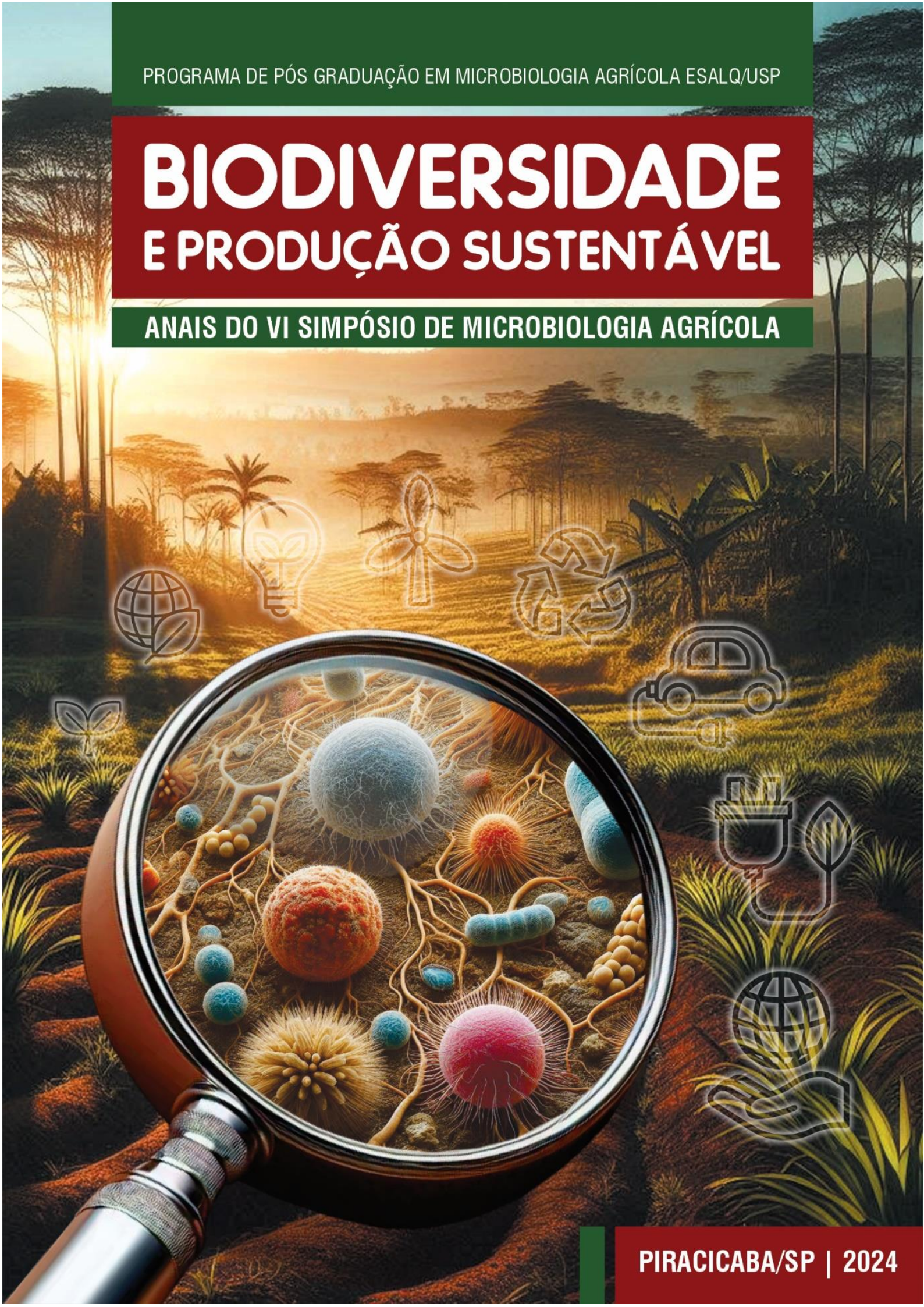
PROGRAMA DE PÓS GRADUAÇÃO EM MICROBIOLOGIA AGRÍCOLA ESALQ/USP

BIODIVERSIDADE E PRODUÇÃO SUSTENTÁVEL

ANAIS DO VI SIMPÓSIO DE MICROBIOLOGIA AGRÍCOLA



PIRACICABA/SP | 2024

Biodiversidade e Produção Sustentável: anais do VI Simpósio de Microbiologia Agrícola

