spanning a range of ecological niches from different continents. Results of the pathway annotation from dispensable gene sets enabled us to elucidate characteristic feature of these genomes.

Keywords: pan genome; core genome; horizontal gene transfer; Azospirillum.

Financial Support: Banaras Hindu University.

Short Note II.III

Paenibacillus riograndensis, a Gram Positive diazotroph PGPB with different mechanisms of action

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Paenibacillus spp. isolated from plant roots have agricultural potential, as several members are nitrogenfixing species. The genus also presents great metabolic diversity and has been found in different ecological niches. The inoculation of plants or seeds with these plant-growth promoting bacteria (PGPB) can help to reduce the use of chemical fertilizers and pesticides, improving agricultural sustainability. In 2010, the species *Paenibacillus riograndensis* and *Paenibacillus sonchi* were described in an interval of only nine days. Recently, using several genome-based metrics and phylogenetic analyses we propose the reclassification of *P. riograndensis* as a genomovar of *P. sonchi*, since this species was firstly described. The complete genome sequence of *P. sonchi* genomovar Riograndensis SBR5^T revealed the presence of genes related to the alternative nitrogen fixation system (*anf* genes). Since there are few studies about *anf* genes in Gram-positive diazotrophs, this species constitutes an interesting model for the study of the regulation of biological nitrogen fixation (BNF) in this group of bacteria. The transcription factor GlnR was proposed to regulate BNF related genes based on *Paenibacillus* comparative genomics. In order to validate this proposal, we investigated BNF regulatory sequences in SBR5^T genome. We identified GlnR-binding sites flanking σ^A-binding sites upstream from BNF-related genes and GlnR-DNA affinity is greatly enhanced when GlnR is in complex with feedback-inhibited (glutamine-occupied) glutamine synthetase (GS). GlnR - GS complex formation is also modulated by ATP and AMP. Thereby, gene repression exerted by the GlnR-GS complex is coupled to nitrogen (glutamine levels) and energetic status (ATP and AMP). Finally, we propose a DNA looping model based on multiple operator sites that represents a strong and strict regulation for these genes.

Keywords: plant-growth-promoting bacteria; nitrogen fixation.
Financial Support: INCT-FBN/CNPq, Newton Fund Grant BB/N013476/1.

Short Note II.IV

Deciphering the molecular bases of the interaction between the diazotrophic endophyte *Kosakonia* sp. UYSO10 and sugarcane plants

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Sugarcane is a multipurpose crop used for sugar, energy, animal feed and bioethanol production. However this crop needs high N-fertilization inputs for optimal growth, which results in high environmental and economic production costs. Plant growth promoting bacteria (PGPB) are of biotechnological interest since they can improve growth of several important crops and, among PGPBs, endophytes are particularly relevant. In order to contribute to the environmental and economic sustainability of sugarcane production system in Uruguay, we focused our attention in the role of the endophytic microbiota associated to this crop. By using the ^{15}N isotope dilution method we demonstrated the contribution of the biological nitrogen fixation (BNF) to Uruguayan sugarcane cultivars (1). Additionally, a collection of putative endophytes associated with these sugarcane cultivars was constructed. The collection was characterised genetically and biochemically, selected diazotrophic strains were identified and their phylogenetic relationships examined (1). *In vitro* and greenhouse inoculation studies demonstrated that the *Kosakonia* sp. UYSO10 isolate is a specific PGP of cultivar LCP 85384 and microscopy studies allowed us to define it as a sugarcane endophyte (2). Genomics and proteomics approaches conducted in *Kosakonia* sp. UYSO10 strain highlighted several features related to the interaction established between UYSO10 strain and sugarcane.

Keywords: plant-growth-promoting bacteria; nitrogen fixation.

Financial Support: INIA-FPTA 275 and 331, PEDECIBA-Biología, ANII, UdelaR.

Short Note II.V

Comparative genomics reveals *Azoarcus* comprises three genera: *Azoarcus sensu stricto*, *Aromatoleum* gen. nov. and *Gallonia* gen. nov.

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The Betaproteobacterial genus *Azoarcus* (s.l.), currently comprises several species and strains which are isolated from diverse environments, particularly from sites contaminated with aromatic and/or petroleum-based compounds and plant rhizosphere too? Several are diazotrophs. It has long been known that *Azoarcus* is paraphyletic, comprising at least two phylogenetically distinct groups. The “plant-associated” group includes strains that were isolated from the rhizosphere or root interior of the C4 plant Kallar grass, but also strains from soil and/or water; all are obligate aerobes and are diazotrophic (Faoro et al. 2017). The other group comprises a diverse range of species and strains that live in water or soil which is contaminated with petroleum and/or aromatic compounds; all are facultative or obligate anaerobes, capable of degrading a wide variety of aromatic compounds under aerobic and anaerobic conditions coupled to nitrate-reduction (denitrification). The present study has performed a comparative genome analysis of 19 *Azoarcus* strains in order to define the genus more precisely. On the basis of average nucleotide identity (ANI), we propose three genera: (i) *Azoarcus* (s.s) comprising *A. communis*, *A. indigens* and *A. olearius*, (ii) *Aromatoleum* gen. nov., comprising *A. anaerobius*, *A. buckelii*, and *A. taiwanensis*, and (iii) *Gallonia* gen. nov., comprising *G. evansii*, *G. toluclasticus*, *G. tolulyticus* and *G. toluvorans*. Each genus also contains strains that have not yet been described to species level. Nif genes are present in *Azoarcus* and *Gallonia*, but they have different evolutionary histories, suggesting that they were obtained from different sources via horizontal gene transfer (HGT); *nif* appears not to be possessed by *Aromatoleum* strains. The genomic data are discussed in terms of the highly varied habitats and lifestyles of *Azoarcus* (s.l).

Keywords: plant-growth-promoting bacteria; nitrogen fixation.

Financial Support: Newton Fund Grant BB/N013476/1.

Short Note II.VI

Structural and functional genomic characterization of *Nitrospirillum amazonense* strain CBAmC, a nitrogen-fixing bacterium isolated from surface-sterilized sugarcane stems

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Nitrospirillum amazonense is a nitrogen-fixing bacterium that shows potential to promote plant growth when inoculated into sugarcane and rice plants. This microorganism has been the subject of biochemical and genetic characterization to elucidate important functions related to host plant interaction and growth promotion, including the determination of genome sequences. CBAmC strain has been isolated from sugarcane (*Saccharum* spp.), and is currently used in a sugarcane consortium inoculant with four other nitrogenfixing bacteria. This presentation describes a significant improvement in the genome sequence and assembly for the *N. amazonense* strain CBAmC, and determination for the first time of a complete genome sequence for this bacterial species, using PacBio technology. In addition, global transcriptional and translational profiles of gene expression were determined when the bacterium was cultured in the presence of sugarcane apoplast fluid. The sugarcane apoplast is a probable colonization niche for that microorganism, thus this study aimed to mimic *in vitro* that possible bacterial life environment in order to obtain information about metabolic pathways and physiological functions determinant for host plant interaction and growth promotion. Genome datamining and results of functional genomics analyses allowed the discovery of genes coding for metabolic pathways and cellular structures that may be determinant for the success of the bacterial establishment and colonization into the host sugarcane plant, besides conferring important characteristics to the inoculant. More importantly, results of the present work shall guide subsequent studies on strain CBAmC as well as the development of a sugarcane inoculant.

Keywords: plant-growth-promoting bacteria; nitrogen fixation.

Financial Support: Newton Fund Grant BB/N013476/1.

Poster II.1

Analysis of the growth and colonization of *Streptomyces* sp. UYFA156 when interacting with its host *Festuca arundinacea*

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Festuca arundinacea (fescue) is a forage grass widely used in Uruguay due to its high productivity and persistence. The strain *Streptomyces* sp. UYFA156 was isolated from inside the seeds of *F. arundinacea* var. Don Tomás, on which it promotes plant growth. However, no growth promotion was observed on another fescue variety, Tacuabé. The aim of this work was to explore the ability of UYFA156 to grow in association with Don Tomás and Tacuabé fescue varieties, under the hypothesis that colonization may be a limiting step in these plant growth promotion differences. Growth curves on root exudates, colonization assays and microscopy were performed. Seedlings were introduced in tubes with Jensen liquid medium with polypropylene beads as support. At 1,7,20 and 30 days, the conditioned Jensen medium was collected. Plates with conditioned medium were inoculated with UYFA156 and growth was analyzed by optical density. Colonization assays were carried out *in vitro* with growing periods of 1,7,20 and 30 days. Plants were weighted and root and aerial parts separated. Each fraction was ground and 0 and -1 dilutions were plated in TSA medium. The growth curves showed that the strain is capable of growing in the presence of exudates of both varieties. A strong dependence of growth on the C/N ratio is also suggested, with N being likely contributed by the root exudates. Colonization was observed on both plant varieties after a day of incubation with UYFA156. In plants incubated 7 days, both plants fresh weight and density of other endophytic bacteria varied significantly between inoculated and non-inoculated plants.These results suggest that the differences in plant growth promotion are not given by an incapability of the isolate of early colonization of Tacuabé plants.

Keywords: plant growth promotion; microscopy; molecular dialogue.

Poster II.2

Indigenous rhizobia from coal mining areas and their contribution to the promotion of growth of herbaceous legumes

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The biological nitrogen fixation (BNF) is a mechanism historically studied, especially for the proven contribution of the use of inoculants as a sustainable alternative in agriculture. In recent decades, studies have also demonstrated the potentialities of rhizobia-leguminous symbiosis in other environments, such as those with low nutrient availability, extreme temperatures, salinity and degraded environments such as in mining areas. In spite of the importance of BNF in these environments, other plant growth promoter characteristics (PGPR) present in rhizobia can help to establish the plants under adverse conditions. In order to characterize the potential of this symbiosis in recovering coal mining areas, six autochthonous rhizobia isolates were selected based on their symbiotic efficiency for *Calopogonium mucunoides*, an herbaceous legume used in revegetation programs in such areas. Five growth promoting characteristics were evaluated, including calcium phosphate solubilization, siderophore production, indole acetic acid (IAA) and ACC deaminase, and the formation of biofilms. The isolates were taxonomically identified via partial sequencing of the 16S rRNA gene. It was verified that the isolates belonging to the genus *Rhizobium* stand out as to the production of IAA and as biofilm formers. The isolates identified as belonging to the genus *Pseudomonas* presented phosphate solubilization capacity and intermediate values of IAA. The isolates identified as *Bradyrhizobium* were shown to have low growth promoting capacity, and only one isolate had the capacity to solubilize calcium phosphate. None of the autochthonous rhizobia presented a mechanism to produce ACC deaminase. In this way, it can be concluded that indigenous rhizobia from coal mining areas have, in addition to nitrogen fixing capacity, different PGPR characteristics with the potential to assist the establishment of *C. mucunoides* under stress conditions in degraded environments.

Keywords: revegetation; plant growth promotion; Calopogonium mucunoides*.*

Financial Support: Universal CNPq 407769/2016-1.

Poster II.3

PGPR traits of isolates obtained from the rhizospheric bacterial community of *Echinocactus platyacanthus*

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The arid and semi-arid regions occupy about 65% of the Mexican landscape. Plant species within the Cactaceae family are highly represented in these regions, some of them threatened by various factors, mainly human activities. In the semi-arid region at Queretaro state, around a third of the local cactus species are endangered in some category; this is the case of *Echinocactus platyacanthus*. Propagation of this plant is difficult due to its slow growth and low survival rate when is reintroduced into its natural habitat. A useful approach to overcome this limitation is the use of PGPR, which contribute to their development, reproduction and survival. The objective of this work is to study, under a microbiological approach, the composition of the bacterial communities associated to *E. platyacanthus* and characterizing *in vitro* the PGPR activities of the rhizospheric isolates. A total of 268 bacterial morphotypes associated with *E. platyacanthus* were isolated and grouped into 45 different 16S rRNA generestriction assay patterns. Representative bacteria of each pattern were selected, their 16S rRNA gene amplified and sequenced to make the phylogenetic analysis. The more abundant genera were *Bacillus* and *Pseudomonas*, although others less abundant (*Staphylococcus*, *Stenotrophomonas* and *Chitinophaga*) were also found. The *in vitro* characterization of the PGPR traits of the *E. platyacanthus* isolates focused on: growth inhibition of phytopathogenic fungi *S. reilianum*, *U. maydis* and *F. solani*; quantification of indoleacetic acid production (48–98 g/ml); qualitative detection of siderophore production (rate 1 - 1.7 in LB-CAS-FeCl3) and ability to solubilize phosphates (rate 1–1.46 in NBRIP). According to these results, the strains that show the highest values are: *Stenotrophomonas*, siderophore production (1.7); *Chitinophaga*, *Pseudomonas* and *Staphylococcus*, IAA production (98, 94 and 93 g/ml, respectively); *Flavobacterium*, solubilizing rate (1.46). Therefore, could be postulated as inoculants.

Keywords: cactus species; endangered; IAA; siderophore production.

Financial Support: CONACYT 221204.

Poster II.4

Transcriptomic responses of *Burkholderia catarinensis* to a phytopathogenic fungus and wheat

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Bacteria from the *Burkholderia* genus are widely isolated from soils and commonly associated with the rhizosphere of economically important grasses as wheat, corn and rice. The new species *Burkholderia catarinensis* was isolated from Brazilian grassland soils and showed plant growth promotion and biocontrol features, as also reported to many other *Burkholderia* spp. In spite of the current restrictions imposed to the use of this genus for inoculation purposes, we are interested in understanding its ecological importance in agricultural systems. Thus, the aim of this work is to study the transcriptome of *B. catarinensis* 89 when in contact with the phytopathogenic fungus *Drechslera tritici-repentis* or with wheat plants contaminated with this fungus. The experiment was performed in three 50 mL Falcon tubes for each condition: bacterium grown in 25% Hoagland solution with 0.2% glucose (OD600= 0.35, control); the control solution plus 10^4 fungal conidiophores (fungus treatment); and three wheat plants per tube (5 days after germination) in contact with the fungus treatment solution (plant treatment). After 3h of incubation under light agitation, at 25ºC, these solutions were mixed with RNA protect®. Bacterial RNA was extracted using the RiboPure™ RNAPurification kit, which yielded nine high quality libraries. Data was sequenced using the Ion Torrent Platform that generated high quality reads for a reliable statistical analysis. After processing the reads with Trimmomatic, Bowtie2, HTSeq-count and DESeq2 tools, we found 347 bacterium genes differentially expressed in the condition with the fungus and 436 in the plant condition (considering a *padj*<0.1 and ±1.5 of log2foldchange). Comparing to the control, we found 83 and 155 up regulated genes and 179 and 291 down regulated genes exclusively in the fungus and plant treatments, respectively. The functional investigation of these genes is currently underway.

Keywords: RNA-Seq; Burkholderia *spp.; biocontrol.*

Financial Support: CNPq; UK-Brazil Nitrogen Fixation Centre (UBNFC).

Poster II.5

Characterization of bacteria associated to bamboo for use in plant micropropagation

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Biotechnologies associated to micropropagation are usefull for the large-scale multiplication of elite-genotypes and for plant germplasm conservation. A technical problem refers to the high rate of microbial contamination, which prevents the establishment of *in vitro* regenerative protocols. In order to evaluate the action of bacteria isolated from the bamboo *Dendrocalamus asper* (Schult. & Schult.f.) Backerex K. Heynee and *Bambusa oldhamii* Munro, an inoculation test using 03 of the 32 bacterial isolates was performed using pineapple var. Pérola *in vitro* cultures. The three bacteria were selected by morphological characteristics and grown in 100 ml of LB (Luria-Bertani) medium for 24 hours. Then, 0.1 ml of each were inoculated into flasks containing MS medium (Murashige & Skoog, 1962) and five pineapple shoots. In the first essay isolates inoculation followed shoot transplanting. At the second, the cultures received the inoculation 30 days later. To the both lots, there were two control treatments: one, with LB medium, without bacterial inoculation and another, without LB addition. Cultures inoculated soon after transplanting suffered competition from bacteria, causing mortality. For cultures inoculated after 30 days, there were higher resistance and survival. Cultures submitted to the control treatment with LB, developed more than those without the medium. It is assumed that LB stimulated the associated bacteria that induced shoot growth. This suggested that the inoculation should be diluted to avoid nutrients competition among bacteria and shoots. Studies on bacteria isolated from bamboos are in progress in order to examine if they stimulate shoot growth and may protect or mitigate contamination by other microorganisms.

Keywords: Endophytic bacteria; in vitro *cultivation; Poaceae.*

Poster II.6

Characterization of plant growth-promoting bacteria associated with canola

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Canola (*Brassica napus* L.) is one of the world's most important oilseed crops. The production of canola in Brazil is modest, but the productivity has potential to be enhanced. The use of plant growth promotion (PGP) rhizobacteria stands out as an economically and environmentally friendly alternative for increasing crop yields. Thus, the aim of this work was to isolate and characterize canola rhizosphere bacteria with different PGP traits. Soil samples from canola rhizosphere were heated (80ºC) for the elimination of non-sporulating bacteria. Isolation was performed using TB and ACA media. The microorganisms obtained were identified by 16S rRNA partial gene sequencing and submitted to a colorimetric assay for auxin detection. The isolates were evaluated for their ability to produce different hydrolytic enzymes by plate assays and their nitrogen fixing ability was evaluated using the acetylene reduction assay. The isolates were also assessed for phosphate (P) solubilization on solid and liquid culture media supplemented with different inorganic sources of phosphorus (TCP, aluminum P, iron P and P rock). Thirty isolates were obtained: 22 belonging to the *Bacillus* genus, 6 to *Paenibacillus*; 1 to *Lysinibacillus* and 1 belonging to the *Microbacterium* genus. Thirteen isolates produced auxins, 15 isolates solubilized P from TCP and 9 isolates solubilized P from P rock in liquid culture. No isolates exhibited P solubilization capacity in media supplemented with iron and aluminum P. The isolates 6PB, 20PB, 27PB and 11TAC, all identified as *Paenibacillus*, yielded the best solubilization performances and pH reduction in liquid medium. These microorganisms produced different enzymes related to rhizosphere competence. The isolates 6PB, 20PB and 27PB were the only ones that showed the ability to fix nitrogen. Thus, these *Paenibacillus* isolates displayed PGP traits and should be used in future greenhouse assays to assess their potential as canola inoculants.

Keywords: phosphate-solubilizing bacteria; Brassica napus *L.; rhizosphere.*

Financial Support: CNPq; UK-Brazil Nitrogen Fixation Centre (UBNFC).

Poster II.7

Diversity of Arbuscular Mycorrhizal Fungi in Areas of Sugarcane Inoculation with Nitrogen Fixing Bacteria

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The diversity of arbuscular mycorrhizal fungi (AMF) was evaluated in two experimentation areas of sugarcane inoculation with N fixing bacteria (NFB), in Quatá, SP, Brazil. The first area (EAI) was being for the first time planted with sugarcane (previous pasture), and the second (EAII) was an 18 years-old sugarcane monoculture. The experiment within each of the areas included two sugarcane cultivars (RB966928 and RB92579) inoculated or not with NFB. A total of 108 soil samples were collected among the two areas in three seasons throughout the crop cycle (three, nine and 16 months after planting). AMF species richness and density of spores in 50 cm^3 of soil were analyzed; and the frequency of occurrence and dominance level of the species were determined. Identification of the AMF species was based on the morphology of the spores. In total, 36 species were verified in the two areas, from which 31 and 29 corresponded to the EAI and EAII, respectively. The best represented genera were *Acaulospora*, *Glomus* and *Scutellospora*. Twenty-three species showed a generalist character, being present in both of the areas. Most of the species were classified as rare, with 12 (39%) (EAI) and 15 (52%) (EAII) species within this category. *Glomus macrocarpum*, *Acaulospora scrobiculata*, *A. mellea*, *Gigaspora* sp. and *Ambispora leptoticha* were dominant in both areas. Spore density was, in general, higher in the EAI compared to the EAII; and showed a tendency to increase towards the end of the crop cycle. In the EAI this variable had mean values of 214 (three months), 156 (nine months) and 618 (16 months) spores/50 cm^3 of soil. In the EAII, the average values were of 144 (three months), 266 (nine months) and 427 (16 months) spores/50 cm^3 of soil. Sugarcane inoculation with NFB did not influence AMF communities, nor did the cultivar. Soil fertility and time of sugarcane mono cropping, and not NFB inoculation or sugarcane cultivar, determine variations in the community of AMF between areas.

Keywords: Saccharum *sp.; Glomeromycota; taxonomic survey.*

Financial Support: PEC-PG CNPq (MSc Grant); Project: Embrapa -QUATA S/A - FUNARBE.

Poster II.8

Functional diversity of the microbial community in the rhizosphere of tomato in response to saline stress and inoculation with PGPB

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Important ecosystems functions are drive by microbial communities, that are space-time shaped accordingly to biotic and abiotic interactions on which they are involved. In this context, this work aimed to estimate the saline stress impact on the functional diversity of microbial communities colonizing the tomato rhizosphere when different plant growth-promoting bacteria (PGPB) are inoculated. Tomato seedlings obtained from surfacesterilized seeds were transferred to rhizoboxes filled with soil: sand (1:4, *v/v*) as substrate to set a 2 x 3 factorial experimental design with 2 saline-stressing conditions (Hoagland's solution supplemented or not with 100 mM NaCl) and 2 PGPB inoculation treatments (*Pseudomonas* sp. 16S or *Enterobacter* sp. 15S) plus a an uninoculated control (C). Plants were grown in a greenhouse for 40 days, and after this period the rhizosphere soil was collected and serially diluted with sterile saline solution (0.85% NaCl) to 10^{-3} which was used to inoculate Biolog EcoPlate^{TM} microplates. Biolog EcoPlates were incubated at 28ºC for a week and the color development on microplates was registered as optical density (OD 590 nm) at regular 24 h-intervals. Microbial activity was expressed as average well-color development (AWCD) for each reading time; data from 96 h-incubation was applied to determine differences in the community functional diversity, expressed as species richness (*R*), Shannon's diversity (*H*), evenness (*E*) and principal component analysis (PCA). Statistically significant differences in utilization of C-substrates was determined by t-test ($P \leq 0.05$). The AWCD values increased accordingly to the incubation time and showed the subsequent trend for most of the time points monitored: 15S+NaCl>C+NaCl>16S+NaCl>16S>C>15S. Significant differences were found between *H* of treatments 16S+NaCl>C>15S; *R* value of treatment C was significantly lower when compared to all other treatments. The PCA allowed to differentiate between treatments and respective preferential C-sources, explaining 55.6% of observed variability in the 2-dimensional analysis. The results demonstrate that either salt stress or PGPB inoculation impacted the functional diversity of microbial communities on the tomato rhizosphere, although such impact differed according to the inoculated strain suggesting a differential stress-protective effect among them.

Keywords: soil functioning; inoculation; stress-protective effect.
Financial Support: Capes, CNPq, Fundação Araucária.

Poster II.9

Culture Collection of Diazotrophic and Plant-Growth Promoting Bacteria of Embrapa Soybean: providing strains for a sustainable agriculture

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In the current scenario, a great challenge is to achieve food production with sustainability, adopting cultural practices to maintain soil quality and biodiversity. Despite the high prokaryotic diversity in the soil, the great limitation is to identify and classify an increasing number of microorganisms and to prospect their biotechnological potential. In this context, the maintenance of culture collections is extremely important for taxonomy, biotechnology and genetics studies. Based on this vision, the "Diazotrophic and Plant Growth Promoting Bacteria Culture Collection of Embrapa Soja" (WFCC Collection #1213, WDCM Collection #1054), located at Londrina, State of Paraná, Brazil, was created in 1991. The bacteria of the collection have been the object of several studies on taxonomy and phylogeny, including the description of 11 new species (*Bradyrhizobium diazoefficiens*, *B. tropiciagri*, *B. embrapense*, *B. viridifuturi*, *B. stylosanthis*, *B. mercantei*, *Rhizobium leucaenae*, *R. freirei*, *R. paranaense*, *R. ecuadorense* and *R. esperanzae*), as well as applied studies, including the development of commercial inoculants in partnership with the private sector. Nowadays, there are 3,700 strains deposited at the culture collection, maintained by cryopreservation (-80ºC and -150ºC) and lyophilized. Protocols of quality following the OECD (Organization for Economic Cooperation and Development) and INMETRO rules for culture collections have been established in the collection. Basic information for the strains characterization includes the morphology of the colonies, the DNA profile by BOXPCR and the sequencing of the 16S rRNA gene. Recently, the culture collection has been recognized by the MAPA (Ministry of Agriculture, Livestock and Food Supply) as an official distributor of authorized and recommended strains for the production of commercial inoculants, including diazotrophic bacteria for non-legume crops and other plant-growth promoting bacteria.

Keywords: taxonomy; phylogeny; inoculants.

Financial Support: CNPq.

Poster II.10

Occurrence of diverse *Bradyrhizobium* spp. in roots and rhizospheres of two commercial Brazilian sugarcane cultivars

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The genus *Bradyrhizobium* harbours many endosymbionts of legumes, but recent research has shown the presence of bacteria from this genus inside roots of sugarcane. This study aimed to investigate the population density of these bacteria associated with the roots of two sugarcane cultivars and to understand if the plant genotype affects the population composition of these bacteria in the rhizosphere and the endosphere. Samples of the rhizosphere and root endosphere compartments of two sugarcane cultivars (RB867515 and IACSP95-5000) were collected at the experimental field of Embrapa Agrobiology, serially diluted and inoculated on gnotobiotic cowpea (*Vigna unguiculata*) seedlings and after growth, presence or absence of nodulation was scored. Although most probable numbers (MPN) could not be reliably determined, it was estimated that there were at least 15625 ($1.6*10^4$) rhizobia g root^{-1} in rhizosphere samples and between 125 and 625 ($1.3\text{-}6.3*10^2$) rhizobia g root^{-1} in the endosphere samples of both cultivars. This showed that the density of rhizobia in the rhizosphere of both sugarcane cultivars is significantly higher than inside roots. From the nodules, 34 isolates were obtained from the rhizosphere samples of each cultivar and 15 and 14 respectively from the roots of cultivars IACSP95-5000 and RB867515. BOX-PCR profiling revealed genetic diversity within this collection, with some dominant (up to 14 representants) and less dominant genotypes. 16S rRNA and ITS sequence analyses showed that the majority pertained to the *B. elkanii* superclade and five isolates were closely related to the recently described *B. sacchari* in the *B. japonicum* clade. No effect was observed for the factors 'plant cultivar' 'root compartment' on *Bradyrhizobium* spp. community composition, that is, the most abundant genotypes occurred bot in rhizosphere and endosphere of both cultivars. These data increase our understanding of the ecology of rhizobia in the sugarcane root system.

Keywords: rhizobia; microbial ecology; non-legumes.

Financial Support: Embrapa; Capes.

Poster II.11

Peanut and maize growth stage affects the rhizobacterial community structure

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Peanut (*Arachis hypogaea* L.) is an economically important leguminous crop in Argentina. The rotation of peanut with other crops, especially cereal crops is crucial to obtain a good yield. In the agricultural area of Argentina, maize (*Zea mays* L.) is the main crop used in rotation with peanuts. Rizosphere bacteria can exert beneficial effects on plant growth grow and development by releasing nutrients into the rizosphere. This soil area is a versatile and dynamic ecological environment of intense microbe plant interactions and the rhizobacterial communities can be affected by a wide range of factors including environmental factors such us soil type and plant species. Considering the importance of peanut and maize crops, in the objective of this study was to analyze bacterial community structure associated with the phenological stages of these plants. For this, a microcosm assay was performed using soil from the peanut growing area of Argentina as plant growth support. Unsterile soil was placed in plastic pots and previously peanut and maize disinfected seeds were sown. Plants were maintained under controlled environmental conditions during 120 and 100 days for peanut and maize respectively. For bacterial rizhosphere analysis, soil samples were taken at the beginning of the assay for both plants and at 60 and 120 days for peanut and at 40 and 100 for maize. Soil DNA from these samples was extracted using a commercial kit. Bacterial community structure of soil rizosphere was analyzed by high-throughput sequencing of 16S rRNA gene (Illumina MiSeq platform). Results obtained indicated that the most abundant phyla, in both peanut and maize rizosphere were Proteobacteria, Acidobacteria, Actinobacteria and Bacteroidetes. Rhizobacterial community structures were different depending on plant species and significant changes were observed through plant growth stages at diverse taxonomic levels (phylum, family and genus). The most significant differences were observed on the rizosphere of late plant growth stages. In conclusion, the structure of the rhizospheric bacterial community is dynamic and is influenced by phenological plant growth stage.

Keywords: bacterial community; plant development; Illumina MiSeq platform.

Financial Support: CONICET; SCyT-UNRC; ANPCyT.

Poster II.12

Insights into the genomes of *Azospirillum brasilense* strains Ab-V5 and Ab-V6, used in commercial inoculants for grasses and legumes in Brazil

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Azospirillum is one of the most studied and used plantgrowth-promoting bacteria (PGPB). Brazil has a long tradition of studies with *Azospirillum*, but it was only in 2009 that the first commercial inoculants entered into the market. Since then, the use of two strains, Ab-V5 (CNPSo 2083) and Ab-V6 (CNPSo 2084), increased exponentially in inoculants for grasses (maize, wheat, rice, brachiarias), and for co-inoculation of legumes (soybean, common bean). In the 2017/2018 crop season, about 5 million doses of inoculants carrying these strains have been commercialized in the country. The genomes of Ab-V5 and Ab-V6 were sequenced on the MiSeq platform at Embrapa Soja and assembled with the A5-MiSeq pipeline (*de novo*), resulting in coverages of 245- and 60-fold, respectively. The genome of Ab-V5 was estimated at 6,934,595 bp, and of Ab-V6 at 7,197,196 bp. Electrophoresis in Eckhart gels indicated that Ab-V5 and Ab-V6 have at least two common plasmids (of about 285,000 and 100,000 bp), and that Ab-V6 carries an extra plasmid of about 150,000 bp. Both genomes are highly similar, with nucleotide average identity (ANI) estimated at 100%. When the genomes were submitted to the annotation (RAST server), 6,349 DNA coding sequences (CDSs) were identified in Ab-V5, and 6,625 CDSs in Ab-V6. Although Ab-V6 synthesizes higher amounts of indole-acetic acid (IAA) than Ab-V5 in culture medium enriched with tryptophan, both strains share high similarity of the genes and of the operons related to the synthesis of auxins. Quorum sensing may represent an important strategy for plant colonization, and both genomes carry several copies of *luxR*, but none of *luxI* genes. One important feature of Ab-V5 and Ab-V6 is their capacity of inducing genes related to tolerance to biotic and abiotic stresses in plants, and both genomes carry several genes related to stress responses, the majority related to oxidative stresses.

Keywords: inoculation; quorum sensing; antioxidant activity.

Financial Support: INCT, CNPq, Capes.

Poster II.13

Increased plant growth promotion performance of *Herbaspirillum seropedicae* in the presence of the saprophytic fungus *Trichoderma longibrachiatum*

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Soil bacteria-fungi interactions play key roles in the ecosystems functionality such as nutrient cycling. Nevertheless, inter- and intraspecific microbial interactions have been neglected as a biotechnological agriculture input. Ecophysiological aspects of the relationship between the fungus *Trichoderma longibrachiatum* UENF 476 (vermicompost isolate) and the nitrogen-fixing bacterium *H. seropedicae* (strain RAM 10: labeled with the green fluorescent protein or strain HRC54) were evaluated. Structural interaction using microscopy and immunolabelling were conducted in vitro o petri dishes containing glass slides or semisolid JNFb medium inoculated with RAM 10 or RAM 10 plus 476. Physiological studies related to diazotrophic activity, effect of the compounds secreted by the fungus, solubility capacity of mineral sources of phosphorus (P-Ca and P-Araxá) and production of indole compounds were measured for bacteria, fungi and their interaction. Our data had shown that there was a clear structural compatibility between the partners with increased populations and rates of biological nitrogen fixation by the bacterium. Also, bacteria number increasing was dependent of the fungi structures, since its metabolites has no effect. Microscopy had shown bacteria colonizing hyphosphere and attached to the hyphae surface as aggregates or biofilms. The P-solubility and the production of indole compounds were increased in the interaction. Greenhouse assay using tomato and papaya seedlings was carried out and the combined use of microorganisms significantly increased plant biomass with values between 70 and 220%. Taken together the results demonstrates the importance of a saprophytic fungus for spatial dispersion and increased survival and activity of diazotrophic bacteria. Combined use of these microorganisms represents a technological alternative for plant growth promotion, being partially justified by the compatibility and synergy of the described ecological relationship.

Keywords: microbial ecology; substrate enrichment; nitrogen fixation.

Financial Support: Capes, CNPq, Faperj, Newton Fund Grant BB/N013476/1.

Poster II.14

Diazotrophic bacteria isolated from sugar cane straw and its biotechnological potential

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Soil bacteria can modulate plant growth and development through different mechanisms. Green harvest of sugarcane includes the deposition of large amounts of plant litter on the soil with a carbon (C) to nitrogen (N) ratio close to Microbial consortium formulations with free living nitrogen-fixing bacteria could decrease the C:N ratio and might be an alternative to accelerate the decomposition process of sugarcane straw, releasing more nutrients in soil. The objective of this study was to isolate and characterize diazotrophic bacteria associated with sugarcane straw collected at LASA Agroindustry company (Linhares-ES). Serial dilution of 10 g samples were inoculated in N-free semi-solid JNFb media and purified colonies were obtained in Dyg's solid medium. Isolates were evaluated in solid media containing 0.1% of zinc oxide, calcium phosphate and carboxymethylcellulose in order to evaluate the solubilizing and degrading capacity. Indolic compounds was determined by the Salkowsky assay, with and without 100 mg/L of tryptophan. PCR technique was used to detect *nifH* gene. A total of 11 bacteria isolates were obtained. After colony purification, typical aerotaxic pellicle and *nifH* gene detection were observed confirming its diazotrophic ability. For P-solubilization, 10 isolates had shown positive capacity with solubilizing index (SI) ranging from 1.1 to 1.6. Six isolates were able to solubilize zinc, with SI between 1.6 to 2.5. None of the isolates were able to degrade cellulose. All isolates produced indolic compounds with values varying between 68.6 μM and 241.4 μM (with tryptophan) and between 74.9 and 228.3 μM (without tryptophan). Despite the lack of cellulose-degrading enzymes, the diazotrophic ability and other PGPR traits point out the potential of these isolates to be part of a microbial consortia to accelerate the decomposition rate of sugarcane straw in green management of sugarcane.

Keywords: microbial bioprospecting; diazotrophic bacteria; microbial decomposition.

Financial Support: CNPq, Faperj, FINEP-PLURICANA and Newton Fund Grant BB/N013476/1.

Poster II.15

Selection of growth promoting bacteria in relation to the production of AIA and nitrogen fixation

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Plant growth-promoting bacteria have been highlighted mainly by their potential to increase crop performance and reduce the use of chemicals products. The aim of this study was to verify the potential of plant growth promotion of 25 bacteria strains. The strains were selected according to genera and species, described in the literature as plant growth-promoting bacteria. Of the bacterial strains, 22 were assigned by the Agricultural Microbiology Culture Collection (CCMAUFLA), Department of Biology, Federal University of Lavras (UFLA), Lavras, Minas Gerais, Brazil and 3 strains (UNIFENAS 100-13, UNIFENAS 100-39 and Ab-V5 provided by EMBRAPA Londrina, Paraná, Brazil) provided by the José de Rosário Vellano University (UNIFENAS), Alfenas, Minas Gerais, Brazil, were used as a positive control. IAA production was quantified in a spectrophotometer at 530 nm and its concentration (μg mL^{-1}) were calculated from a standard curve created with pure IAA. The nitrogen fixation was determined for the NFb (Ab-V5 – positive control) and JMV medium (UNIFENAS 100-13 – positive control), both semi-solid media. The experiments were conducted in triplicate. The best results for IAA production were observed for *Bradyrhizobium japonicum* CCMA 0088, *Burkholderia cenocepacia* CCMA 0101, *Burkholderia cepacia* CCMA 0056, *Enterobacter cloacae* CCMA 1285 and *Azospirillum brasilense* Ab-V5 strains, producing 3.70, 3.31, 3.10, 2.86 and 2.48 μg mL^{-1} IAA, respectively. All strains, except *Paenibacillus amylolyticos* CCMA 0448 and *Paenibacillus illinoisensis* CCMA1269, were capable of fixing nitrogen in NFb medium. In JMV medium, only UNIFENAS 100-13 *Burkholderia cenocepacia*, UNIFENAS 100-39 *Burkholderia cenocepacia* and CCMA 1269 *Paenibacillus illinoisensis* strains had positive results. Ab-V5 *Azospirillum brasilense*, CCMA 0056 *Burkholderia cepacia* and CCMA 1285 *Enterobacter cloacae* are the strains selected in this work with higher potential to be used as plant growth-promoting bacteria.

Keywords: PGPB; microorganisms; Azospirillum brasilense*.*

Financial Support: FAPEMIG, Capes and CNPq.

Poster II.16

Characterization of plant growth promoting bacteria isolated from wild rice grown in the Pantanal Sul Matogrossense

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The objective of this work was to evaluate the occurrence of diazotrophic bacteria associated to wild rice *Oryza latifolia* and *O. glumaepatula* and analyze the plant growth promotion features of the isolates. Three samples were collected from each wild rice genotype, at the flowering stage, grown at two sites of the Paraguay River. The number of diazotrophs in the roots of both wild rice was counted. Vials from the highest dilutions showing characteristic veils of diazotrophs were selected and successively transferred to the semi-solid media. Characteristics related to biotechnological potential were analyzed: cellulolytic, proteolytic, amylolytic activities, siderophore production, Indole acetic acid (AIA) and solubilization of calcium inorganic phosphate and zinc oxide. In addition, the 16S rRNA was sequenced and a phylogenetic tree was constructed. Thirty-three isolates were selected for the greenhouse inoculation experiment planted in plastic trays containing commercial substrate (sand, vermiculite and commercial vegetable substrate). Plants were harvested after 52 days after transplanting and analyzed for biomass accumulation. A high number of diazotrophs associated to roots of both rice genotypes harvested in two areas was detected: *O. latifolia* ($1.55x10^7$ and $1.71x10^7$ bacteria/g root) and *O. glumaepatula* ($1.01x10^7$ and $0.68x10^7$ bacteria/g root). A total of 201 diazotrophs were isolated and 80% presented some type of functional activity: 66% proteolytic (21%), amylolytic (16%), siderophores (55%), production of AIA (40%), solubilization of calcium phosphate (52%) and zinc oxide (50%). The 16S rRNA sequencing identified strains belonging to the genera *Pantoea*, *Achromobacter*, *Enterobacter*, *Stenotrophomonas*, *Pseudomonas* and *Bacillus*. Among the tested isolates, the inoculation of *O. latifolia* with the isolate 110 CAL increased up to 59% the fresh biomass the increased 214% the dry mass. In the case of *O. glumaepatula* the inoculation with the isolate 180 CAG increased up to 54% the fresh biomass the increased 260% the dry mass. The results showed that these two strains belonging to the genus *Pantoea* showed great potential for dry and fresh biomass of the wild rice species.