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Editores

Me. Mauricio Junior Machado
Dr. Rafael Barty Dextro
Dra. Simone Raposo Cotta

Capa

Rafael Dantas Barbosa

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Carta aos Participantes

É com grande satisfação que agradecemos a presença de todos no VI Simpósio de Microbiologia Agrícola (SMAGRO), promovido pelo Programa de Pós-Graduação em Microbiologia Agrícola da Escola Superior de Agricultura “Luiz de Queiroz” da Universidade de São Paulo, realizado entre os dias 09 a 12 de abril de 2024 na cidade de Piracicaba - SP. O VI SMAGRO, com o tema "Biodiversidade e Produção Sustentável," proporcionou um espaço para a troca de conhecimentos e discussões sobre a relevância da microbiologia agrícola na conservação da biodiversidade e na promoção de práticas sustentáveis na agricultura, na indústria e na preservação ambiental.

Além do SMAGRO, tivemos o prazer de sediar três eventos igualmente importantes para a nossa área: o IV Congresso Brasileiro de Microbiologia Agrícola, Agropecuária e Ambiental (CBMAAA); o XIII Encontro Nacional de Alunos de Pós-Graduação em Microbiologia da área de Ciências Agrárias (ENAP); e o XV Fórum de Coordenadores de Programas de Pós-Graduação em Microbiologia Agropecuária. O CBMAAA e o ENAP, eventos itinerantes realizados a cada dois anos, reúnem programas de pós-graduação de Microbiologia Agrícola, Agropecuária e Ambiental da área de Ciências Agrárias I da CAPES, envolvendo instituições como a Universidade Estadual Paulista “Julio de Mesquita” de Jaboticabal e Rio Claro, Universidade Federal de Lavras, Universidade Federal do Rio Grande do Sul, Universidade Federal de Viçosa, além da Universidade de São Paulo. Essa diversidade de instituições reflete a importância e a força da nossa comunidade acadêmica, que se dedica a promover a interação e o avanço dos conhecimentos em microbiologia no campo das Ciências Agrárias.

Esperamos que o evento tenha proporcionado momentos enriquecedores, com debates e colaborações que possam impulsionar novas pesquisas e contribuir para o fortalecimento da nossa área de atuação.

O Programa de Pós-Graduação em Microbiologia Agrícola da ESALQ-USP, juntamente com a comissão organizadora do VI SMAGRO, gostaria de expressar nossa sincera gratidão a todos os participantes.

Cordialmente,

Profª Dra. Maria Carolina Quecine Verdi
Vice - Coordenadora do Programa de Pós Graduação em Microbiologia Agrícola ESALQ/USP e Coordenadora da Comissão Organizadora do VI SMAGRO

Me. Mauricio Junior Machado
Representante Discente do Programa de Pós Graduação em Microbiologia Agrícola ESALQ/USP e Representante da Comissão Organizadora do VI SMAGRO

Minicursos

Análise Metagenômica de Comunidades Microbianas Usando Plataformas Web
Dr. Thierry Alexandre Pellegrinetti (CENA/USP)

Bioinsumos Inovadores: da Prospecção dos microrganismos ao Registro de Produtos Biológicos
Dra. Marta Olievira (ESALQ/USP)
Dra. Claudia Paz (ESALQ/USP)
Dra. Janaina Seibert (ESALQ/USP)
Dr. Jonathan Rodriguez (ESALQ/USP)
Me Isabela Rocha Pires (ESALQ/USP)
Laryssa Marinho (ESALQ/USP)
Dra. Natasha Iwanicki (ESALQ/USP)
Me. Raphaelle Valle (ESALQ/USP)

Coleção de culturas microbianas: isolamento, preservação, manutenção e aplicações
Dra. Letícia Bianca Pereira (ESALQ/USP)