Keywords: Oryza*; BFN; Diazotrophs.*

Financial Support: Fundect/MS, Capes.

Poster II.17

Evaluation of IAA producer and phosphate solubilizer rhizosphere yeast *Torulaspora globosa* to promote lettuce growth in field conditions

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The soil has an abundant microbial diversity, which the majority remains unknown. Among the identified microorganisms that inhabit the soil, one group stands out as beneficial to the agricultural activities: the microorganisms that promote plant growth (PGPM). They can aid the development of plants through, mainly, the production of phytohormones, nutrients solubilization and control of phytopathogens. Among the PGPM, the yeasts present diminutive prominence, because they are present in less quantity in the soil; however, literature reports indicate that this group presents great potential, since it showed excellent results in *in vitro* experiments. Considering the above, this work had the objective of evaluating the inoculation of rhizosphere yeast *Torulaspora globosa* (strain 5S55) in seeds and lettuce seedlings, and evaluation of the potential to plant production increase. Half of the lettuce seeds was inoculated ($1x10^8$ cells / kg of seed) by mixing the seeds with the cell suspension into the plastic bag to homogenize; sowing occurred in trays on coconut fiber substrate. The seedlings (from inoculated and uninoculated seeds) were divided into 3 treatments which received a new inoculation of $1x10^6$ cells, 7 days after emergence, 15 days after emergence or 7 and 15 days after emergence. Before transplant, ten seedlings were evaluated (length, number of leaves, leaf width, dry mass), and another was transplanted to the field, being evaluated after harvesting (shoot length, stem length, width, length and number of leaves, aerial dry mass). The results showed that inoculation of the yeast did not cause a significative increase or damage to plant development. Although yeast *T. globosa* (5S55) has been isolated from rhizosphere, there is no evidence of its survival ability when inoculated on seeds or roots in non-sterilized substrates, and its establishment and association with the plant. In this way, despite the good results obtained with the yeast as a producer of the indoleacetic acid *in vitro*, it is necessary other studies, evaluating new forms of inoculation of the seeds, increase the cellular concentration of the inoculum, and the evaluation of the presence of yeast in the rhizosphere of inoculated plants.

Keywords: microorganisms; yeast.

Poster II.18

Potential of plant growth promotion and Pb^{2+} resistance by bacteria isolated of mining tailings

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Microorganisms isolated of contaminated areas with heavy metals are important biotechnology tools because it's resistance mechanisms can perform soil or water decontamination function. Allied to these mechanisms, there is also the capacity of these microorganisms in promoting the plant growth, like in biologic nitrogen fixation and hormones production like the indolacetic acid (IAA). These function, is of the high importance for degraded areas recuperation, since in revegetation process the symbiotic relation with these microorganisms is very important, ensuring the high efficiency and low cost for vegetation restoration. Therefore, the present work had the objective of test the plant growth promotion potential of 10 strains of bacteria isolated of mining tailings of Mariana – MG. The bacteria has initially identified by MALDI TOF technique as: *Lysinibacillus sphaericus* 1.1; *Lysinibacillus sphaericus* 1.2; *Pantoea dispersa* 1.4; *Pantoea dispersa* 2.1; *Pantoea dispersa* 2.2; *Pantoea dispersa* 2.3 and four Isolated not identified (1.3; 1.5; 1.6 and 1.7); The tests realized in the microorganisms were the capacity of Pb^{2+} resistance, IAA production and biologic nitrogen fixation. The results demonstrated growth in up to 3.5 mM of Pb^{2+} for strains *Lysinibacillus sphaericus* 1.1; *Lysinibacillus sphaericus* 1.2; Isolated 1.3; Isolated 1.6 and Isolated 1.7 and growth up to 4.0 mM for strains *Pantoea dispersa* 1.4; *Pantoea dispersa* 2.1; *Pantoea dispersa* 2.2 e *Pantoea dispersa* 2.3. No microorganisms produced IAA and the isolated 1.6 and 1.7 showed capacity of nitrogen fixation. The results demonstrated a possibility of use principally of the isolated 1.6 and 1.7 in studies of vegetal growth promotion in degraded area recuperation contaminated with heavy metals. However, more studies should be realized, evaluating others desirable characteristics, well as the microorganisms and plants interactions.

Keywords: nitrogen fixation; microorganisms; heavy metals.

Financial Support: Fapemig, CNPq e Capes.

Poster II.19

Ecotypes theory for the identification of bacterial groups with potential PGPR traits in *Bacillus subtilis/amyloliquefaciens*

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Endospore-forming plant growth-promoting rhizobacteria (EF-PGPR) have a great potential in agriculture, both for it is effectiveness in biocontrol and biofertilization, and for its advantages of commercial formulation. Processes such as the prospecting of new and better isolates, the design of multi-strains inocula or the identification of factors that cause variability in their effect require the delimitation of groups of isolates that are sufficiently homogeneous to provide explanatory and predictive power. Today, the delimitation of species according to the official concept for bacteria does not provide such homogeneity. Under the hypothesis that the delimitation of groups of isolates by ecological similarity (concept of Ecotype) can provide the level of homogeneity required for the studies, this work aimed to evaluate the effectiveness of the theory of Ecotypes for the identification of bacterial groups with potential PGPR traits in the clade *Bacillus subtilis/amyloliquefaciens*. Because of this, EF-PGPR were isolated associated to the native plant (*Carludovica palmata*) and another cultivated (*Zea mays* var. 109), established in two contrast fertility soils in the Urabá Antioqueño. Isolates belonging to the clade of interest were selected according to their rDNA 16S sequence and, based on the sequence of the gyrA gene, a demarcation analysis of Putative Ecotypes (PE) was carried out using the Ecotype Simulation (ES) and AdaptML algorithms. In the demarcation, 11 PE were identified by ES and 4 by AdaptML. A significant association was found between the bacterial Ecotype and the plant species, reflecting specificity in the association. Likewise, a significant association was found between the demarcated Ecotypes and the *in vitro* production levels of indoles, revealing differences in biochemical traits with effect on plants. Taken together, these results suggest that the identification of bacterial Ecotypes may be useful in the study and application of EF-PGPR.

Keywords: endospore-forming PGPR; ecological homogeneity; speciation.

Poster II.20

Occurrence of diazotrophic bacteria in *Jacaratia corumbensis* O. Kuntze (Caricaceae)

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The ability of nitrogen biological fixation by diazotrophic bacteria boosts the studies of occurrence, isolation, characterization and selection of efficient strains for plant growth promotion. Our aim was to quantify and compare populations of diazotrophic bacteria isolated from the aerial part, root and rhizospheric soil of *Jacaratia corumbensis* O. Kuntze (Caricaceae). Three individuals of *J. corumbensis* were collected in two areas in the Pantanal region: Taquaral (plants 1, 2 and 3) and Jacadigo settlement (plants 4, 5 and 6), which lie about 15 km from Corumbá city, MS. The Most Probable Number technique (MPN) using the NFB and LGI semisolid culture media (N-free) semi-selective for *Azospirillum* and *Nitrospirillum* was used to isolate bacteria from each selected part of each plant. Counting was performed based on the presence of a pellicle, following the table of McCrady for three replicates per dilution. No significant differences were observed in the MPN of diazotrophic bacteria between both sampled areas and culture media. However, there was a significant difference for the samples of aerial part, root and rhizospheric soil, where the root samples presented MPN log values of cells per gram of root ranging from 0.0 to 5.39, in the NFB medium and 3.1 to 4.15 in the LGI medium. Although the rhizospheric soil samples of plants 1 and 3 presented the highest log values of the MPN of cells per gram of rhizospheric soil, both with 6.73, followed by plants 2 and 5, with 5.51 and 5.4, no bacteria were observed in plants 4 and 6 (NFB medium). In the LGI medium, the log values of NMP per gram in the rhizospheric soil ranged from 0.0 to 5.92. In the aerial part samples, the highest values of log of the MPN were 3.91 and 3.0, while in the LGI medium we observed no bacteria. In conclusion, bacteria naturally inhabit *J. corumbensis*, and are mainly associated with the plants' roots. Sampled areas did not influence the number of bacterial populations in the plant species.

Keywords: biological fixation; Nitrospirillum*; pantanal region.*

Financial Support: CNPq and Fundect.

Poster II.21

Errors in taxonomic attribution of genome sequences of *Paenibacillus polymyxa* deposited in Genbank

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The species *Paenibacillus polymyxa* is composed of diazotrophic Gram-positive free-living bacteria that can promote the growth of plants with economic relevance. Identification of its isolates is essential to guide its appropriate study and use. The 16S rRNA gene marker does not present enough resolution to discriminate *Paenibacillus* isolates at the species-level. With the advent of high-throughput sequencing technologies, the possibility of identifying organisms through genomic metrics such as Average Nucleotide Identity (ANI) has arisen. Genomic analyses are showing that many taxonomic attributions do not correctly express phylogenetic relationships among *Paenibacillus*. The aim of this work is to validate the taxonomic attributions of *P. polymyxa* strains deposited in GenBank through genomic metrics. More than three hundred genome sequences of the genus *Paenibacillus* were compared with that from the *P. polymyxa* type-strain ATCC 842 through the computation of the ANI with the PYANI software. Nine genome sequences attributed to *P. polymyxa* presented ANI values higher than or equal 95% (species circumscription threshold). Fifteen genome sequences assigned to *P. polymyxa* were classified as false positives (FP), because they presented ANI values lower than 95% in relation to the type strain. Besides these sequences, one genome sequence was not attributed to *P. polymyxa*, although it presented an ANI value higher than the species circumscription threshold. The accuracy of the *P. polymyxa* species name attribution was 37.5%, and the FN rate was 10%, with FP genomes representing 62.5% of the genomes classified as *P. polymyxa*. Many attribution errors were found in GenBank, therefore it is recommended the use of genomic metrics before submitting genome sequences to this database.

Keywords: ani; genomics; bioinformatics.

Financial Support: UK-Brazil Nitrogen Fixation Centre (UBNFC) and CNPq.

Poster II.22

Paenibacillus gauderius sp. nov., a nitrogen fixing species isolated from the rhizosphere of *Helianthus annuus* L.

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Plant growth and productivity are largely influenced by soil microbes, especially the beneficial bacteria known as PGPB (Plant Growth Promoting Bacteria), which are found around roots or associated with plant tissues. Members of the genus *Paenibacillus* present broad metabolic diversity and have been isolated from different plants and habitats. In previous studies, three facultative anaerobic endosporeforming bacteria (designated P3E, P26E, and P32E) were isolated from the rhizosphere of sunflower grown in fields of Rio Grande do Sul State (Brazil). These isolates presented positive results for nitrogen fixing and were identified as members of *Paenibacillus* genus, according to preliminary analyses of 16S rRNA gene. The genomes of these isolates were sequenced and genomic analyses (average nucleotide identity and orthologous ANI) revealed similarity values between 84 and 85% when they were compared to their closest relatives *Paenibacillus graminis*, *Paenibacillus jilunlii*, and *Paenibacillus sonchi*. *In silico* DNA–DNA hybridization (dDDH) also showed similarity ranges below the recommended threshold of 70%. Phylogenetic reconstructions based on 16S rRNA gene and core-proteome showed that P3E, P26E, and P32E form a distinct clade, which did not included any type strain of the current *Paenibacillus* species. The isolate P26E produced ellipsoidal endospores terminally located in swollen sporangia, under anaerobiosis growth. Major cellular fatty acids were anteiso-$C_{15:0}$ and iso-$C_{15:0}$, which represent about 58 and 14% of the total fatty acids in P26E, respectively. The value found for the DNA G + C content of P26E was 49.4 mol %. Based on different genomic metrics, phylogeny, and phenotypic data, we propose that P26E represents a type strain of a novel species within the genus *Paenibacillus*, for which the name *Paenibacillus gauderius* sp. nov. is proposed.

Keywords: Paenibacillus*; plant growth promoting bacteria; nitrogen-fixing; 16S rRNA phylogeny; genomic metrics.*

Financial Support: CNPq, Fapergs, Newton Fund Grant BB/N013476/1.

Poster II.23

Bacillus spp. isolated from garlic and their potential to the growth promotion and biocontrol

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The rhizobacteria are a group of microorganisms that lives around the roots of the plants, associatively or in free life. Some bacteria have the ability to promote plant growth, and they are called Plant Growth Promoting Rhizobacteria (RPCPs). This is due to the production of metabolites that help root growth, phosphate solubilization and biological control.Thus, the objective was to evaluate some mechanisms of rhizobacteria isolates from *Allium sativum* to induce plant, such as phosphate solubilization, IAA production and antibiosis against the fungus *Sclerotium cepivorum* (causing “white rot”) *in vitro*. Initially, 27 isolates of *Bacillus* spp. From the Laboratory of Microbiology collection of the Universiadade Federal de Santa Catarina, Curitibanos *campus* were tested to phosphate solubilization. They were grown in 5mL of liquid LB medium (pH 7.2) at a temperature of 25°C for 72 hours. After growing, these were transferred to Petri dishes containing calcium phosphate (Ca_3PO_4) at pH 7.0, with five replicates. For analysis of IAA production test, the isolates were grown in 5mL of liquid LB medium, enriched with tryptophan (0.05 g/mL), followed by incubation with Salkowski Reagent and subsequent analysis by spectrophotometry. For antibiosis trial, inhibition halos were observed around colonies grown on PDA medium with *Sclerotium cepivorum* at the center. Four out of the 27 isolates had higher levels of phosphate solubilization. For the IAA production test, two isolates produced larger amounts of the compound. The white rot inhibition analysis was statistically significant among the isolates and the control. Thus, it can be concluded that the RPCPs have potential for growth induction for further analysis.

Keywords: Rhizobacteria; plant growth; Alliaceae; biological control.

SESSION 3: What to seek in PGP

Key Note III.I

Nitrogen-fixing bacteria do not live alone

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Legume root nodules are plant organs where atmospheric nitrogen is converted to ammonia by the activity of bacteria known as rhizobia. Both alpha-rhizobia (*Rhizobiaceae*) and beta-rhizobia (some *Burkholderia* and *Cupriavidus* strains) initiate the development of nodules. However, numerous other bacteria also can be isolated from nodules, but in the past, these nonrhizobial microbes were thrown away as contaminants. Nevertheless, studies in many countries demonstrated that a number of the nodule isolates had plant growthpromoting traits or biocontrol activity, and hence might be useful as inoculants for enhancing crop production or biocontrol. To test this possibility, we isolated bacteria from both nodules as well as soil and performed coinoculation experiments with rhizobia on roots grown in N-depleted medium to determine if legume growth was enhanced over and above that following inoculation with the nitrogen-fixing strain alone. We also investigated the mechanisms whereby the non-rhizobial bacteria stimulate plant growth in plants in addition to legumes by examining their sequenced genomes, when available, for specific PGPB traits, e.g., hormone synthesis, siderophore production, or phosphate solubilization, and also by performing biochemical or physiological tests to determine whether any increases observed in plant growth were mediated by the expression of genes underlying these traits. By combining PGPB and nitrogen-fixing bacteria, we hope to develop consortia that will be useful for sustainable agriculture and to reduce soil and water contamination brought about by the overuse of chemical fertilizers and fungicides.

Keywords: microbiome; PGPR.

Short note III.I

Genome Wide Association Studies uncovers genes associated with plant growth promotion driven by endophytic bacteria (*Azoarcus olearius*)

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It is well documented that specific rhizosphere bacteria have the ability to promote plant growth. However, the molecular mechanisms that underlie the ability of these bacteria to associate with plant roots (both as epiphytes and endophytes), as well as to promote plant growth, are still poorly understood. In this study, we surveyed natural variation in a population of 360-ecotypes of *Arabidopsis thaliana* responding to the plant growth promoting bacteria (PGPB) *Azoarcus olearius* DQS^{4T}. All of the ecotypes within this population have been extensively genotyped. The panel showed a wide phenotypic variation to inoculation. Combining plant development traits (primary root length, lateral root number, root fresh weight and shoot fresh weight), we identified 8 genomic regions that showed strong correlation with variation in one or more of the phenotypic traits measured. In several cases, this genome wide association analysis led to the prediction of specific genes that might underlie the trait. Based on these results, we are now evaluating the function of each of the identified genes using CRISPR/Cas9 gene editing. Thus far, our findings suggest that the ability of the plant to respond to PGPR inoculation is controlled by multiple genes that do not predict simple mechanisms (e.g., changes in the levels of single plant hormones). The goal of our research is provide a more mechanistic understanding of bacterial plant growth promotion, which we believe will expand interest in these systems both for basic research and field application.

Keywords: microbiome; PGPR.

Short note III.II

Importance of quorum sensing signals in the communication of endophytic PGP-bacteria with their host plants

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Beneficial endophytic bacterial colonization of plants depends on specific microbe-plant communication, as is established for well-known symbioses. However, for beneficial endophytic bacteria in non-legume crops, much less is known in this respect. The objective or our studies was to further elucidate the possible involvement of *N*-acylhomoserine lactones (AHLs), the quorum sensing signals of Gram-negative bacteria, in the interaction with crop plants. We examined the colonization patterns by confocal laser scanning microscopy using fluorescently tagged bacteria and gene expression profiles of bacteria and plants with RNA seq and quantitative RT-PCR. Wild type and *luxI*-mutants (unable to synthesize AHLs) of the diazotrophic endophyte *Gluconacetobacter diazotrophicus* PAL5, and Acidovorax radicis N35, a beneficial endophyte of wheat and barley, were studied. A GFP-labelled *luxI*-mutant of *G. diazotrophicus* PAL5 was unable to colonize rice endophytically, in contrast to the AHLproducing wild type. Compared to the wild type strain, superoxide dismutase (*sodA*), and gum biosynthesis (*gumA*) were only weakly expressed in the *luxI*-mutant during plant colonization. In *A. radicis* N35, a *luxI*-mutant also showed less efficient endophytic colonization. Surprisingly, the expression profile of plant genes during colonization by the *luxI*-mutant showed clear defense responses, like the expression of flavonoid biosynthesis genes and increased flavonoid production, while the wild type caused only some priming responses. When 10μM pure C8- or C12-AHL compounds were applied to roots of barley seedlings, morphological and physiological changes in roots were observed. In addition, priming of systemic plant defense responses was recorded, which was accompanied by increased levels of salicylic acid and nitric oxide (NO) in plant tissues. Thus, AHL compounds of beneficial rhizobacteria play an important role in endophytic plant colonization and improved plant performance.

Keywords: quorum sensing; N-acyl-homoserine lactones; plant responses.

Poster III.1

Transcriptome analyses of maize roots inoculated with *Herbaspirillum seropedicae*

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Herbaspirillum seropedicae is an endophytic diazotrophic bacterium used in agricultural bioinoculant. Deciphering gene expression and metabolic pathways is critical to improve efficiency in promoting plant growth. This work was design to elucidate how colonization by *H. seropedicae* modulates gene expression in maize roots. Maize seeds (Dekalb 7815) were disinfected and germinated for 72h. Seedlings with 2.5 cm radicles were transplanted into 2L pots with $CaCl_2$ 2mM and kept in a growth chamber. After 24h, the inoculum containing $2x10^7$ cells mL^{-1} of *H. seropedicae* HRC54 strain was applied. Five days after inoculation, samples were collected for biometric analysis (8 replicates) and for RNA extraction (3 biological replicates, each formed by 6 seedlings). For RNA-Seq and RT-qPCR, total RNA extraction was used to synthesis cDNA library. Total DNA extraction was used for verifying inoculum establishment through the RT-PCR. Positive biostimulation phenotype was observed with root (242 and 73%) and shoot (166.7 and 188%) biomass increase, respectively for fresh and dry mass. *H. seropedicae* quantification in roots of inoculated was higher than in the control, approximately 10^6 and 10^2 bacterial cells per plant, respectively. The analysis of the RNA-Seq showed that 949 genes were differentially expressed, been 454 genes up-regulated and 495 genes down-regulated. The validation by RT-qPCR showed that the expression of the up-regulated genes gibberellin-regulated protein and phosphate transporter protein were increased 3.5 and 2.68 folds more than control and the down-regulated genes early responsive to dehydration protein and heavy metal transport detoxification protein were slightly decreased - 0.31 and - 0.40 fold than control, respectively. Inoculation with *H. seropedicae* promotes the plant growth and change the gene expression of the roots of maize seeds. These data may be used to further elucidate various mechanisms in plant bacteria interaction.

Keywords: plant-bacteria interaction; maize; transcriptomic; qPCR.

Financial Support: Capes; Faperj; Newton Fund Grant BB/N013476/1.

Poster III.2

Genetic, physiological and metabolic characterization of *Pseudomonas* sp. strains exhibiting biofertilizer traits and broad-spectrum biocontrol potential

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Fluorescent pseudomonads have widely been exploited for their use as biofertilizers and biocontrol agents and are considered as key players in sustainable agriculture for their eco-friendly applications, potential roles in food safety and sustainable crop production. However, very few studies comprehensively characterize the putative biocontrol genes, antagonistic metabolites, and biofertilizer traits, simultaneously. Following study evaluated four different species of *Pseudomonas* for their *in vitro* biocontrol potential against fungal phytopathogens of wheat, rice, chickpea and sugarcane, role in wheat growth promotion, characterization of antagonistic genes and antimicrobial compounds for their broadspectrum application as biocontrol biofertilizers. Molecular characterization of *Pseudomonas* sp. isolates was based on 16S rRNA gene. Antifungal activity of these strains was determined by agar diffusion method based on inhibition of mycelial growth. Secondary metabolites were extracted by solvent partitioning and subjected to LC/ ESI-MS/MS for confirmation of compounds and $[M+H]^+$ and $[M+Na]^+$ ions were monitored for phenazines, siderophores, pyrroles, aromatic acids, cyclic lipopeptides, phenols, quorum sensing signals and respective genes were detected by PCR. All strains were also screened for plant growth promoting traits and plant experiments were conducted on wheat in climate control room. On average, all strains exhibited 0.8 cm inhibition zones against the causal agents of root rot, seedling blight, red and stem rot. All eleven strains showed the production of six phenazine derivatives, 2-acetamidophenol, pyochelin, pyocyanin and *N*-acylhomoserine lactones while pyrrolnitrin, WLIP, 2-hydroxyphenazine and 2,8-dihydroxyphenazine were unique to *P. aurantiaca* strains only. This study demonstrated the production of three new ortho-dialkyl-substituted aromatic acids; Lahorenoic acid A, B, C and WLIP from *Pseudomonas chlororaphis*. Indole-3-acetic acid, HCN, protease and lipase production were observed by all strains while cellulase production and zinc solubilization was variable. Only *P. aeruginosa* and *P. putida* could solubilize inorganic phosphate. *P. aurantiaca* strains; ARS-38, RP-4, PB-St2 and *P. putida* RS-1 considerably increased wheat shoots and roots biomass and lengths. These findings indicate the potential of these *Pseudomonas* strains to be used as user-friendly single-strain bioinoculums with multifaceted biocontrol and biofertilizer traits.

Keywords: fluorescent pseudomonads; antimicrobial metabolites; bioinoculum.

Financial Support: Higher Education Commission (HEC), Pakistan.

Poster III.3

Antioxidant activity of *Allium sativum* L. with yeasts and bacteria in *Sclerotinia sclerotiorum* control

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Phytopathogen attack influences the production of secondary metabolites in plants due its defense response, and the use of benefic microorganisms to induce plant resistance also shares this principle, because the plant starts to produce secondary metabolites that will protect it from the attack of the pathogens, making it resistant. Thiosulfinates and phenolic compounds are associated with the protection of the garlic plants, acting as a nonenzymatic antioxidant, and with its defense response, acting like antifungal compounds. The purpose of this study was to evaluate the antioxidant activity DPPH, total antioxidant capacity - TAC and oxygen radical absorbance capacity - ORAC, reducing power and chelating power) measured in extracts from garlic cloves submitted to different treatments with biological control agents for the purpose of *Sclerotinia sclerotiorum* control. To evaluate the effect of yeasts and bacteria of the genus *Bacillus* on *Sclerotinia sclerotiorum* control were used garlic cloves from cultivar Gigante roxo. The experiment was carried out based on the methodologies described by Rahman et al. (2016) and Mello et al. (2011) with some adaptations. The species of microorganisms used were *Saccharomyces cerevisiae* (T1), *Pichia kudriavzevii* (T2), *Candida labiduridarum* (T3), *Bacillus acidiceler* (T4), *Bacillus macauenses* (T5), *Bacillus amyloliquefaciens* (T6), *Bacillus pumilus* (T7) and *Acidithiobacillus ferrooxidans* (T8). The *Bacillus macauenses* (treatment 7) showed the best chelating power and antioxidant activity measured by (DPPH and TAC) where as, *Bacillus pumilus* (treatments 9) and *Bacillus acidiceler* (treatment 6) showed the best ORAC antioxidant activity. Results indicate that the use of biological control agents have potential to improve quality to the final product in addition to promote the control of the phytopathogen.

Keywords: garlic; Sclerotinia sclerotiorum*; thiosulfinates; total phenolic compounds.*

Poster III.4

Microbial prospection and combination of N_2-fixing and cell-wall degrading properties to increase sugarcane straw decomposition

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Sugarcane straw is valuable raw material for bioenergy and nutrient cycling management. Among factors affecting its decomposition rates, nitrogen avaliability play pivotal role. Microbial prospection and screening for cell-wall degradation were performed for future design of a microbium consortium to speed up straw decomposition under field condition. Soil-straw from a long-term sugarcane green harvest area was sampled for chemical and microbial evaluation. A soil without straw deposition was analised as reference (Rf). A total of 325 isolates were obtained (102 bacteria and 223 fungi) with population size associated to straw>soil covered with straw>soil wihout straw. Straw deposition increased soil pH, P/K/Mg/Na contents, sum of bases and CTC related to Rf soil. Based on colony co-existence compatibility, diazotrophic and cellulose degradation ability, twelve bacteria and twelve fungi isolates were selected for screening interaction in liquid medium containing sugarcane straw as C-source using total reducing groups (GRT) method. One bacteria-fungal combination: *Bacillus safensis* isolate 59 and fungal isolate 395 was selected for futher in vitro straw decomposition assays. To perform it, single and combined microorganisms were tested for microbial activity on agar-water plates with sugarcane straw (1g/L). After 72h-growth at 30ºC, the treatments were processed for Fluorescein Diacetate (FDA) analisys. For straw treated with bacteria, the microbial activity was noted after 60 min incubation. For Fungus treated straw, highest activity was observed at 60 and 120 min after incubation. Microbial combination had shown ealier and greater activity when compared to singles, and its highest increase was at 30 and 90 min. Taken together, the results suggest that bacteria-fungal consortia with nitrogen-fixing and cell-wall degrading abilities are potencial biotecnological tools to increase sugarcane straw decompostion and nutrient avaliability for sugarcane crop.

Keywords: microbial screening; decomposing microorganisms; nutrient cycling.

Financial Support: Capes; Faperj; Projeto Bilateral Capes/JSPS; Newton Fund Grant BB/N013476/1.

Poster III.5

RNA-seq analysis of the *Cupriavidus-Mimosa* symbiotic interaction

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Rhizobia comprise a group of α and β proteobacteria known for their ability to establish symbiosis with leguminous species. This symbiotic interaction is often characterized by a high level of host specificity and generally requires an exchange of diffusible signals between legumes and rhizobia. The molecular dialogue begins with the exudation of flavonoids and other inducers, by the host legume roots, which are recognized by a transcriptional regulator in the bacterium, the NodD protein, which in turn triggers the expression of the *nod* genes. Enzymes codified by *nod* genes are responsible of the Nod factors which induce the formation of root nodule primordia and play essential roles in the infection process. Nowadays, most of the information about the factors involved in the establishment of the symbiosis between legumes and rhizobia comes from models where alpha-rhizobia have been use. In the present work we employ an RNAseq approach in order to have a global view of the molecular mechanisms involved in the symbiotic interaction process between the beta-rhizobium *Cupriavidus necator* UYPR2512 and its legume host *Mimosa pudica*. To study the first steps of the interaction, total RNA was isolated from bacteria cultures induced by luteolin, as this flavonoid is known to induce the expressionof nod genes. To study the mature symbiotic interaction; RNA was obtained from nitrogenfixing nodules of *Mimosa pudica*. Ribosomal depleted samples were used to generate whole transcriptome libraries using the NGS platform NovaSeq (Illumina). Bioinformatic analyses showed differential expression of genes implicated in early symbiotic interaction, in quorum sensing and in bacterial survival. The identification of these genes, would us to know with more detail the metabolic pathways involved and bring up a global view of the establishment of symbiotic associations between beta-rhizobia and host plants.

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Poster III.6

Highlighting the plant growth promoting and interaction mechanisms in the genome of the endophytic bacteria *Kosakonia* sp. UYSB139 and Pantoea sp. UYSB45

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The endophytic bacteria are those that actively colonize plants tissues and establish associations without causing apparent damage. The study of plant growth promoting endophytic bacteria (PGPEB) associated with crops of agronomic interest, is a field in constant development due to the biotechnological potential as bioinoculants. Bacterial genome sequences have the potential to provide valuable information to understand the plant-endophyte interaction for biotechnology application. Previously a collection of putative bacterial endophytes associated with sweet sorghum cultivar was obtained and characterized biochemically as well as physiologically. Strains *Kosakonia* sp. UYSB139 and *Pantoea* sp. UYSB45, came up as PGPB in *gnotobiotic* and in greenhouse trails. In addition, both strains were defined as “true endophyte” by using microscopy and molecular biology techniques. The aim of the present work is to deepen in the knowledge about the pathways involved in the plant-endophyte interaction, particularly those related with the PGP; as well as to correlate the genomic information with the *in vivo* and *in vitro* data previously obtained. Illumina Hiseq2500 system was used for genome sequencing of the strains under study. Subsequently, the data obtained was assembled and annotated using the RAST service and the Prokka software. The annotations of some of the systems involved in the interaction with the plant such as flagella, chemotaxis, nutritional adaptation, secretion systems among others, were manually cured. The results showed that both strains have a set of genes possibly involved in the interaction with the plant, as well as in the PGP traits which will be presented in depth.

Keywords: endophytes; sweet sorghum; genomics; Ilumina.

Financial Support: ANII and PEDECIBA.

Poster III.7

Rhizobium sp. (BR 10268) colonizes internal sugarcane tissues, produces phytohormones and accelerates mini-sett germination

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The use of pre-germinated mini-setts instead of planting large stem-pieces is a new technique to improve the efficiency of sugarcane production systems in Brazil. With the goal of developing bioproducts to improve the germination process of mini-setts, the present study evaluated plant colonization and growth-promotion traits of a novel strain of *Rhizobium* sp. (BR 10268) during sugarcane mini-sett germination. When grown under controlled conditions, inoculated setts demonstrated improved germination, with significantly ($p<0.05$) increased shoot length at 3 days after inoculation (DAI) and increased shoot dry mass at 6 DAI. In another experiment, bacteria could be re-isolated from surfacedisinfected setts [$(1.8\pm0.92)*10^7$ CFU.g^{-1} at 7 DAI and $(4.9\pm2.7)*10^6$ CFU.g^{-1} at 20 DAI] and roots [$(2.1\pm0.75)*10^5$ CFU.g^{-1} at 7 DAI and $(5.6\pm4.59)*10^4$ CFU.g^{-1} at 20 DAI]. BOX-PCR analyses confirmed the identity of the recovered bacteria and the strain was not detected in the non-inoculated control. Endophytic colonization at 3 and 8 DAI was also confirmed by confocal laser scanning microscopy using a mCherryexpressing derivative of BR 10268. The specific promotion of shoot growth - in contrast to roots - led us to study the production of plant growth affecting substances by the strain. Gas chromatography-mass spectrometry/selective ion monitoring (GC-MS/SIM) applied to culture supernatant after growing the strain in a defined medium enabled the detection of the following substances (ng.mL^{-1}): indole acetic acid (30.23), cytokinin (3.39) and active and inactive giberellic acids GA_3, GA_4, GA_7, GA_9, GA_{12} and GA_{20} (22.52, 33.45, 20.59, 5.71, 10.11, 3.22 and 13.66, respectively). Therefore, the present study confirms that *Rhizobium* sp. BR 10268 is an effective sugarcane endophyte, which probably stimulates sett germination and shoot growth via plant hormone production. Biotechnological applicability will be evaluated under field conditions.

Keywords: plant growth; endophytic colonization; confocal microscopy.

Financial Support: CNPq grants 420746/2016-1 and 308898/2017-6; Newton Fund grant BB/N013476/1; Capes.

Poster III.8

Plant growth promoting capability of endophytic-diazotroph isolates associated with sweet sorghum (*Sorghum bicolor*)

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Sweet sorghum is a multipurpose crop used in Uruguay for bioethanol production. To achieve its optimal growth, this crop requires high amounts of chemical fertilizers, which has a negative impact on ecosystems and in the production costs. This concern led us to evaluate new technologies for a more sustainable production. In this sense the use of plant growth promoting bacteria (PGPB) as biofertilizers, is a sustainable alternative that may reduce the use of chemical fertilizers. The isolates UYSB89 (*Kosakonia radicincitans*), UYSB119 (*Bacillus toyonesis*) and UYSB139 (*Kosakonia cowanii*), were obtained from the internal tissues of stem, seed and root of adult plants of sweet sorghum, cv. M81E. These isolates shown several PGP characteristics *in vitro*, including the ability to fix atmospheric nitrogen, and were defined as an endophytes of sweet sorghum plants. Furthermore, it was proved that they are PGPB of sweet sorghum plants, under *gnotobiotic* conditions. The objective of this work was to evaluate under greenhouse conditions, the PGP capacity of those isolates in sweet sorghum plants (cv. M81E and ADV2010). After 4 months the biometric parameters plant height, stem diameter and dry weight of the aerial and root portion were evaluated. The results reveal that all the isolates had significant differences with the negative control (uninoculated), in at least one of the parameters evaluated. Particularly, the isolate UYSB139 inoculated in the cv. ADV2010 stands out, since it showed significant differences in comparison with the control in all the parameters evaluated. These results are of high relevance for the development of a biofertilizer for sweet sorghum, specifically adapted to the varieties, soils and climates of Uruguay.

Keywords: endophytes; sweet sorghum; genomics.

Financial Support: ANII, PEDECIBA, CAP-UdelaR.

Poster III.9

The effect of sugarcane apoplastic fluid on the proteome of HCC103 strain of *Herbaspirillum rubrisubalbicans*

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Endophytic diazotrophic bacteria, able to perform biological N fixation inside the sugarcane tissues, could in part supply the large amounts of nitrogen fertilizer required for sugarcane production. It is believed that the plant apoplast, defined as the set of extracellular compartments containing carbohydrates, mineral salts, proteins, lignin and water, is an appropriated niche for the establishment of endophytic diazotrophic bacteria. To increase the knowledge on mechanisms associated to the apoplastic colonization by a sugarcane associated diazotrophic bacterium, HCC103 strain of *Herbaspirillum rubrisubalbicans* was grown in JNFb medium in the presence or absence of RB867515 sugarcane apoplastic fluid, using a gel-free proteomic approach. Seven hundred and forty-two proteins were identified in this proteome experiment, of which 570 presented an unchanged expression profile while 172 were considered as differentially expressed proteins (DEPs). In the group of DEPs, 104 of them were considered up-regulated and 61 down-regulated. DEPs were annotated with Gene Ontology (GO) terms using Blast2Go platform. The most abundant GO terms for biological processes of proteins identified as down-regulated are: organic substance biosynthetic process, organonitrogen compound metabolic process, cellular nitrogen compound metabolic process, cellular biosynthetic process, cellular aromatic compound metabolic process and organic cyclic compound metabolic process. On the other hand, up-regulated proteins were related to carboxylic acid metabolic process, organonitrogen compound biosynthetic process, cellular nitrogen compound biosynthetic process and aromatic compound biosynthetic process. To validate the proteome data, a RT-qPCR assay will be done. These proteomic data in association with data from a transcriptome can help in better understand the association of diazotrophic bacteria with sugarcane apoplastic fluid.

Keywords: plant growth-promoting bacteria; functional genomics; plant-bacteria interaction.
Financial Support: Embrapa and Newton Fund grant BB/N013476/1.

Poster III.10

Isolation, characterization and selection of endophytic bacteria from nut grass (*Cyperus rotundus* L.) rhizomes

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Nut grass (*Cyperus rotundus* L.) is an herbaceous plant present in more than 90 tropical and subtropical countries, spontaneously growing and potentially competing with plants of economic importance, mainly due to its rusticity. This plant species also has relevant auxin contents in its rhizome, whose extract is used for root cuttings in asexual plant propagation. Thus, the competition capacity of *C. rotundus* and the effects of its extract highlight the potential of the microbiota associated with this plant species to promote plant growth and induce rusticity. Therefore, the aim of this work is to isolate and identify endophytic bacteria from nut grass rhizomes, as well as to test *in vitro* their potential for direct plant growth promotion by auxin production and biological nitrogen fixation. For this purpose, bacteria from *C. rotundus* rhizomes were isolated in NA (Nutrient Agar Medium) and YMA (Yeast Mannitol Agar Medium) and identified by MALDI-TOF MS (protein profile) and by biochemical tests. The isolates were also submitted to auxin production capacity and biological nitrogen fixation in vitro tests. We obtained 56 isolates, which were separated by colony morphology. Bacteria were identified as belonging to the genera *Acinetobacter* (5%), *Enterobacter* (8%), *Chryseobacterium* (9%), *Bergeyella* (5%), *Flavobacterium* (18%) and *Burkholderia* (43%). Among the obtained isolates, 78% were able to fix nitrogen, 41% were able to produce auxin and 36% were able to perform both.

Keywords: plant growth-promoting bacteria; biological nitrogen fixation; bacteria isolation.
Financial Support: Capes, CNPq, Programa de Pós-Graduação em Microbiologia Agrícola/UFLA.

Poster III.11

An efficient barley and wheat colonization is achieved by *Paraburkholderia tropica* after seed inoculation

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Root colonization is a key factor in the successful interaction of Plant Growth Promoting Bacteria with plants. The study of bacterial population dynamic in plant tissues is a direct measure of their efficiency as colonizers. The aim of this study was to determine the colonization patterns of the PGPB *Paraburkholderia tropica* MTo-293 after its application to barley and wheat seeds to evaluate the efficiency of root colonization by the bacteria under gnotobiotic conditions using different approaches: i-culture-dependent techniques by counting bacterial colonies in suspension of disinfected and non-disinfected plant tissues and ii-culture-independent techniques by microscopic observations of plant tissues inoculated with marked strains with *gfp* and *gus* reporter genes, and by molecular biology techniques. The results of this work showed that: 1- *P. tropica* was able to colonize roots and stems of barley and wheat, both superficial and endophytically, and a similar behaviour was observed for both grasses. Root surface and endophytic bacterial population were moderately stable throughout the experiment, reaching values ranging from 7 to 10 and 4 to 5 log CFU/g of fresh weight, respectively. *P. tropica* was also able to colonize aerial tissues reaching endophytic stem populations greater than that found in roots (5 to 7 log CFU/g of fresh weight) for both grasses. 2- *P. tropica* could be localized by microscopy on roots of both grasses. It was observed an extensive surface colonization with rows and groups of bacteria on the junction of the epidermal root cells, on root hairs and on root tips. 3- It was also possible to detect *P. tropica* in disinfected and non-disinfected roots and stems tissues from barley and wheat plants using a nested-PCR as an alternative and easier way to determine the presence of the bacterium after inoculation of barley and wheat seeds. This work provides useful knowledge for the application of *P. tropica* as an inoculant in grasses.