Dereplicação e Anotação de Produtos Naturais em dados de LC-MS/MS
Dra. Rhuana Valdetário Médice (FCF/USP)
Me. Márcio Barczyszyn Weiss (FCF/USP)

Microscopia eletrônica de varredura e processamento de imagens
Prof. Dr. Elliot Watanabe Kitajima (ESALQ/USP)
Me. Pedro Renato Bodo de Paiva (ESALQ/USP)

Teoria à prática de extração, colonização, montagem e exame de esporos de Fungos Micorrízicos Arbusculares (FMAs).
Téc. Denise de Lourdes Colombo Mescolotti (ESALQ/USP)
Dr. Felipe Martins do Rêgo Barros (ESALQ/USP)

Programação

DIA 10/04/2024

- **Mesa redonda: Biodiversidade Microbiana e Produção Sustentável**

Dr. Rodrigo Mendes (EMBRAPA); Dr. Acácio A. Navarrete (Universidade Brasil); Dr. André Rodrigues (UNESP)

- **Palestra: Microbiota na restauração de ecossistemas degradados e sua contribuição para a sustentabilidade ambiental.**

Dra. Kelly Hidalgo Martinez (UNICAMP)

- **Palestra: Aplicações biotecnológicas sustentáveis de bactérias magnetotáticas**

Dra. Fernanda de Ávila Abreu (UFRJ)

- **Re-imaginando a sustentabilidade para impactar o mundo de verdade**

Mariana Yuri Yama (UPL)

- **Palestra: Economia Circular: biodiversidade e bioprodutos renováveis**

Dra. Ayla Sant'anna (INT – RJ)

- **Manejo Raízen**

Marcela Bruscagin (RAÍZEN)

DIA 11/04/2024

- **Mesa Redonda: Microrganismos para o controle biológico de pragas e doenças**

Dr. Ítalo Delalibera Júnior (ESALQ/USP); Dr. Éverton Kort Kamp Fernandes (UFG); Dra. Janaína Brandão Seibert (ESALQ/USP); Dra. Natasha Sant'Anna Iwanicki (ESALQ/USP)

- **Palestra: Biodiversity – role of microorganisms in global change**

Dr. Ashish Malik (University of Aberdeen- Escócia)

- **Palestra: Produtos naturais biossintetizados por microrganismos**

Drª. Taicia Pacheco Fill (UNICAMP)

- **Ingredientes para nutrição microbiana em fermentação líquida**

Paulo Fernandes (AUNARE)

- **XIII Encontro Nacional de Alunos de Pós-Graduação em Microbiologia da área de CiênciasAgrárias (ENAP)**

Me. Mauricio Junior Machado (ESALQ/USP); Me. Cinara Ramos Sales (UNESP-FCAV); Matheus Victor Maso Lacôrte e Silva (UNESP-Rio Claro); Me. Lutécia Rigueira Medina (UFV), Dr. Danilo José Machado de Abreu (UFLA)

- **XV Fórum de Coordenadores de Programas de Pós-Graduação em Microbiologia nas Áreas de Ciências Agrárias**.

Dra. Maria Carolina Quecine Verdi (ESALQ-USP); Dr. Daniel Guariz (UNESP); Dr. Fabrício Souza Campos (UFRGS); Dr. Victor Pylro (UFLA); Dr. Wendel Batista (UFV).

DIA 12/04/2024

- **Bioinsumos: os novos protagonistas da agricultura**

Carlos Mendes (BIOMIX)

- **Mesa Redonda: Ecologia do solo – microrganismos para um futuro sustentável**

Dra. Joana Falcão Sales (Groningen University - Holanda); Dr. Arthur Prudêncio de Araujo Pereira (UFC); Dr. Thierry Alexandre Pellegrinetti (CENA-USP)

RESUMOS

Os autores são os únicos e totalmente responsáveis pela veracidade, exatidão e precisão das informações que forneceram para publicação nestes anais.