Keywords: endophytes; grasses; plant growth promoting bacteria.

Financial Support: This research was supported by grants provided by CIC-PBA.

Poster III.12

Efficacy of *Azospirillum brasilense* on biological control of garlic (*Allium sativum* L.) phytopathogens

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Control of phytopathogens in agriculture is still carried out by application of agrochemicals, which has several environmental impacts, besides increasing the cost of food production. Therefore, many studies have been dedicated to researching alternative methods of plant pathogens and parasites control. Some microorganisms that interact with plants have biological control capacity. These microorganisms present various mechanisms of indirect growth promotion, which involve -1,3-glucanase, chitinase, cellulase and pectinase, hydrocyanic acid, antibiotics, antifungal metabolites and siderophores production and plant defense induction. Thus, the aim of this work was to evaluate the ability of *Azospirillum brasilense* to perform biological control of fungi that affect garlic (*Allium sativum* L.) *in vitro* culture. For this purpose, mini-garlic bulbs were cultivated on MS medium (Murashige and Skoog). Fungi that contaminated *in-vitro* culture were identified by macro and microscopic characteristics. Physical effect of bacteria on fungal hyphae was evaluated by scanning electron microscopy. Contamination by *Sclerotium* and *Penicillium* was observed, but *A. brasilense* inoculation allowed less fungal contamination. Scanning electron microscopy images allowed the observation of fungal hyphae colonization by bacteria, evidencing the potential of this species to promote plant growth indirectly.

Keywords: biological control; fungi; Scanning electron microscopy; plant growth-promoting bacteria.

Financial Support: Capes, CNPq, Programa de Pós-Graduação em Microbiologia Agrícola/UFLA.

Poster III.13

Physiological keys to elucidate the differential response of *Lotus spp.-Fusarium solani* interaction

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The interactions between plants and fungal endophytes could improve the host performance. Nevertheless, some agents such as the environment, plant species and age could modify the development of the system, changing the balance from mutualism to pathogenesis. This variability among fungal effect on plants has led the “mutualism-parasitism *continuum*” theory. *Lotus tenuis*, is a widely legume used as forage in Argentina, due of its capacity of growth in restrict soil conditions. Additionally, the model *Lotus japonicus* has been used for the study of responses to biotic and abiotic stresses. Our aim was focused in to determinate the main factors involved in the response of two *Lotus* species (*L. japonicus* and *L. tenuis*) to the symptomless infection of *Fusarium solani*. This strain has been capable to infect roots of *L. tenuis* and *L. japonicus*. Both infections were intra-cellular, restricted to the root surface, epidermis and cortex. The characterization of the physiological response of both plant species to the effect of *F. solani*, was performed through physiological analysis of photosynthesis and chlorophyll fluorescence parameters. In addition, through the analysis of metabolic profile, using GC-EI/TOF-MS technology, we were determinate the main compounds involved in the primary metabolism. Our results have shown a growth improvement in plants of *L. japonicus*, displaying as the increase of biomass of shoots and roots; while in *L. tenuis*, there was a decrease in the same parameters. Photosynthetic rates did not to be affected by the infection, in both species. The metabolic profiling has shown the differences in the amount of some sugars, phosphates, phenylpropanoids and polyols. Taking together, our results indicate a differential response of *Lotus* species to the infection by a *Fusarium* endophyte. This evidence could elucidate the base of the study of some mechanisms that could explain the switch between mutualism and pathogenesis, for this proposed biological model.

Keywords: symptomless infection; mutualism; pathogenesis.

Poster III.14

Interspecific communication in vitro between *Bradyrhizobium* and *Azospirillum* via acyl-homoserine lactones mediators

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Co-inoculation of soybean with *Bradyrhizobium* and *Azospirillum* has been used to stimulate root growth, nodulation and increase in yield. A co-culture of these microorganisms would facilitate the production of inoculants. In this case, the density of cells can be regulated by interspecific communication through acylhomoserine lactone molecules (AHLs), a mechanism that regulates the gene expression in a density-dependent way known as *Quorum Sensing* (QS). The objective of this study was to evaluate the QS mechanism involved in the interaction among *B. japonicum* strain CPAC 15 and *A. brasilense* strains Ab-V5 and Ab-V6 on growth and on bacterial biofilm formation. For this, a transconjugant (CPAC 15-QS) of the wild-type strain (CPAC 15) was obtained by conjugation with *Escherichia coli* possessing the plasmid (pME6863) involved in the degradation of AHL. The AHLs communication molecules found in the wild type CPAC 15 were 3-OH-C12-HSL; 3-OH-C14-HSL and 3-oxo-C14-HSL. The growth of both *Azospirillum* strains did not change with the presence of the wild-type or transconjugant *Bradyrhizobium* strains. However, the growth of the strains CPAC 15 and CPAC 15-QS was reduced in the presence of *Azospirillum*, although it was still above 1 x 10^9 CFU/mL, the minimum cell count for inoculants in Brazil. The strain CPAC 15-QS had less growth in the presence of Ab-V5 compared with the wildtype, whereas the difference was smaller in the presence of Ab-V6. The co-cultures produced biofilms, mainly with Ab-V6, showing that the QS via AHL did not interferes in the formation of biofilm. We concluded that, even not expressively, AHL communication occurs between *A. brasilense* strain Ab-V5 and *Bradyrhizobium*, triggering or repressing phenotypes related to bacterial growth.

Keywords: co-cultive; co-inoculation; Quorum sensing.

Financial Support: CNPq, Capes.

Poster III.15

Effect of heterologous AHLs on the phosphate solubilizing ability of *Serratia* sp S119 and *Enterobacter* sp J49

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The bacterial phosphate solubilizing ability in the plant rhizosphere is influenced by several factors being one of them the presence of other microorganisms that share the same environment. It is widely described that bacterial communication is subject to the release of molecules of the acyl homoserine lactone (AHLs) type. The objective of this work was to evaluate the influence of heterologous AHLs potentially present in this environment on the phosphate solubilizing capacity of *Serratia* sp S119 and *Enterobacter* sp J49. Heterologous AHLs were obtained from the phosphate solubilizer strain *Pseudomonas fluorescens* P3, the free-living N_2 fixer and producer of phytohormones *Azospirillum brasilense* Az39, and the soybean microsymbiont, *Bradyrhizobum japonicum* E109. The effect of heterologous AHLs on the phosphate solubilizing activity was analyzed by qualiand quantitative assays. For the former, Petri dishes containing NBRIP-BPB culture medium supplemented with AHLs were used and the phosphate solubilization halo formation was analyzed. For the quantitative estimation assay the modified colorimetric technique of Fiske and Subbarow (1925) was used. Bacteria were grown in liquid cultures of NBRIP supplemented with the heterologous AHLs at 28°C under agitation. Samples were taken at different times to determine soluble P content and pH. Results obtained from both assays indicated the heterologous AHLs showed no effect on the phosphate solubilizing ability of the bacteria. The low pH values obtained from the bacterial supernatants in the quantitative test, suggests that the bacteria would be expressing the main mechanism of solubilization by releasing organic acids. The results obtained indicate that the phosphate solubilization phenotype is not dependent on quorum sensing.

Keywords: plant growth promoting bacteria; rhizosphere.

Poster III.16

Cumulative effect of co-inoculation with *Bradyrhizobium* and *Azospirillum* on corn yield and biometric components

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Nitrogen fertilizers are the main way of supplying nitrogen to grasses, however there are diazotrophic bacteria like *Azospirillum* that have the ability to fix atmospheric nitrogen making it available to plants. Microorganisms such as *Bradyrhizobium* can contribute to the production of secondary metabolites and together with *Azospirillum* could result in increased productivity in grasses. The objective of this work was to evaluate the cumulative effect of the co-inoculation with *Bradyrhizobium* and *Azospirillum* (BA) on yield and biometric components of maize in succession to wheat and soybean. The experiment was carried out in Campo Mourão-PR, Brazil, in a Latossolo Vermelho distroférrico (Oxisol). The experimental design was a randomized complete block, consisting of 8 treatments (TRAT) with 5 replicates and experimental units of 36m^2.In wheat, all TRAT received fertilization of 24 and 70 kg of N ha^{-1} in the subsurface band and in broadcast, respectively. In the TRAT 1, 3, 4 and 6 seeds were inoculated with (BA) and TRAT 2, 5, 7 and 8 were not inoculated (NI). In soybean, TRAT 1, 2, 3, 7 were inoculated with *Bradyrhizobium* (BRAD) and TRAT 4, 5, 6 and 8 were inoculated with BA. In maize, all TRAT were fertilized with 48 and 70 kg of N ha^{-1} in the subsurface band and in broadcast, respectively. The TRAT 1, 2, 4 and 5 were NI and TRAT 3, 6, 7 and 8 were inoculated with BA. Finally, in the succession of wheat, soybean and maize, the inoculation was TRAT 1 (NI+BRAD+NI), 2 (BA+BRAD+NI), 3 (NI+BRAD+BA), 4 NI+BA+NI), 5 (BA+BA+NI), 6 (NI+BA+BA), 7 (BA+BRAD+BA) and 8 (BA+BA+BA). The data collected were submitted to analysis of variance and averages compared by the Tukey test ($p > 0.10$) using the SAS statistical program. No significant statistical differences were observed for yield and thousand kernel weight. The yield ranged from 8603 to 8761 kg ha^{-1} and thousand kernel weight ranged from 318.7 to. No significant statistical differences were also observed for the biometric components, where the number of rows in the spike ranged from 16.48 to 17.32, the number of grains per row ranged from 29.32 to 31.92, the length of the spike ranged from 14.80 to 15.43 cm and the spike diameter ranged from 52.31 to 53.24 mm. In the treatments studied there were no significant increases in yield and biometric components of maize after continuous using of BA.

Keywords: PGPR; Zea mays*; Nitrogen.*

Financial Support: Capes.

SESSION 4: The Holobiont Plant: multitrophi interactions

Key Note IV.I

Regulatory cues contributing to endophyte establishment in rice

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The diazotrophic model endophyte of grasses, *Azoarcus olearius* BH72, colonizes roots of its original host plant Kallar grass and rice in similar patterns. The lifestyle of these endophytes is remarkable, as they establish in the root apoplast in high numbers; however the molecular mechanisms by which diazotrophic endophytes interact with their host are not well understood. How do bacteria adapt to their endophytic lifestyle in comparison to free-living growth, and which regulatory cues contribute to expression of genes required for the endophytic state? Cell-density and quorum-sensing like regulatory pathways were suspected. Previous transcriptomic analyses revealed that around 8% of protein-coding genes of strain BH72 were differentially regulated in conditioned supernatants in comparison to standard growth conditions, however the autoinducer could not be identified. Mutations in functionally diverse genes of this regulon revealed that encoded proteins were contributing to endophytic competence in *Azoarcus*-rice colonization assays: PilA the pilin of type IV pili, PilX a pilus assembly protein, 1544 a diguanylate cyclase/phosphodiesterase with PAS/PAC sensor, or Azo1684 an attachment factor. Here we show that expression of several genes was also upregulated in the endophytic state in rice roots, as demonstrated by single-cell gene expression studies using transcriptional fusions to *tdtomato* and CSLM. However, the regulatory signals appeared to differ from expectations. Rather than accumulation of an autoinducer, the depletion of complex nitrogen sources from the conditioned culture medium led to elevated gene expression of putatively quorum-sensing regulated genes. The level of the alarmones guanosine tetraphosphate (ppGpp) and guanosine pentaphosphate (pppGpp) result in reprogramming of transcription during so-called stringent response. Mutation of *relA*, encoding for an alarmone synthesis enzyme, altered the expression pattern of target genes. This and further data suggest that stringent response is involved endophyte establishment.

Keywords: rice; endophytes; plant bacteria interactions.

Short Note IV.II

Communication between Plants and Beneficial Bacteria: what the plant genes can tell us?

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Economically important crops, especially monocots, can obtain a substantial part of their N needs from biological nitrogen fixation (BNF) by interacting with associative and endophytic diazotrophic bacteria that either live near the root surface or endophytically colonize plant tissues. One of the best-reported outcomes of this association is the promotion of plant growth by direct and indirect mechanisms, as well as increase in tolerance against biotic and abiotic stresses. Inoculants of associative and endophytic diazotrophic bacteria had been shown to lead to positive results on sugarcane yields, which are dependent on the plant genotype and soil conditions. Our group has been studying sugarcane and maize genes involved in the establishment of a beneficial type of association with nitrogen-fixing bacteria, aiming to assist in the development of more responsive cultivars to inoculants of beneficial diazotrophs. An integrated differential transcriptome was generated by Illumina RNAseq and it provided an overview of sugarcane and maize metabolism, growth and development controlled by nitrogen, water and endophytic nitrogen-fixing bacteria during a successful association. All together, the data suggest that an important control of the efficiency of the association is already set in the early stages of plantbacterium recognition, when specific plant genotypes sense the environment and regulate several plant signaling pathways involved in microorganism recognition and plant defense. We propose that "soil-rhizosphererhizoplane-endophytes-plant" could be considered as a single coordinated unit with dynamic components that integrate the plant with the environment to generate adaptive responses in plants to improve growth. The homeostasis of the whole system should recruit different levels of regulation, and recognition between the parties in a given environment might be one of the crucial factors coordinating these adaptive plant responses.

Keywords: microbiome; PGP; plant-bacteria interaction.

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Short Note IV.III

The sugarcane microbiome profile unravels the structure, diversity and colonization pattern of plant-beneficial microbial communities

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The sugarcane-associated microbial community has long been explored for its capability of supporting plant development under diverse conditions. These surveys have mostly focused on specific bacterial groups such nitrogen fixing bacteria by approaches based on isolation on defined culture media and inoculation of a single bacteria. However, the solely use of methods based on cultivation are strongly biased and may not reflect the real composition of bacterial community in plant. Furthermore, the inoculation of single bacteria does not represent the highly complex dynamic of a plant microbiome. As a result, we lack fundamental information regarding the microbial assemblage and its functional role in association to sugarcane plant. Our group has adopted a strategy that concomitant use culture-dependent and -independent techniques to target dominant microbial groups from the sugarcane microbiome. By using culture independent techniques, we found a core microbiome composed of less than 20% of the total microbial richness and that sum up for over 90% of the total microbial relative abundance in roots, stalks and leaves of sugarcane. We designed a synthetic bacterial community by choosing naturally dominant groups in the sugarcane microbiome, mostly poorly explored in terms of association to sugarcane plant. Bacterial candidates were derived from a collection of microorganisms with over 5k isolated communities from roots and stalks of sugarcane. Our results shows that the synthetic community robustly colonized plants, stimulated the root development and tripled plant biomass. Community profile shows that each microbe in the synthetic community display a pattern of colonization that do not correlate with phylogenetic affiliation. Genome sequencing of the synthetic community shoed that the robust colonizers have a specific set of enriched functions that enable efficient plant colonization.

Keywords: sugarcane; microbiome; PGP; microbial diversity.

Short Note IV.IV

Maize miRNAs in response to diazotrophic bacteria association and nitrogen deficiency

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Plants have a complex mechanism of gene expression regulation involving miRNAs that influences their development, adaptation and response to stress. miRNAs are regulated in the interaction between plants and nitrogen-fixing bacteria. Gramineous plants establish an association with diazotrophic bacteria, colonizing plants without causing damage to the host. This association is influenced by the available environmental nitrogen. However, the regulation of miRNAs in gramineous plants inoculated with diazotrophic bacteria under different nitrogen concentrations has not been described. In order to understand the role of maize miRNA in these different conditions, we performed an experiment using maize plants cultivated in hydroponic medium inoculated with *Herbaspirillum* (Zae94) and *Azospirillum* (SP245). Further, the inoculated plants were divided into two different nitrogen contents (3 mM and 0.3 mM). Five biological replicas of each sample groups were collected after 1 and 7 days of treatment with the nitrogen conditions, and the roots and shoots were collected separately. Bacterial colonization was confirmed measuring the amplification of species specific rRNA with qRT-PCR. We selected four miRNAs candidates to analyse the expression profile in these samples. These miRNAs are related to copper homeostasis, because in a previous study, we proposed a hypothetic model involving these miRNAs. The up-regulation of miR397, miR398, miR408 and miR528 in maize inoculated with diazotrophic bacteria leads to the inhibition of their targets. Targets of these miRNAs are defence related, so down-regulation of these could facilitate the plant-bacteria association. Literature data show that in low/absent soil nitrogen conditions these miRNAs are down-regulated. A similar effect occurs during plant-pathogen interaction. Our findings suggest important roles of miRNA regulation in maize during association with diazotrophic bacteria under different nitrogen conditions.

Keywords: Herbaspirillum*;* Azospirillum*; plant-bacteria interaction.*

Financial Support: Newton Fund grant BB/N013476/1, Capes, FINEP, INCT, CNPq and Faperj.

Poster IV.1

Transcriptome analysis of *Gluconacetobacter diazotrophicus* PAL5 strain in response to iron

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Gluconacetobacter diazotrophicus has been the focus of several studies aiming to understand the mechanisms behind diazotrophy. In this process, iron is an essential cofactor for many enzymes involved in cellular processes and electron transport chains, including Biological Nitrogen Fixation (BNF). A transcriptome comparison was carried out between iron-starved cells of *G. diazotrophicus* treated with iron and untreated controls. The present study is the first global analysis of the early transcriptional response of exponentially growing *G. diazotrophicus* to iron. The analysis of the RNA-Seq data showed that the 3,700 genes present in the genome of the bacterium, 104 were differentially expressed, with 23 induced and 81 repressed in the presence of iron. These data revealed that genes encoding functions related to iron homeostasis, including iron transport systems, TonB-dependent receptors, as well as regulatory proteins, were significantly up-regulated in response to iron limitation. Certain genes involved in biosynthesis of secondary metabolites such as FAD-binding oxidoreductase, NAD(P)H dehydrogenase (quinone), were over-expressed under iron-limited conditions. In contrast, we observed that expression of genes involved in Fe-S cluster biosynthesis such as iron-sulfur cluster assembly accessory protein and flagellar biosynthesis such as flagellar biosynthesis protein FliQ were down-regulated in an iron-depleted culture medium. Notably, some genes encode hypothetical or conserved hypothetical proteins of unknown function, suggesting that they are involved directly or indirectly in iron metabolism or metabolic adaptation to different iron-availability conditions. These data will also be confirmed by RT-qPCR, enabling to suggest a possible metabolic model and providing new insights into the effects of iron on metabolism in *G. diazotrophicus*.

Keywords: diazotrophic bacteria; RNA-Seq; RT-qPCR.

Financial Support: Capes, CNPq/INCT-FBN, Embrapa.

Poster IV.2

Microbiome of maize seed: source of beneficial bacteria for germination and seedling growth?

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Plant microbiome revealed a complex microbial community and seed-born bacteria is part such complexity, which the role for plant performance is largely unknowing. The present study was designed to access the diversity of maize seed-born bacteria community and to evaluate their effect on germination and seedling growth. Germination tests were carried out in axenic condition, where maize seeds (*Zea mays* var. SHS 5050) was submitted to: a) disinfestation in alcohol 70% for 5 min and in hypochlorite (NaClO 1.25%) for 30 min; b) absence of disinfestation. Seeds were placed in petri dishes containing agar-water (0.5%). Daily counts of germinated seeds (radicle>5mm) were carried out over 8 days and percentage and germination speed (%G; GSI) were calculated, as well time and average germination speed (AGT; AGS). The bacteria number were estimated in NB solid medium and JNFb and LGI semi-solid medium. Inoculation assays were performed with a mixture of 9 seed-born bacteria isolates that were reinoculated (10^7 cells.mL^{-1}) in disinfested maize seeds. Controls consisted of seeds that were not disinfested and disinfested without reinoculation. After 6 days, seed-germination, population size and bacteria colonization were evaluated by microscopy. Non-seed disinfestation resulted in higher GSI and AGS. The %G of the disinfested seeds did not differ from the treatment with absence of disinfestation and presented superior AGT. Total and diazotrophic bacteria associated to maize root was higher in the non-disinfested seeds (NB: $8.3x10^8$ CFU.mL^{-1}, JNFb and LGI: >10^7 cells.g^{-1}) in relation to disinfested seeds (NB: $3.4x10^7$ CFU.mL^{-1}; JNFb and LGI: undetected). Higher bacteria colonization was observed in non-disinfested maize roots with biofilm formation. Disinfested seeds, colonization occurred as isolated cells and small aggregates. It is concluded that maize seed disinfestation can affect its germination pattern and reduce its colonization by the bacterial microbiome.

Keywords: beneficial bacteria; germination; maize root.

Financial Support: Capes, CNPq, Faperj, Newton Fund Grant BB/N013476/1.

Poster IV.3

Genes involved in Nitrogen signaling and metabolism in sugarcane associated with beneficial diazotrophic bacteria

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The diazotrophic endophytic bacterium *Gluconacetobacter diazotrophicus* can promote plant growth and increase productivity when associated with important crops as sugarcane. The objective of this work is to identify genes involved in signaling and metabolism of nitrogen in sugarcane, participating in a beneficial association with endophytic diazotrophic bacteria. For this, differential transcriptome databases generated from 32 samples sequenced by Illumina RNAseq were integrated. The generated database contains sugarcane genes expressed in a beneficial association for the plant, in different environmental conditions and plant developmental stages, including data of plants inoculated with *G. diazotrophicus* in high NO_3 (5mM) and low NO_3 (0.5mM). By in silico analysis, it was possible to find 13 differentially expressed genes involved in nitrogen signaling and metabolism, being five nitrate transporters, one ammonium transporter, two asparagine synthase, one receptor-like quinase and three members of transcription factor families (RWP-RK, GATA and LOB37). Gene expression patterns are being validated by RT-qPCR. The results suggest that the endophytic diazotrophic bacteria might be able to reprogram the metabolism of N in plants, when associated in a beneficial way. Therefore, the regulation of N nutrition may be an important way by which these diazotrophic bacteria can benefit plants. Furthermore, these genes may be expression markers and/or functionally crucial for the establishment of an efficient association with diazotrophic bacteria. Potential molecular markers identified in sugarcane as differentially expressed in an efficient association can be used not only to identify sugarcane genotypes potentially responsive to inoculants with beneficial diazotrophic bacteria but also to characterize more efficient endophytic bacteria as well as to support in the understanding of regulated metabolic pathways by the diazotrophic bacteria that lead to benefits to the plant.

Keywords: Gluconacetobacter diazotrophicus*; nitrate; transcriptome.*

Financial Support: INCT, CNPq, Faperj, Capes, FINEP.

Poster IV.4

Expression profile of nitrogen regulation during maize association with diazotrophic bacteria

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Fixed nitrogen is one of the most common limiting factor for crop productivity. Although the demand for fixed nitrogen can be met by the use of chemical fertilizers, impacts on nitrogen cycling result in large environmental and economic damages. In this context, the Biological Nitrogen Fixation (BNF) presents itself as a sustainable alternative for the supply of this nutrient. Several grass crops can be benefited of the association with endophytic diazotrophic bacteria that live in the intercellular spaces and vascular cylinder of plant tissues. Since establishment of this beneficial association depends in part on nutritional conditions of the soil, the aim of this work is to study the effects of nitrogen concentration combined with specific diazotrophic bacterial species for the efficiency of the association. Maize nitrogen signaling and metabolism were initially analized in differential transcriptomes generated of plants inoculated with two diazotrophic bacteria, *Azospirillum brasilense* strainBR11005 and *Herbaspirillum seropedicae* strain HRC54. Gene expression profile showed that some plant responses are specific to the inoculation with distinct diazotrophic bacteria. Maize responses to NO_3- were largely induced by both bacteria, whereas assimilation of NH_4^+ into amino acids was repressed by *H. seropedicae*. A second study is being now conducted with maize plantlets inoculated with one of four diazotrophic bacteria: *A. brasilense* strain FP2, *A. brasilense* strain Sp245, *H. seropedicae* strain ZAE94 and *Gluconacetobacter diazotrophicus* PAL5; and submitted to different nitrate concentrations (0.3 or 3mM Mg $(NO_3)_2$). All diazotrophic bacteria promoted plant growth 7 days after treatment (dat), however higher levels of increse were observed among low nitrate growing plants. RNA samples were extracted and gene expression profiles are currently being analyzed, searching for regulatory mechanisms involved in the plant perception of different environmental conditions, so that a beneficial association with the diazotrophic bacteria is established.

Keywords: nitrogen; diazotrophic bacteria; maize.

Financial Support: INCT, FINEP, CNPq, Faperj, Capes, Newton Fund grant BB/N013476/1.

Poster IV.5

Transcriptome and proteome approaches for the diazotroph *Nitrospirillum amazonense* grown in sugarcane apoplast fluid

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Nitrospirillum amazonense is a nitrogen-fixing bacterium isolated from several plants such as rice, maize, sorghum and sugarcane. The CBAmC strain of *N. amazonense* was isolated from sugarcane stalks variety CB45-3 and it has been reported that this bacterium promotes growth of sugarcane variety RB867515 when inoculated under field conditions in a consortium with four other nitrogenfixing bacteria. The sugarcane apoplast represents a probable niche for the establishment of several bacteria, including *N. amazonense*. The present work had the objective to access the influence of sugarcane apoplast fluid on the global transcriptomic and proteomic profiles of *N. amazonense* cultured *in vitro*. The bacterium was subjected to high-throughput RNA sequencing (RNASeq) analysis and protein identification through liquid chromatography-tandem mass spectrometry (LC-MS/MS). The comparative analysis between the transcriptome and proteome profiles showed strong consistency between the results of the two approaches. Data analysis revealed that expression levels of transcripts and proteins related to cell motility and signal transduction were strongly under-expressed in the presence of the apoplast fluid. In contrast, transcripts and proteins were overexpressed for posttranslational modification, protein turnover, chaperones, and multidrug efflux transporters. In conclusion, the results showed that the metabolic state of *N. amazonense* during cultivation in the presence of apoplast is directed to proteins capable of supporting adaptation in a complex environment such as the sugarcane apoplast. The transcriptome and proteome comparative analysis will guide future studies with the CBAmC strain as well as the potential development of an inoculant for sugarcane.

Keywords: RNA; proteins; bacteria-plant interaction.

Financial Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes) and Embrapa.

Poster IV.6

Genome-wide association analysis reveals candidate genes for tropical maize responsiveness to *Azospirillum brasilense*

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The inoculation of Plant Growth-Promoting Rhizobacteria (PGPR) with mechanisms of biological nitrogen (N) fixation and production of phytohormones is one of the main strategies to supplement the chemical inputs of N and to increase the root development in maize. An intriguing issue is how the genes in cereals are involved in the recognition of this beneficial microorganism allowing its colonization and growth promotion. In this context, the aim was to find candidate genes for maize-*Azospirillum brasilense* association via Genome-Wide Association Study (GWAS). We evaluated 118 maize hybrids in November 2016 and February 2017 in Piracicaba, São Paulo, Brazil under greenhouse conditions using unsterilized soil without input of N fertilizer. The experimental design adopted was the randomized complete block with three replications and two treatments: non -inoculated and inoculated seeds with *A. Brasilense*. Several shoot and root traits were evaluated at the V7 stage of development. The phenotypic analyzes were performed using mixed model equations (REML/BLUP). Traits with significant inoculation effect were subject to GWAS analysis using 52,215 SNP (Single Nucleotide Polymorphism) markers with additive and heterozygous (dis)advantage models using the FarmCPU-R package. There was a significant effect of the inoculation only for dry root mass, root volume, root mean diameter, root specific length, and specific root surface area. The association mapping allowed the identification of 21 significant SNPs for the inoculated treatment, in which only two was coincident to 22 found in the control treatment (N stress). The candidate genes in these regions are involved in signaling pathways of the plant's defense system, regulation, and production of phytohormone. Our findings are a benchmark towards the understanding of the genetic variation among maize genotypes for the association with *A. Brasilense*. Furthermore, it allows starting the marker-assisted selection in maize concerning this association.

Keywords: plant growth-promoting rhizobacteria; nitrogen stress; heterozygous model.

Financial Support: FAPESP (Process: 2015/01188-9), CNPq and Capes.

Poster IV.7

Transcritptome profile of the interaction between mild phytopathogen *Herbaspirillum rubrisubalbicans* M1 and *Sorghum bicolor*

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Herbaspirillum rubrisubalbicans causes mild disease in a few varieties of sugar cane and sorghum resulting in loss of the infected leaves and minor loss in productivity. The transcript profile of *H. rubrisubalbicans* M1 colonizing sorghum roots, and the profile of sorghum leaves noculated with *H. rubrisubalbicans* were determined. We found genes potentially involved in both beneficial and pathogenic interactions of this microorganism with graminaceous species. Among the 545 differentially expressed genes of sorghum inoculated with *rubrisubalbicans* M1, 303 were up-regulated and 242 down-regulared. The sorghum up-regulated genes were probably related to response against pathogens, impairing growth and propagation of pathogens, indicating activation of the plant immune system by *H. rubrisubalbicans*. The bacterium seems to modulate the immune response of the plant when in association, decreasing the hypersensitive response and increasing the scattering power in the tissue. 1592 genes of *H. rubrisubalbicans* M1 were differentially expressed during the interaction with sorghum, being 457 Upregulated and 1135 down-regulated. Extensive cell wall modification was apparent by up-regulation of genes involved in peptidoglycan turnover and lipopolysaccharide synthesis, porins, secretion systems and other membrane proteins. Up-regulated genes for two MAMPs, flagellin and peptidoglycan, were found. The observed changes suggest that *H. rubrisubalbicans* is highly efficient in colonizing the plant rhizosphere, expressing several traits important for survival in such a competitive environment. The ability to over-colonize plant xylem together with expression of few pathogenicity factor genes may shift the interaction from benign or neutral to pathogenic in susceptible cultivars. According to our data, both *H. rubrisubalbicans* and sorghum gene expression profile are regulated by the interaction in such way that the bacteria is able to modulate the plant immune response.

Keywords: Herbaspirillum*; regulation, PGPR.*

Financial Support: INCT-FBN/CNPq, Newton Fund Grant BB/N013476/1 and Capes.

Poster IV.8

LncRNA involved in the response to nitrogen deficiency stress and diazotrophic bacteria in maize

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LncRNA recently emerged as functional non- protein coding RNA transcripts, longer than 200nt, most of it undergoing post-transcriptional processing similar to mRNA. Besides their similarity, lncRNA exhibit relatively low expression pattern when compared to coding transcripts, presenting specific profile depending on the tissue, cell type, developmental stage and environmental stress response. In plants there is functionally identified lncRNAs responsive to auxin, Phosphorous starvation, drought and salt stress, and also in *Populus* sp. and *Zea mays* was observed evidence of lncRNA involved in nitrogen deficiency adaptative response. However, the understanding of lncRNA involvement in beneficial bacteria response and its correlation to the expression profile caused by nitrogen deficiency is limited even though the mRNA and sRNA expression regulatory response is extensively reported. In order to analyze the possible lncRNA involvement in the response to beneficial bacteria, we perfomed an experiment with maize inoculated with *Azospirillum brasilense* (SP245) and *Herbaspirillum seropedicae* (Zae94), the samples were extracted using Trizol and 8 libraries were sequenced by Illumina HiSeq, being two libraries from *A. Brasilense*, two libraries from *H. seropedicae* and four mock inoculated controls. The colonization was confirmed by qRT-PCR with species specific rRNA primers. The lncRNA was identified using CPC2 and PLEK coding predition tools, filtering out the transcripts shorter than 200nt and with ORFs longer than 100aa. In the analysis we were able to identify transcripts only expressed in the inoculated libraries, in addition some transcripts were differentially regulated according to the microrganism inoculated. To compare the lncRNA regulation between nitrogen deficiency and beneficial bacteria treatments, we downloaded the transcriptome available at SRA database from a experiment aiming to identify the lncRNA regulated by nitrogen starvation. It observed 637 nitrogen-responsive lncRNAs believed to be acting, by co-expression enrichment analysis, in specific pathways such as NADH dehydrogenase activity, oxidative phosphorylation and others. Considering the literature reviews in mRNA and some classes of ncRNA stress response, there is a sizable overlap in the regulation profile activated by nitrogen starvation and beneficial bacteria association, suggesting lncRNA may play a role in the plant response to such conditions.

Keywords: long noncoding intergenic RNA; plant-bacteria interaction.

Financial Support: Newton Fund grant BB/N013476/1, FINEP, CNPq, Faperj and Capes.

SESSION 5: Bacterial and Plant Physiology

Key Note V.I

Manipulating Biological Nitrogen Fixation for Agricultural Benefit

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The practice of applying chemical fertilisers to increase crop yields has had an enormous impact on world agriculture but has resulted in severe agronomic and environmental penalties, including eutrophication of aquatic systems and atmospheric pollution. In order to circumvent this nitrogen conundrum, it has been proposed that biological nitrogen fixation (BNF) could be exploited to provide a more sustainable alternative to the use of nitrogen fertilisers in agriculture. Using synthetic biology approaches, it is feasible to design engineered bacteria with enhanced nitrogen fixation in which the process of ammonia biosynthesis by nitrogenase is uncoupled from its assimilation, thus enabling export of surplus fixed nitrogen. Since, disabling ammonia assimilation and enhancing nitrogenase levels is likely to incur a significant growth penalty for the bacteria, plant specific signals could be incorporated to ensure that ammonia excretion only occurs after the plant-microbe association is established. In this talk I will describe our recent progress in exploiting fundamental knowledge of gene regulation in diazotrophs to engineer bacteria with potential advantages for PGPR. If time permits, I will also describe our latest results on manipulating nitrogen fixation systems to facilitate direct engineering of nitrogenase into cereals.

Short Note V.I

Metabolic pathways due to loss of GlnB signaling in *Azospirillum brasilense*: a proteomic approach

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PII proteins are the main signal transducers of cellular and nitrogen metabolism, interacting with a variety of cellular targets. *Azospirillum brasilense* has two PII proteins, called GlnB and GlnZ, which regulate nitrogen metabolism in response to changes in extracellular ammonium concentrations. Although several studies have discovered the functions of PII under conditions of low and high ammonium concentrations, these data are still insufficient to define their function in the complex Ntr system. Wild-type and glnB mutant (LFH3) strains of *A. brasilense* were cultured under high (+) or low (-) ammonium and the proteome of these cells were compared using label free quantitative LC-MS/MS analysis. To determine Differentially Abundant Protein Species (DAPs) a p value <0.05 and a fold change > 1.8 or <0.5 was used. 2165 proteins were identified when the *glnB* mutant strain LFH3 was compared to the FP2 control, of which 379 were upregulated and 720 were down regulated in high concentration of ammonium (20mM), 338 were upregulated and 199 were down regulated in low concentration of ammonium (2mM). Gene ontology (GO) enrichment analysis revealed terms involved in oxydoreductase activity, transport, NADH dehydrogenase activity, signal transducer activity and nitrogen cycle metabolic process, to be increased in high concentration of ammonium while terms associated with transferase activity and lipid biosynthetic process, to be increased in low concentration of ammonium. KEGG pathway analysis indicated that the upregulated proteins in LFH3+ vs. FP2+ comparison were mostly involved in oxidative phosphorylation, transport, cysteine and methionine metabolism, whereas the down regulated proteins were largely associated with carbon metabolism, two-component system, biosynthesis of secondary metabolites and carbohydrate metabolism. Among these, Rrf2 family transcriptional regulator (A0A060DJY4, A0A060DHM9) was one of the most significantly upregulated proteins. STRING network analysis showed that the Rrf2 is correlated with the CysK (Q2PZM5, [Cysteine synthase]) that is upregulated in both high and low concentration of ammonium in the *glnB* mutant strain. These results suggested GlnB might participate in cysteine biosynthesis.

Keywords: proteomic; GlnB; Azospirillum brasilense*.*

Financial Support: CNPq.

Short Note V.II

RNA-seq Reveals New Functions of NtrY/NtrX Two-component System of *Herbaspirillum seropedicae*

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The nitrogen-fixing and Betaproteobacterium *Herbaspirillum seropedicae* colonizes aerial parts of crops of economic importance, including maize, and sugar cane. Genes required for nitrogen fixation are under control of the members of the two-component system family, NtrB/NtrC system. *H. seropedicae* genome also contains genes coding for proteins homologous to NtrB and NtrC, named NtrY and NtrX respectively. NtrY, the histidine kinase, is essential for nitrate assimilation, but not required for nitrogen fixation; NtrX, the response regulator, binds to promoter regions of genes related to nitrate catabolism. So far, the function of the NtrY/NtrX system of *H. seropedicae* has been related to nitrate metabolism. Aiming to explore other functions of this system, we conducted a transcriptional profiling by RNAseq with wild-type *H. seropedicae* and *ntrY* mutant grown under nitrogen-limiting condition, and then submitted to nitrate- or ammonium-shock. The transcriptional profiling of wild-type and *ntrY* mutant was compared to each other at each nitrogen condition showing that 266, 962 and 431 genes were regulated by NtrY under nitrogenlimiting, nitrate-shock or ammonium-shock, respectively. Both RNA-seq and β-galactosidase analyses suggested that NtrY was required to repress *nas* genes (involved with nitrate assimilation) under nitrogen limitation, and to partially activate them in response to nitrate. NtrY was also required to activate the qor gene, which encodes a NADPH-quinone reductase, and to repress genes encoding structural components and maturation proteins of *cbb3*-type heme-copper oxidase (*fixNOP* and *hemNHsero_3206fixIS* genes). NtrY was also important to activate genes related to flagellar assembly and bacterial chemotaxis. In conclusion, the NtrY/NtrX system of *H. seropedicae* is not restricted to fine-tuning the regulation of nas genes, but also regulates other general cellular functions, including swarming motility, chemotaxis, and electron transport chain.

Keywords: bacterial physiology; nitrate assimilation.

Financial Support: CNPq and Capes.

Short Note V.III

The effect of sugarcane apoplastic fluid on the global gene expression pattern of strain HRC54 of *Herbaspirillum seropedicae*

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Sugarcane is one of the most important energetic crops in Brazil. In order to improve its yield, the use of nitrogen fertilizers is common, but these can be harmful for the environment and elevate the cost of the production. The inoculation with plant growth-promoting bacteria (PGPB) has become an alternative to increase productivity and reduce negative environmental impacts. The diazotrophic endophytic bacterium *Herbaspirillum seropedicae* is one of this bacteria usually suggested for inoculation of non-leguminous plants. Even with the promising results, the low efficiency of the inoculant when compared to use of the fertilizers is still considered a problem. Studying the molecular mechanisms related to an efficient plant-bacterium interaction can show important genes and pathways that can be determinant for the bacterial inoculant effect. To get closer to the scenario found during the interaction process, an alternative is the bacterial growth in the presence of some tissue or plant fluid. Considering this, the total RNA of strain HRC54 from *H. seropedicae* grown in JNFb medium supplemented with the apoplastic fluid from sugarcane RB867515 variety was used to identify differentially expressed genes. This RNA-Seq experiment permitted to identify 88 differentially expressed genes, in which 43 were considered up-regulated and 45 down-regulated. The up-regulated genes are related to translation process, metabolism and transport of carbohydrates, which could be an indicative of a possible communication between the organisms. The down-regulated genes are related to cell motility and mechanisms from signal transduction, which could demonstrate the bacteria response to the presence of the fluid. The future validations of the expression pattern found could provide insight about the interaction process.