SUMÁRIO

Activity of Beta-Glucosidase enzyme and biometric parameters in Brachiaria inoculated with *Bacillus subtilis* for potassium solubilization

G. M. Castilho[1], R.L. Oliveira[1], M.O. Barbosa[1], E.H. Rocha[1], M.D. Ramire[1], F.D. Andreote[1]

[1] Luiz de Queiroz College of Agriculture and Av. Paduá Dias, 11

castilho.gabriel@usp.br

The solubilization of rock powder, providing nutrients slowly, can be a solution to the low efficiency of nutrient absorption in fertilizing. The objective of this study was to evaluate Beta-glucosidase activity in the soil and biometric parameters in *Brachiaria* using 2 types of rock powder and *Bacillus subtilis* inoculation. The experimental design was completely randomized with five replicates in a 3X2 factorial scheme. *Brachiaria* plants were allocated to the following block treatments: 1 - Soil only (T1); 2 - Soil without *B. Subtilis* (T2); 3 - Soil with *B. Subtilis* (T3); 4 - Soil + Granite (T4); 5 - Soil + phonolite (T5); 6 - Soil + *B. Subtilis* + granite (T6); 7 - Soil + *B. Subtilis* + phonolite (T7). The seeding of *Brachiaria B.* cv. Marandu occurred on 13/07/2023 in 5 kg pots. The first collection was performed 37 days after emergence (DAE) and the second at 92 DAE. The Activity of Beta-glucosidase showed that, in the first collection, treatment T6 obtained 30,4, T7 26,25 and T3 21,25 µg PNG, with a statistical difference between T6-T3 and T7-T3. However, the second collection showed a activity of 6 µg PNG higher in T3 than T6 and 8 µg PNG in T3 than T7, without statistical difference. Regarding the influence of Beta-glucosidase, only root weight, in the second collection, correlated with 0.6. In both biometric parameters, there was an increase in weight in treatments with rock powder compared to the control, but no differentiation regarding inoculation between treatments.

KEYWORDS: rock powder, *Bacillus subtilis*, Beta-glucosidase, solubilization

Cocoa fermentation using a mixed of hybrids

Q. DA SILVA[1]*, M. C. NAHIME[1], S. R. DIAS[1], N. N. BATISTA[1], S. J. MARTINEZ[1], R. F. SCHWAN[1]

[1]Federal University of Lavras, Trevo Rotatório Professor Edmir Sá Santos, Lavras - MG, 37203-202

* queren.silva@estudante.ufla.br

Cocoa fermentation is one of the post-harvest processes that has the greatest impact on the final quality of chocolate. Its involves the action of various microorganisms such as yeasts, lactic acid bacteria (LAB), and acetic acid bacteria (AAB). This study aimed to estimate the microbial population during the spontaneous fermentation of cocoa using a mixture of hybrids (PS 1319, PH 16, BN 34, CEPEC 2002, SJ02, CCN 10, and Ipiranga) and their influence in chemical composition of cocoa. Fermentations were conducted for 144 hours. The dominant microbiota was estimated from samples at 0, 72, and 144 hours. YEPG, MRS, AN, and GYC media were used to estimate the microbial population. Chemical analyses were performed using high-performance liquid chromatography. Yeasts were dominant during the cocoa fermentation process. At the end of the process, AAB (5.24 log CFU/g) and yeasts (5.45 log CFU/g) showed the highest population. Microbial diversity varied with time and microbial group. All carbohydrates were consumed. Organic acid with the highest content at the beginning of fermentation was citric acid (13.79 g/kg), which was not detected at the end of the process. The ethanol (2.28 g/kg) and acetic acid (6.66 g/kg) was detected in highest concentration after 72 hours. The concentrations of methylxanthines increased throught the fermentation (caffeine 43.51% and theobromine 42.24%). Thus, yeast populations may have prevailed during the fermentation process due to positive interactions between yeast and bacteria, contributing to the consumption of carbohydrates and the production of metabolites such as organic acids, ethanol, and bioactive compounds.

KEYWORDS: Fermentation; Bacteria; Yeasts; Cocoa.