Keywords: plant growth-promoting bacteria; RNA-Seq; plantbacteria interaction.

Financial Support: CNPq.

Poster V.1

The PII interactome in *Azospirillum brasilense*

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PII proteins are signal transducer proteins, ubiquitous in bacteria. They are involved in nitrogen metabolism regulation and act mostly by direct protein-protein interaction in response to effector binding of ATP, ADP and/or 2-oxoglutarate (2-OG). Effectors binding by PII cause conformational changes, which reflect directly on PII interaction with target proteins. In *Azospirillum brasilense* there are two PII proteins named GlnB and GlnZ, involved with transcriptional activation of nitrogen metabolism genes and nitrogenase post-translational regulation in response to variation of ammonium and 2-OG cellular levels. Since PII proteins are able to sense 2-OG, which is directly involved with carbon and nitrogen cellular levels signaling, a role in carbon metabolism by PII proteins have been addressed. Here we show that PII appears to exert a pleiotropic effect in *A. brasilense* metabolism. The proteins identified in interactome assays were mainly related with fatty acids metabolism, nucleotides metabolism, and proteins involved in various metabolic pathways, like NAD+ synthetase, malic enzyme, and RNAses.

Keywords: nitrogenase; regulation; genes.

Financial Support: INCT-FBN/CNPq, Newton Fund Grant BB/N013476/1. Fundação Araucária and Capes.

Poster V.2

The GlnR regulon in *Paenibacillus riograndensis*

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Paenibacillus species are Gram-positive endosporeforming bacteria frequently found associated with roots of diverse plant species. Many paenibacilli are diazotrophs, and for this reason they are often investigated as potential plant growth-promoting bacteria. However, the molecular mechanisms that underlie nitrogen fixation in these bacteria are still scarce. Recently, we have found that operators of the repressor GlnR are located upstream of nitrogen fixation genes in *Paenibacillus riograndensis* SBR5. GlnR is a key regulator of nitrogen metabolism in *Bacillus subtilis*, nevertheless its role in *Paenibacillus* spp. should be further elucidated. For this purpose, a de novo positional weight matrix (PWM) was built after detecting GlnR operators-like motifs upstream of nitrogen fixation related-genes and the gene glnR of different *Paenibacillus* strains through MEME software. Subsequently, using MAST software, the matrix was utilized to search operators upstream the CDSs of *Paenibacillus* strains. In *P. riograndensis* SBR5, forty-four genes presented GlnR operators. Most of these operators are conserved upstream of ortholog genes among *Paenibacillus* spp. Based on RNAseq data, we found that from these genes, twenty are co-expressed with the gene *nifB* in *P. riograndensis* SBR5 under nitrogen fixation conditions. Many of these genes encode proteins that are clearly implicated with nitrogen metabolism, such as the following: an ammonium transporter, a glutamine transporter, an alanine dehydrogenase, a nitrite reductase, a dipeptide transporter, and the transcriptional regulator PucR. In *B. subtilis*, PucR activates the expression of enzymes implicated in scavenging alternative nitrogen sources, notably a guanine deaminase and an urease. These results indicate that GlnR also plays a central role in nitrogen metabolism of *Paenibacillus riograndensis*.

Keywords: GlnR; Paenibacillus*; nitrogen metabolism.*

Financial Support: Capes, CNPq, UK-Brazil Nitrogen Fixation Centre (UBNFC).

Poster V.3

The genetic relationship between *Azospirillum brasilense* and maize during the inhibition of indole-acetic production by the plant

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Maize is among one of the most used plant species in human food, for this reason ways to increase its production without increasing the planted area are always necessary. One way of achieving this is using plant growth promoting bacteria. Among them are the bacteria of the genus *Azospirillum*. These bacteria are found in the rhizosphere of grasses and cereals both in the tropics and in temperate climates. They have as main characteristics the ability to fix the gaseous nitrogen and to produce hormones that can be used by the plants. It is believed that both processes are responsible for stimulating the increase of the final mass of the plants. The present study aimed to better understand the genetic relationship between *Azospirillum brasilense* and maize during a situation of inhibition of indole acetic acid (IAA) production by the plants. For this, the chemical compound Yucasin [5-(4-chlorophenyl)-4H-1,2,4-triazole-3-thiol] was applied to the plants. It is an inhibitor of one of the IAA production pathways in plants. The genetic expression of the bacterium and plant during this situation was analyzed by Dual RNA-Seq. The data obtained by the transcriptome sequencing of both plant and bacterium were analyzed using a combined analysis approach. With this methodology it was possible to identify sequences for both the organisms. Through the mapping with the respective annotated genomes, the presence of genes expressed in both organisms was detected.

Keywords: Azospirillum*; plant-bacteria interaction.*

Financial Support: CNPq, Capes and Newton Fund Grant BB/N013476/1.

Poster V.4

A mechanism for the regulation of *Herbaspirillum seropedicae* NifA by its GAF domain in response to ammonium levels

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Herbaspirillum seropedicae is an endophytic diazotrophic β-proteobacterium found in the xylem vessels of economically important grasses, where it supports plant growth through nitrogen fixation and phytohormone production. Biological nitrogen fixation in this organism is regulated by the transcriptional activator NifA, which controls the expression of the *nif* genes. NifA activity is repressed by both oxygen and ammonium ions; the ammonium repression is mediated by its own N-terminal GAF domain and alleviated by the interaction of the GAF domain with GlnK. We employed site-directed mutagenesis of *glnK* to probe the importance of uridylation and the relevance of GlnK interaction with ATP and 2OG for the derepression of NifA activity; random mutagenesis of *glnK* was also performed in an attempt to locate residues important for protein-protein interaction. The results show that the interaction of GlnK with ATP is essential and the interaction with 2OG is the main requirement for the activation of NifA by GlnK. Unlike what is seen in other organisms, the uridylation of GlnK seems to have no effect on the activation of NifA. Random substitutions inactivating GlnK were concentrated on the upper face of the PII trimer, near the T-loop, suggesting this surface to be the region responsible for interaction with NifA. Random mutagenesis of the GAF domain of an oxygen-insensitive NifA variant allowed the identification of 26 individual NifA-inactivating substitutions located between residues 14-164 of NifA and 11 NifA-activating substitutions concentrated between residues 176-193, a region predicted to form a connection helix between the GAF and AAA+ domains. The spatial distribution and identity of the substitutions, together with the characterization of the NifA variant oligomerization state in solution, led to the proposal of control of the oligomerization state as a mechanism of NifA regulation, similar to what was proposed for *Aquifex aeolicus* Nlh1.

Keywords: Herbaspirillum seropedicae*; NifA; GlnK.*

Financial Support: CNPq, Capes, INCT-FBN, BBSRC, and Newton Fund grant BB/N013476/1.

Poster V.5

Effect of biochar on the mirobial enzymes activity of soil with eucalyptus

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Biochar is a carbon-rich material obtained from carbonized biomass under a low oxygen atmosphere, used as soil conditioner. It has potential to alter physical, chemical and microbiological properties of the soil, increasing the availability of water and nutrients to the plants. This work evaluated the effect of biochar produced from eucalyptus residues in the soil microbial activity of beta-glucosidase and urease enzymes of a commercial cultivation area of *Eucalyptus urograndis* installed in a Red-Yellow Latosol in São Jerônimo da Serra, Paraná. The experiment was designed in randomized blocks varying the dose of biochar (0 or 1.11 mg/ha) and fertilization (0, 120 or 150 g of NPK 4:42:6 per tree) with four replicates. The rhizospheric and non-rhizospheric soil were collected 90 days after planting and used for determining the most probable number of diazotrophs and the microbial betaglucosidase and urease activity enzymes. The highest beta-glucosidase activity was recorded in the absence of fertilization and biochar in the rhizospheric soil (2, 173 ug/g of soil). In general, fertilization and biochar reduced beta-glucosidase activity and the number of nitrogen-fixing bacteria. In the non-rhizospheric soil, the beta-glucosidase activity was 50% to 75% lower than in the rhizospheric soil; this indicates that the closer the bacteria are to the root, the greater is the breakdown of carbohydrates and the production of glucose, providing more energy for their proliferation. In contrast, the highest urease enzyme activity was recorded in presence of medium fertilizer and biochar in non-rhizospheric soil (18.5 ug NH_4-N/g). In general, the presence of biochar generated higher urease activities, indicating that more NH_3 was being produced, thus there was more availability of N for eucalyptus plants in this condition. Both enzymes showed to be good indicators of soil quality and fertility, since they were sensitive to soil management as well as nutrient availability variations.

Keywords: Beta-glucosidase enzyme; urease enzyme; biological nitrogen fixation.

Financial Support: Brazilian Program of National Institutes of Science and Technology (INCT-FBN), CNPq, Capes and Fundação Araucária of the Parana State.

Poster V.6

Responses to elevated c-di-GMP levels in *Azospirillum brasilense* Az39

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The Alphaproteobacteria *Azospirillum brasilense* is a nitrogen-fixing plant-growth-promoting rhizobacteria (PGPR) which have beneficial effects in many agronomically important plants. Its beneficial properties depend on an efficient attachment and colonization of the plant root surface, which implies a genetic and metabolic adaptation to stablish an intimate association with the plant host. Cyclic diguanilate (c-di-GMP) has emerged as an ubiquitous second messenger in bacteria, probably best known for controlling the transition from a planktonic/motile lifestyle to a sessile biofilm mode of growth. *Azospirillum*, as many other plant-associated bacteria, encode dozens of c-di-GMP metabolic enzymes involved on its synthesis and degradation. The likely functional redundancy evidences a complex and evolved signal transduction systems on these bacteria but often hinder genetic approaches to reveal c-di-GMP networks and components affecting a particular function. In this work, we have exploited the overexpression of a well characterized diguanylate cyclase to artificially raise the intracellular levels of c-di-GMP in *Azospirillum brasilense* Az39. The altered c-di-GMP levels generate a substantial colony morphology change evidencing the overproduction of at least one c-di-GMP-promoted polymer when was grown on solid media supplemented with different dyes. Furthermore, c-di-GMP promotes flocculation in liquid media and different forms of biofilm formation. The swimming motility was also altered and GFP-labelled versions allowed us to study the impact of high levels of this bacterial second messenger on the colonization of the plant host.

Keywords: A. Brasilense*; mobility; colonization.*

Financial Support: grant BIO2017-83533-P to J.S.

Poster V.7

Quorum sensing and other communication strategies in *A. brasilense*

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The mechanism known as quorum sensing (QS) based on N-acyl-L-homoserine lactones (AHL) allows cell-to-cell communication to control the expression of certain genes under high cell density conditions. QS systems are found in gramnegative bacteria and used for controlling various phenotypes that are often essential for interaction with both, prokaryotic and eukaryotic cells. *Azospirillum* sp. may indirectly regulate several physiological mechanisms by a quorum system. However, the AHL-mediated QS mechanism seems to be unusual in *A. Brasilense*. On the other hand, the quorum quenching (QQ) refers to all processes involved in the interruption of the QS mechanism. The QQ is a natural mechanism, either to degrade their own QS signals, or to establish competitive relationships with other microorganisms by degradation of their signal molecules. The QQ activity depends of AHL-lactonase and AHL-acylase. *A. brasilense* Az39 is a comprising versatile plant growth promoting rhizobacteria (PGPR), which have been shown remarkable capacity to benefit a wide broad of crops under agronomic conditions. This strain has been used to produce commercial inoculants for cereals as maize and wheat or legumes as soybean in Argentina and Mercosur. Despite their agronomic importance, few studies have been performed to unravel the quorum system mechanism in such strain. In this work, we examined *in silico* and *in vitro* the whole mechanisms related with the QS and QQ system in Az39. The results obtained showed that although this strain does not produce molecules of the AHL type, it is capable of degrading them by enzymatic activity. Interestingly, the QQ activity is due to the action of an AHL-acylase and not to an AHL-lactonase, associated to the bacterial cell. Moreover, the Az39 genome encodes a single key LuxR *SOLO* for bacterial communication probably at the rhizospheric level. These results suggesting that Az39 have capacity to degrade the exogenous AHLs of other bacteria sharing the same environment and respond to like-AHLs molecules by the presence of the LuxR *SOLO* modulating the interaction between microbial communities and plants.

Keywords: plant growth-promoting rhizobacteria; quorum quenching; LuxR SOLO*.*

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Poster V.8

Role of PQQ cofactor of *Serratia* sp. S119 in the modulation of bacterial antioxidant mechanisms

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Serratia sp. S119 is a plant growth promoting bacteria (PGPB) that improves peanut and maize growth. In early stages of interaction between these bacteria and plants they are initially detected as pathogens because plants response to its infection by an increase in ROS production. It is assumed that PGPB are able to avoid this response in order to achieve a successful rhizospheric and endophytic colonization, and to finally express its growth promotion ability. Bacteria present different antioxidant strategies to avoid the damage of ROS being both no enzymatic mechanisms (like gluthathione) and enzymatic mechanisms (specific enzymes activities). Cofactor PQQ is a prosthetic group in bacterial dehydrogenases involved in sugars and alcohol oxidation. It is capable of catalyzing continuous redox cycling and was reported as an efficient scavenger of O_2- and OH^-. In addition, it has been reported that PQQ could have a role in bacterial antioxidant enzymes activities during plant-bacterium interaction. The aim of this work was to study the role of PQQ molecule in the modulation of bacterial antioxidant mechanisms (enzymatic and no enzymatic). The strains used in this study were *Serratia* sp. S119 and the PQQ minus- mutant *Serratia* sp. RSL. Peroxide was used as ROS response molecule to evaluate antioxidant modulation in bacteria. Different concentrations of H_2O_2 (0, 5, 7, 10 mM) were added to the culture medium and CFU ml^{-1} was determined during 48 hours of growth. Enzymes activities; SOD, CAT and PX were determined according to Beauchamp and Fridovich (1973), Aebi (1984) and Alderete et al. (2009), respectively and GSH content was determined according to Anderson, (1985). All determinations were done during bacterial exponential growth phase. *pqq*E gene expression analysis was analyzed by qRT-PCR following methodology described by Ludueña et al. (2017). Results obtained indicated that 7 mM of H_2O_2 produced no differences between CFU ml^{-1} of both strains so this concentration was selected for further assays. Similar CAT, SOD and PX activities were observed when both bacteria were grown in the presence of H_2O_2. On the contrary, RSL showed an increase in its GSH content when it was grown in the presence of H_2O_2 meanwhile S119 showed a decreased in the level of this molecule. The expression of *pqq*E gene showed no differences in all conditions assayed. In conclusion it is possible to suggest that PQQ cofactor is not involved in these antioxidant mechanisms under these experimental conditions.

Keywords: plant growth promoting bacteria; PQQ; antioxidant enzymes.

Financial Support: CONICET; SCyT-UNRC; ANPCyT.

Poster V.9

Construction and characterization of *Azospirillum brasilense* strains containing nifA gene deleted in the GAF domain

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Azospirillum brasilense is a nitrogen-fixing bacterium found associated with several plants of agricultural interest, presenting potential application as a biofertilizer. Biological nitrogen fixation is a very energy-demanding process requiring at least 16 mol of ATP per mol of N_2 reduced. At the transcriptional level, regulation of nitrogen fixation in *A. brasilense* is centered on the activity of NifA, the specific activator of *nif* genes transcription. This protein presents a regulatory N-terminal GAF domain, which prevents NifA activity in the presence of ammonium. The objective of this work was to construct *A. brasilense* strains containing chromosomal deletion of the 5' region of the *nifA* gene. The regions located upstream and downstream of the target deletion region (coding for the first 187 residues of NifA) were amplified by PCR and cloned into plasmid pSUP202 along with a *sacBKm* cassette, which functions as a negative selection marker. The plasmid was transferred to the wild-type FP2 and the ammonium ion excreting HM053 *A. brasilense* strains by biparental conjugation, yielding the TAFP and TAHM strains, respectively. Nitrogenase activity assays revealed that chromosomal deletion of NifA N-terminal domain resulted in a protein unable to activate the transcription of nif genes even at low ammonium levels, which was confirmed by the absence of *nifB::lacZ* transcriptional fusion expression. These results suggest that the deleted region of the *nifA* gene seems to be important for protein stability or active conformation. The mutant strains were complemented for nitrogenase activity with plasmids expressing the native or an N-truncated (lacking the first 201 residues) version of the NifA protein. The latter transconjugant showed activity even in the presence of high levels of ammonium. Mutant complementation assays have shown that truncation of the 5' region of *nifA* gene results in active NifA even in the presence of ammonium when expression is from the lac promoter.

Keywords: biological nitrogen fixation; transcriptional regulation; chromosomal deletion.

Financial Support: CNPq, Capes, INCT-FBN, and Newton Fund Grant BB/N013476/1.

Poster V.10

Characterization of *ipdC*, *flcA* and *narL-like* mutant strains of *Azospirillum brasilense* in the interaction with grasses

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Azospirillum brasilense is an alpha-proteobacterium apable of associating with several economically important grasses and promoting plant growth and productivity. For *A. brasilense* to exert this growth promoting effect it is necessary the establishment of a successful interaction with the host plant. However, the factors involved in the *Azospirillum*-plant interaction are not completely known. The objective of this work was to construct mutant strains of *Azospirillum brasilense* strain FP2 in the *flcA*, *narL-like* and *ipdC* genes and characterize their involvement in the interaction with grasses. The phenotypes of the mutant strains *ΔflcA*, *ΔnarL-like* and *ΔipdC*, were evaluated for nitrogenase activity, indole acetic acid production, flocculation, red congo binding, glass fiber biofilm formation and the ability of colonization of wheat roots. The capacity to fix nitrogen was not affected in mutant knockouts and interestingly the *ΔnarL-like* mutant showed a higher *in vitro* nitrogenase activity level than the wild-type strain. The levels of indol acetic acid production in the *ΔipdC* mutant was significantly lower than that of the wild-type strain. The mutant unable to flocculate, *ΔflcA*, and the *ΔnarL-like* mutant presented lower capacity to connect with the congo red than the wild strain, suggesting a change in the composition of surface polysaccharides in these mutants. The biofilm formation in *ΔflcA* and *ΔnarL-like* mutants was 40% and 50% lower than that of the wild strain, respectively. However, the colonization phenotype of the *ΔflcA* mutant did not differ from the wild strain. Interestingly, the *ΔnarL-lik*e mutant presented an epiphytic population larger than that of the wild strain in the wheat root colonization assay, suggesting that possibly this mutant is more efficient in root colonization.

Keywords: bacteria-plant interaction; biofilm; biological nitrogen fixation; biofertilizer.

Financial Support: INCT-FBN, CNPq, CAPES, UFPR.

Poster V.11

Maize root exudates improves the response to *Azospirillum brasilense* Ab-V5 inoculation

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The root exudates produced by plants are thought to mediate plant-bacteria interactions, and to shape the composition and function of rhizosphere microbiome. The quali-quantitative composition of root exudates rules the chemotactic response of soil organisms and can influence the establishment of selected plant growthpromoting bacterial strains (PGPB) used in commercial inoculants. Thus, the purpose of the present study was to evaluate whether root exudates from commercial maize hybrids could upgrade the growth-promotion effects of *Azospirillum brasilense* Ab-V5 inoculation on the early growth of maize plants. The experiment was carried out in a greenhouse in a completely randomized design, using surface-sterilized seeds (hybrids DKB 240 and P30F53H) treated with the following inoculant solutions: Ab-V5 suspension in water (1×10^6 cells mL^{-1}); Ab-V5 suspension in root exudate (1 mg mL^{-1}) from DKB 240; and Ab-V5 suspension in root exudate from DKB 390 (1 mg mL^{-1}). Plants were watered with N-depleted (2 ppm N) nutrient Hoagland's solution each four day, with an additional control treatment at full-strength Hoagland's solution (20 ppm N). After 30 days of growth, physiological and biometric evaluations were carried out. Supplementation of Ab-V5 inoculants with the root exudates extracts boosted the inoculation response, regardless the extract added or the plant genotype. DKB 240 genotype had increased most of the growth parameters above the values observed in control plants grown at high N-fertilization. This same effect was observed for the P30F53H genotype, although differences regarding the source of the root exudate could be observed. Few parameters related to the photosynthetic apparatus were affected by the fertilization or inoculation treatments. This research highlights that root exudates are involved in the behavior of inoculant bacteria, therefore the use of these compounds may lead to inoculant formulations of higher efficiency than regular inoculant formulations.

Keywords: biofertilizers; chemotaxis; inoculant technology.

Poster V.12

Metabolic pathways due to loss of GlnB signaling in *Azospirillum brasilense*: a proteomic approach

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PII proteins are the main signal transducers of cellular and nitrogen metabolism, interacting with a variety of cellular targets. *Azospirillum brasilense* has two PII proteins, called GlnB and GlnZ, which regulate nitrogen metabolism in response to changes in extracellular ammonium concentrations. Although several studies have discovered the functions of PII under conditions of low and high ammonium concentrations, these data are still insufficient to define their function in the complex Ntr system. Wild-type and *glnB* mutant (LFH3) strains of *A. brasilense* were cultured under high (+) or low (-) ammonium and the proteome of these cells were compared using label free quantitative LC-MS/MS analysis. To determine Differentially Abundant Protein Species (DAPs) a p value <0.05 and a fold change> 1.8 or <0.5 was used. 2165 proteins were identified when the *glnB* mutant strain LFH3 was compared to the FP2 control, of which 379 were upregulated and 720 were down regulated in high concentration of ammonium (20mM), 338 were upregulated and 199 were down regulated in low concentration of ammonium (2mM). Gene ontology (GO) enrichment analysis revealed terms involved in oxydoreductase activity, transport, NADH dehydrogenase activity, signal transducer activity and nitrogen cycle metabolic process, to be increased in high concentration of ammonium while terms associated with transferase activity and lipid biosynthetic process, to be increased in low concentration of ammonium. KEGG pathway analysis indicated that the upregulated proteins in LFH3+ vs. FP2+ comparison were mostly involved in oxidative phosphorylation, transport, cysteine and methionine metabolism, whereas the down regulated proteins were largely associated with carbon metabolism, two-component system, biosynthesis of secondary metabolites and carbohydrate metabolism. Among these, Rrf2 family transcriptional regulator (A0A060DJY4, A0A060DHM9) was one of the most significantly upregulated proteins. STRING network analysis showed that the Rrf2 is correlated with the CysK (Q2PZM5, [Cysteine synthase]) that is upregulated in both high and low concentration of ammonium in the *glnB* mutant strain. These results suggested GlnB might participate in cysteine biosynthesis.

Keywords: Proteomic; GlnB; Azospirillum brasilense*.*
Financial Support: CNPq.

SESSION 6: Applications of PGPR in Agriculture

Key Note VI.I

Interaction of Plant Growth-Promoting Bacteria and microalgae: from basic studies of plant-bacteria interaction to potential biotechnological applications

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A simple, quantitative experimental model, offering a convenient and basic approach to studies of plant–bacterium interactions, is proposed. This involves immobilizing a unicellular, freshwater microalga, a species of *Chlorella* that serves as the plant and a plant growth-promoting bacterium (PGPB), a strain of a species of *Azospirillum* of agricultural origin. The two micro-organisms are co-immobilized in small alginate beads to allow close interaction and avoid external interference from bacterial contaminants. The interaction resulted in a significant increase in several growth parameters of the microalgae, such as fresh and dry weight, pigment concentration, lipid concentration and variety of fatty acids, concentration of carbohydrates and starch, and increase in concentration of vitamins. In addition it has been found that there is an effect on the activity of enzymes related to the metabolism of ammonium (glutamate dehydrogenase, glutamine synthetase), phosphorus (alkaline phosphatase), fatty acids (acetyl carboxylase) and starch (ADP glucose pyrophosphorylase). All these significant metabolic changes during co-immobilization of the two micro-organisms are interlinked and offer several biotechnological applications. These include wastewater treatment, carbohydrate and lipid production, photosynthetic pigments, and food for human and animals. This model is also a simple and easy to handle tool for basic science biological studies.

Short Note VI.I

Co-inoculation using the PGPR species of *Azospirillum brasilense* - Brazilian case

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In Brazil, in the last harvest, the average inoculation rate in Soybean was of 78%, 10% higher than the crop of 15/16. This growth evidences that the farmer is increasing his/her perception about the impact of the Biological Fixation of N on the crop productivity. Still, the search for higher levels of production per cultivated area is incessant, and for this Nitrogen, it is the indispensable element, since it is fundamental in vital processes throughout the plant cycle. Given the circumstances, the use of tools that allow greater efficiency of N biological fixation is extremely relevant, which is why the co-inoculation technique has been gaining more and more space in the agricultural production system. The co-inoculation is the association of two or more species, and the most widely studied is the association of bacteria *Bradyrhizobium* with *Azospirillum brasilense*. In addition to being an N fixing bacterium, *Azospirillum* is capable of producing hormones, such as AIA, which auxiliate in the best development of plants, especially of the root system. With that said, it will facilitate the communication of the *Bradyrhizobium* sp with the plant and will guarantee the anticipation in the formation of nodules, greater number of nodules and more nodules located in the main root of the plants, which are more efficient. All these benefits have been observed in the field, in the most diverse edaphoclimatic conditions and has been highlighting the success of the co-inoculation technique. This is clearly evidenced by the number of doses of *Azospirillum*-based inoculants sold by companies associated with ANPII, which in the crop of 17/18 were almost 6 million doses, a growth of 32% compared to the previous harvest, while in the corn area, the cultivation until now have a greater use of this inoculant, presenting a decrease of 6%, which infers the growth of the adoption of the co-inoculation. Certainly, this is a strategy of total relevance for the continuous breach of production records in our country.

Keywords: nitrogen; Azospirillum*; coinoculation.*

Short Note VI.II

A brief history of microbiology. The history of *A. brasilense* Az39 in agriculture

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Azospirillum is one of the most studied bacterial genera in the last 60 years; However, the history of the appearance of biological products formulated with this bacterium began in the 1980s, but intensified in the last 20 years in Argentina, Brazil and the rest of South America. In the case of Argentina, *A. brasilense* Az39 is the strain that has been recommended for more than 40 years for the production of biofertilizers for wheat, sorghum, corn and soybean (co-inoculation). This strain has demonstrated a great capacity to promote plant growth with average yield increases greater than 10.0% and a success rate higher than 70% in different crops in thousand experiments. Despite the immense amount of information available at the agronomic level, until a few years ago very little was known about the molecular basis that determined the ability of this strain to promote plant growth. In 2012, in the Laboratorio de Fisiología Vegetal y de la Interacción Planta-Microorganismo of the Universidad Nacional de Río Cuarto, a international consortium was initiated with the aim to sequence the genome of *A. brasilense* Az39 and *B. japonicum* E109, the most used strains for formulation of inoculants in Argentina. Using a combined sequencing strategy, it was established that the Az39 genome has a size of 7.39 Mpb distributed in 6 replicons [1 chromosome, 3 chromides and 2 plasmids]. Through the use of comparative bioinformatics tools, numerous genes and putative proteins involved in the expression of plant growth promotion mechanisms and other related with the rhizosphere lifestyle were identified, among them those related to the biological nitrogen fixation, biosynthesis of phytohormones and the presence of several secretion systems. The decoding of this information has provided a solid basis for the elucidation of new mechanisms of interaction and growth promotion, as well as some specific components that would determine the agronomic success of this microorganism. In this presentation we will address some of the new biological models recently identified for this bacterium and how they affect their rhizosphere lifestyle.

Keywords: genomic; plant growth promoting rhizobacteria; bioproducts.

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Short Note VI.III

Induction of plant-stress tolerance and defense genes by *Azospirillum brasilense* cells or their metabolites in maize plants

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Azospirillum spp. are plant-growth-promoting bacteria used worldwide as inoculants for a variety of crops. In Brazil, the application of inoculants containing *A. brasilense* strains Ab-V5 and Ab-V6 to cereals has grown exponentially in the last years. We investigated the effects of inoculation with Ab-V5 or Ab-V6 strains on the maize by applying it on seeds or by leaf spray of cells or their metabolites at the V2.5 growth stage, a strategy to relieve incompatibility with pesticides used for seed treatment. Experiments were performed with the hybrid DKB330 VT PRO2 in greenhouse at Embrapa Soybean, Londrina, PR, Brazil. Treatments consisted of mineral-N fertilizer (50% N) and methods of inoculation: SI (standard seed inoculation at sowing), ILS (inoculation by leaf spray) and MLS (metabolites of *A. brasilense* Ab-V5 and Ab-V6 by leaf spray). At 32 days after transplanting, maize leaves and roots were sampled for assessment of expression of genes related to oxidative stress (*APX2*, *CAT1* and *SOD2*) and plant defense (pathogenesis-related *PR1* and *prp4*) by RT-PCR. Data obtained were analyzed using the Rest2009 software package. There were differences according to the gene, plant tissue, strain and method of inoculation, but, in general, inoculation with *Azospirillum* or its metabolites resulted in up-regulation of genes related to oxidative stress in leaves and down-regulation in roots; conversely, in general, PR genes were down-regulated in leaves and up-regulated in roots. Emphasis should be given to the application of metabolites, especially Ab-V5 + Ab-V6, which in general resulted in the highest up-regulation of oxidative-stress and PR genes both in leaves and in roots. We hypothesize that the benefits of inoculation of *Azospirillum* on seeds or by leaf spray, as well as of leaf spraying of *Azospirillum* metabolites, are strongly correlated with eliciting genes related to plant-stress tolerance and defense against pathogens.

Keywords: leaf spray; oxidative stress; Zea mays *L.*

Financial Support: CNPq, Capes.

Short Note VI.IV

Development of Biofertilizers – A journey from Associative Nitrogen Fixation to PGPR

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For more than one hundred years, rhizobia are known to be traditional biofertilizers. It was with the advent of acetylene reduction assay (ARA) technique in 70’s that nitrogenase activity associated with roots of non-legume was reported. Dr. Johana Dobereiner was the first to report nitrogen fixing ability in sugarcane, following which there have been series of reports on nitrogen fixing bacteria associated with roots of grasses using ARA. This established associative nitrogen fixation as a field which led to the first nitrogen fixation conference with non-legumes in 1979. The renewed interest in plant rhizosphere resulted in isolation of large number of bacteria associated with roots of non-legumes. Majority of such bacteria have been characterized phylogenetically by using 16S rRNA technology. Subsequently many plant inoculation studies using these bacteria have indicated beneficial effects not only due to nitrogen fixation but also by the ability of inoculated organisms to produce phytohormones and to solubilize insoluble soil phosphorus. For such bacteria, Joseph Kloepper and Milton Schroth introduced the new term Plant Growth Promoting Rhizobacteria (PGPR) in 1978. Based on these evidences, Biofertilizers are defined as preparations containing live, non-pathogenic micro-organisms which are beneficial to agricultural production in terms of nutrient supply. A brief review of the studies conducted in our lab related to microbiological diversity and Metagenomic analysis of Rhizosphere of Plants growing in extremely Halophytic Environment will be presented. The main objective of the present work was to study plant growth promoting potential of selected halophilic bacterial strains and their role in enhancing growth of wheat and maize under salinity stress conditions.

Poster VI.1

Root architecture of pre-sprouted sugarcane seedlings inoculated with five strains of diazotrophic bacteria

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In addition to growth promotion, diazotrophic bacteria also act in the biological nitrogen fixation process (BNF), allowing productivity gains and reducing costs with N-inputs. The objective of this work was to evaluate the influence of a mixture of diazotrophic bacteria on root architecture of pre-sprouted sugarcane seedlings. The experiment was conducted in a 1x2 factorial scheme in a randomized complete block design, with ten replications. The treatments were: control without inoculation and inoculation using a mixture of five strains of bacteria mixed at the time of application: *Gluconacetobacter diazotrophicus* BR11281 (Gd), *Herbaspirillum seropedicae* - BR11335 (Hs), *H. rubrisubalbicans*, BR11504 (Hr), *Paraburkholderia tropica* - BR11366 (Pt) and *Nitrospirillum amazonense* - BR11145 (Na). Peat inoculant was prepared with each strain mixed at planting diluted in water (1:100 (w / v) applied by immersion for 30 min. Control used gems were submerged in water with the same time. Nodes were planted in boxes containing sterile substrate and 20 days after were transferred to tubes containing commercial Multiplant™ substrate. At 30 days after planting, digitized roots images, previously stored in 50% (v / v) alcohol, were evaluated for morphology using WinRhizo Pro™ software. The evaluated variables were length, root volume, length / volume ratio, tip number, ramifications and root overlap. Data were submitted to analysis of variance using the Scott-Knott ($P \leq 0.05$). A mean increase of approximately 610.60 cm in the root length of the inoculated seedlings was observed in comparison to the control. As for the volume of roots, there was an addition, in cm³, of 27.89%. The length / volume ratio (cm / cm³) between inoculated and control, increased by 26.5%. Regarding the tips, roots and root overlap, there was an increase of 20.24%, 44.40% and 47.46%, respectively. Therefore, inoculation with the use of the five strains of bacteria is effective in the root development of sugarcane.

Keywords: plant-bacterial interaction; Saccharum *sp.; BNF.*

Funding: INCT/CNPq, Faperj, Capes.

Poster VI.2

Biomass accumulation in sugarcane inoculated with diazotrophic bacteria at two levels of N and P

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Inoculation with diazotrophic bacteria responds differently to plant genotype as well as the type of soil and environment conditions, including N and P levels available on the soil. The objective of the present work was to evaluate the influence of the inoculation of a mixture of five diazotrophic bacteria sugarcane variety RB867515 cultivated under hydroponic conditions and two N and P levels. The experiment was conducted in a 2x2x2 factorial scheme in a randomized block design, with five replications. The treatments were: uninoculated control and inoculated with a mixture of *Gluconacetobacter diazotrophicus* – PAL5, *Nitrospirillum amazonense* CBAmC, *Herbaspirillum rubrisubalbicans* – HCC103, *H. seropedicae* – HRC54 and *Paraburkholderia tropica* – Ppe8. The Hoagland's solution was used to grew the plants under N and P levels 0,3 e 3 mM) during 30 days. N restriction was performed during 72 h before the treatments were applied. After 15 days the experiment was collected and evaluated the biomass accumulation of leaves, stems and roots. Data were submitted to analysis of variance using the Scott-Knott 5% test. Under 3 mM N and P, leaf dry mass increased 130% over the control, followed by inoculated in high dose of P and low of N. Stem dry mass there was an increase of 30.6% in the inoculated in high P and low N, in relation to the control and the same was observed for dry root mass, with an increase of 50% in the inoculated in high dose of P and low in N. In all the variables was observed a smaller development in the treatments without inoculation and high doses of N and P. Therefore, the inoculation was an important factor in the development of the plant, focusing on those in high P and low N.

Keywords: plant bacteria interaction; plant growth, FBN.

Financial Support: Faperj, Capes, CNPq and Newton Fund Grant BB/N013476/1.

Poster VI.3

Colonization of maize and tomato seedlings by plant growth-promoting *Bacillus* sp. (ZK) and *Rhizobium* sp. (8.1.2.1)

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Recent inoculation studies have demonstrated that the bacterial isolates from the rhizosphere of sunflower (*Bacillus* sp. strain ZK) or maize (*Rhizobium* sp. strain 8.1.2.1) have ability to promote the growth of maize plants. Although the potential of both strains were demonstrated high in such studies, no information about the ecological plant-bacteria relationships are available. The knowledge of colonization mechanisms and respective colonized sites are fundamental to understand how these bacteria interact with the host plant, and helps to develop strategies to optimize its inoculation in field crops. In this context, this work aimed to determine colonization sites of the plant-growth-promoting bacteria (PGPB) *Bacillus* sp. (ZK) and *Rhizobium* sp. (8.1.2.1) in maize and tomato. To this, maize or tomato seedlings obtained from surface-sterilized seeds were grown in test tubes (90 mL) containing 40 mL of agar-water (1% m/v). Inoculation of seedlings were carried out at the 7th day of germination by transferring 1 mL of each bacterial suspension (1×10^6 cells mL^{-1}) to the bottom of the test tube. Plants were grown at constant temperature of 25±2ºC and 12 h photoperiod for additional 14 days (maize) or 21 days (tomato). After growth period, the roots were collected evaluated for endophytic colonization by CFU counts of surface disinfested roots, and rhizoplane colonization by CFU counts of washed roots. In both cases, root material was macerated in sterile saline solution (1:10 m/v) and serially diluted to 10^{-6} (g mL^{-1}). CFU counts were carried out in solid Dygs medium with five replicates per treatment, and data were submitted to analysis of variance and means were compared using the Tukey's test ($p < 0.05$). Both bacteria evaluated were able to colonize the rhizoplane of maize and tomato in high populations (above 1×10^8 CFU mL^{-1}), but none of them were observed in surface sterilized roots suggesting the inability to reach the endophytic habitat. In addition, *Rhizobium* sp. 8.1.2.1 colonized the maize rhizoplane in higher populations than *Bacillus* sp. ZK, while in tomato the opposite trend was observed. Microscopic studies are under way to determine the colonization pattern of both PGPB strains in maize and tomato.

Keywords: plant colonization; plant-bacteria interaction; inoculation.

Financial Support: Capes, CNPq, Fundação Araucária.

Poster VI.4

^{15}N influx and growth of contrasting sugarcane genotypes inoculated with plant growth promoting diazotrophic bacteria

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Plant growth promoting bacteria are capable of stimulating plant growth through various mechanisms, such as phytohormones production, biological nitrogen fixation and nutrient solubilization processes. These bacteria can be found naturally in association with sugarcane or can be inoculated in order to increase promoter stimulus. This work aimed to assess the joint inoculation of five diazotrophic bacteria species on growth, N accumulation and ^{15}N-NH_4+ and ^{15}N-NO_3- influx in two contrasting sugarcane genotypes (IACSP95-5000 and RB867515) previously selected based on their demand on soil fertility level. Two greenhouse experiments were conducted on a hydroponic cultivation system. Plants were grown in a 3 mM solution of N for 40 days, and thereafter the following concentrations of N-NH_4+ or N-NO_3-: 25, 75, 150 and 300 µM of ($^{15}NH_4$) 2 SO_4 (98% ^{15}N atoms - 10 min) or $K^{15}NO_3$ (60% ^{15}N atoms - 20 min) to evaluate the N influx. The varieties accumulated dry mass and total N in a differentiated and inverse way, IACSP95-5000 used the available N for producing 15.5% more shoot dry weight in the presence of NH_4+ in the control and obtained the lowest values of Km of this ion. Variety RB867515 responded positively to inoculation with diazotrophic bacteria increasing by 21% the total aerial dry mass in the presence of NO_3-. There was found that the inoculation treatment in the variety RB867515 increased of NH_4+ influx by 13% in comparison to the non-inoculated treatment. In the case of IACSP95-5000 variety, the inoculation treatment increased by 29% in the NO_3-influx, compared to the control. In the uninoculated plants, the NO_3- influx was higher in RB867515 than IACSP95-5000.

Keywords: ^{15}N-fertilizer; N uptake kinetics; nitrogen.

Financial Support: Capes, CNPq, Faperj and Newton Fund Grant BB/N013476/1.

Poster VI.5

Influence of the substrate on pre-sprouted seedling of sugarcane inoculated with a mixture of diazotrophs

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The pre-sprouted seedlings method using one note stem piece of sugarcane as a vegetative plant part brought many advantages to production, enhancing developed of sugarcane under field conditions. The plantlets were produced using different substrates and inoculation can be modified by the chemical and physical composition of the product used. This study has the objective to evaluate the root system of one node sugarcane plantlets, variety RB92579, grown in tubes with different substrates and inoculated with a mixture of five strains of diazotrophic bacteria. The experiment was setup at a growth chamber using a factorial scheme 2x2. The factors were: with and without bacteria inoculation of *Nitrospirillum amazonense*, *Herbaspirillum seropedicae*, *H. rubrisubalbicans*, *Gluconacetobacter diazotrophicus* and *Paraburkholderia tropica* and the commercial substrates Coconut Fiber™ (No.11) and Multiplant™, with three replicates. The stem cuttings with one bud were heat treated during 30 min in water bath at 52ºC and then treated with fungicide Comet™ for 3 min. The nodes were inoculated using peat inoculant mixing the five strains at planting time by immersion in water in a proportion of 1:50 (w/v) during 30 min. The roots were collected 30 days after planting and analyzed the morphology using the software WinRhizo Pro™ measuring: length, surface area, root diameter and volume, root length/volume ratio, number of forks, tips and crossings. After variance test, mean values were separated using Tuckey ($P \leq 0.05$). The results for length, surface area and forks obtained when planting in coconut fiber were superior to that of Multiplant™ substrate. The buds inoculated and planted in Multiplant substrate were superior in length, surface area, length/volume ratio and tips when compared to control plants.

Keywords: inoculation; substrate; plant growth promotion.

Financial Support: Faperj, CNPq-INCT and Newton Fund Grant BB/N013476/1.

Poster VI.6

Effects of maize inoculation with ethylenediamine-resistant mutants of *Pseudomonas* sp

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Plant growth-promoting bacteria (PGPB) comprise part of plant microbiome of biotechnological interest due to their potential to decrease the use of agrochemicals in agriculture. Among the commonly found PGPB species, the *Pseudomonas* genus is known for high competitiveness and efficiency in expressing growthpromotion traits. In this sense, the objective of this study was to evaluate the effectiveness maize growthpromotion by a diazotrophic *Pseudomonas* sp. and its mutant derivatives with altered N-metabolism, when plants grown under limited N supply. A total of 13 ethylenediamine (EDA) resistant mutant derivatives from *Pseudomonas* sp. AZM-01, an isolate obtained from maize rhizosphere, were selected after random mutagenesis with methyl methanesulfonate (MMS). The selected mutants showed to be altered in N-metabolism and auxin production when compared to the wild strain, and were used to inoculate maize plantlets (2B610 hybrid, Dow AgroSciences) grown in pots filled with 2 kg of a soil: sand (1:2, v/v) mixture as substrate. Inoculation was carried out at the 5th day of germination by transferring 1 mL of each bacterial suspension (1 x 10^6 cells mL^{-1}) on site of epicotyl emergence. The experiment was set in a complete randomized design with 16 treatments and five replicates as follow: 14 treatments of inoculated plants grown under limited N supply (wild strain and 13 mutant derivatives), and 2 uninoculated controls (plants grown with limited or full N supply). Variations in N supply were accomplished by the application of Hoagland's nutrient solution at 2 ppm or 20 ppm of N in a weekly basis. The plants were grown for 30 days and the response to inoculation with wild and mutant strains were determined through biometric parameters: root volume, root dry weight, shoot dry weight and plant dry weight. Data were submitted to analysis of variance and means were compared using the Scott-Knott's test ($P<0.05$). *PSEUDOMONAS* sp. AZM-01 increased biomass of maize plants under N-limiting conditions, although several mutant strains overcome the wild AZM-01 performance, especially the EDA-5 strain which supports biomass accumulation at equivalent amount of plants grown under full N supply. The random mutagenesis constitutes an important tool to improve the performance of wild PGPB strains.

Keywords: biofertilizer; plant inoculation; methyl methanesulphonate; plant growth-promoting.
Financial Support: Capes, CNPq, Fundação Araucária.

Poster VI.7

Isolation and characterization of plant growth promoting rhizobacteria and their effects on growth of tomato seedlings

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Nowadays the use of Plant Growth Promoting Rhizobacteria (PGPR) is considered a component for sustainable agriculture. The efficiency of inoculation depends of factors such as plant species, soil type, inoculum density, and environment conditions. Native bacteria are better adapted to a local plant and its environment than exogenous microorganisms. On the other hand, tomato (*Solanum lycopersicum* L.) is one of the most important horticultural crops worldwide and Mendoza is the major producer in Argentina. However, there is little information available regarding the use of rhizobacteria in this crop. The aim of this work was to isolate and characterize PGPR at different soil depths from roots and rhizosphere of tomato crops, and to study the effect of inoculation with selected PGPR isolates on growth of tomato seedlings in order to reduce the fertilizer's rates. After isolation and characterization according to their PGPR characteristics, 5 bacteria strains were selected. All of them were able to solubilize phosphate, produce siderophores, fix nitrogen and tolerate high NaCl. Then, seedbed experiment was conducted in growth chamber with 15-days-old tomato seedlings inoculated at the root level. The seedlings were inoculated with the strains 25X1, 64S1, 53F, 42P4, 60I1, inoculation buffer alone (control without bacteria) and N-P-K (18:18:18; control fertilized). Inoculation with all bacteria increased ($p<0.05$) plant height, stem diameter, roots dry weight (DW) and shoots DW as compared with control without bacteria and in some cases equaled the control fertilized. Also inoculation with 64S1, 60I1 and 42P4 increased roots DW as compared with control fertilized. The results suggest that native PGPR may be used as bio-inoculant to improve the nutrient integrated management reducing the utilization of fertilizers.

Keywords: PGPR; tomato; seedling.

Financial Support: SECTYP UNCUYO, PIP CONICET, PICT (ANPCyT).

Poster VI.8

Application of *Gluconacetobacter diazotrophicus* increases tolerance of red rice variety to water stress

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Plant-growth-promoting rhizobacteria (PGPR) are associated with plant roots and augment plant productivity and immunity; however, recent work by several groups shows that PGPR also elicit socalled "induced systemic tolerance" to water stress. This work aimed to evaluate the inoculation effect of *Gluconacetobacter diazotrophicus* strain PAL5 on alleviation of water stress in red rice (*Oryza sativa* L.) variety (genotype 405 Embrapa Meio Norte). In this sense, it was measured the expression patterns for the Ethylene-responsive transcription factor 4 (OsAP37), Drought-Induced lipid transfer protein (OsDIL) betaine aldehyde dehydrogenase (BADH) genes of red rice plants during the interaction with *G. diazotrophicus* submitted to two soil moisture level conditions: 50% and 100% of field capacity. Biochemical analyzes (monodehydroascorbate, electrical conductivity and chlorophyll content) and gene expression quantified by qRT-PCR was carried out 15 days after establishing the moisture stress levels. The results showed that *G. diazotrophicus*-treated red rice plants presented much darker green leaves and substantially lighter wilt symptoms than control plants. The *G. diazotrophicus* inoculation treatment decreased the leaf monodehydroascorbate content and relative electrical conductivity by 40% and 15%, respectively; increased the leaf chlorophyll content and the root recovery intension by 3.45-fold and 50%, respectively. In addition, the inoculation mitigated the drought-triggered up-regulation of the expression of the OsAP37, OsDIL and BADH genes, in red rice leaves. These results indicated that *G. diazotrophicus* conferred induced systemic tolerance to drought stress in red rice plants, by protecting plant cells, maintaining photosynthetic efficiency, membrane integrity and increasing some osmoprotective solutes.

Keywords: mitigation drought stress; Oryza sativa *L.; Diazotrophic bacteria.*

Financial Support: CNPq Universal – MCTI n° 483547/2013-1, Capes and UEPB.

Poster VI.9

Self-regulation of exopolysaccharide production in *Gluconacetobacter diazotrophicus* by a tyrosine kinase

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The endophytic bacterium *Gluconacetobacter diazotrophicus* produces exopolysaccharides (EPS) that are required for biofilm formation and colonization of rice seedlings. Bacterial tYrosine (BY) kinases are known to undergo autophosphorylation as well as phosphorylate other proteins. Because BY kinases are frequently associated with EPS biosynthetic production pathways in bacteria, we speculate that *G. diazotrophicus*-like selfregulation may be a general feature of EPS production. Here we investigated whether EPS produced by the *G. diazotrophicus* strain PAL5 is a signaling molecule that controls its own production. The wild type PAL5 assisted in the verification of glycosyltransferase (gumD) phosphorylation and a gumD$^-$ (EPS defective) mutant strain in BY kinase autophosphorylation. For *in vivo* phosphorylation assays, the cells were grown in the presence of ^{32}P-orthophosphate and analysed by the, pulsed labeling technique. The BY kinase protein of interest was immunoprecipitated with a specific antibody and the retained radioisotope quantified by a scintillation counter, visualized on SDS-PAGE and, exposed to an X-ray film. Here, we present evidence that the BY kinase (GDI2549, Wzz) of *G. diazotrophicus* strain PAL5 is able to phosphorylate an endogenous enzyme, glicosyltransferase (GDI2547, gumD), which participates in the synthesis of the EPS. EPS interacts with the extracellular domain of Wzz, which is a receptor, to control kinase activity. In the absence of EPS, the kinase is inactivated by autophosphorylation. The presence of EPS inhibits autophosphorylation and instead promotes the phosphorylation of a glycosyltransferase in the biosynthetic pathway, thereby stimulating the production of EPS. Thus, EPS production is subject to a positive feedback loop that ties its synthesis to its own concentration. Tyrosine kinase-mediated self-regulation could be a widespread feature of the control of EPS production in bacteria.

Keywords: Exopolimer synthesis; autophosphorylation; Diazotrophic bacteria.

Financial Support: CNPq Universal – MCTI n° 483547/2013-1, Capes and UEPB.

Poster VI.10

Paraburkholderia tropica is able to growth in chemostat under N_2-fixing conditions

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The use of Plant Growth Promoting Bacteria (PGPB) as inoculants is a promising way to reduce the use of agrochemicals products. Plant growth stimulation by PGPB can be a consequence of phytohormones production, phytopathogens inhibition or facilitation in nutrients supply, such as Nitrogen (N_2) fixation. In this work, physiological aspects of the diazotroph endophyte *Paraburkholderia tropica* growing in chemostat under limited carbon conditions have been defined. The results of this work showed that: 1- *Paraburkholderia tropica* MTo-293 was able to growth in chemostat with N_2 as the sole nitrogen source provided that the dissolved oxygen was maintained at 1-2% air saturation, but it was not a stable state: a small increase in the dissolved oxygen resulted in the inhibition of the nitrogenase and the culture washing. Nevertheless, a stable state of N_2-fixing conditions was achieved with a starter dose of ammonium in the medium, that could be the growth condition that most resembles what it is found in its natural environment. 2- The biomass yields of *P. tropica* growing with glucose and a starter dose of ammonium were lower than those grown under non-N_2-fixing conditions (0,39 vs 0,49 Cmol biomass/C-mol substrate), and the specific rates of oxygen consume were higher under N_2-fixing conditions (3,0 vs 1,8 mol/g biomass.h). 3- The extracellular glucose oxidation via a PQQ-linked glucose dehydrogenase was expressed only under N_2-fixing conditions, as it was also found in batch cultures. Carbon-limitation conditions were not enough to active the expression of this enzyme, suggesting that its synthesis is regulated by an efficient utilization of the Carbon source under circumstances where the energetic demand for growth functions are high (N_2-fixing cultures). This work contributes to the knowledge of physiological aspects of an endophytic PGPB that are necessary to consider for its application in agriculture and to enhance plant-microbe interaction in non-legumes.

Keywords: cultures; diazotroph; endophyte.

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Poster VI.11

Effect of isolated indigenous PGPR and chemical fertilizers on growth and productivity of pepper (*Capsicum annum* cv Calafyuco INTA)

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The excessive use of fertilizers has shown a negative impact on crop productivity, soil and water contamination, together with enhanced crop susceptibility to diseases. The inoculation of crops with plant growth promoting rhizobacteria (PGPR) might also help to overcome these problems by diminishing fertilizers utilization. Pepper (*Capsicum annum* L) is the most popular of fresh vegetables worldwide due to its combination of color, flavor, and nutritional value. The aim of this work was to study the effect of inoculation with different indigenous PGPR (25X1, 64S1, 53F, 42P4, 60I1) isolated from *Solanum licopersicum* crops on germination, growth, and yield of pepper cultivar Calafyuco INTA. 1) We evaluated speed and percentage of germination of seeds in petri dishes inoculated with five strains of PGPR or with water (negative control). 2) Twenty five-days-old seedlings grown in chamber growth (12 h of photoperiod, 100 μmol m^{-2} s^{-1}, 24±2ºC) were inoculated with the five PGPR strains or not (negative control without bacteria) or fertilized with Red Hakaphos® Base (N-P-K, 18:18:18, positive control). In forty five-days-old seedlings the height, root and shoot dry weight (DW) were evaluated. Then, seedlings were transplanted to plastic pots filled with sterile soil and transferred to greenhouse (natural photoperiod, Temp 24±2ºC). Treatments were arranged in a completely randomized design. Height, root and shoot DW, foliar area and fruit number were determined. Only the strain 60I1 increased the speed of germination and plant growth. All PGPR strains increased plant height, roots and shoots DW as compared with the negative control. PGPR inoculation equaled control fertilized effects on growth and production ($p<0.05$). The two best performers were 60I1 and 25X1. These results, indicates the potential of this indigenous PGPR strains might be used to reduce the utilization of chemical fertilizers.

Keywords: PGPR; pepper; bioinoculant.
Financial Support: SECTYP UNCUYO, PIP CONICET, PICT (ANPCyT).

Poster VI.12

Can *Herbaspirillum* seropedicae alleviate plant water stress?

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Severity and frequency of drought is expected to increase with global climate changes affecting crop yields. Inoculation with endophytic bacteria have been reported to reduce the negative impact from water stress. In the present study we evaluate in vitro the potential of *H. seropedicae* (Hs) to mitigate plant water stress in two biological systems: (a) stress simulated by polyethylene glycol 6000 on tomato plants cv Santa Clara and (b) stress after water suspension on maize plantlets (var SHS 5050). For tomato, *Hs* inoculation reduced the negative impact of PEG on root growth and lateral root emission. Inoculated plants improve photosynthesis and relative water content in either in the presence or absence of PEG. Using epifluorescent microscopy and *Hs* strain RAM 10 linked with gfp we had shown distinct colonization pattern of root surface under PEG-stress and changes density of root hairs. For maize, the plants were analyzed after leaf wilt to determine fresh weight, dry weight, electrolyte extravasation, proline content and in situ location of O_2- and H_2O_2. Hs-Inoculation reduced the negative impact of the water stress by reduction of electrolytes extravasation and increasing of proline content. Also, stressed plants treated with bacteria had shown lower accumulation of ROS in relation to stressed and non-inoculated plants. Ultrastructural analysis from maize leaf revealed that the water deficit affected the chloroplast membrane, but there was less damage in *Hs*-treated plant. Collectively, the results point that *H. seropedicae* can alleviate water stress by modulation of root architecture, increasing water uptake and contents, keeping highest photosynthetic rates as well reducing reactive oxygen species accumulation and chloroplast membrane damage.

Keywords: beneficial bacteria; drought stress; cellular ultrastructure.

Financial Support: Faperj, Capes, Newton Fund Grant BB/N013476/1.

Poster VI.13

Effect of the association of endophytic bacteria in *Urochloa ruziziensis* submitted to osmotic stress

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Urochloa ruziziensis presents high quality of forage, with high food value, but is not tolerant to drought. Plants constantly subjected to stresses have the ability to modulate defense responses to get back to their normal metabolism, these responses can neutralize the cytotoxicity of reactive oxygen species and are known as antioxidant mechanisms. Plant growth promoting bacteria (PGPB) can aid plants to develop mechanisms involved in responses to abiotic stresses. The objective of this study was to evaluate the effect of PGPB on plants submitted *in vitro* to water stress. *Urochloa ruziziensis* and the bacteria *Azospirillum brasilense*, *Herbaspirillum seropedicae* and *Leclercia adecarboxylata* were used. The experiment was entirely randomized with 8 treatments. Pre-germinated seeds were placed in test tubes containing 25ml of MS medium with Polyethylene glycol (-0.3MPa) and inoculated with 10^6 CFU.seed^{-1} of each of the strains. After 13 days of cultivation, the length, fresh and dry weight of shoot and root biomass, microbial counts, relative water content (RWC) and membrane stability index (MSI), roots morphology by staining with blue 1% methylene, stomatal morphology and enzymatic analysis were evaluated. Polymerase chain reaction (PCR) was performed to confirm the presence of the adhered bacteria. Data were submitted to ANOVA and compared by the Tukey test ($p>5\%$). As result, the strain *A. brasilense* had positive effect on the maintenance of the cellular metabolic activity observed by the maintenance of the RWC and MSI in plants under water restriction, and showed the capacity to promote the greatest increases in the aerial and root part and the stomatal area maintenance under both conditions. *L. adecarboxylata* strain was shown to be promising for use in forages due to increase shoot and root biomasses, and the MSI and RWC maintenance at high levels. The enzymatic activity was modified by the presence of the bacterium and even more by the stress imposition, and SOD (*A. brasilense* and *H. seropedicae*) and GST (*L. adecarboxylata*) could be used as response markers. *A. brasilense* can be considered the best strain for commercial purposes.

Keywords: Brachiaria*; water deficit; antioxidant enzymes.*

Poster VI.14

Effect of *Azospirillum brasilense* on nitrogen use efficiency in maize under limited nitrogen supply

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Development of varieties more efficient to nitrogen use in association with plant growth-promoting bacteria is considered the main strategy to reduce nitrogen fertilizer uses. The aim of this study was to identify efficient maize inbred lines (*Zea mays* L.) in the use of nitrogen and evaluates the effect of *Azospirillum brasilense* inoculation on nitrogen use efficiency. Twenty-six inbred lines and one hybrid (Dow® 2B587PW) were evaluated. The experiment was carried out in pots filled with sand and clay soil in proportion of 3:1 (v/v) under greenhouse conditions. The experimental design applied was completely random with four replicates and treatments disposed in factorial scheme (27 x 2), constituted by combination of 27 genotypes under low nitrogen and low nitrogen inoculated with *A. brasilense* strain Ab-V5. Twenty-eight days after planting, shoot nitrogen and nitrogen use efficiency was measured. Through analysis of variance was observed significant effect of genotype x inoculation interaction inferring differential response of genotypes to inoculation with *A. Brasilense*. The Scott and Knott test showed that 19 genotypes were more nitrogen use efficiency when inoculated with *A. brasilense* in comparison with noninoculated condition. The inbred line L15 showed lowest nitrogen use efficiency when inoculated with com *A. Brasilense*. Genotypes L7, L12 and 2B587PW showed highest nitrogen use efficiency when associated with *A. brasilense* Ab-V5.

Keywords: Zea mays *L.; abiotic stress; plant growth-promoting bacteria.*

Poster VI.15

Growth-promoting bacteria associated with nitrogen top-dressing in the agronomic performance of maize in the second harvest

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The use of inoculants, consisting of plant growthpromoting bacteria (PGPB), is one of the alternatives available to reduce the use of chemical fertilizers, and it can greatly increase the efficiency of these inputs in agricultural production systems. PGPB comprise a group of microorganisms that can stimulate plant growth and development by direct and indirect mechanisms. Regarding maize, several studies have demonstrated higher grain yield when the crop was inoculated with these microorganisms. Therefore, the purpose of the present study was to evaluate the effect of PGPB under different levels of nitrogen (N) top-dressing in the agronomic performance of maize. The experiments were conducted in Londrina and Maringá, Paraná state, using a randomized block design, in a factorial scheme, with six replications. The factors were N fertilizer (0, 40 e 80 kg.ha^{-1}) and inoculants (*Azospirillum brasilense* Abv-5 and Abv-6, *A. brasilense* Abv-5 and *A. brasilense* Abv-5 + *Rhizobium* sp.). All treatments received basal fertilization (300 kg.ha^{-1} of the formula 8-28-16). Concerning inoculation, significant effect was observed for the presence of inoculants in comparison to its absence, whereas no effect of inoculation on grain yield was detected when N top-dressing was applied, suggesting that these technologies compete with each other and are non-additive. Furthermore, in the absence of N topdressing, inoculation with *A. brasilense* Abv-5 and *A. brasilense* Abv5 + *Rhizobium* sp. resulted in higher grain yield in relation to the other treatments evaluated, with average increases of 9.98 and 16.44%, respectively.

Keywords: Azospirillum brasilense*;* Rhizobium *sp.; inoculant; grain yield.*

Poster VI.16

Genome-guided isolation of a novel derivative of the antifungal metabolite burkholdin from the PGPR *Burkholderia catarinensis*

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The increased availability of genome sequences has enabled the development of valuable tools for the prediction and identification of bacterial natural products directly or indirectly involved in plant growth. The new species *Burkholderia catarinensis* was isolated from Brazilian grassland soils and produces phytohormones, siderophores, and an unknown antifungal metabolite. In greenhouse assays, this bacterium was able to reduce the fungal disease caused by *Colletotrichum gloeosporioides* in apple plants. The aim of this work is to identify and purify natural products of *B. catarinensis* 89 through a genome-guided approach. The genome was sequenced using the MiSeq Illumina platform and assembled with the A5 pipeline. The genome had a total length of 8,198,254 bp comprised in 84 contigs and 66.5% of G+C content. A genome analysis using the antiSMASH tool revealed 16 clusters putatively related to secondary metabolism and antibiotics production. In particular, this tool identified a nonribosomal peptide synthetase (NRPS) cluster for production of the siderophore ornibactin, a hybrid NRPS-Type 1 polyketide synthase (PKS) cluster for the production of the antifungal glycolipopeptide burkholdinlike compound and a gene cluster encoding homoserine lactones (HSL), probably involved in the regulation of both metabolites. After *in silico* prediction, purification of the metabolites was performed by employing DADHPLC, and metabolites were analyzed using a QTRAP-LCMS spectrometer. We were able to purify the ornibactin derivatives D (*m/z*= 761) and F (*m/z*=789) and to detect the derivative B (*m/z*= 733). The quorum sensing metabolites C6-HSL and C8-HSL were observed in a LCMS/MS analysis. Moreover, a group of metabolites with masses of 1240, 1254, 1268, 1216, 1244, and 1272 Da could be detected in MALDI-ToF experiments, which are probably new derivatives of the antifungal burkholdin produced by *B. catarinensis*. Thus, this methodology was useful to identify metabolites probably related to plant growth.

Keywords: genome-guided isolation; secondary metabolites; antifungal compounds.

Financial Support: CNPq-Brazil; Deutscher Akademischer Austauschdienst (DAAD); UK-Brazil Nitrogen Fixation Centre (UBNFC).

Poster VI.17

Integrating high-throughput phenotyping to monitor nitrogen stress mitigation processes induced by diazotrophs in sorghum

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Diazotrophs are the microbes that are capable of reducing gaseous nitrogen (N) into ammonia, making N available for plant uptake. Diazotrophs mitigate N stress in plants by interacting intimately with plants, yet the mechanisms for how diazotrophs relieve N stress may go beyond just N fixation. This research aims to study the changes in plant traits and N accumulation induced by a diverse set of diazotrophs over time and ultimately establish a stable relationship between plants and diazotrophic microbes under N stress. *Sorghum bicolor* is used in this study because it is widely grown in developing countries and its increasing importance in biofuel production. To determine if diazotrophs enhance sorghum growth under low N conditions, plants were inoculated with synthetic communities (SynComs) consisting of at least nine different diazotrophs and grown in an automated highthroughput phenotyping facility. The diazotrophs were originally isolated from the roots and soil of field-grown sorghum. Acetylene reduction assays were used to verify isolates with N fixation abilities. A SynCom consisting of non-diazotrophic strains and a non-microbe treatment were included as the controls. Sorghum morphological and physiological traits were monitored daily using RGB (Red Green Blue) and hyperspectral cameras. After two months of growth, the DNA from roots and rhizosphere was isolated and prepared for 16S amplicon sequencing to determine which microbes from the different SynComs colonized sorghum roots and rhizosphere. The transcriptional responses in roots were studied to assess the changes in transcript level from the microbial and N treatments. Selected microbes from these SynComs will be genome sequenced to determine other mechanisms of growth promotion in addition to N fixation. This study will allow for a deeper understanding of both plant- and microbe-based mechanisms behind the N stress mitigation that result from diazotroph inoculation.

Keywords: stress; microbe interaction; nitrogen.

Poster VI.18

Bacteria that promote plant growth of *Panicum maximum* BRS Zuri

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The use of alternative strategies that promote improvements in animal production, especially management that associate sustainability with profitability should be given priority. Thus, the use of growth-promoting and nitrogen-fixing bacteria in forage grasses may be an important management alternative in the present day for improved pasture production and, consequently, animal production. Thus, the work aims to evaluate the productive characteristics and mass of roots, of *Panicum maximum* BRS Zuri inoculated in sowing with associative diazotrophic bacteria in Argissolo and nitrogen (N) fertilization. The experiment was carried out in pots, in a greenhouse, consisting of eleven treatments and five replicates in a randomized block design. The composition of the AB-V5 and AB-V6 strains of *Azospirillum brasilense*, *Pseudomonas fluorescens* CNPSo 2719 and the composition of AB-V6 and *Rhizobium tropici* CIAT 899, associated or not with nitrogen fertilization and co-inoculation of the plants after cutting, were tested. Bacteria were applied to the seed in the establishment. The evaluation was performed through three cuts every 4 weeks for the determination of forage mass production, morphological composition, number of tillers and dry mass of roots. All analyzes were submitted to analysis of variance at 5% probability and the Scott-Knott averages comparison test was applied when the mean difference was significant. For the accumulated dry mass (DM) of forage and roots, DM of leaf, DM stem plus sheath, DM tiller, number of tillers and chlorophyll content the highest averages were presented by treatments AB-V6 and *Rhizobium tropici*, *Pseudomonas fluorescens*, AB-V5 and AB-V6 of *Azospirillum brasilense* associated with N. The treatments submitted to co-inoculation of the aerial part after the cut did not present significant difference ($P > 0.05$) in relation to the other inoculated for all variables analyzed. We concluded that co-inoculation was not effective to express a greater response in terms of forage dry mass production, and growth-promoting bacteria did not show significant differences among them when associated with N. Diazotrophic bacteria applied without N application were significantly lower in all evaluated parameters.

Keywords: sorghum; microbe interaction; nitrogen.

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Poster VI.19

Azospirillum brasilense Vi22 and its potential as a sunflower growth promoter

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Sunflower (*Helianthus annuus* L.) is one of the most important oleaginous produced in the world. Sunflower seeds are rich in oil of high nutritional quality, and this plant also has great utility for beekeeping, ornamental use, animal feed, and biofuel production. In Brazil, sunflower crop has declined in recent years due to lack of economic incentive and control of disease, such as root rot induced by *Sclerotinia sclerotiorum*. In previous studies, bacteria from the rhizosphere of sunflower grown in fields of Rio Grande do Sul State (Brazil) were isolated and tested for various beneficial characteristics. The isolate designated Vi22 was identified as *Azospirillum brasilense* according to 16S rRNA gene, and presented positive results for nitrogen-fixing, production of siderophore and indolic compounds. Vi22 isolate was also able to degrade oxalic acid *in vitro*, precursor molecule and the main virulence factor of *S. sclerotiorum*. To evaluate the performance of Vi22 in a field experiment, different treatments were tested using seeds of sunflower cultivar BRS 321 in a completely randomized block design (four replicates), with plots measuring 4.2m×6.0m and spaced by rows of 1.0 m. High yields of grains (about 4,000 kg ha^{-1}) were observed at 120 days post sowing in the treatments with (i) full dose of nitrogen and without inoculation, and (ii) inoculant along with half dose of nitrogen fertilizer (Anova, Skott-Knott). Additional field experiments should be carried out in soils with and without the presence of *S. sclerotiorum*, to evaluate the biocontrol potential of strain Vi22. Further studies should also be performed to obtain the genomic sequence of strain Vi22, in order to identify genomic elements related to plant-microbe interaction, and also those that enable bacteria to survive in environmental biotic/abiotic stress.

Keywords: Azospirillum brasilense*; plant growth promoting bacteria;* Sclerotinia sclerotiorum*; sunflower.*

Financial Support: Capes, CNPq, Fapergs, Newton Fund Grant BB/N013476/1.

Poster VI.20

Survival evaluation of *Azospirillum brasilense* FP2 in wheat seeds treated with agrochemicals and cell protectors

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Inoculants containing *Azospirillum brasilense* have been used intensively in agriculture worldwide. This rhizobacterium is inoculated on seeds previously treated with fungicides and insecticides, however, there is a limited knowledge about the effect of seed dressing compounds on bacterial viability. Thus, the objective of this work was to monitor the viability of *A. brasilense* strain FP2 after inoculation on wheat seed (cv. TBIOToruk) treated with agrochemicals and cell protectors. Bacterial counts were evaluated 4, 12, 24, 36, and 48 hours after seed inoculation with seven different dressing treatments (two fungicides, two insecticides, two treatments with an association between fungicide and insecticide and a control without any chemical treatment). Besides, two cell protectors were added with two seed dressings from different companies. Two-hundred seeds from each treatment were washed in saline solution (0.85% NaCl pH 7.0) for 20 minutes at 170 rpm on an orbital shaker. The resultant suspensions were submitted to 10-fold serial dilutions down to 10^{-8}, followed by drop plating in RC medium. The experiment was carried out with biological and technical triplicates. The data were submitted to analysis of variance and means were compared by the Tukey test (0.05) using the MiniTab Express program (version 1.5.1). All chemicals tested caused a negative effect on the viability of *A. brasilense* FP2. No viable cells were detected 36 h after inoculation and, in general, treatments with fungicides were more harmful than treatments with insecticides. However, the addition of cell protectors extended cell viability throughout the 48 h of analysis. Based on these results, to guarantee viable bacteria cells on the seed at sowing, it is recommended to add protectors in the chemical seed dressing and if not, to sow right after inoculation or up to 36 hours.

Keywords: fungicides; insecticides; plant-bacteria interaction.

Financial Support: Brazilian Program of National Institutes of Science and Technology-INCT, Brazilian Research Council-CNPq, Fundação Araucária of the Paraná State and Petrobras.

Poster VI.21

Growth of micropropagated sugarcane seedlings inoculated with fifteen strains of diazotrophic bacteria

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Inoculation with diazotrophic bacteria promotes growth in sugarcane, resulting in productivity gains and reduction of inputs use, especially nitrogen fertilizers. The objective of this study was to evaluate the influence of diazotrophic bacteria on micropropagated sugarcane seedlings of IACSP91-1099 variety. The experiment was carried out in a greenhouse under a completely randomized design, with 5 replicates and 15 strains from 7 species as follows: *Paraburkholderia silvatlantica* (Ps), strain BR12166 (T1), *Paraburkholderia tropica* strain BR11366T (T2) ; *Gluconacetobacter diazotrophicus* (Gd), BR11280T (T3), Gd BR11200 (T4), Gd BR11281 (T5); *Herbaspirillum rubrisubalbicans*, BR11192 (T6), Hr BR11197 (T7), Hr BR11506 (T8), Hr BR11504 (T9); *H. Seropedicae*, BR11774 (T10), Hs BR11335 (T11); *Nitrospirillum amazonense*, BR11145 (T12), Na BR11140 (T13); *Bradyrhizobium sacchari*, BR10280 (T14); *Rhizobium* sp. BR10268 (T15), the mixture of 15 strains (T16) and the control without inoculation (T17). Micropropagated seedlings were inoculated for 30 min by the immersion of roots in diluted peat inoculant (1:100 v/v) or in the control with distilled water. Seedlings were planted in tubes containing Coconut Fiber substrate nº 11™. Two evaluations of fresh and dry shoot mass (FSM - DSM), fresh and dry mass of roots (FRM - DRM) and DSM/DRM ratio were made at 28 and 43 days after planting (DAP). Data were submitted to the Scott-Knott test ($P \leq 0.05$). Only at 43 DAP there was a significant difference between the treatments. For FSM and DSM, 13 inoculated treatments were superior to the control with average increase of 35% (except for T5, T13 and T14 for DSM). For DRM, treatments 3, 4, 6, 7, 8, 12 and 16 were higher by 29.8%, on average over the control. The DSM/DRM ratio showed no difference.

Keywords: Saccharum *sp.; endophytic bacteria; interaction plant-bacteria.*

Financial Support: Capes, CNPq, Faperj and Projeto Pluricana.

Poster VI.22

Growth evaluation of the sugarcane varieties RB966928 and RB975201 inoculated with a mixture of diazotrophic bacteria

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Apart from a raw material for sugar and ethanol, sugarcane biomass is also an important energy source. The use of pre-selected bacterial strains as inoculant can improve plant growth, but such response seems to be dependent on sugarcane variety. The objective of this study was to evaluate the response of sugarcane varieties to the inoculation with a mixture of 5 diazotrophic bacteria. A completely randomized experimental design was used in a triple factorial scheme (2x2x2) with 5 replications. The treatments were plant variety (RB966928 and RB975201); inoculation, or not, with diazotrophic bacteria (*Gluconacetobacter diazotrophicus* – PAL5^{T}, *Nitrospirillum amazonense* - Cbamc, *Herbaspirillum rubrisubalbicans* HCC103, *H. seropedicae* – HRC54 and *Paraburkholderia tropica* – Ppe8^{T}); and use, or not, of a short heat treatment (52ºC for 30 min). Two plant samplings were made at 25 and 45 days after planting (DAP). All sugarcane buds were treated with Comet™ fungicide (0.1%) for 3 min, followed by their inoculation by 30 min immersion in the inoculant solution. At 25 DAP, plants were evaluated after sprouting and initial growth on a sterile substrate. Then, plantlets were transferred to tubes containing Fertilized Coco Fiber™ substrate (No. 11) for further growth up to 45 DAP, when they were again evaluated. After analysis of variance, different means were separated using Tukey test ($P \leq 0.05$). Analysed variables were aerial (SDW) and root dry mass (RDW), SDW/RDW ratio, plant height (PH), total chlorophyll (TC) and root architecture by using WinRhizo Pro™ software. The heat treatment showed a negative effect on root growth reducing length and volume by 32 and 42%, respectively. Without heat treatment, inoculation increased plant growth only for the variety RB966928 at 45 DAP. Increased RDW and reduced SDW/RDW ratio were observed for the variety RB975201 when inoculated.

Keywords: Saccharum *sp.; plant growth promotion; bacteria.*

Financial Support: Faperj, CNPq and Newton Fund Grant BB/N013476/1.

Poster VI.23

Responses of inoculated soybean and wheat to metabolites from *Rhizobium tropici*

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The use of rhizobia and plant growth promoting rhizobacteria is recommended for the main crops of agronomic interest in Brazil, allowing total or partial reduction on the use of industrial nitrogen fertilizers. However, new strategies can be adopted to make the response to inoculants even more effective, with more consistent results in the field. The objective of this work was to evaluate the effect of the application of a *Rhizobium tropici* metabolic extract (ME) and its fractions, in combination with the inoculation of *Bradyrhizobium japonicum* and *B. diazoefficiens* in soybean (Desafio 8473RR) and *Azospirillum brasilense* in wheat (BRS 394 and BRS 264). After induction the ME was isolated from *R. tropici* supernatant grown in a defined medium and further purified/fractionated in MEF1 and MEF2. The experiment was carried out in greenhouse using Leonard jars. Five treatments were tested: control without inoculation, inoculated control and the others with the inoculum plus the molecules (ME, MEF1 and MEF2), for both crops. Thirty days after germination, shoot dry mass (SDM) and root dry mass (RDM) were measured. Number of nodules and nodules dry mass were also evaluated in soybean plants. No differences were observed among the treatments for wheat BRS 394. However, when inoculated and treated with ME and MEF1, cultivar BRS 264 presented SDM 40% and 35% higher than the control, and 20% and 13% superior than the treatment inoculated only with *A. Brasilense*, respectively. For soybean, compared to the treatment exclusively inoculated with *Bradyrhizobium* spp., RDM and SDM were, respectively, 11% and 5% higher when MEF2 was applied. The results indicate the biotechnological potential of rhizobia secondary metabolites and their fractions to improve the performance of commercial inoculants.

Keywords: biological nitrogen fixation; plant growth promotion; secondary metabolites.

Acknowledgments: Embrapa (02.13.08.003.00), INCT (MCTI / CNPq / Capes / FAPS) / (MPCPAgro 465133 / 2014-2), Science without Borders - CNPq (400205 / 2012-2) and MINECO AGL2016-77163-R.

Poster VI.24

Growth of sugarcane variety CTC9003 inoculated with five strains of diazotrophic bacteria

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Sugarcane is an important crop for Brazil as it is used for production of sugar and ethanol fuel and other cogeneration of energy from the bagasse, among other purposes. Solutions that improves crop productivity or N utilization can be achieved by the inoculation with selected diazotrophic bacteria. The objective of this work was to measure the initial growth of sugarcane variety CTC9003 inoculated with five diazotrophic bacteria strains. The treatments were: control, inoculation with *Gluconacetobacter diazotrophicus* - Gd (BR11281), *Nitrospirillum amazonense* – Na (BR11145), *Herbaspirillum seropedicae* – Hs (BR11335), *H. rubrisubalbicans* – Hr (BR11504) and *Paraburkholderia tropica* – Pt (BR11366) applied as a single inoculant or in a mixture of five. Individualized buds were thermally treated (52ºC for 30 min), immersed in fungicide (Comet™ 0.01%; 3 min) inoculated by immersion the buds in peat inoculant diluted in water (30 min; 1/50 w/v) and planted in tubes containing commercial substrate (Multiplant™). The design was in randomized blocks with 10 replicates. After 20 days, plants were transferred to pots containing 6 kg of soil and the plant growth was measured after 20 days. The variables measured were: dry and fresh mass of the shoot and root, dry mass from leaf +1, leaf area, besides root evaluations such as: length, protection area, surface area, diameter, volume, length/volume, number of tips, forks and crossings; using software WinRhizo Pro™. After variance analysis, the averages were submitted to Tukey's test ($P \leq 0.05$). Inoculation with Hr strain and the mixture improved shoot dry mass, leaf area. Hr had a positive effect between 19 and 25% on roots variables compared to the control and other inoculated treatments except to tips and forks.