FUNDING: CNPQ, FAPEMIG AND CAPES

Inhibition of saprophytic microorganisms by endophytic fungi in *Coffea arabica* fermentation

Maria Isabela Arruda Santana1* , Henrique Lemos[2] , Ana Paula de Carvalho Alves[3] , Flávio Meira Borém[3] , Patrícia Gomes Cardoso[1]1

1 Departament of Biology, Federal University of Lavras (UFLA), MG, Brazil.
2 Departament of Agriculture, Federal University of Lavras (UFLA), MG, Brazil.
3 Departamento of Agricultural Engineering, Federal University of Lavras (UFLA), MG, Brazil.
maria.santana2@estudante.ufla.br

The microbial community during coffee fermentation is diverse. The presence of filamentous fungi in this process influences the quality and food safety of the drink. Endophytic fungi are microorganisms that colonize the interior of plant tissues and can produce secondary metabolites of biotechnological interest and have antimicrobial action. The aim of this work was to evaluate the action of an endophytic fungus on saprophytic fungi in fermented coffee. Fruits of *Coffea arabica* cv. Topázio were inoculated with the fungus DIS01, previously cultivated in liquid medium containing parchment as the only source of nutrients. Samples were collected after 0, 24, 48, 72 and 96 hours of fermentation and plated on Potato Dextrose Agar (PDA) medium plus the antibiotic cefotaxime. The experiment was conducted with a Completely Randomized Design (DIC), in triplicates and the control treatment was carried out with coffee fruits immersed in a culture medium with parchment only. The number of the Colony Forming Unit (CFU) was determined, followed by the morphological characterization of fungi. The population of saprophytic fungi varies with fermentation time, being 32.76% lower in 24 hours in coffee inoculated with endophytic fungus compared to the control. 61 different morphotypes were isolated, characterized as the genus *Penicillium, Aspergillus, Cladosporium, Fusarium, Rizhopus* and *Rhizhoctonia*. The variation in the number of saprophytic fungi during fermentation may have been caused by the presence of secondary metabolites produced by the fungus or by variations in the physical/chemical fermentation conditions, such as temperature and pH.

KEYWORDS: Coffee, biological control, filamentous fungi, parchment

FUNDING: Minas Gerais Research Foundation (FAPEMIG), National Council for Scientific and Technological Development (CNPq) and Coordination for the Improvement of higher Education Personnel (CAPES)

Influence of local environmental variables on the community of Arbuscular Mycorrhizal Fungi in *Araucaria angustifolia*

M. Barbosa[1]*, B. Francisco[2], T. Timo[3], P. Ferreira-Filho[1]

[1]Federal University of São Carlos (UFSCar), Sorocaba, SP, Brazil
[2]State University of Western Paraná (UNIOESTE), PR, Brazil
[2]Federal University of São Carlos (UFSCar), Lagoa do Sino, SP, Brazil
*mbarbosa@estudante.ufscar.br

Araucaria angustifolia (Bertol.) Kuntze has an intrinsic symbiotic relationship with arbuscular mycorrhizal fungi (AMF), microorganisms responsible for the acquisition of essential nutrients and tolerance to abiotic stresses, however, the presence or absence of AMF can be influenced by environmental variables of the local landscape. In this work, we determined indicators that can predict AMF composition in areas with the presence of the then endangered *A. angustifolia*. We collected soil and litter samples in eight randomized plots in four different areas in southern Minas Gerais State, Brazil. All AMF of both materials were grouped into morphospecies. We collected data for 27 environmental variables distributed across landscape, forest structure, and soil chemistry and used Pearson's correlation to verify which environmental variables have the greatest potential to explain the composition of AMF. These analyses were performed in the R environment using the DescTools and Corrplot packages. Our analyses resulted in 12 potential predictor variables, namely: distance to the edge, percentage of grasses, shrubby and herbaceous layers, height of shrubby and herbaceous layers, density of araucaria individuals, litter input, diversity of the tree layer, pH, organic matter content and phosphorus content in the soil. Subsequent analyses by Bayesian modeling with evaluation of probabilities calculated in Monte Carlo simulation via Markov chains (MCMC) will be used to obtain random effects models capable of inferring whether the environmental variables collected are modulators of AMF richness and abundance in *A. angustifolia* (alternative hypothesis) or if there is no relationship (null hypothesis).

KEYWORDS: Mycorrhiza, Forests, Bayesian Models, Landscape Ecology.

FUNDING: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

Production of proteases from solid-state fermentation of *Bacillus subtilis* CCMA 0085

N. Ribeiro[1]*, C. Castro[1], C. Silva[1]

[1] Universidade Federal de Lavras (UFLA)- Instituto de Ciências Naturais- Departamento de Biologia- Setor de Microbiologia Agrícola- Caixa Postal 3037-37200-000 – Lavras, MG- Brasil.
*nayribeiro2325@gmail.com/nayara.ribeiro2@estudante.ufla.br

Enzyme production by microorganisms has broad application in various industrial sectors due to its biological and biodegradable nature. This study evaluated the proteolytic enzyme production of *Bacillus subtilis* CCMA 0085 through solid-state fermentation (SSF) utilizing sugarcane bagasse as a substrate. Enzyme production was monitored at different fermentation times (24, 48, 72, 96, and 120 hours). The experimental design was completely randomized with three replications per treatment, totaling 15 plots. In SSF, an inoculum of 10^7 CFU mL-1 of fermentative liquid media was added, maintained under agitation at 35°C for 24 hours. The culture was homogenized in 20 grams of dry and sterile sugarcane bagasse in polypropylene bags, incubated at 35°C for the proposed times. Samples were extracted in distilled water and kept under agitation in an ice bath for 1 hour. Subsequently, they were filtered, centrifuged and subjected to enzymatic quantification and determination of total proteins for spectrophotometry, and then the specific activity of proteases was obtained. The enzymatic determination results were subjected to analysis of variance and the averages were compared using the Scott-Knott test at a significance level of 5%. It was observed that the 24 hour time point showed superior production, with 57 U mL-1. Further identification and characterization of the proteases produced by *B. subtilis* CCMA 0085 may reveal potential applications in different industrial sectors, providing an important alternative to conventional chemical processes at low cost.

KEYWORDS: bacteria, enzyme production, sugarcane bagasse, fermentation time, protease characterization, low-cost application.

FUNDING: COORDENAÇÃO DE APERFEIÇOAMENTO DE PESSOAL DE NÍVEL SUPERIOR (CAPES)

Nematicidal Effect of *Bacillus subtilis* CCMA 0085 Crude Extract via Solid-State Fermentation

N. Ribeiro[1]*, C. Castro[1], C. Silva[1]
1 Universidade Federal de Lavras (UFLA)- Instituto de Ciências Naturais- Departamento de Biologia- Setor de Microbiologia Agrícola- Caixa Postal 3037-37200-000 – Lavras, MG- Brasil.
*nayribeiro2325@gmail.com/nayara.ribeiro2@estudante.ufla.br

Nematodes can damage plant roots in agricultural crops, decreasing their yield. Although chemical nematicides are commonly used to control them, they may pose environmental and human health risks. This study evaluated the nematicidal potential of *Bacillus subtilis* CCMA 0085 through solid-state fermentation (SSF) using sugarcane bagasse as a substrate. In SSF, an inoculum of 10^7 CFU mL-1 of fermentative liquid media was added, maintained under agitation at 35 °C for 24 hours. The culture was homogenized in 20 grams of dry and sterile sugarcane bagasse in polypropylene bags, incubated at 35°C for 120 hours. The crude extract was extracted, filtered and centrifuged. To evaluate the nematicidal potential, the crude extract, denatured crude extract (boiled) and distilled water (control) were tested in a completely randomized design with three replications per treatment, totaling 15 plots. Each plot consisted of a microtube containing 10 µL of nematodes from the Panagrellus sp. genus and 20 µL of the treatment, maintained at 28 °C in the dark for 24 hours. Live juvenile counts were performed using optical microscopy (4x) to quantify population reduction. The results were subjected to analysis of variance and the averages were compared using Tukey's test at a significance level of 5%. It was observed that the crude extract was superior to the others, with a 22% reduction in the nematode population. Therefore, this discovery provides a basis for future investigations aimed at applying the compound as a sustainable, biodegradable and low-cost alternative for nematode control in agricultural systems.