Keywords: Herbaspirillum*; root; inoculant.*

Financial Support: Faperj, Capes, CNPq and Newton Fund Grant BB/N013476/1.

Poster VI.25

Agronomic evaluation of *Herbaspirillum seropedicae* as inoculant that improves maize yields in Brazil

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The current study aims to test the agronomic efficiency and contribution of *Herbaspirillum seropedicae* strain ZAE94 (BR 11417) to maize under field conditions. Eighteen field assays were conducted in four different locations during consecutive years using two hybrids and two varieties of maize in peat-based inoculant in a random block design with four repetitions. The inoculant containing the ZAE94 strain was applied in the absence of N and in the presence of 40 kg N ha^{-1}. The application of 40 and 80 kg N ha^{-1} was also tested without inoculation. Crop productivity and nitrogen accumulated in the grain were evaluated in addition to values of ^{15}N, BNF in the treatments without N. The N-fertilizer dose of 40 kg ha^{-1} of N associated with the bacterial inoculant produced similar crop yields to the treatment containing 80 kg of N ha^{-1} and increased the grain N content, especially in the off-season. The inoculated treatments have smaller values of ^{15}N than non-inoculated and this is most evident in the off-season. The BNF contributed about 30% of N accumulated in plants inoculated with ZAE94. This work revealed that the application of the *Herbaspirillum* inoculant increases the input of BNF to the plants and there is a better chance of yield responses to inoculation under low N fertilizer application at off-season.

Keywords: inoculation; maize; nitrogen; diazotrophs.

Financial Support: Faperj, Capes, CNPq.

Poster VI.26

Root development of micropropagated sugarcane inoculated with different species of diazotrophic bacteria

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Brazil is the world's largest producer of sugarcane for sugar and ethanol and productivity of this crop can be modified by the inoculation of diazotrophic bacteria selected for this purpose. The objective of this work was to evaluate the influence of 15 strains of bacteria on micropropagated plantlets of sugarcane variety IACSP91-1099. The experiment was carried out in a greenhouse in a randomized design with 5 replicates. Species (strains) tested were: *Paraburkholderia silvatlantica* (Ps/BR12166), *P. tropica* (Pt/BR11366T), *Gluconacetobacter diazotrophicus* (Gd/BR11280, BR11200 and BR11281T), *Herbaspirillum rubrisubalbicans* (Hr/BR11192, BR11197, BR11506 and BR11504), *H. seropedicae* (Hs/11774 and BR11335), *Nitrospirillum amazonense* (Na/BR11145 and BR11140), *Bradyrhizobium sacchari* (Bs/BR10280) and *Rhizobium* sp. (Rz/BR10268), a mixture of 15 strains and a noninoculated control treatment. Micropropagated plantlets were inoculated for 30 min by the immersion of the roots in diluted peat inoculant (1:100 w/v) and a control with distilled water. Plantlets were planted in tubes containing Coconut Fiber. Root morphology was analysed using the software WinRhizo Pro™ considering: length (L), volume (V), projected area (PA), surface area (SA), diameter (D), number of tips (T), forks (F) and crossings (C). Two evaluations were made at 28 and 43 days after planting (DAP). The data were submitted to the test of Scott-Knott ($P \leq 0.05$). Root length was enhanced by 200% when inoculated with Gd/BR11200 and Na/BR11145. V, PA, SA, T, F and C achieved the highest values with Gd-BR11280 and BR11200. *G. diazotrophicus* showed to be the species that changes root morphology in several variables compared to the others tested. All strains tested affected root architecture showing growth effects. Only two strains, Na/BR11140 and Bs/BR10280, reduced root diameter, which is a desirable effect.

Keywords: sugarcane; root architecture; bacteria.

Financial Support: Capes, CNPq, Faperj, Funarbe and Newton Fund Grant BB/N013476/1.

Poster VI.27

Morphological changes in maize roots induced by inoculation with *Azospirillum brasilense* and/or its metabolites

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Rhizobacteria of the genus *Azospirillum* can stimulate root growth through the production of phytohormones and signaling molecules, which can increase plant growth and yield. In Brazil, the use of *Azospirillum brasilense* strains (Ab-V5 and Ab-V6) in the production of maize (*Zea mays* L.) has resulted in higher yields. However, inoculation with *A. brasilense* can be hampered by the incompatibility of this bacterium with pesticides employed for seed treatment. Alternative inoculation methods, such as leaf spray, have been studied. Moreover, recent studies have shown that the application of *A. brasilense* metabolites (free of cells) has resulted in shoot growth promotion and other metabolic responses in maize. However, evaluations in the root system have not been performed yet. The objective of our study was to assess the effects of inoculation of *A. brasilense* and/or its metabolites, via seeds or leaf spray, on root morphology of maize. A greenhouse trial was performed using Leonard jars, with the following treatments (6 replicates): uninoculated control (C), seed inoculation with Ab-V5 and Ab-V6 (SI), seed application of metabolites of Ab-V5 and Ab-V6 (SM), leaf spray inoculation with Ab-V5 and Ab-V6 (LSI), and leaf spray application of metabolites of Ab-V5 and Ab-V6 (LSM). After 35 days of plant growth, the root parameters: dry mass, total and specific length, area, volume, diameter, tissue density and number of branches were assessed. Data were analyzed by Anova and Tukey's test at 5%. Specific length, diameter and tissue density of the roots did not differ among the treatments. SI, SM, LSI and LSM did not differ from each other for any root parameter. Root dry mass, total length, area, volume and number of branches were higher in SI, SM and LSI than in C. Root area was higher in LSM than in C. Both the inoculation with *A. brasilense* and the application of metabolites, via seeds or leaf spray, can increase the soil exploration potential of the maize root system.

Keywords: leaf spray; root length; root volume.

Financial Support: Capes, Fundação Araucária-STI and INCTMPCPAgro (CNPq 465133/2014-2). Rondina ABL is post-doc fellow from Capes. Nogueira MA and Hungria M are fellows from CNPq.

Poster VI.28

Agronomic efficiency of diazotrophic bacteria for nitrogen fixation in irrigated rice BRS Pampa

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The agronomic efficiency of new accessions of diazotrophic bacteria for nitrogen fixation with the cultivar BRS Pampa of irrigated rice constitutes stage official and the objective of this work. Six accessions of the Multifunctional Temperate Microorganisms Collection, consortium (C), evaluated in an experiment conducted under Planossolo Háplico, Pelotas, RS, 2015/16 crop. The experimental design was in randomized blocks, with four replications, and with direct sowing of rice seeds. The treatments included: (1) absence of nitrogen fertilizer and inoculation; (2) 100% N-mineral [120 kg ha^{-1} N: 15 kg N ha^{-1} at seeding + 10^5 kg N ha^{-1} in coverage (stages: 50% V3/V4 + 50% R1)]; (3) C1 (accesses CMM 174, CMM175, CMM179); (4) 75% N-mineral [15kg N ha^{-1} at seeding + 90 kg N ha^{-1} in coverage (stages: 50% V3/V4 + 50% R1)] + C1; (5) C2 (CMM 197, CMM 205, CMM 176); (6) 75% N-mineral [15 kg N ha^{-1} at seeding + 90 kg N ha^{-1} in coverage (stages: 50% V3/ V4 + 50% R1) + C2. Peat based inoculants prepared with concentration of 8.4 x10^8 (C1) and 7.4 x 10^8 (C2) CFU g of $inoculant^{-1}$. N foliar (NF) and relative index of chlorophyll (IRC), grain yield (GY), dry matter production, N accumulation in leaves and grains (NG), number and mass of panicles evaluated. The data submitted to analysis of variance, comparing the means by the Tukey test at 5% of significance. Significant differences observed for the effect of the consortia on GY, IRC and NG. The GY obtained with C2 + 75% N were equal to the control (13000 kg ha^{-1}), differing in 1000 kg ha^{-1} from C1 + 75% N (12000 kg ha^{-1}). Consortia + 75% N had sufficient NF (3.2%) and NG equal to the control. IRC from C2 + 75% N (71) was highlighted in the increase of chlorophyll in rice leaves compared to C1 + 75% N (65), which was similar to the control (50). There is efficiency of C2 to fix 15% N and to contribute to the increase of GY of BRS Pampa. These consortia will be validated under other soil and climatic conditions.

Keywords: inoculant; Oryza sativa *L.; profitability.*

Financial Support: Embrapa, Total Biotecnologia.

Poster VI.29

Polymeric CMC/starch mixtures as alternative carriers of bacterial formulations for sugarcane inoculation

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Production systems have sought sustainable alternatives that are ecologically viable associated with reduced production costs. Inoculation technology in legume crops is a well accepted practice that is gradually being extended to other crops with significant increases in production. Inoculation contributes to the promotion of biological nitrogen fixation (BNF) and other characteristics associated with plant growth promoting rhizobacteria (PGPR). The development of bacterial formulations is an essential part in ensuring product efficacy. Researches with biodegradable polymer carriers aim to guarantee the maintenance of cellular viability. Three carboxymethylcellulose (CMC)/starch-based formulations (protected under patent PI 0506338-8), prepared as mixtures 80/20, 60/40 and 40/60 and compatibilized with magnesium oxide (0.3% or 1% MgO), were tested as carrier for sugarcane inoculant strains *Gluconacetobacter diazotrophicus* (BR11281), *Herbaspirillum seropedicae* (BR11335), *H. rubrisubalbicans* (BR11504), *Nitrospirillum amazonense* (BR11145) and *Parabulkholderia tropica* (BR11366). Twenty grams of each carrier were packed in polypropylene bags, autocaved and inoculated with 2 ml of each bacterial suspension. For 150 days, cell viability in culture media specific for each strain was evaluated monthly. It was observed over the period that the best results were found in the 40/60 formulation with 0.3% MgO, except for strain BR11335, which showed similar response in the three ratios, and BR11504 where the best results were obtained in formulations of 40/60 and 60/40 with 0.3% MgO. The selection of the CMC and starch ratio and the concentration of MgO are critical factors that must be adjusted during the development of efficient bacterial formulations based on CMC/starch.

Keywords: biological nitrogen fixation (BNF); sustainability; growth promoting rhizobacteria.

Financial Support: Capes.

Poster VI.30

Genetic control and heterosis for maize-*Azospirillum brasilense* association

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Several studies have shown differential ability between maize genotypes to allow or to impede the *Azospirillum brasilense* colonization and receive benefits from this association. In this sense, the knowledge about genetic inheritance is critical for plant breeders select the best strategies aiming the development of genotypes more efficient in the association with these microorganisms. Hence, our objective was to study the genetic control and heterosis effect (or hybrid vigor) of maize-*A. brasilense* association. We evaluated 118 maize hybrids from nineteen inbred maize lines in an incomplete diallel mating design, during November 2016 and February 2017 in Piracicaba, São Paulo, Brazil. The experiments were conducted in greenhouse conditions and the plants were grown in pots containing soil without N fertilizer. It was used a randomized complete block with three replications for testing two treatments: non-inoculated seeds and inoculated seeds with *A. Brasilense*. At the V7 stage of development, root, and shoot dry weight, and a serial of root morphological traits were evaluated. The diallel analysis was performed using mixed model equations (REML/BLUP). The genotypes showed significant inoculation performances for five root traits. For them, both general and specific combining ability (GCA and SCA, respectively) were significant. However, the GCA in inoculated treatment presented a greater magnitude than in control. Considering that GCA is related to the portion of inheritable genetic variance, it suggests that the effects on the plant caused by *A. brasilense* are persistent over time and can support the evolutionary potential of species. On the other hand, heterosis was weakly influenced by the inoculation. Nevertheless, based on this result and the diallel analysis theory, it is possible to infer that epistasis could be relevant to the genetic control of this association. Finally, our findings suggest that the selection to improve maize-*Azospirillum* association may be based on the *per se* inbred lines performance, which could be usined to develop new superior hybrids.

Keywords: Plant growth-promoting rhizobacteria; biological nitrogen fixation; Diallel analysise.
Financial Support: FAPESP (Process: 2015/01188-9), CNPq and Capes.

Poster VI.31

Beans (*Phaseolus vulgaris* L.) development and productivity after furrow inoculation: greenhouse and field trials

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Bean (*Phaseolus vulgaris* L.) is a legume of great importance since it is considered the base of the Brazilian food. This Fabaceae is able to associate with microorganisms capable of promoting plant growth, which is one of the most viable alternative to increase agricultural productivity without damaging the environment or increasing costs. This work evaluated the effect of the furrow inoculation of microorganisms by biometric and production measuring parameters, besides productivity. Ten treatments were designed in a randomized block in triplicate, varying the dose of nitrogen (N) in coverage (0 or 150 kg/ ha) and the microorganism: commercial bean inoculant (*Rhizobium tropici* SEMIA4088); *Bacillus subtillis* QST713; *Trichoderma asperellum* SF04 or *Burkholderia* sp. 10N6, alone or in consortium. In greenhouse, the treatment with N in coverage, the inoculation with the *R. tropici* SEMIA4088 or *B. subtillis* QST713 increased dry mass, productivity, and the number of pods per plant. Furthermore, the inoculation with *R. tropici* SEMIA4088 + *T. asperellum* SF04 or *R. tropici* SEMIA4088 + *Burkholderia* sp. 10N6 increased the number of nodules. In the field, the treatment with N in coverage was the most effective for all parameters, but the inoculation with *R. tropici* SEMIA4088, *R. tropici* SEMIA4088 *T. asperellum* SF04 or *R. tropici* SEMIA4088 + *B. subtillis* QST713 + *T. asperellum* SF04 also increased the number of nodules and pods per plant, besides productivity. Although the results obtained in greenhouse were not totally congruent with the field ones, the inoculation with *R. tropici* SEMIA4088, *R. tropici* SEMIA4088 + *T. asperellum* SF04 and *B. subtillis* QST713 induced the best results in both conditions. These results amplify the number of efficient inoculants for the bean culture, as well as the method of inoculation.

Keywords: Beans cultivation; plant growth promoting microorganisms; inoculants.

Financial Support: Brazilian Program of National Institutes of Science and Technology (INCT-FBN), CNPq, Capes and Fundação Araucária of the Parana State.

Poster VI.32

Agronomic efficiency of soybean bioinductive inoculants and fertilizers in the lowlands of Rio Grande do Sul

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Soil cultivation in lowland lands faces limitations such as poor natural drainage of soils and poses challenges for the efficiency of inoculation. The objective of this work was to evaluate the agronomic efficiency of inoculants and bioinductive fertilizers (FBIO): three liquids and two peat moss composed of *Bradyrhizobium* strains [SEMIA 5019 + SEMIA 587 (*B. elkanii*) + SEMIA 5079 (*B. japonicum*) + SEMIA 5080 (*B. diazoefficiens*), co-inoculation with *Azospirillum brasilense* strains (Ab-V5 and Ab-V6), pre-inoculation (2 days) and two FBIO, in experiment conducted under Planossolo Háplico, Capão do Leão, RS, in the 2016/17. The experimental design was in a randomized complete block, with 12 treatments and four replicates, distributed in 4 x 5 m plots (3 m apart), one control without inoculation and one with 100% nitrogen fertilizer and ten with commercial products of the company Total Biotechnology. The cultivar BMX Potencia RR sown on 11/24/2016, in the conventional system. Flood irrigated plots at 18 days post-emergence (DPE) and, at 21 DPE, there was 50 mm of rainfall, causing soil to be flooded. Evaluated the parameters: dry mass of nodules (MSN), dry shoot mass (MSPA) and productivity. The results submitted to analysis of variance and the means compared by the Tukey test at 5% of significance. Significant differences observed for the effect on the MSN treatments, with the highest inoculants being the peat inoculum composed of micronized substrate (TESM) with *B. elkanii* and the liquid with a high concentration of bacteria *B. japonicum* (10^9 CFU mL^{-1}) value in N-mineral treatment. There was no significant influence on the accumulation of MSPA, which presented an average value of 2.8 g $plant^{-1}$. The yields in the inoculated treatments were higher than those obtained in the N-mineral treatment (6,430 kg ha^{-1}) with emphasis on treatments with TESM peat inoculants (8,860 kg ha^{-1}) and preinoculation with inoculant that allows higher survival of bacteria (7,854 kg ha^{-1}).

Keywords: Glycine max*; biological nitrogen fixation; hydromorphic soil.*
Financial Support: Embrapa, Total Biotecnologia.

Poster VI.33

Understanding the apoplast bacterial community from sugarcane inoculated with a consortium of five diazotrophic bacteria

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The apoplast has been described as a potential niche for the endophytic bacterial establishment. In sugarcane, this site occupies ~ 3% of the volume of stems and is a significant sugar storage location. The characteristics of the apoplast, such as: pH, amount of sugars, amino acids and temperature are near the optimum for bacterial growth, which suggests a greater number of bacterial cells in this niche. Studies have detected the presence of ~10^5 bacterial cells per mL of apoplast fluid including for example the N_2-fixing (diazotrophic) bacterium *Gluconacetobacter diazotrophicus*. To date, there is no broader evaluation of bacterial diversity in the apoplast fluid, neither the effect of bacterial inoculation on it, so the present study focused on the identification of the apoplast bacterial community of two sugarcane varieties: RB867515 (responsive to inoculation) and RB927515 (non-responsible). In addition, the effect of inoculation of a consortium with five N_2-fixing bacteria on this community was analyzed. A culture-independent approach and large-scale sequencing of 16S rRNA genes was used for the taxonomic analysis of the bacterial community. The results revealed the presence of nine abundant and common phyla for all samples. Some less represented phyla were suppressed in the inoculated treatment, and this effect was observed as the relative abundance of main OTUs increased in inoculated samples of variety RB867515. A possible apoplast fluid core microbiota has been observed in this work, represented by 321 OTUs common to all treatments and sugarcane varieties. These results bring valuable advances in understanding the bacterial community in the apoplast fluid of sugarcane and the effect of inoculant application.

Keywords: endophytic bacteria; next-generation sequencing; N_2-fixing bacteria.

Financial Support: Faperj, CNPq and Newton Fund Grant BB/ N013476/1.

Poster VI.34

Selection of *Bradyhizobium* strains that benefit different sugarcane growth variables

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Studies have shown that *Bradyrhizobium* spp., well known as leguminous microsymbionts, may play a role in biological nitrogen fixation (BNF) when associated with sugarcane. Recently, we obtained a collection of *Bradyrhizobium* spp. from sugarcane roots and rhizosphere. The present study aimed to select sugarcane growth promoting bacteria among 13 representative strains from this collection, selected based on BOX-PCR fingerprinting. The screening occurred in a greenhouse experiment with 1L pots with natural soil and sugarcane mini-setts (cv. RB867515) as planting material. The soil was fertilized with the equivalent of 40 kg N/ha and planted with sugarcane mini-setts inoculated with each of the selected strains individually. Non-inoculated setts were planted on the same soil or on soil with a higher N dosage (eq. 80 kg/ha) as negative (NI) and positive (+N) controls respectively. Thirty (30) days after inoculation (DAI), fresh and dry mass of roots and shoots, root volume, chlorophyll content, shoot length and diameter and leaf area were evaluated. Inoculation with AG48, a non-symbiotic *Bradyrhizobium* strain lacking *nod*-genes, but with *in vitro* nitrogenase activity, led to an increase of 16% in clorophyll content when compared to the NI control (t-test 10%). The cowpea-nodulating strain P7-6 stood out in increasing root volume (21%), fresh (37%) and dry mass (25%) when compared to the NI control (t-test 5%). Inoculation with the rhizosphere isolate RBR134b increased leaf area in 37% in relation to the NI control. These results suggest that these *Bradyrhizobium* strains promoted sugarcane growth via different mechanisms and were able to compete with the natural soil microbiota. The selected strains will be tested in field experiments to confirm their effectiveness as inoculants.

Keywords: non-legumes; endophytic rhizobia; plant growth promoting bacteria.

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Poster VI.35

Evaluations of sugarcane variety CTC9001 inoculated with five diazotrophic bacteria on nitrate assimilation

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Sugarcane has a great importance for the Brazilian economy. Nitrogen is an essential macronutrient for its growth and the assimilation of N-fertilizer can be improved by the inoculation of plant growth promoter diazotrophs. The objective of this work was to evaluate the nitrate content, nitrate reductase and free amino N in the sugarcane variety CTC9001 inoculated or not with a mixture of five strains of diazotrophic bacteria under hydroponic condition using 2 N-levels (0.3 and 3 mM NO_3-). The treatments were distributed in factorial arrangement 2x2x4, the first factor was dose of N (0.3 and 3.0 mM), inoculated or not with a mixture of five bacterial strain/species: *Gluconacetobacter diazotrophicus* – PAL5^T, *Nitrospirillum amazonense* CBAmC, *Herbaspirillum rubrisubalbicans* – HCC103, *H. seropedicae* – HRC54 and *Paraburkholderia tropica* – Ppe8^T. Inoculation was performed using peat inoculant applied diluted in water (1/50 w/v) by immersion (30 min) 20 days before the hydroponic transferring of the plantlets. The assay was evaluated at 48, 51 and 66 days after, with five replications. The studied parameters were: nitrate content and accumulation, free amino N, nitrate reductase and soluble sugars. The highest values of soluble sugars were observed at 66 days in 3.0 mM NO_3- in the inoculated treatment compared to the non-inoculated treatment. Highest N amino acids concentration was observed in high N level with no effect of the inoculation. Nitrate content and accumulation and nitrate reductase activity were not modified by the inoculation treatments in all 3 harvests.

Keywords: nitrite; FBN, bacteria, nitrogen.

Financial Support: Capes; EMBRAPA.

Poster VI.36

Survival and viability of *Azospirillum brasilense* Ab-V5 immobilized on injection-molded biodegradable plastic and effect on tomato seedlings

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The immobilization of Azospirilla in biodegradable materials has been proved to increase bacterial viability along time and its expected growth-promotion effects when used as inoculants. This study reports the immobilization of *Azospirillum* in a biodegradable material, developed to substitute the non-biodegradable plastic tubes traditionally used in nurseries to produce seedlings. The survival and viability of immobilized *Azospirillum brasilense* Ab-V5 along time was evaluated as well as the growth-promotion effect on tomato seedlings. Biodegradable plastic material was produced with poly (lactic acid), sugarcane bagasse, starch, glycerol, tannin and nutrients, which followed the extrusion and injectionmolding to produce conical tubes (12 cm x 3-1.5 cm). The tubes were immersed in a starch-gelatin solution containing Ab-V5 at 10^9 cells mL^{-1} for 1h, dried at 25ºC and immersed in a second starch solution of Ab-V5 at 10^9 cells mL^{-1} for 1h, dried and stored in cellophane bags. The survival and viability of the immobilized bacteria were evaluated at 1, 7, 14, 28 and 56 days of storage at room temperature, by determining the number of colony forming units (CFU). The tubes containing immobilized Ab-V5 cells were evaluated for its growth-promotion capability, by using 7-days stored bacterized tubes filled with organic substrate to growth tomato seedlings and comparing them to seedlings grown in unbacterized tubes. The CFU counts demonstrated that immobilized Ab-V5 cells sustained (1×10^9 CFU cm^2) for up to 7 days of storage, when decreased continuously, but remaining viable in high populations (up to 2.3×10^6 CFU cm^2). The development of tomato seedlings was evaluated after 35 days of planting, with plants grown in bacterized tubes showing increases in length (36% increase over control plants), number of leaves (100% increase), stem diameter (50%), root volume (137%), root dry mass (170%) and shoot dry mass (70%).

Keywords: biological input; inoculant; sustainability.

Financial Support: Capes, CNPq, Fundação Araucária, PROPPG/UEL.

Poster VI.37

Pseudomonas fluorescens Rt6M10 and *Azospirillum brasilense* AZ39 inoculation increases yield and fruit quality of two tomato varieties for industry

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Tomato (*Solanum lycopersicum*, L) is one of the most important and widely distributed horticultural vegetable crops in the world. During 2016 and 2017 season Argentina reached the historical record of 77 ton ha^{-1}. Mendoza is one of the major industry tomato producers, but the internal market demand of the country is still unsatisfied. Fertirrigation is necessary for the crop development, however, overuse of fertilizers cause environmental problems. The objective of this work was to evaluate the effect of tomato root inoculation with *P. fluorescens* Rt6M10 and *A. brasilense* Az39 on yield and fruit quality of two varieties of industrial tomato in a field trial. Uco 14 and Harris Moran 3861 were the selected tomato varieties. Seedlings with two fully expanded true leaves were bacterized and the treatments were: 1) Rt6M10, 2) Az39, 3) consortium (Rt6M10+Az39), 4) fertilized, 5) control (without bacteria). Forty days old plants were transplanted to the field and drip irrigated. Ninety days after transplant, steam diameter, photochemical efficiency of photosystem II and chlorophyll relative amount were evaluated. The yield, size and quality of the fruit were also evaluated at the time of harvest. Inoculation increased the stem diameter, the number and fresh weigth of fruits per plant; as well as the equatorial and polar fruit diameter in both verieties. Also, inoculation increased pH and decreased titulable acidity in both varieties. However, only the soluble solids of Uco 14 fruits were increased with the inoculations. The results of the work suggest that Rt6M10 or Az39 inoculation could be useful to reduce the use of chemical fertilizer, mantaining and even improving the industrial tomato yield and quality. This can lead to economical and enviromental benefits in a sustainable agriculture.

Keywords: PGPR; industrial tomato; root inoculation.
Financial Support: SECTYP UNCUYO, PIP CONICET, PICT (ANPCyT).

Poster VI.38

Initial Growth of Sugarcane Genotypes in Response the Biostimulant Containing Endophytic Diazotrophic Bacteria and Humic Acids

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The use of biostimulants in agriculture have been increasing worldwide. Combined application of beneficial bacteria and humic acid had shown remarkable effect for different crops increasing growth and development rates and productivity. However, genotypic variation response is poorly explored, being necessary for future plant breeding programs. The present study was designed to evaluate the growth performance of eight sugarcane genotypes treated with endophytic diazotrophic bacteria and humic acids. The genotypes tested were RB951541, RB058046, RB855536, RB739735, SP813250, RB92579, RB969017 and RB966928 that was obtained from in vitro propagation and were transplanted and inoculated at nursery stage. The experimental designed was completely randomized with 12 replications and two treatments: non-inoculated control and the biostimulant application composed by a bacteria mixture (*Herbaspirillum seropedicae* HRC54, *H. rubrisubalbicans* HCC103 and *Gluconacetobacter diazotrophicus* PAL5) suspended in humic acid extracted from sugarcane filter cake at 48 mg C.L^{-1}. The experiment was evaluated biweekly during 60 days after inoculation (DAI) measuring: height, diameter, number of leaves, SPAD, and fresh and dry mass of the aerial part and root (FMAP; FMR; DMAP; DMR) at 60 DAI. For the first evaluation (15 DAI), five genotypes had shown positive responses. However, differences were not statistically significant at harvest. For RB058046, an increase of 28.45% and 11.31% height and diameter were observed. Negative results in FMAP and DMR were obtained for only RB739735. For DMAP there was no significant difference and for the FMR there was gain of 94.01% in relation to the control in the variety SP813250. It is concluded that the genotypes behave differently in relation to the biostimulant response.

Keywords: biostimulation; growth promotion; plant seedlings.

Financial Support: PLURICANA, Faperj, UENF and Newton Fund Grant BB/N013476/1.

Poster VI.39

Phosphate solubilization, indole acetic acid synthesis and effect on soybean (*Glycine max*) biomass inoculated with *Pochonia* spp

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The fungus *Pochonia chlamydosporia* has been highlighting to have a potential as a biological control agent and promising as a plant growth promoter. The objective of this study was to evaluate the phosphate solubilization capacity and the production of indole acetic acid (IAA) *in vitro*, as well as select isolates in greenhouse with potential to promote plant growth of soybean crop. The inoculum sources of *P. chlamydosporia* were isolated from soils of different cultures and identified. For *in vitro* phosphate solubilization assay, half-strength potato dextrose broth added with K_2HPO_4 and $CaCl_2$ solution was used. For the IAA production assay, the isolates were transferred to Erlenmeyer flasks with culture medium in the absence (control) and presence of L-tryptophan. As for the phosphate solubilization test, all the isolates were able to solubilize phosphate, with emphasis on the UFT-P02 isolate, which presented a high phosphate solubilization index. For the production of indole acetic acid, the best results were higher in the presence of the L-tryptophan inducer, with UFT-P04 (12.3 μg mL^{-1}) being the best isolated. In greenhouse evaluations, dry matter of the aerial part, root dry matter, total dry matter and plant height at 45 and 56 days post emergence were evaluated, among the strains which showed the best relative efficiency in relation to the control were strains UFT-P05 and UFT - P03 with 77% and 75% increase respectively. The result of the present work proves the efficiency of the isolates of *P. chlamydosporia* as plant growth promoter.

Keywords: fungi; growth promoter; phytohormone.

Financial Support: Capes.

Poster VI.40

Growth promotion in legumes, *Glycine max* and *Vigna unguiculata*, by *Pochonia chlamydosporia* isolate in field

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Cowpea (*Vigna unguiculata*) is a very important food crop, especially on Brazilian northeast; however, it has low productivity due to the low level of technology. Soybean (*Glycine max*) cultivation has great socioeconomic and food importance, Brazilian and worldwide. In order to achieve better productivity, several studies related to the selection of microorganisms have been carried out. Thus, the present work aimed to evaluate the effect of the doses of the strain *Pochonia chlamydosporia* UFT-Pc10, through the agronomic efficiency to be used as inoculant for cowpea and soybean crop. In a field experiment, treatments consisted of five doses of *P. chlamydosporia* inoculant (0, 1, 2, 3 and 4 kg of *Pochonia* inoculant per 50 kg of seeds), being the cowpea cultivar Sempre Verde BRS Rouxinol and soybean cultivar M8615 IPRO. For the cowpea experiment, it was observed in both evaluations that the doses provided an increase in the dry mass of the cowpea plants when compared to the control. The best dose was 3 kg for each 50 kg of seed, which favored a higher grain yield (1083,7 kg ha^{-1}). For the experiment with soybean, the results showed that the different inoculant doses were efficient, and the best dose was also 3 kg for each 50 kg of seed, which favored a higher grain yield (4115,7 kg ha^{-1}). Thus, this study shows that *P. chlamydosporia* UFT-Pc10 has potential for use as a biofertilizer incrementing growth and consequently biomass in cowpea and soybean plants, besides favoring productivity.

Keywords: fungi; biomass; productivity.
Financial Support: Capes.

Poster VI.41

Effect of different strains and concentrations of *Bacillus* and *Azospirillum* on maize grown under hydroponic and field conditions

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Plant growth-promoting microorganisms can improve crop yield and quality, and are key for a sustainable agricultural. This work aimed to investigate the effect of different bacteria and their concentrations on maize growth under nutrient solution and field. Nine strains of bacteria, genera *Bacillus* and *Azospirillum*, belonging to the Collection of Multifunctional Microorganisms of Embrapa Maize and Sorghum were used for maize root inoculation in a concentration of 10^5-10^7 colonyforming units. mL^{-1}. Maize seeds were grown in a floating system with ½ Hoagland´s nutrient solution. After the acclimatization period, the maize roots were incubated with the bacteria for six hours and then transferred to the nutrient solution for ten more days. The maize roots were photographed and the root traits were analyzed and quantified with RootReader2D and WhinRhizo softwares. Six treatments increased total dry weight and three increased total root surface area. Regarding macro and micronutrients, two treatments presented a significant increase in the shoot and fourteen in the root. A principal component analysis (PCA) divided the microorganisms in three main groups, two that performed better than the control and one that kept the same plant growth as control. The capacity of *tryptophan-dependent production* of indole-3-acetic acid (*IAA*) of all strains were measured *in vitro*. All strains that produced IAA, presented a positive effect on plant growth by stimulating root elongation. In the field, maize plants inoculated with two strains (B116 and B119), that also presented an increase in total dry weight in hydroponics, had higher yield and grain P content in soil with TSP, and only B116 presented an increase when no P was added. Overall, our results showed that bacterial inoculation had positive effects on maize and promoted higher root surface area, plant growth and yield.

Keywords: biostimulant; root; Zea mays*; plant growth promoting bacteria.*

Poster VI.42

Maize growth under low and high N-levels and inoculated with four different strains of diazotrophic bacteria

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Maize (*Zea mays* L.) is one of the world's leading cereals. Its inoculation with diazotrophic bacteria can reduce the costs associated with nitrogen fertilization in its production. The objective of this work was to evaluate the inoculation of four diazotrophic bacteria on biometric parameters of maize plants grown with two N levels. The experimental design was in randomized blocks, with 4 replicates. The treatments were distributed in a factorial arrangement (2 x 5 x 4). The first factor was N level (0.3 or 3.0 mM), the second was inoculation (inoculated or not with *Herbaspirillum seropedicae* ZAE94 or *Azospirillum brasilense* Sp245 or FP2 or HM053), and the third was sampling date (four dates). Surface disinfected seeds of maize hybrid SHS5050 were pregerminated and inoculated three days after germination by immersion of the roots for 1 hour in the inoculant solution with 10^9 cells mL^{-1}; the control treatment consisted of the same process but in sterile saline solution. The seedlings were transferred to a hydroponic system set up in a greenhouse, which consisted of pots filled with Hoagland's nutrient solution modified by using the two N levels and maintained under intermittent aeration. At 19, 24, 30 and 38 days after inoculation plants were collected and evaluated for shoot dry mass (SDW), leaf area (LA), stalk diameter (SD), plant height (PH), root length (RL) and number of leaves (NL). Data were submitted to analysis of variance and means were compared by the LSD test ($P \leq 0.05$). There was interaction between N levels and inoculation treatments, and the treatments with the highest N level presented the highest means for the evaluated variables, exception made to RL. In addition, treatments with the highest N level inoculated with the strain SP245 performed superiorly to SDW, LA, SD and PH. Under low N level, strain inoculation did not result in significant differences for the tested variables.

Keywords: biological nitrogen fixation; endophytic bacteria; hydroponic system.
Financial Support: Faperj, Capes, CNPq and Newton Fund Grant BB/N013476/1.

Poster VI.43

Inoculation of wheat seeds with plant growth promoting bacteria

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Wheat crop is characterized as the most important winter crop of southern Brazil. Nitrogen (N) is the fundamental nutrient for its production. In order to reduce the use of chemical fertilizers, a promising alternative is the use of Plant Growth Promoting Bacteria (PGPB). PGPB provide benefits due to their ability to fix nitrogen, solubilize nutrients, produce phyto-hormones etc. In this sense, the objective of the work was to evaluate the development and productivity of the wheat crop after seed inoculation with PGPB. The experimental field trial was performed under a randomized block design with 10 treatments: three treatments varying nitrogen fertilization doses (0, 50 and 100 kg/ha of N) without inoculation and the additional seven treatments, without N and varying the inoculant (either the commercial bacterium *Azospirillum brasilense* Abv5/Abv6, or the new bacterial isolates, *A. brasilense* HM053, *Burkholderia* sp. 5V3, *Burkholderia* sp. 10N6, *Pseudomonas* sp. H2D and *Agrobacterium* sp. C7D) alone or in consortium. The evaluated variables were: plant emergence and tillering, grain yield, hectolitre weight (HW), a thousand seeds weight (TSW) and, leaves and grain N content. The inoculation of *Pseudomonas* sp. H2D significantly increased (Duncan $p<0.05$) plant emergence and tillering. All inoculants, except *Burkholderia* sp. 5V3 and the consortium, provided higher grain yield than the 100 kg/ha of N treatment. The highest productivity was reached by *A. brasilense* Abv5/Abv6 (36.4% increase) followed by *A. brasilense* HM053 (20.9% increase) and *Pseudomonas* sp. H2D (17.8% increase), when compared to the control without inoculation and N in coverage. For the variables HW, TSW and leaves and grain N content, there was no statistical difference. Our results confirm the potential of the commercial inoculant *A. brasilense* AbV5/AbV6 to increase wheat growth and put in a highlight position the strain HM053 of *A. brasilense* that is known due to its ability to excrete ammonium.

Keywords: diazotrophic bacteria; biological nitrogen fixation; promotion of plant growth.

Financial Support: Capes; Brazilian Program of National Institutes of Science and Technology (INCT-FBN); CNPq; Petrobras; Fundação Araucária.

Poster VI.44

Development of low-cost biofertilizers targeted to the urban, periurban and peripheral agriculture: a social technology

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Urban and periurban agriculture contributes to the food security in big cities. Such systems are intensive and faces the need for a regular input of nutrients, which is not always accomplished by farmers due to their economic restrictions. Here we report the application of a low-cost culture medium to growth the plant growth-promoting bacteria *Azospirillum brasilense* and its application by urban farmers working at Londrina, Paraná state, Brazil.The composition of the culture medium was the following: 5% soy textured protein (w/v), 10% glycerol (w/v) and 5% crystal sugar. Firstly, the soy protein was boiled together with glycerol for 1 h, following the addition of crystal sugar and the fast cooling of the broth to room temperature. A total of 10 L of this culture medium was used to growth *A. brasilense* Ab-V5 cells in a plastic bucket with lid (30 L), prepared to be used as fermenter by adding a plastic spigot, an airlock grommet and an ordinary aquarium air pump; all these items purchased from local stores and supermarkets. Previously to the use, the fermenter was sanitized with detergent and ethanol 70%. The broth was transferred from the pan to the fermenter, and hence inoculated with *A. brasilense* growth in DYG's medium to a population of 10^4 cells mL^{-1}. The bacterial growth stands for 48 hours, and during this period a silicone antifoam was used each 24 h (5 drops). The quality of the broth was evaluated by CFU count, presence of contaminants and the amounts of exopolysaccharides (EPS) and polyhydroxybutyrate (PHB). At the end of bacterial cultivation, the Ab-V5 populations were up to 1.11 x 10^8 CFU mL^{-1} presenting 12.3% of PHB (cellular dry mass); the EPS content was up to 3.73 g L^{-1} and contaminants were below 10^2 cells mL^{-1}. This technology was transferred to urban farmers, which applied the biofertilizer in lettuce in substitution to the fertilizer formulation 4-14-8 (N-P-K); the produced plants do not differed in weight or appearance.

Keywords: sustainability; inoculants; culture medium.

Financial Support: Capes, CNPq, Fundação Araucária, PROPPG/UEL.