KEYWORDS: bacteria, nematodes, biocontrol, biodegradable, low-cost application.

FUNDING: COORDENAÇÃO DE APERFEIÇOAMENTO DE PESSOAL DE NÍVEL SUPERIOR (CAPES)

Isolation and characterization of bacteriophages for different species of *Klebsiella* spp.

J.J.F. Soares[1]*, T.F. Lopes[1], A.J.C. Santos[1] , J.D. Da Silva[1], R.S. Dias[1], S.O. De Paula[1]

[1]Federal University of Viçosa, Department of General Biology; Molecular Immunovirology Laboratory, University campus - Viçosa, MG - Brazil 36570-900
*jose.j.soares@ufv.br

Bacteria of the *Klebsiella* genus are commensals in the gastrointestinal tract of humans and animals. However, when there is an imbalance in the intestinal microbiota, particularly in patients with compromised immune systems, it can lead to severe conditions such as pneumonia, bacteremia, and cholecystitis. Many *Klebsiella* species are already resistant or multidrug-resistant to common antibiotics. In this scenario, phage therapy—an therapeutic approach that utilizes bacteriophages (viruses that infect bacteria)—emerges as a promising alternative. Phages are widely found in the environment and highly specific to the bacteria they infect during their infection cycle. Thus, this study aimed to isolate phages for three different *Klebsiella* species: *K. oxytoca* (strains ATCC 13182 and 7170-2), *K. pneumoniae* (strains KPC+, 144, 58B, KP13, and 5B), and an isolate of *K. variicola* (26E). Environmental samples collected from pig farming and domestic sewage were used for viral isolation, followed by biological and genomic characterization of the virus. As a result, two phages were isolated for K. oxytoca ATCC 13182, one for 26E, one for KP13, one for 7170-2, and one for 583. Based on successful isolations, the Ko22 phage that infects ATCC 13182 was chosen for biological and genetic characterization. Results showed its ability to inhibit biofilm formation, stability in the pH range of 5-10, a latency period of 20 minutes, and a burst size of 22 PFU/cell. This study demonstrates that phage therapy has the potential to be an interesting tool for enhancing alternatives in pathogen control.

KEYWORDS: *Klebsiella*, phage therapy, antibiotics, antimicrobial resistance.

Selection of *Papiliotrema laurentii* strains with improved xylose uptake by adaptative laboratory evolution

J.S.C. Fonseca[1]* , E.L.M. Almeida[1] , R.Z. Ventorim[1] , W.B. Silveira[1]

[1] Department of Microbiology, Universidade Federal de Viçosa, Minas Gerais, Brazil
*juliana.carneiro@ufv.br

The increasing demand for biodiesel has boosted the use of alternative oil sources, including the lipids produced by oleaginous yeasts using lignocellulosic hydrolysates as carbon source. Since these hydrolysates contain inhibitors generated during the pretreatment step, such as acetic acid, we have previously used Adaptive Laboratory Evolution (ALE) to select *Papiliotrema laurentii* UFV-I strains tolerant to acetic acid. Among them, the ATS I stood out, however the xylose uptake rate is lower compared to the parental strains. As such, we conducted herein a new round of ALE to enhance xylose consumption and lipid production for potential use in lignocellulosic biorefineries. We conducted the ALE experiments using two xylose concentrations (20 and 30 g/L) in Yeast Nitrogen Base (YNB) minimal medium, with triplicates of each concentration. After 90 days, we characterized the evolved strains, referred to as Xylose Uptake Improved Strains (XUIS), regarding their kinetic and physiological parameters, as well as acetic acid tolerance. All evolved strains, except XUIS II, maintained acetic acid tolerance. XUIS I, III, and IV presented the highest specific growth rates (0.401, 0.373, and 0.399 h-1 , respectively), representing increases of 57.25, 56.47, and 46.27% compared to ATS I. XUIS I displayed the highest final biomass yield, lipid titer, lipid productivity, and specific xylose uptake rate (80.15% higher than ATS I), highlighting its biotechnological potential. Therefore, XUIS I is the most promising strain due to its improved xylose consumption, acetic acid tolerance, and oleaginous phenotype.