Poster VI.45

Productive characteristics of grass-paiaguás inoculated with plant growth-promoting bacteria and N-fertilizer

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It was intended to evaluate the inoculation of growthpromoting bacteria and N-fertilizer in the productive characteristics of the grass-paiaguás. The experiment was developed in an entirely randomized design, in the greenhouse, with a trial period of 12 months. The treatments consisted of not inoculated; *A. brasilense* Ab-V5, *A. brasilense* Ab-V6; *P. fluorescens*-CCTB03; *P. fluorescens*- ET76; and *Pantoea ananatis*-AMG521. Each inoculated treatment was evaluated according to three levels of N (zero, 50 and 100 kg ha^{-1} of N). The test was conducted in a 6 x 3 factorial with 6 repetitions. The pots were filled with 15 dm^3 of sandy texture soil and all pots were corrected the phosphorus, potassium and applied 20 kg ha^{-1} of N. When the plants reached 35 cm, the cut was performed and left residue of 15 cm. At the end, the roots of each vase were washed individually and dried in the greenhouse for the determination of the dry matter. When the interaction effect of the factors was identified, the responses to each type of bacterium were compared within each dose of nitrogen fertilization. In all statistical analyses, the PROC GLM of the SAS statistical package at 5% was used. There was interaction between inoculation and N-fertilizer for mass production of total fodder, leaf ratio: thatch and mass production of grass roots-Paiaguás. For the production of total fodder mass (TFM) and N0, the bacteria CCTB03, ET76 and AMG521 promoted increment of 54, 41 and 46%, respectively, in relation to the non-inoculated treatment. In N100, the Ab-V5 promoted greater TFM among the strains. However, inoculation of the Ab-V6 at this same level of N-fertilizer provided less TFM. In the production of roots mass (RM) it was observed that the two strains of *P. fluorescens* and AMG521 promoted average increments 42% in the N0 and above 100% in the N50. However, at both levels of N-fertilizer. The other bacteria (Ab-V5 and Ab-V6) also provided increased mass of paiaguás grass roots. At the highest level of N-fertilizer (N100), the use of inoculation (independent of the strain) has doubled the RM in relation to the non-inoculated. The use of and *Pantoea* benefited the production of fodder and roots.

Keywords: Brachiaria*; endophytic microoganisms; rhizobacteria.*

Financial Support: Fundação Agrisus e CNPq.

Poster VI.46

Elongation of leaves and tillering of grass-araes inoculated with plant growth-promoting bacteria and fertilization nitrogen

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It was intended to evaluate the use of growth-promoting bacteria and N-fertilizer in the rate elongation of leaf and tillering the *U. brizantha* cv. Xaraes. The experiment was developed in an entirely randomized design, in the greenhouse, with a trial period of 12 months. The treatments consisted of not inoculated; *A. brasilense* Ab-V5, *A. brasilense* Ab-V6; *P. fluorescens*-CCTB03; *P. fluorescens*- ET76; and *Pantoea ananatis*-AMG521. Each inoculated treatment was evaluated according to three levels of N (zero, 50 and 100 kg ha^{-1} of N). The test was conducted in a 6 x 3 factorial with 6 repetitions. The pots were filled with 15 dm^3 of sandy texture soil and all pot were corrected the phosphorus, potassium and applied 20 kg ha^{-1} of N. Once a week the length of the leaf blade was measured, registering the occurrences of senescence, cut, death and expansion of the leaf blade of the demarcated tiller to determine the leaf elongation rate (LER) and every 28 days was performed the tiller count of each pot. When the interaction effect of the factors was identified, the responses to each type of bacterium were compared within each dose of nitrogen fertilization. In all statistical analyses, the PROC GLM of the SAS statistical package at 5% was used. For xaraés grass, it was observed interaction between nitrogen fertilization and the PGPB in the parameters of LER and basal tillers. In the absence of nitrogen fertilization, the bacteria ET76 and AMG521 promoted increments of 51% and 52%, respectively, in the LER in relation to non-inoculated treatment. The other strains, CCTB 03, Ab-V5 and Ab-V6 also resulted in stretching of the upper leaves to the non-inoculated treatment, at 48, 43 and 34%. When associated with the dose of 50 N, no strain promoted increase of LER in relation to the non-inoculated treatment, and there was a decrease for Ab-V6. Finally, using 100 N, CCTB 03 provided greater, statistically higher (16%) to the non-inoculated control receiving 100 N. The ET76 and AMG521 strains promoted greater leaf elongation and number of tillers.

Keywords: endophytic microoganisms; morphogenetic and structural characteristics; rhizobacteria.

Financial Support: Fundação Agrisus e CNPq.

Poster VI.47

Co-cultivation of plant growth promoting bacteria and their incidence on symbiotic performance in *Glicyne max*.

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The response to the increasing demand for food should be given in a sustainability context. So, the use of biofertilizers is a overcoming practice, since it reduces the use of chemical fertilizer, that negatively impact on the agro-ecosystems. The aim of this work was to develop a soybean biofertilizer formed by three bacterial genera, through a biotechnological development in order to culture the microorganisms in the same fermentative process. The interactions among the microorganisms was established through phenomenon of *quorum sensing* (QS) and were assessed by bioassays. The tests on plants were carried out in greenhouse, using sterile vermiculite as substrate. Data were analyzed using ANOVA, to determine the effect of each treatment. The significant minimum difference (DMS) test was used to determine differences between means. All the studied strains: *Bradyrhizobium japonicum* E109, *Azospirillum brasilense* Az39 and *Pseudomonas fluorescens* P3, produced signaling molecules of the acyl homoserine lactones (AHLs) type. In *B. japonicum*, the presence of heterologous AHLs induced changes in the homologous AHLs synthetized. On the other hand, the plant growth promoting capacity of the strains was analyzed, being *A. brasilense* the main acid indol acetic (AIA) producer. Its production was influenced by QS. In addition, *P. fluorescens* showed siderophores production and phosphorous solubilize capacity, independently of QS. Finally, the best symbiotic performance of *B. japonicum* was achieved by the use of triple co-cultured consortium. This system represents a biotechnological alternative, where the plant growth promoting bacterial synergically complement each other, achieving results that were not observed when they were produced and applied individually.

Keywords: biofertilizers; quorum sensing*; soya.*
Financial Support: Universidad Nacional de La Pampa.

Poster VI.48

Evaluation of the bacterial isolate *Burkholderia* sp. 10N6 in the promotion of plant growth in different cultures

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One of the biggest challenges is to cope with the growing demand for food and at the same time to reduce the environmental impact and costs of agricultural production. A highly promising alternative is to use Plant Growth Promoting Bacteria (PGPB) as biofertilizers. In the present work, a potential PGPB that showed to fix nitrogen, to solubilize mineral and to produce protease and indole acetic acid in vitro, was used as biofertilizer of different crops in greenhouse and field trials. *BURKHOLDERIA* sp. 10N6 was isolated from the rhizosphere of maize by the Microbial Molecular Biology Laboratory (LABMOM) group of the State University of Ponta Grossa (UEPG). Bean, soybean and wheat were evaluated varying the nitrogen (N) dose, including or not the inoculant, which was either the bacterium indicated for the specific crop or the *BURKHOLDERIA* sp. 10N6. Evaluations were carried out such as shoot and root dry mass, production components and productivity. Beans inoculated with 10N6 showed productivity higher (26.1% increase) than the ones that received the commercial inoculant (18.5% increase) in greenhouse trial. However, in the field trials, inoculation induced similar values of productivity: 9.9% and 12.4% increase, respectively. In wheat, the seeds inoculated with 10N6 showed higher levels of emergence in relation to the ones inoculated with the commercial bacterium in greenhouse trials, but the last provided more productivity (36% of increase) in the field. In soybean, even though there was a low number of nodules per plant after the inoculation of the commercial bacterium (1.5 nodules/plant), the productivity was higher (7.7% increase) than the ones inoculated with 10N6 (2.6% increase). The different responses found to the inoculation of PGPB may be due to several key factors, among them the interaction between the microorganism and the different cultivars, the environmental factors, besides the nutritional and water conditions in which the plant was found.

Keywords: plant growth promoting bacteria; biofertilizers; biotechnology.

Financial Support: CNPq, Fundação Araucária, Petrobrás.

Poster VI.49

Azospirillum brasilense seed inoculation (*Urochloa ruziziensis* and/or upland rice) on microbial activity, soil fertility and yield

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Microbiological indices have been sugested to observe the soil changes caused by management systems, such as microbial biomass-C and respiratory activity (release CO_2-C). An alternative to reduce the use of fertilizers, increasing the soil chemical quality and plant nutrients, is the biological fixation of atmospheric nitrogen (FBN). Among these nitrogen-fixing bacteria, *Azospirillum* spp. can be associated to grasses. The study aimed to evaluate the effect of seed *Azospirillum brasilense* inoculation (*Urochloa ruziziensis* as cover crop and/or upland rice), on the microbial activity, soil fertility and yield. The project carried out in an experimental area belonging to UNESP/ Ilha Solteira Campus, municipality of Selvíria-MS, in the Cerrado region. The soil is an Oxisol and the area is under no-till (NT) system for more than 10 years. In the past five years, different summer species (soybean, corn and / or Urochloa) and winter species (beans/wheat, corn and/or Urochloa and beans) were grown. In all these years, millet preceded the summer crops. To this summer season study, the experimental design was a randomized blocks in 2 x 2 factorial scheme with four replications, i.e., two seeds inoculation treatments (with or without) and two seed crops (Urochloa and/or rice plants). The *A. brasilense* Abv5 Abv6 strains were seed applied. The rice cv. BRS Esmeralda was saw in plots of 10 rows of 0.35m x10m. The N fertilization occurred 30 days after emergence, using ammonium sulfate, at 60 kg ha^{-1} of N. At the beginning of the rice filling grain period, soil samples were collected (0.0-0.1 to evaluate the fertility, microbial biomass carbon by the fumigation-extraction method, and the released CO_2-C. There was no influence of seeds inoculation with *A. brasilense* (Urochloa and/or rice or for the interactions, on the microbial activity or soil fertility. The possible influence of inoculated bacteria on improving soil fertility and microbial activity by nitrogen fixation, or for increasing of root development by phytohormones production (auxins) in the rice plants, as expected, were not observed. In this special case, no differences among treatments maybe occurred due to the high rates of OM, P, H+Al and SB in the soil, reducing the importance of seed inoculation for the plants development.

Keywords: Oryza sativa*; microbial biomass; diazotrophic bactéria; cerrado.*

Financial Support: Capes - Coordination and Improvement of Higher Education Personnel and CNPq - National Council of Scientific and Technological Development.

Poster VI.50

Response of the C4 energy *Pennisetum purpureum* variety PCEA to inoculation with diazotrophic bacteria

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The fast-growing C4 grass *Pennisetum purpureum*, commonly known as elephant grass, produce large amount of biomass and usually does not demand large quantities of N-fertilizer. Furthermore, the biological nitrogen fixation (BNF) quantification of several *Pennisetum* varieties showed that N derived from the biological process varied from 14 to 40%. Several nitrogen-fixing bacteria including *Herbaspirillum*, *Azospirillum*, *Gluconacetobacter* and *Burkholderia* have been isolated from elephant grass genotypes. Among them, we isolated the strain LP343 (*Gluconacetobacter diazotrophicus*) that increased up to 30% the biomass in the genotype Cameroon compared to no-inoculated plants. Here, we compared the inoculation effect of strains LP343 and Sp245 of *G. diazotrophicus* and *A. brasilense*, respectively, in the elephant grass variety PCEA (seed propagated- Embrapa Gado de Leite). Seeds were coated with peat containing the bacterial strains at concentration of 10^8 cell/ml and planted in plastic boxes (plantagil) filled with soil from the same field experimental area. The experiment was installed in randomized block design in factorial 22 scheme (two factors each at two levels) besides the N fertilized and uninoculated treatments. The 60 days old plants were transferred to field plots fertilized according to the chemical soil analysis. The harvests were realized at 4 and 8 months after transplanting to the field. The results showed an increase in the fresh and dried biomass with inoculated of strains LP343 and Sp245 superior to the N fertilizer treatment in the first cut while in the second cut the biomass accumulation was smaller than the N fertilization although in both conditions there was no statistical significant differences among treatments. There was a tendency of better response of the plant to leaf spray inoculation with the strain LP343 in contrast to strain SP245 that promoted more biomass accumulation when inoculated on the seeds. Analysis of the N content of leaf +3 (first harvest) showed a higher N accumulated in the leaves in comparison to other treatments. Additional evaluation including the biomass and nutrient contend along the plant cycle should provide a clear picture about the inoculation effect on elephant grass variety that propagate by seeds.

Keywords: endophytic bacteria; leaf spray; biofertilizer.

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Poster VI.51

Yield of maize grown at different technological levels submitted to inoculation with *Azospirillum*

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In conditions of high technological level (high levels of soil organic matter, consolidated no-tillage system and high productive potential of the genotype), it is not recommended to reduce the side dressing nitrogen fertilization, but positive responses to inoculation with *Azospirillum* were observed. The objective of this work was to evaluate the yield of maize grown at different technological levels submitted to inoculation with *Azospirillum*. The mapping of the area was carried out using the Farm Works™ Mapping software. The mesh used was 142 m x 142 m, totaling 1 point per 2.02 ha with an average of 20 sub samples per point. Soil was collected at depth of 0 to 10 cm, since the experimental area was characterized as no-tillage system, with crop succession: intercropping (grass/legume x maize). The treatments of the research were: T1: Low organic matter (OM), without *Azospirillum* and expected yield grain (EYG) of 12 t ha^{-1}; T2: Low OM, with *Azospirillum* and EYG of 12 t ha^{-1}; T3: Mean OM, without *Azospirillum* and EYG of 12 t ha^{-1}; T4: Mean OM, with *Azospirillum* and EYG of 12 t ha^{-1}; T5: High OM, without *Azospirillum* and EYG of 12 t ha^{-1} and T6: High OM, with *Azospirillum* and EYG of 12 t ha^{-1}, with four replicates. The seeded hibrid was AS1666®. The dependent variable analyzed was yield grain. The data collected were compared by the Tukey test ($p \leq 0.05$). The comparative analysis of the treatments revealed a significant difference of the T6 treatment (higher technological level) in relation to the others, confirming the hypothesis that the higher the technological level increases the efficiency of the use of the nitrogen fertilization in coverage, resulting in larger income. Based on the above, it can be concluded that the inoculation of maize with *Azospirillum* cultivated at a high technological level increases the yield grain significantly.

Keywords: agrotechnology; precision agriculture; efficiency of nitrogen.

Poster VI.52

Growth and contribution of BNF to different species of *Brachiaria* inoculated with *Azospirillum brasilense*

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Most of the planted pastures in Brazil belongs to the genus *Brachiaria* (Trin.) Griseb. (syn. *Urochloa* P. Beauv.), which is explained by its good adaptation and yield in the relatively poor soils commonly found in tropical environments. The already proven plant growth promoting effect of some diazotrophic bacteria led to the formulation of the hypothesis that the initial *Brachiaria* establishment after seeding and growth can be improved by inoculating seeds with plant growth promoting diazotrophic bacteria. Based on this, the current study aimed at understanding the inoculation response of *Azospirillum brasilense* strain Sp245 in 14 genotypes of *Brachiaria*: *B. brizantha* (cvs. Marandú, Xaraés, Paiaguás, Piatã and B140); *B. decumbens* cv. Basilisk; *B. humidicola* (cvs. Comun, Tupi, H47 and Llanero); *B. ruziziensis* and the hybrids (cvs. Ipyporã, Mulato and Convert HD364) compared to the respective non-inoculated control. Two experiments using soil and one using sterile substrate were carried out. Plant dry weight, nitrogen accumulation and biological nitrogen fixation (BNF) contribution by using the ^{15}N natural abundance technique were measured. Inoculation brought about positive results in five of the 14 genotypes tested. The BNF contribution was variable, but an average of 10% of N accumulated in the shoots was observed for the responsive genotypes. Root architecture was also modified by inoculation, which was positively related with dry weight and N content of Marandú, Llanero, Ruziziensis and Convert HD364 genotypes, but negatively in Xaraés.

Keywords: pasture; diazotrophs; growth promotion; nitrogen.

Financial Support: Capes, CNPq-INCT.

Poster VI.53

Normalized difference vegetation index of maize inoculated with *Azospirillum* at different levels of technology

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Studies carried out in the western region of Santa Catarina state indicate that inoculation in maize with *Azospirillum* increases the efficiency of the side dressing nitrogen fertilization. This efficiency depends mainly on the technological level available (soil organic matter content, crop system and genotype yield potential). The objective of this work was to evaluate the normalized difference vegetation index (NDVI) of maize grown at different technological levels submitted to inoculation with *Azospirillum*. The experimental area was conducted with Precision Agriculture (AP). The mapping of the area was carried out using the Farm Works Mapping software. The mesh used was a square of 142m x 142m totaling 1 point per 2.02 ha with an average of 20 sub samples per point. Soil was collected at depth of 0 to 10 cm, since the experimental area was characterized as a system of no-tillage system, with crop succession: intercropping (grass / legume x corn). The treatments of the research were: T1: Low organic matter (OM), without *Azospirillum* and expected yield grain (EYG) of 12 t ha^{-1}; T2: Low OM, with *Azospirillum* and EYG of 12 t ha^{-1}; T3: Mean OM, without *Azospirillum* and EYG of 12 t ha^{-1}; T4: Mean OM, with *Azospirillum* and EYG of 12 t ha^{-1}; T5: High OM,without *Azospirillum* and EYG of 12 t ha^{-1} and T6: High OM, with *Azospirillum* and EYG of 12 t ha^{-1}, with four replicates. The seeded hybrid was AS1666®. The dependent variable analyzed was the NDVI (TRIMBLE, 2018). The data collected were compared by the Tukey test ($p \leq 0.05$). The comparative analysis of the treatments revealed a significant difference of the T6 treatment (higher technological level) in relation to the others, resulting in higher values of NDVI in the VT (panning). Thus, it can be concluded that the inoculation of maize with *Azospirillum* cultivated at high technological level significantly NDVI increases.

Keywords: NDVI; precision agriculture; agrotechnology.

Poster VI.54

Eucalyptus monoculture reduces the bacterial diversity of grasslands from different soil types in Brazil's Pampa biome

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The Pampa biome is predominantly composed by grasslands in a transition zone between tropical and temperate climates of the Rio Grande do Sul State (Brazil). Most of Pampa's soil has an extremely sandy texture, although composed by a wide variety of types originated from complex geological formations. The monocultures expansion has challenged the sustainability of this biome, which became one of the most important Brazilian regions for flora and fauna preservation. The microbial diversity can be an appropriate biological indicator for soil quality because microbes support the maintenance of biogeochemical cycles, and variations in their taxonomic abundance may signalize important changes in the environment. According to this, the impact of *Eucalyptus* on the soil of the Pampa biome was evaluated in this work, in order to explore the relations between microbial compositions and environmental disturbances. Bulk soil samples were collected and evaluated as to biological and chemical properties from *Eucalyptus* (E) and native grasslands (G) areas. Three different samples were obtained in triplicate for each type of vegetation (E or G), totalizing eighteen libraries. The microbial taxonomic composition was achieved by pair-end sequencing of the V4 region from 16S rRNA gene using the Illumina platform. Bacterial dominance indexes were significantly increased in *Eucalyptus* fields regardless of soil type, especially for the orders Bacillales and Rhizobiales, microbial groups commonly found in association with plant roots. In addition to changes in biodiversity indexes, many chemical characteristics were negatively altered in *Eucalyptus* soils, such as decreased of pH, ammonia and phosphorus content. Ultimately, our results provide interesting estimate regarding the soil microbial diversity of native grasslands and *Eucalyptus* areas in Pampa biome and can help us to better understand the afforestation challenges and impact on threatened grasslands in South America.

Keywords: Pampa biome; Eucalyptus *monoculture; biological indicators.*

Financial Support: Capes; CNPq.

Poster VI.55

Yeasts as plant growth promoter for beans (*Phaseolus vulgaris* L.)

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Yeasts are single-celled fungi that can be found naturally in the rhizosphere and phylosphere of plants. Some species have the ability to participate in several important biotechnological processes such as: biological control of fungi for their ability to produce killer toxins, production of siderophores and other compounds known as plant growth promoters. This work aimed to evaluate the action of promoting plant growth by yeasts from natural environments. *In vitro* germination tests were carried out on bean seeds. The experiment was conducted in a completely randomized design, with 6 replicates, 2 treatments (inoculated and not inoculated) and 15 yeasts were evaluated. Yeasts were grown in BDA liquid medium at 120 rpm, 28ºC for 3 days. The bean seeds were distributed in Petri dishes containing Hoagland nutrient solution. For the inoculated treatment, the seeds were immersed in liquid medium of BDA with yeasts for a period of 1h and for the control not inoculated the seeds were immersed in liquid culture medium without yeasts. Growth was evaluated for 7 days after inoculation. The evaluation of root and shoot length showed that 4 yeasts (*Occultifur Braziliensis*, *Yamadazyma riverae*, *Hyphopichia homilentoma*, *Hyphopichia homilentoma*) provided higher root growth by 23% and 18% in shoot and 25% in total fresh biomass compared with the control, indicating that these yeasts perform mechanisms that provides greater plant growth. This is relevant considering that further development in the root system provides better conditions for absorption of nutrients and other plant compounds to develop their growth cycle. The next steps will be to study the mechanisms, phytohormones or other substance make possible this difference in the increase of these variables.

Keywords: microorganisms; rhizosphere; biological control.

Financial Support: CNPq, Embrapa.

Poster VI.56

Potential use of Dark Septate to promote the plant growth

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Dark septate endophytic (DSE) fungi are facultative biotrophs that associate with hundreds of plant species, contributing to their growth. However, several ecological functions of DSE fungi need further understanding. With the aim to study the potential of DSE to promote the growth of plants, several trials were performed under laboratory and glasshouse conditions with different fungi inoculated in tomato and rice. In these experiments the ability was evaluated of fungi to contribute to plant growth, nutrient uptake and nutrient recovery efficiency, using organic (finely ground plant material [*CANAVALIA ENSIFORMIS* (L.)]) or inorganic (ammonium sulfate) N source, being both enriched with ^{15}N. We observed significant plant growth effects after inoculation with DSE, increasing plant dry matter in more than 30%. Additionally, it was observed that inoculated rice plants accumulated more N, P, K, Ca, Mg, Fe, Mn, and Zn and demonstrated increased height, leaf number and tillering. In the inoculated treatment, a significantly higher recovery efficiency of ^{15}N, P, and K was shown, but more benefit was obtained in tomato when the N-source was organic. The results indicated a biotechnological potential of DSE as inoculant for different crops.

Keywords: fungi; rice; tomato.

Financial Support: CNPq, Capes, Faperj, Embrapa.

Poster VI.57

Effect of seed inoculation on Paiaguás grass tolerance to water deficit stress

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Inoculation of plant growth promoting bacteria (PGPB) in grasses is an innovative technology able to mitigate the effect of drought on pastures constantly submitted to this stressor agent. This study aimed to evaluate the effect of seed inoculation on the photosynthetic pigments of Paiaguás grass submitted to water deficit stress. The experiment was conducted in a greenhouse, in 9 dm3 pots containing sandy soil Caiuá formation, for an experimental period of seven months. The study was conduted as a factorial experiment base on randomized block design, with four replications: non-inoculated, (1) *Azospirillum brasilense* Ab-V5, (2) *Pseudomonas fluorescens* total and (3) *Pantoea ananatis* AMG521 associated with four levels of soil water availability (20, 40, 60 and 80%). The inoculants were prepared in the concentration of 10^8 cells mL^{-1} the substrate and applied at a rate of 15 $mL\ kg^{-1}$ of seeds. The cuts were made when the plants reached between 35 to 40 cm, leaving a residue of 15 cm. Before each cut, the SPAD was read on the last fully expanded leaf of three tillers per pot using a non-destructive method, trough the chlorofiLOG® Chlorophyllometer. There was no interaction between inoculation and water stress. It was found that water stress not significantly influenced photosynthetic indices ($P>0.05$), except for chlorophyll b ($p<0.01$). Seed inoculation did not result in higher amounts of Chlorophyll a (Chl a), Chlorophyll b (Chl b) and total chlorophyll (Chl t), compared to the control. However, it was revealed that inoculation of bacteria 3 resulted in lower photosynthetic indices. There was a linear relationship between Chl b and water stress ($y=0.0191x+9.5R^2=0.94$) so the increase in water stress level resulted in a decrease in Chl b content. Leaf Chlorophyll content is a key parameter in determining photosynthesis. Our results suggest that the use of PGPB did not confer better photosynthetic indices to Paiaguás grass submitted to water deficit stress.

Keywords: diazotrophic bactéria; drought stress; forage.
Financial Support: CNPq, Agrisus, Sementes Facholi.

Poster VI.58

Growth promotion of strawberry plants inoculated with microorganisms

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The cultivation of strawberry plants is generally carried out intensively with high consumption of agrochemicals, which can cause several impacts on the environment. As an alternative to this farming system, the use of plant growth promoting bacteria can be incorporated to the organic production system, reducing the impact on the environment. The objective of this study was to evaluate the influence of different plant growth promoting bacteria on strawberry plants growth. The experiment was conducted in a completely randomised design, with a 8 × 2 factorial scheme, involving eight combinations of bacteria (*Azospirillum brasilense* Ab-V5, *Burkholderia cepacia* CCMA 0056, and *Enterobacter cloacae* CCMA 1285) inoculated in pairs, in threes and alone and two levels of nitrogen fertilizer application (0 and 50%), totaling 16 treatments. Six aliquots of 1-mL of the bacterial suspensions (10^9 CFU mL^{-1}) were inoculated into the roots of the strawberry plants according to each treatment. After 75 days of growing in a greenhouse, root and shoot length (cm), root and shoot dry weight (g) and number of leaves were recorded. Analysis of variance were performed and the mean values were compared using the Scott-Knott test at 5% probability level. Strawberry plants significantly responded to the inoculation of the strains *Azospirillum brasilense*, *Burkholderia cepacia* and *Enterobacter cloacae*, and best growth parameters were observed when the plants were inoculated with more than one strain. The combination of the three bacteria together (*Azospirillum brasilense* + *Burkholderia cepacia* + *Enterobacter cloacae*) plus 50% nitrogen fertilizer stood out from the other treatments. Therefore, it is possible to infer that co-inoculation of the strains with the use of nitrogen fertilizer is better for strawberry plants, resulting in better plant growth responses.

Keywords: PGPB; microorganisms; Azospirillum brasilense*.*

Financial Support: FAPEMIG, Capes and CNPq.

Poster VI.59

Plant growth promoting microorganisms effects on banana plant *in vitro* growth

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Banana plant (*Musa* sp.) is the second most cultivated fruit tree in the world and the mainly cultivated in Brazil among the tropical fruits. Tissue culture is the main propagation method used for this crop. In this sense, the optimization of this technique can bring great economic benefits to the farmer. The inoculation of growth promoting bacteria has already shown benefits in different crops such as increased biomass production, greater resistance to stress conditions and changes in physiological properties. The aim of this study was to evaluate the influence of plant growth promoting microorganisms on the growth of banana plant during the *in vitro* culture. Explants were inoculated in MS medium with three strains of microorganisms [*Azospirillum braziliense* (Ab V5), *Azospirillum* sp. (CCMA 1291 – positive control) and *Burkholderia* sp. (CCMA 0056)] at $1x10^8$ CFU mL^{-1}, the absence of the microorganism was the negative control. After 30 days in a growth room at 27ºC with luminous intensity of 36 μMol $m^{-2}s^{-1}$ and photoperiod of 16 hours, the growth traits were assessed (length, number and fresh weight of roots, number and area of the leaves, length and fresh weight of the shoots). The experimental design was completely randomized with 4 treatments. There was no difference on leaf number and shoot length between the treatments; CCMA 0056 caused the death of the explants; the number and fresh weight of roots and the shoot length were similar to the negative control. We can conclude that there is no positive effect on banana plant growth cultivated *in vitro* in response to the inoculation with microorganism.

Keywords: Musa *sp.; PGPB; plant tissue culture.*

Financial Support: FAPEMIG, CNPq and Capes.

Poster VI.60

Growth stimulation of perennial ryegrass by plant growth promoting bacteria under limited nutritional conditions

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Ryegrass has a nutritional profile suitable for animal feed in high tropical regions; however, a limiting factor for its establishment is the high demand for fertilization. The objective of our research was to study the influence of the inoculation of 12 bacteria isolated from silvopastoral systems of Nariño-Colombia on the growth of perennial ryegrass (*Lolium perenne*) var. One 50. Additionally, the strains were identified molecularly (16S rRNA) and characterized by their plant growth promotion (PGP) traits. The results showed that strains A2 belong to Stenotrophomonas sp., N_2 to *Rhizobium* sp., P3 to *Pantoea* sp., and A1, A6, N1, N3, A7, N9, N8, N5 and N7 to *Pseudomonas* sp. The microorganisms exhibited qualitatively diverse activities of PGP as fixation of nitrogen, solubilization of phosphorus, potassium and zinc, production of indolic compounds and synthesis of siderophores. In *in vitro* bioassays implementing Hoagland solution without nitrogen and insoluble phosphorus (phosphate rock), the ryegrass shoot length was measure 24 days after planting. We observed that the bacterial inoculation significantly promotes the total length of the ryegrass under limited nutritional conditions (absence of N and insoluble P). An increase between 29 and 52% was observed when the plants were inoculated with respect to the treatment without inoculation. Interestingly, the inoculated grass grew on average 13% more than the treatment with complete Hoagland solution. The strains A2, A5, P1, A6 and N1 were selected for future studies in greenhouse and field conditions because they have the greatest beneficial vegetal influence to reduce the mineral fertilization dose.

Keywords: phosphate-solubilizing bacteria; diazotrophic bacteria; indolic compounds.

Financial Support: Corporación Colombiana de Investigación Agropecuaria - AGROSAVIA.

Poster VI.61

Evaluation of growth promoting activity of two strains identified as *Bacillus amyloliquefaciens* in tomato culture

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Plant growth promoting rhizobacteria (PGPR) are currently viewed as a sustainable alternative to achieve increases in crop production. The term PGPR includes a group of free living bacteria, capable of colonizing the plant´s rhizosphere, resulting in positive effects on their growth and development. The mechanisms through which they interact positively with plants are diverse, and they include the production of growth regulators such as abscisic acid (ABA), gibberellins (GAs), indole acetic acid (IAA); nitrogen fixation, and solubilisation of nutrients, among others. The objective of this work was to evaluate the growth promotion capacity of two strains of *Bacillus amyloliquefaciens*, in tomato plants. These strains, designed in this work as*Ba*1 and *Ba*2, were selected according to biochemical characteristics that would indicate a possible positive effect on plants. For the purposes of this work, *Moneymaker* tomato cultivar was selected, and an essay was carried out under greenhouse conditions. Tomato seeds were previously subjected to a bio-priming process, inoculating them with *Ba*1 and *Ba*2 for 24 h at 28 ± ºC in BHI (Brain Heart Infusion) culture medium. As a control, seeds were soaked in equal medium and conditions but without bacteria. After 60 days from sowing, plantlets were subjected to a second inoculation. At 80 days post-emergence, moment in which the crop was fructifying; length of roots, length of the aerial part and dry weight were evaluated. Strain *Ba*2 showed a clear plant growth promoting activity, manifesting statisticaly significant differences between the treatments in the variables dry weight and length of the aerial part.

Keywords: growth promotion; tomato; PGPR.

Financial Support: UE “IRNASUS” UCC – CONICET. INTA.

Poster VI.62

Genomic studies of microorganisms in phytoremediation of contaminated soils with hydrocarbons under *Populus* sp. culture

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The contamination of soils by hydrocarbons has a marked effect on the modification of their properties and the development of microorganisms. Furthermore, the persistence of these components in soils is prolonged over time. In recent years, the use of biological methods in the remediation of contaminated sites has increased. The implementation of the phytoremediation technique implies the use of plants and their associated microorganisms at the rhizosphere level, capable of tolerating and detoxifying persistent contaminants in the soil.Certain groups of bacteria under certain conditions are capable of intervening in the degradation of hydrocarbons and have the metabolic capacity to convert these agents into CO_2 and H2O. Microorganisms that use hydrocarbons as a carbon source must have enzymes systems such as the oxygenases group, capable to produce metabolites that lead to hydrocarbons degradation. *Gordonia* sp. possesses the ability to tolerate and remove compounds containing hydrocarbons, aromatic, and aliphatic. The objective of this work was to carry out a genomic analysis study of a bacterial strain of the genus Gordonia sp, isolated and identified from contaminated soil with diesel fuel. The identification was made by a high performance sequencing system to obtain the complete genome. *Gordonia* sp was associated with *Populus deltoides*, in agricultural land. The concentration of the contaminant was 10,000 mg. kg^{-1} ss. On the basis of the results of the complete obtained sequence was inferred that the bacteria has the capacity to produce enzymes such as aldehyde and alcohol dehydrogenases, monooxygenases and hydroxylases; all able to metabolize different hydrocarbons using different metabolic pathways. Among those identified, Alcohol dehydrogenase (EC 1.1.1.1), with an identity range between 69 to 91%; Salicilate hydroxylase (EC 1.14.13.1), with a range between 66 to 81% and the AlkaneSulfonatemonooxygenase (EC 1.14.14.5), with an identity range between 68 to 87% respect to those corresponding to other microorganisms cited by the GenBank. These results of genomic sequencing, would indicate the bacterial potentiality in the enzymes production which could be actively involved in compounds degradation presents in the hydrocarbons mix as is the case of diesel fuel.

Keywords: Gordonia *sp; hydrocarbons; enzymes.*

Financial Support: Universidad Católica de Córdoba.

Poster VI.63

Evaluation of the inhibition capacity of five different *PGPR*s on the pathogenic fungi *Rhizoctonia solani*

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Rhizoctonia solani is an aggressive soil-borne pathogen distributed throughout the world, causing a wide variety of diseases in crops of agricultural importance such as cotton, potato, tomato and soybean among others. Primary methods for managing the diseasehave included planting resistant cultivars, the use of fumigant chemicals and agronomic measures such as crop rotation. However, none of these approaches hasproven to be effective enough.As a sustainable alternative, the use of PGPR (*Plant-Growth Promoting Rhizobacteria*) as a biological control agent of the pathogen has been proposed. Mechanisms of biocontrol by PGPR include competition of niches, production of antifungal metabolites and enzymes, and the induction of resistance as well as growth promotion.The aim of this work was to evaluate the biocontrol efficiency of five different PGPR species against one isolate of *R. solani*. Three strains of *Bacillus amyloliquefaciens* (PS01, PS02 and PS03), one strains of *Bacillus cereus* (PS04) and one of *Paenibacillus* spp. (PS05) were evaluated. Co-culture tests on Potato Dextrose Agar (PDA) plates were used as a mean of testing the PGPRs inhibition capacity. Antagonism was evaluated by measuring the radial growth of the pathogen after 3-4 days of incubation at 28ºC ± 2. The strains identified as *Bacillus cereus* and *Paenibacillus* spp.did not show inhibitory capacity, while strain PS03 showed an inhibitory capacity superior to 74%, followed by strains PS01 and PS02 with values superior to 60%.

Keywords: soil-borne-pathogens; biocontrol; PGPR.

Financial Support: UE "IRNASUS" UCC – CONICET. INTA.

Poster VI.64

Preliminary assay on the biocontrol capacity of two *Bacillus* species on the causal agents of two limiting soil borne diseases of onion crops

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Onion (*Allium cepa* L.) is one of the most important horticultural crops in Argentina, with an estimated production of 450 thousand tons per year. Argentinean onion supplies the domestic market and it is also know worldwide as a culinary product of good flavor and high quality. Brazil is the main destination Argentinean onions are exported mainly to Brazil but, in recent years, there has been growing demand from European countries, especially Spain and Belgium, representing a need to increase onion production. Plant diseases caused by fungi, represent one of the most importance losses in onion production. Among these diseases, "onion pink root" and "basal plate rot" both caused by soil-borne fungi; *Setophoma terrestris* and *Fusarium spp.* respectively, are responsible of severe damage and yield lost. Management of soil borne diseases is not an easy task and normally requires of a combination of different strategies like crop rotation, resistant varieties and use of biocontrol agents among others.The screening and identification of PGPRs with antagonistic activity against fungal pathogens is an environmentally friendly alternative to protect crops. In this work, we present preliminary results of two *Bacillus spp.* as a candidates for biologically control *S. terrestris* and *Fusarium spp.* Co-culture essays between the pathogens and the *Bacillus* isolates were carried out in PDA (Potato Dextrose Agar) plates. Both strains showed a good antagonistic activity against both pathogens, with aninhibitory capacity superior to 75% in the case of *S.terrestris*, and over 65% in the case of *Fusarium spp.* Next steps in our research will be to test this PGPR in field assays to validate this results.

Keywords: soil-borne-pathogens; biocontrol; PGPR.

Financial Support: UE "IRNASUS" UCC - CONICET. INTA

Poster VI.65

Selection of *Bacillus* spp. for biological control of diseases and growth promotion of soybean

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Plant growth promoting rhizobacteria (PGPR) are able to promote biocontrol, induce plant growth and improve crop yield. The genus *Bacillus* is one of the most studied biocontrol agent, but information about native *Bacillus* from Mato Grosso do Sul are scarce. The purpose of this study was to evaluate *Bacillus* strains isolated from bromeliads for biocontrol of soybean white mold disease and in addition to promote plant growth. Initially, 32 bacilli isolates were analysed by PCR for the presence of the genes *bamD*, *ituD*, *fenF*, *bacAB*, *mrsA* and *sfP* coding for antimicrobial molecules. *In vitro* bioassays with isolates positive for the presence of the target genes were carried out against four pathogenic fungi *Sclerotinia sclerotiorum*, *Macrophomina phaseolina*, *Rhizoctonia solani* and *Colletotrichum truncatum*. The best isolates were the assessed *in vivo* to control the white mold disease in Potencia RR and M6210 IPRO soybean cultivars. The same soybean cultivars were used for the plant growth promotion test inoculated individually with the commercial product or co-inoculated with 8 different bacilli strains, besides the uninoculated control. The results showed that the genes *bamD*, *ituD* and *fenF* were detected in 25% of the tested isolates while *bacAB* was observed in 53% of bacillus strains. No PCR amplified product was observed for the genes *mrsA* and *sfP*. The *in vitro* bioassays showed that two isolates, VBE19 and VBE57, were able to inhibit all four pathogens tested while only two other isolates, VBE05 and VBE01 provided a reduction of 39.1% and 37.5%, respectively of the disease progress during the in vivo bioassays. The coinoculation treatments presented higher number of nodules in the cultivar Potencia RR while higher speed rates of emergency, differing statistically from control and the commercial product, were observed in the cultivar M6210 IPRO. Individual inoculation of the isolate VBN02 or co-inoculated with *Bradyrhizobium* increased fresh shoot mass, fresh root mass, root dry mass and root length. In conclusion, our results showed that the *Bacillus* tested showed potential for biocontrol of white mold and promoted growth of soybean plants.

Keywords: rhizobacteria; inoculation; biocontrol.

Financial Support: Fundect/MS.

Poster VI.66

Root-promoting rhizobacteria in *Acacia mearnsii* cuttings

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Cloned *Acacia mearnsii* spp. plantations are based on greenhouse production of plants generated by vegetative propagation. Several studies have demonstrated that rhizospheric bacteria can stimulate plant growth and, more recently, that they can increase rooting in vegetative material. Considering this, the objective of this study was to verify the effect of bacterial strains on the rooting of *Acacia mearsii*. The trial was performed in greenhouse of the forestry company Tanagro S.A., in January 2018, with four treatments: (i) control treatment; (ii) application of 6.000 ppm of indolbutyric acid (IBA) and two bean-nodulating *Rhizobia* strains pre-selected according to their ability to produce indole-acetic acid (IAA) and named here as R6 (18.01 μg IAA mL^{-1}) and R9 (36.5 μg IAA mL^{-1}), both belonging to the SEMIA strain collection (DDPA / SEAPI). Five replicates were performed and the experimental unit consisted in 90 cuttings. The bacterial inoculums, in a concentration of 10^9 $CFUml^{-1}$, were applied to the cutting base for 3 min and were set in substrate to rooting in greenhouse with intermittent misting. After 30 days, rooting, dry root matter, root area and root volume were evaluated. The application of bacterial inoculums R9 and R6 promoted gains in the rooting rate of 19.9% and 28.3%, respectively, compared to the control treatment. Bacterial strains R6 showed the highest root biomass production with 12 mg $plant^{-1}$, twice the obtained by the cuttings of the control treatment. Although the bacterial strains presented lower root length than the treatment with IBA application, the root volume was superior in relation to the control treatments and the IBA. These results show that the use of these IAA producing bacteria increases rooting rates, root biomass as well as root volume, which indicates a good formation of the root system, aiding in a greater absorption of water and nutrients, and, consequently, a better development of seedlings.

Keywords: seedlings; black wattle; indole-acetic acid.

Poster VI.67

Lettuce and rhizosphere microbiome responses to *Pseudomonas* species

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The use of plant growth promoting rhizobacteria (PGPR) may be more successful in crops that undergo a stage of production of seedlings in substrate, such as lettuce. However, one of the most common problems in research with PGPR is the difficulty in obtaining reproducible results. Furthermore, few studies have evaluated plant growth promotion and soil microbial community composition resulting from bacterial inoculation under field conditions, regarding lettuce culture. Due to the importance of this culture in the national market, we evaluated the effect of *Pseudomonas* strains on lettuce growth. The most promising strains were phylogenetically and physiologically characterized for plant growthpromoting traits, including phosphate solubilization, IAA and HCN production and their effect on plant growth under farm field conditions. Additionally, the impact of beneficial strains on the rhizospheric bacterial community was evaluated for inoculated plants. The strains IAC-RBcr4 and IAC-RBru1, with different plant growth promoting traits, improved lettuce plant biomass yields up to 30%. These two strains also impacted rhizosphere bacterial groups including *Isosphaera* and *Pirellula* (phylum Planctomycetes) and *Acidothermus*, *Pseudolabrys* and *Singusphaera* (phylum Actinobacteria). This study demonstrated consistent results for the effects of *Pseudomonas* strains on lettuce growth promotion for seedlings and plants grown under tropical field conditions.

Keywords: field experiment; plant growth promting rhizobacteria; bacterial community.

Poster VI.68

Physiological aspects of *Methylobacterium mesophilicum* endophyte inhibition of *Xylella fastidiosa* phytopathogen

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Methylobacterium is composed by pink-pigmented methylotrophic bacteria (PPFMs), which are one of the most abundant endophytes in plants. This bacterium is able to promote plant growth directly by producing phytohormones and providing nutrients to the plant and indirectly by reducing phytopathogen growth and pathogenicity. Therefore, little is known about the physiological and molecular mechanisms involved in *Methylobacterium* interaction with other bacteria. *M. mesophilicum* strain *SR1.6/6* was isolated from citrus, previous studies show it is able to promote citrus growth and inhibit citrus phytopatogen *Xylella fastidiosa in vivo*. Therefore, the present work aimed to analyze physiological aspects of *M. mesophilicum SR1.6/6-X. fastidiosa 9a5c* interaction. Quantification of both bacteria growth were performed during co-cultivation using qPCR after 24h, 48h and 72h of interaction. Moreover, it was evaluated *X. fastidiosa 9a5c* biofilm influenced by different bacteria exudate: *X. fastidiosa 9a5c*, *M. mesophilicum SR1.6/6* and co-cultivation. Results showed that during co-cultivation both bacteria reduce its number, *M. mesophilicum SR1.6/6* inhibit *X. fastidiosa 9a5c* growth only after 72h. While after only 24h, the presence of co-cultivation exudates increase *X. fastidiosa* biofilm. Theses results indicate a decrease in pathogen mobility after 24hh and growth after 72h, which could explain why citrus plant does not present disease symptoms. Understanding these interaction, along with gene expression which is also been investigated, can help to improve methods used to increase citrus growth and productivity, as well as plant pathogen resistance.

Keywords: endophytic biofilm; citrus; phytopatogen - endophyte interaction.

Financial Support: CNPq and FAPESP.

Poster VI.69

Rhizobium strains in the biological control of the phytopathogenic fungi *Sclerotium (Athelia) rolfsii* on the common bean

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To identify *Rhizobium* strains' ability to biocontrol *Sclerotium rolfsii*, a fungus that causes serious damage to the common bean and other important crops, 78 previously isolated rhizobia from common bean stored at *SEMIA* culture collection were assessed. Dual cultures, volatiles, indole-acetic acid (IAA), siderophore production and *16S rRNA* sequencing were employed to select strains for pot and field experiments. Thirty-three antagonistic strains were detected dual cultures, 16 of which were able to inhibit $\geq$84% fungus mycelial growth. Antagonistic strains produced up to 36.5 μg mL^{-1} of IAA, and a direct correlation was verified between IAA production and mycelium inhibition. *SEMIA* 460 inhibited 45% of mycelial growth through volatile compounds. *16S rRNA* sequences confirmed strains as *Rhizobium* species. In pot conditions, common bean seeds grown on *S. rolfsii* infested soil and inoculated with *SEMIA* 4032, 4077, 4088, 4080, 4085, and 439 presented same disease percentage of uninfested controls. A field trial corroborated the efficacy of the biocontrolling *SEMIA* strains detected on pot test. The most efficient strains detected, *SEMIA* 439 and *SEMIA* 4088, decreased 18.3% and 14.5% of the *S. rolfsii* - promoted disease incidence, respectively. The remnants strains were able to decrease disease incidence by 12.5% to 8.7%. *SEMIA* 439 and *SEMIA* 4088 treatments presented the area under the disease progress curve (AUDPC) values statistically significantly lower from the uninoculated treatment. *SEMIA* 439 and 4088 decreased 19.9% and 17.5% of the AUDPC comparing to uninoculated controls, respectively. In conclusion, *Rhizobium* strains could be strong antagonists towards *S. rolfsii* growth. Bacterial IAA has a function in soil competitiveness. *SEMIA* 4032, 4077, 4088, 4080, 4085, and 439 are effective biological control of *S. rolfsii* on common bean plants.

Keywords: crop improvement; indole-acetic-acid; pathogenic fungus.

Poster VI.70

Co-inoculaiton, macronutrient contents and dry mass production in corn after two co-inoculated seasons

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Nitrogen is one of the essential elements to the vegetal development. This element can be supplied by biological fixation by associative microorganisms such as *Azospirillum*. However microorganisms such as *Bradyrhizobium* can contribute with plant development by synthesis of secondary metabolites. The objective of this work was to evaluate the macronutrient contents (N, P and K) and dry mass production of corn after one season of wheat and soybean co-inoculated with *Bradyrhizobium* and *Azospirillum* (BA) in succession to wheat and soybean. The experiment was conducted in Campo Mourão-PR, Brazil, in Oxisol. The experimental design was a randomized complete block, consisting of 8 treatments (TRAT) with 5 replicates and experimental units of 36 m^2. In wheat, all TRAT received fertilization of 24 and 70 kg of N ha^{-1} in the subsurface band and in broadcast, respectively. In the TRAT 1, 3, 4 and 6 seeds were inoculated with (BA) and TRAT 2, 5, 7 and 8 were not inoculated (NI). In soybean, TRAT 1, 2, 3, 7 were inoculated with *Bradyrhizobium* (BRAD) and TRAT 4, 5, 6 and 8 were inoculated with BA. In maize, all TRAT were fertilized with 48 and 70 kg of N ha^{-1} in the subsurface band and in broadcast, respectively. The TRAT 1, 2, 4 and 5 were NI and TRAT 3, 6, 7 and 8 were inoculated with BA. Finally, in the succession of wheat, soybean and maize, the inoculation was TRAT 1 (NI+BRAD+NI), 2 (BA+BRAD+NI), 3 (NI+BRAD+BA), 4 (NI+BA+NI), 5 (BA+BA+NI), 6 (NI+BA+BA), 7 (BA+BRAD+BA) and 8 (BA+BA+BA). The data collected were submitted to analysis of variance and averages compared by the Tukey test ($p > 0.10$) using the SAS statistical program. Significant statistical differences were observed for SPAD in which TRAT 1 (62.50) was significantly higher than TRA 6 (57.33). The content of phosphorus in leaves was different just for the TRAT 6 (2.35 g kg^{-1}) being lower than the other TRAT (2.35 g kg^{-1}). No statistical differences were observed for the corn dry mass production that ranged from 5936 to 4961 kg ha^{-1}, for leaf N content ranged from 36.86 to 35.69 g kg^{-1}, the K leaf content ranged from 17.02 to 15.40 kg kg^{-1} and N content in grain ranged from 12.27 to 11.75 g kg^{-1}. However, the levels of N, P and K (TRAT 1 and 4) were considered adequate. The continuous using of BA in two seasons and in corn do not increase the significantly the variables analyzed, but the TRAT 6 increase de SPAD and P.

Keywords: nitrogen; BNF; Azospirillum.
Financial Support: Capes.

Poster VI.71

Evaluation of the antagonistic activity of metabolites produced by the culture of *Bacillus halotolerans* to control *Rhizoctonia solani*

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The use of antagonistic metabolites in soils where their natural presence can be reduced by the agricultural activity it self, represents a sustainable environmental friendly technology to control *Rhizoctonia solani*. Our objective is to evaluate the antagonistic activity of metabolites produced by the strain *Bacillus halotolerans* IcBac02.1 cultivated in mineral medium in a batch system at the level of stirred tank bioreactor. The presence of antibiotic metabolites was determined from cellfree supernatant, for which it was concentrated and fractionated with organic solvents of increasing polarity; butanolic phase was concentrated, freeze-dried and fractionated using the Flash chromatography technique with different systems of acetonitrile: water. F3 fraction showed greater inhibition of mycelial growth against *R. solani* under in vitro conditions using the plate diffusion technique. Also, the presence of hydrolytic enzymes such as cellulases, proteases and amylases was determined by colorimetric techniques. At greenhouse level, it was used common bean cv Canario Centenario plants inoculated with one strain of *Rhizobium* at beginning of the assay. The Treatments were: plants inoculated with Cell-free supernatant and F3 fraction, untreated plants and with the pathogen. Cell-free supernatant and F3 fraction were inoculated at the base of 1-week-old plants before infecting the plants with *R. solani*. Our results showed that both treatments significantly reduce the incidence of the disease by 50% after ten days of evaluation, due to the action of antibiotic metabolites and hydrolytic enzymes. Therefore, the use of antagonistic metabolites produced by the culture of *B. halotolerans* represents a promising alternative for phytopathogens control.

Keywords: Rhizoctonia solani*; antagonistic metabolites; fermentation.*

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Poster VI.72

Beneficial microorganisms in sugarcane pre-sprouted seedling productions

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The search for increasing productivity and decreasing pests and diseases in sugarcane crop has conducted to changes in its production system, such as the inclusion of presprouted seedlings production ("MBP – mudas pré-brotadas"). This system allows the inoculation of beneficial microorganisms, improving seedling development. The objective of this work was to evaluate the effects of plant growth-promotion bacteria (PGPB), endophytic and rhizospheric, and arbuscular mycorrhizal fungi (AMF) on sugarcane seedlings production by the "MPB" system and later transplanting to soil. The experiments were carried out under greenhouse condition, using commercial substrate with different fertilization levels. *Kosakonia radicincitans* (IAC/BECa090) and *Bacillus* sp. (IAC-RBca10) co-inoculated with AMF mixture increased shoot dry matter seedlings, up to 25%, in a partial-fertilized substrate. *Burkholderia caribensis* (IAC/BECa-088), *Pseudomonas* sp. (IAC-RBca5) and *Bacillus* sp. (IAC-RBca10), improved shoot dry matter up to 50%, without additional fertilization. Co-inoculation of *K. radicincitans* (IAC/BECa-090) and AMF increased shoot and root biomass, up to 27 and 75% respectively, in the conventional fertilized substrate, showing synergistic effect of inoculation. *Pseudomonas putida* (IACRBcr4) promoted root growth of IACSP91-1099 seedlings up to 135%. "MPB" transplanted and grown in a proper fertilized soil increased shoot and root dry matter up to 20%, when *Herbaspirillum frisingense* (IAC/BECa152) and *Pseudomonas putida* (IAC-RBcr4) were coinoculated with AMFs. Inoculation of *Burkholderia tropica* (IAC/BECa135) increased about 25% and 47% shoot and root dry matter of seedlings, respectively, after transplantation to soil. Beneficial microorganisms inoculation may be a feasible management in "MPB" system production. However, the response of the plants to inoculation depends on the fertility level of the substrate, the cultivar and species and bacterial isolate.

Keywords: plant growth-promoting bacteria; arbuscular mycorrhizal fungi; rhizobacteria; endohytic bacteria.

Poster VI.73

Response of the inoculation of *Azospirillum brasilense* strains AbV5 and AbV6 in wheat lines

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Positive responses has been observed in the association of Plant Growth Promoting Bacteria of the genus *Azospirillum* with wheat crop. The aim of this study was to evaluate the effects of inoculation (AbV5 and AbV6 strains) of *Azospirillum brasilense* on agronomic characteristics in wheat lines under field conditions. The experiment was carried out in Ponta Grossa, PR, in the randomized block design with 3 replicates, and treatments were arranged in a subdivided plot scheme. In plots the effect of inoculation with *A. brasilense* (AbV5, AbV6 and non-inoculated) was studied and wheat genotypes (18 UEPG new lines and 2 Safira® and Quartzo® controls) in subplots. Nitrogen fertilization was carried out at sowing (15 kg ha^{-1}) and topdressing at tillering stage (60 kg ha^{-1}). The evaluations were performed for the following characteristics: number of tillers m^{-2}, vegetative and reproductive cycle (days), height of plants (m), number of viable ears m^{-2}, thousand grain weight (g), weight of hectoliter (kg hl^{-1}), grain yield (kg ha^{-1}), chlorophyll content (ICF), leaf N content (g kg^{-1} leaf) and protein content (g protein kg^{-1} grain). The results showed highly significant differences among wheat genotypes for all traits evaluated. However, the interaction wheat genotypes x inoculation was significant only for the tillering variable. The L 3, L 6, L 14 and Quartzo® cultivar when inoculated with AbV6 strain showed an increase in the number of tillers, as well as L 9 line and Safira® cultivar when inoculated with AbV5 strain. The results corroborate with available literature that attributes positive responses to the specificity between wheat genotype and strain. In addition, more conclusive field experiments about real positive/negative effect of *A. brasilense* inoculation on wheat genotypes are required.

Keywords: wheat breeding; plant growth-promoting rhizobacteria (PGPR); adaptive characteristics.

Financial Support: Capes.

Poster VI.74

Comparative effect of single and dual inoculations of *Azospirillum brasilense* and *Bacillus* sp on root growth of maize seedlings

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Plant growth promoting rhizobacteria (PGPR) have been used as biofertilizer to enhance the plant growth, quality and yield. Thus, they are crucial for a sustainable agriculture. The objective of this work was to analyze the effect of single and dual inoculations of *Azospirillum brasilense* (strains 2142 and 1626) and *Bacillus* sp. (strains B116 and B2084) on the root growth of maize seedlings cultured in nutrient solution. The four bacterial strains from the collection of Multifunctional Microorganisms of Embrapa Maize and Sorghum were cultivated in liquid LB medium, separately and in combinations during 72h. Then, the cultures were centrifuged, resuspended in 0.85% NaCl, and the final concentration adjusted to 10^7 colony forming units (UFC) / ml. The maize seeds were germinated between two sheets of filter papers (Whatman 1) moistened with distilled water and incubated during four days at 30ºC. After germination, the seedlings were transferred to Hoagland's nutrient solution for seven days at 30ºC, and then the root part of the plantlets were immersed in the bacterial suspensions for six hours. Afterward, the plantlets were transferred to tray containning a complete Hoagland's solution and incubated at 30ºC for 10 days. After this period, the roots of each plant were photoghraphed, and the morphology of the roots was analized by the softwares rootreader2d and *whinrhizo*. The results showed that the strain B2084 of *Bacillus* sp. and the combination of 2142x1626 significantly improved root growth (root length, total root surface, fine root surface area and dry weight), in addition to increasing the N, P, and K content of both the aerial part of plants and roots. Other strain combinations (B20842x2142, B2084x1626, and B116xB2084) also showed positive effects on root morphology and N, P, K content. The combination of two strains of *A. brasilense* (2142x1626) was superior for all parameters analyzed compared with the results of each of these strains isolated. The present result highlights the potential of the tested PGPR as bioinoculants to improve maize productivity under the field conditions.

Keywords: growth promoting bacteria; biostimulants; nutrient absorption.

Financial Support: Embrapa Milho e Sorgo, FAPEMIG, CNPq.

Poster VI.75

Inoculation of plant growth promoting bacteria (PGPR) increases the protein and macronutrient content in rice (*Oryza sativa* L.)

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The cultivation of rice in Peru is an important economic activity. The excessive use of fertilizers and pesticides, as well as improper practices, not only causes environmental pollution, it also reduces the quality of the soil. This degradation decreases the productivity per hectare, impact on the quality of life of the rice farmer. An alternative to the problem is the selection of native PGPR bacteria to reduce the use of fertilizers and improve their efficiency of use, increasing productivity. The purpose of this study was to evaluate the effect of the individual and consortium inoculation of five PGPR bacteria on the content of glutelin, assimilation of nitrogen (N), phosphorus (P) and potassium (K) in the grain of rice, variety La Esperanza, under greenhouse conditions. Five strains of PGPR bacteria were selected for this study: *Citrobacter bitternis* p9a3m, *Micrococcus yunnanensis* la2b2, *Staphylococcus hominis* p7b1m, *Bacillus megaterium* la1a4 and *Burkholderia ubonensis* la3c3. In an experimental design of completely randomized blocks, inoculation was carried out individually and in a consortium of bacteria receiving increasing doses of nitrogen fertilization (NF) and phosphate fertilization (PF) (0, 25, 50, 75 and 100%). The evaluation was of 135 days and the content of glutelin, N, P and K (%) in grain was analyzed. The individual inoculation of la3c3 at 25% NF and la2b2 at 25% PF, increased the content of glutelin by 4.1 and 10.9%, respectively. The bacterial consortium at 50% NF and the strain p9a3m at 50% PF, increased by 18.5 and 13.6%, the assimilation of N respectively. The individual inoculation of the p9a3m strain at 0% NF and PF, increased the P% by 3.9 and 10.9%, even fertilized at 25% PF, increased by 28.9% the K%. All compared to the 100% NF and PF control. The use of PGPR bacteria in rice increases the nutritional profile of the grain, so promising strains will be evaluated under field conditions.

Keywords: glutelin; consortium.

Financial Support: INNOVATE PERU, Convention n° 101-INNOVATEPERU-IDIBIO-2018.

Poster VI.76

Assessment of PCR with specific primers target to *A. brasilense* Az39 in complex samples

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A.brasilense Az39 is the selected strain for inoculant formulation for non-legumes in Argentina. This strain was isolated in 1982 from surface-sterilized wheat seedlings in Marcos Juarez (Córdoba, Argentina). After 40 years of use, the ability of Az39 to increase crop yield of maize and wheat under agronomic conditions has been demonstrated. At present, there was not protocol in Argentina that allow to identify and authenticate *A. brasilense* Az39 strain in commercial formulations or isolates obtained from soil samples. Until now, the identification of this microorganism was carried out using classical methodologies, accompanied by molecular tools, but these are not enough to ensure identity at strain level. Recently, in the Laboratorio de Fisiología Vegetal e Interacción Planta-Microorganismo from the UNRC, we developed a molecular technique based in single sequences obtained from the genome of *A. brasilense* Az39. Using the genome sequence of Az39, an *in silico* PCR-AFLP (PCR-amplified fragment length polymorphism) was performed. The results allowed to recognize unique DNA fragments in the genome of Az39. These sequences were used for the design four pairs of primers and perform the PCR amplification to identify Az39 in pure cultures. In this work, genomics DNA were extracted from complex samples of culture mediun or soil inoculated and not inoculated with Az39. Templates DNA were used for the specific PCR. The amplification products were visualized by agarose gel electrophoresis. Our findings show that only the Az39 inoculated samples were positives for PCR amplification. The results indicate that specific primers can detect *A. brasilense* Az39 in a complex sample, even when there is more than one kind of microorganism.

Keywords: Azospirillum*; PGPR.*

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Poster VI.77

Extraction of N in sugarcane under increased N-fertilizer use and inoculation with five diazotrophs

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The aim of this work was to evaluate nitrogen (N) extraction and ^{15}N-fertilizer recovery efficiency by sugarcane as a function of nitrogen fertilization and inoculation with a mixture of five diazotrophic bacteria. An experiment was installed in an Ultisol localized at the experimental area of Embrapa Agrobiologia, Seropédica, RJ, Brazil. The study was conducted during the plant cane growing season using the variety RB92579 and seven harvest time. The experimental design was a randomized complete block design with four replications and four treatments: control without N-fertilizer and without inoculation (-N-inoc), no N-fertilizer and with inoculation (-N+inoc), N-fertilizer without inoculation (50kg ha^{-1} N-urea: +N-inoc), and N-fertilizer and inoculation (+N+inoc). The mixed inoculant was prepared to obtain 10^8 cells g^{-1} of *Herbaspirillum rubrisubalbicans* strain HCC103, *H. seropedicae* - HRC54, *Nitrospirillum amazonense* - CBAmc, *Gluconacetobacter diazotrophicus* - PAL5T and *Paraburkholderia tropica* - Ppe8T. The mixture was prepared using five bags of the peat inoculant diluted in water in a proportion of 1/50 (w/v) and applied by immersion of the stem cuttings during 30 min before planting. Cane yield and N content were evaluated. Inoculation (-N+inoc) increased dry mass accumulation and N content at 168 days after planting (DAP). The PGPR inoculation did not influence the N-fertilizer recovery efficiency as well as the FBN contribution. The N derived from soil were enhanced by the inoculation when associated with 50 kg of N-fertilizer during the period of high vegetative growth.

Keywords: isotopes; sugarcane nutrition; bacteria; plant growth promotion.

Financial Support: Capes; CNPq-INCT and Faperj.

Poster VI.78

Benefits of co-inoculation of *Bradyrhizobium japonicum* BR 1602 and plant growth-promoting rhizobacteria in *Calopogonium mucunoides*

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Calopo (*Calopogonium mucunoides*) is an herbaceous legume that has been recommend for the revegetation of degraded areas, since it is highly adapted to acid soils with low fertility. Currently, *Bradyrhizobium japonicum* (BR 1602) is the recommended strain for inoculation of this legume, and recent studies have shown that the benefits of that strain can be enhanced by co-inoculation with plant growth promoting bacteria (PGPR). Previous works in our laboratory isolated and characterized rhizospheric and/ or endophytic bacteria that present several mechanisms for promoting plant growth in vitro. Thus, the present work evaluated the influence of co-inoculating the strain BR 1602 with three PGPR isolates on the growth of calopo in low fertility soils, collected in a coal mining area in the initial stages of recovery. The experiment was conducted in a completely randomized design in which six treatments were tested: non-inoculated control, BR 1602 alone, and the consortium between BR 1602 with each of the three PGPRs evaluated - *Pantoea* sp. (MSR2), *Pseudomonas oryzihabitans* (MS8), and *Pseudomonas thivervalensis* (SC5), with five replicates each. After 50 days, the number (NN) and dry mass of nodules (NDM), shoot (SDM) and root dry matter (RDM), and the content and accumulation of N in MSPA were determined. Coinoculated treatments increased NN, NDM, SDM and accumulation of N by 570%, 800%, 212% and 153% respectively, in relation to BR 1602 applied alone. The results evidenced that co-inoculation of BR 1602 with selected PGPR isolates in calopo is a promising alternative for the revegetation of degraded areas.

Keywords: degraded areas; microbial inoculants; calopo.

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Poster VI.79

Biosolubilization efficiency of *Azospirillum brasilense* and *Bacillus* sp. in single and mixed cultures

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Phosphorus (P) is one of the most important macronutrients, although the total P content in soils frequently exceeds the required amount for plant development, most of the P complexed with minerals in the soil, which is unavailable to plants. Phosphorus solubilizing microorganisms represent a viable alternative to this problem. The objective of this study was to evaluate the potential of two strains of *Azospirillum brasilense* (1626 and 2142) and two strains of *Bacillus* sp. (B116 and B2084) inoculated as single and combined cultures to test their phosphate solubilization ability. The strains were grown in liquid culture medium containing calcium phosphate (P-Ca), iron (P-Fe) or sodium phytate (P-Fit) during nine days. For the determination of soluble P, the colorimetric method and spectrophotometry readings at 880 nm were used. A significant difference was observed for the strains tested in isolated and mixed forms, and the treatments with addition of P-Ca showed, on average, the better solubilizing activity. The strains B116, B2084, and combinations of B116x2142, B116x1626, B2084x2142, and 2142x1626 showed highest P release from P-Ca. For the combination 2142x1626, the P release was higher. The mixed forms B116x2142, B116x1626, B2084x2142, and 2142x1626 also showed high values of P release from P-Iron, with values ranging from 36 mg P L^{-1} to 41 mg P L^{-1}. The combination B2084x2142 was superior with respect to this treatment. The P-Fit rate ranged from 3.64 mgP L^{-1} to 28.67 mgP L^{-1}, and the highest values occurred for the combinations B2084xB116, B2084x2142, and for the pure strains of *Bacillus* sp. (B116 and B2084) with the release of 28.67 mgP L^{-1}, 16.15 mg L^{-1}, 12.15 mg L^{-1}, and 24.67 mg L^{-1} respectively. The combinations of *Bacillus* sp. and *A. brasilense* presented greater ability for P biosolubilization compared with the isolated strains. These P-solubilizing combinations will be tested as plant growth promoter inoculants.

Keywords: microorganisms; phosphate rock.
Financial Support: Embrapa Milho e Sorgo, FAPEMIG, CNPq.

Poster VI.80

Revegetation approaches of degraded soils by open-pit mining through useful plants and beneficial microorganisms

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Degraded soils by open-pit mining require rehabilitation processes, in which establishing plant cover and microbial communities is essential to reactivate the nutrient cycling. For this, the use of key plant species, beneficial rhizobacteria and symbiotic nitrogen-fixing bacteria (BFNs) has shown utility, by stimulating the aerial and root growth of plants and, in some of them, greater tolerance to stress factors, as well as an impact in microbial communities. Consequently, the aims of this work were the prospecting and selection of bacteria capable of promoting the establishment and development of plants suitable for the revegetation of degraded soils by mining and additionally observe the impact of plant growth on microbial communities and soil health in this type of materials. For this, rhizobacteria associated to *Brachiaria decumbens* and BFNs to *Acacia farnesiana* were isolated and the best ones were selected in greenhouse tests. Next, these bacteria in addition to mycorrhizal fungi were evaluated in field experiments where soil health data (active carbon, respiration, phosphorus and protein in soil), nitrogen and foliar phosphorus were taken and analysis of bacterial diversity through extraction of total soil DNA was done. In greenhouse assays, 2 promising strains (*Bacillus subtilis* and *Sinorhizobium fredii*) were found promoting plant growth. In field experiment, little effect from the addition of microorganisms in the development of plants was observed, but the growth of natural grasses was perceived, which improved the soil health parameters. Furthermore, the introduced legume presented effects on microbial diversity, especially of the *Rhizobiaceae* family. This study showed the possibility of recovering degraded soils from the use of key plants and microorganisms inoculation, but it is still necessary additional strategies to optimize it.

Keywords: degraded soils; revegetation; microorganisms.
Financial Support: Argos S.A.

Poster VI.81

The influence of microorganisms on the productivity and quality of radish plants

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The radish, although little consumed by the Brazilians, undergoes a lot of price variation in the market that technologies that improve the production are necessary. In the organic system, the challenge is even greater, since we must adopt less costly management in the production system. The present study aims to improve and increase production, without major investments and technical workmanship through the interaction between microorganisms and the radish culture. The experiment was carried out in pots and a randomized block design with four replicates and five treatments: T1 (*Bacillus subtilis*), T2 (*Bacillus cereus*), T3 (*Bacillus subtilis* + *Bacillus cereus*), T4 (*Environmental microorganisms*) and T5 control) and two cultivars of radish, namely A, cultivar Margaret Queen Kobayashi and B, cultivar Cometa. The experiment was conducted for a period of 49 days and evaluated in relation to plant height, leaf number, root diameter, shoot dry weight and root weight. In the conditions of this study there were significant differences between the cultivars Cometa and Margaret, but not among the treatments within the same cultivar for the variables: height, number of leaves and diameter. All treatments presented higher averages in comparison with the control, evidencing the positive effect of the microorganisms on the radish development. The cultivar Cometa presented a superior result compared to Margaret cultivar in relation to the height (10%) and the number of leaves (40%). Regarding the diameter variable, there was a significant difference between cultivars for the interaction of the microorganisms *Bacillus subtilis* and *Bacillus cereus* (BS * BC). The results of dry matter (DM) of the aerial part were smaller for the control, which shows the positive effect of the microorganisms in the cultivation of radish. The best result for DM was the cultivar Cometa, with a value of 1.22 g and in the Margaret cultivar of 0.96 g being the best treatment for the two cultivars the interaction of the microorganisms BS * BC.

Keywords: Raphanus sativus *L.; organic agriculture; interaction of microorganisms.*

Financial Support: FAPEMIG, CNPq and Capes.

Poster VI.82

Functional activities of *Bacillus* isolated from bromeliads and growth promoting activity of maize exposed to drought stress

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The search for drought-tolerance in plants has increased and numerous strategies has been evaluated, and one of them is the use of plant growth promoting rhizobacteria (PGPR). Among the beneficial mechanisms, the hydrolysis of 1-aminocyclopropane-1-carboxylate (ACC) it's an example. ACC is the precursor of ethylene in plants that in high concentrations slow root growth and cause senescence. The objective of this work was to characterize *Bacillus* spp. isolates regarding their enzymatic properties and the effect of inoculation of these isolates on germination of maize under drought stress. The isolates were evaluated for catalase, cellulase, protease and amylase activity, indole-3-acetic acid (IAA) production, solubilization of phosphorus (P) and zinc (Zn), detection of ACC deaminase by PCR of the *acdS* gene and measurement of the enzymatic activity. The ACC deaminase-producing were evaluated for growth under dry and production of exopolysaccharides (EPS). The best isolates were inoculated in maize to analyze the effect on germination under stress by polyethylene glycol (PEG). Selected isolates were assessed in a pot experiment in which the effects on mass accumulation in maize were observed in single inoculation and co-inoculation experiments with *Azospirillum brasilense* (Ab-V5/Ab -V6) under conditions of water stress (dry). According to the results, 52%, 84%, 60% and 52% produced catalase, cellulase, protease and amylase, respectively; while 32% produced IAA, 76% and 64% solubilized P and Zn. 22 isolates showed amplicons for the *acdS* gene. However, only 10 isolates were able to use ACC as only N-source. All bacteria grow under dry and produce EPS. In the germination assay, inoculation of the VBE23 isolate had a higher percentage of germination than the treatments without inoculation. In the pot experiment, in drought conditions the single inoculation of VBE23 and its coinoculation provided the highest values of fresh and dry mass of shoot and root, showing the potential of this isolate for growth promotion under drought stress.

Keywords: ACC deaminase; inoculation; germination.

Financial Support: Fundect and CNPq.

Poster VI.83

Inoculation of a native bacterial consortium improves yield and milling quality in rice (*Oryza sativa* L.)

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Rice is the most important cereal for more than 50% of the world population, however it is highly dependent on nitrogen fertilization to reach the maximum yield potential. The excessive use of nitrogenous chemical fertilizers causes pollution and its lack of use reduces the productivity of rice per hectare. The design and use of native bacterial inoculants is a successful proposal within an integrated nutrient management program. The purpose of this study was to evaluate the effect of individual and consortium inoculation of three strains of plant growth promoting bacteria: *Burkholderia ubonensis* la3c3, *Bacillus megaterium* la1a4 and *Citrobacter bitternis* p9a3m, under different doses of nitrogen fertilization (NF) (0, 25, 50, 75 and 100%), on the grain yield (GY) and milling quality as whole grain (WG) and broken grain (BG) of rice, variety La Esperanza. The experimental design of divided plots was carried out in the district of Juan Guerra, San Martín, Peru. The inoculation of the bacteria (10^9 CFU/ml) was carried out in two stages, prior to the seedbed and during the transplant. The maintenance of the crop was carried out until the harvest (135 days). The GY (Mg ha^{-1}) was determined as well as the WG% and BG%. The GY obtained in the treatment inoculated with the consortium and fertilized with 100% NF (8.15 Mg ha^{-1}) was 13.5% higher than that obtained in the control treatment that received 100% NF (7.18 Mg ha^{-1}). An increase of 2.5% in the GY was also obtained in the treatment inoculated with the consortium and fertilized with 75% of NF, likewise, in this treatment the proportion of whole grain was increased by 4.8% and reduced 30.9% the proportion of grain broken in relation to the control. It is evident that the use of a native bacterial consortium in the rice variety La Esperanza increases the efficiency in the use of nitrogen fertilizers and even reduce the dose of NF up to 25%, constituting an alternative to improve crop productivity of rice in the San Martín.

Keywords: biofertilizer; nitrogen fertilization; liquid inoculant.

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Poster VI.84

Organic fertilization and *Bacillus subtilis* inoculation influence the growth of *Mentha arvensis* L.

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The management of soil with organic fertilization from the use of composting is considered the base of medicinal plants mineral nutrition. However the inoculation of microorganisms, such as the rhizobacteria promoters of growth which act as elicitors, promoting several benefits to the production through the phosphorus solubilisation, production of phytohormones and antibiosis. Technique of great importance since the use of synthetic products is limited and the rhizobacteria can influence and or increase the production of secondary metabolites. Therefore, it was aimed in the present work to test the efficiency of organic fertilization (composting) combined to the inoculation of *Bacillus subtilis* in promoting the growth of the *Mentha arvensis* L., providing changes in the plant metabolism, morphology and physiology. The experiment was conducted in a greenhouse in the Department of Agriculture at UFLA, in randomized design, containing 4 treatments (1- soil without fertilization (witness); 2- soil + 10 kg m^{-2} of composting; 3- soil + *Bacilus subtilis*; and 4- soil + composting + *Bacillus subtilis*) and six repetitions (four plants/repetition and one plant per vase). After 60 days of cultivation the number of sprouts, number of stolons, plant height, dry biomass of aerial part, dry biomass of roots and total dry biomass were evaluated.The treatment 4 was the most efficient in the ontogeny of *Mentha arvensis L.* and the results shown that the inoculation of *Bacillus subtilis* potentialized the development of species in the analysed variant, it increased the number of yolks by stolon, main method of specie propagation, it was also observed the increase of the dry biomass in the aerial portion, as well as influenced the production of total phenols. Therefore the use of *Bacilus subtilis* in the production of *Mentha arvensis* L. was efficient and can be used as subsidy for further testing in medicinal plants.

Keywords: rhizobacteria; secondary metabolism; medicinal plant.

Fomentation Agency: Capes, FAPEMIG e CNPq.

Poster VI.85

Maize induced selection of plant-growth-promoting rhizobacteria

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Brazilian Atlantic Forest (BAF) soil is a potential source for isolating unknown bacteria which could lead to growth promoting of relevant agricultural crops. In this work, a stepwise bacterial enrichment approach was carried out using two maize cultivars and decrease in soil/vermiculite ratio as substrate, accompanied by bacteriome analyses and isolation. Two maize cultivars, with different growth responses upon inoculation with *Azospirillum brasilense* were grown in greenhouse pots using a mix of sterile vermiculite and BAF soil (25% w/w) as substrate without nitrogen input. After 30 days, rhizosphere soil and roots were collected for bacterial isolation and DNA extraction. The remaining substrate and plant roots were homogenized, mixed with sterile vermiculite (25%ratio), and the mixture used as substrate for a second cycle of maize growth. This procedure was repeated for five cycles. Bacteria biodiversity of each cycle was determined by 16S rRNA V4 deep-sequencing of metagenomic DNA. Isolates were identified by 16S rRNA sequencing, and tested for plant-growth promotion traits. Rhizospheric microbiome shifted over the cycles on both cultivars with a decrease in abundance of Acidobacteria, and an increase of Actinobacteria, Bacteroidetes, and Archaea. Different genera composition were observed within microbiome of the maize cultivars. About 1700 bacteria were isolated from each cultivar. Analyses of 40 isolates showed that 17.5% solubilized phosphate, 20% produced siderophores, 20% showed fungal biocontrol activity, and 65% fixed nitrogen. These data suggest that successive growth cycles of maize is an effective method to select PGPRs, and such selection is dependent of the plant cultivar.

Keywords: maize, plant-growth-promoting bacteria, nitrogen fixation.

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Poster VI.86

Control of soil pathogenic fungi using selected rhizobacterias strains isolated from common bean plants

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The common bean is a legume of economic, cultural and nutritional importance. The use of microorganisms that have the ability to promote plant growth (PGP) and biocontrol represents a sustainable alternative in crop management. The aim of the work was to evaluate the effect of fifteen bacteria strains between *Bacillus* and *Pseudomonas* genus in the reduction of the disease incidence cause by *Rhizoctonia solani* and promotion of plant growth. This bacteria were isolated from the rhizosphere of common bean var. Centenario plants. All the strains produced AIA in different values in a range of 16.29 to 51.68 μg/ml and, 60% presented solubilization halo in bicalcium phosphate and 20% in tricalcium phosphate after 15 days of evaluation. At the in vivo level, all the bacteria strains promoted the germination of common bean seeds, of which, the strain TrB02.40 increased the germination at 97% of seeds with statistical differences compared at 57% germination of the control. At the greenhouse level, 7 treatments were considered: plants inoculated with two strain of the genus *Bacillus* (TrB02.32 and TrB02.40), two of *Pseudomonas* (LbPs02.15 and LbPs03.1), all of them in interaction with the pathogen, as well, chemical control, untreated plants and plants inoculated only with the pathogen. All the plants were inoculate with *Rhizobium* E10 except those of the chemical control and without inoculation. The TrB02.40 strain in interaction with E10 showed only 13% of disease incidence compared with the other microbial treatments. The plants inoculated only with the pathogen presented 100% of disease incidence. Also, TrB02.40 strain in interaction with E10 presented the highest values in height (14 cm) and fresh and dry weight (6.82 g and 0.84 g) with significant differences compared to plants without inoculation. However, no significant difference was observed with the treatment based on chemical control. In addition, the plants treated with TrB02.40 strain and *Rhizobium* E10 present 51 nodules / plant compared with plants inoculated only with *Rhizobium* (38).

Keywords: Disease incidence; PGP bacteria; biocontrol.

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