KEYWORDS: xylose uptake, oleaginous yeast, lignocellulosic hydrolysates

FUNDING: FAPEMIG, CNPQ

Analysis of intrisecic defects of *Coffea arabica* cv. Topazio inoculated with endophytic fungus

Maria Isabela Arruda Santana[1]* , Henrique Lemos[2] , Ana Paula de Carvalho Alves[3] , Flávio Meira Borém[3] , Patrícia Gomes Cardoso[1]

1 Departament of Biology, Federal University of Lavras (UFLA), MG, Brazil.
2 Departament of Agriculture, Federal University of Lavras (UFLA), MG, Brazil.
3 Departamento of Agricultural Engineering, Federal University of Lavras (UFLA), MG, Brazil.
maria.santana2@estudante.ufla.br

Endophytic fungi colonize interior plant tissues, synthesizing secondary metabolites with bioactive activities beneficial to their host. The quality of the drink of the coffee is influenced both by the sensorial profile and by defects in raw beans. The aim of this work was to evaluate the physical properties of *Coffea arabica* after fermentation with endophytic fungus inoculation. The coffee samples separated by density were subjected to fermentation for 96h, in different treatments: inoculation with solid DIS01 fungus (InS) and liquid inoculation (InL), solid control (CS), liquid control (CL) and control. The fruits of coffee were placed on a patio for drying to a humidity level of 11%, followed by resting for 30 days and processing. The quantification of intrinsic defects was carried out in accordance with Normative Instruction no. 8/2003, from the Ministry of Agriculture, Livestock and Food Supply. Statistical analysis was performed using SISVAR®. The defect analysis demonstrated an increase in defects in relation to the control, especially in the CS treatment, with the burnt defect being the most frequently found. The defects can be attributed to the failure to completely remove the mucilage during fermentation, interfering with the drying process, facilitating the growth of spoilage bacteria and fungi. It is concluded that the presence of the DIS01 changes the physical properties studied, it necessary to complement the studies to understand the origin of the changes and the influence on the final quality of the drink.

KEYWORDS: Fermentation, coffee, saprophytic microorganisms, burnt defect

FUNDING: Minas Gerais Research Foundation (FAPEMIG), National Council for Scientific and Technological Development (CNPq) and Coordination for the Improvement of higher Education Personnel (CAPES)

Phosphate rock-solubilizing Bacillus, a promising resource to enhance the efficiency of phosphate fertilizers

Ádila Natália França de Almeida[1], Gladys Angélica Apaza-Castillo[2], Gislaine Cristina Barro[1], Renan Fantine[2], Maria Carolina Quecine Verdi[2], Paulo Sérgio Pavinato[1]

[1]Departament of soil science, Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba-Brazil.
[2]Genetics of Microorganisms Laboratory "Prof. João Lúcio de Azevedo", Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba-Brazil.
* adilaalmeida@usp.br

The low efficiency of phosphate fertilization in tropical soils demands the search for sustainable and economically viable strategies to increase phosphorus (P) availability for crops and improve the efficiency of phosphate fertilizers. Therefore, this study aimed to select and identify strains of *Bacillus* spp., either isolated or in consortia, based on their ability to solubilize phosphate rock. For this purpose, an in vitro bioassay was conducted with 40 strains of *Bacillus* spp. using SP medium supplemented with low-solubility Bayovar phosphate rock, and P solubilization capacity was determined using the molybdenum blue colorimetric method at 882 nm. After selection, potential consortia were formed among the elite strains through an antibiosis assay to observe antagonistic activity between them, and their ability to solubilize P was determined as described previously. For identification, genomic DNA was extracted using the DNeasy® Blood & Tissue kit (Qiagen®) and the 16S rDNA was amplified using the primers PO27F and R1387. As selected elite strains MGB289 (A) and MGB3314 (B) solubilized 20.97 mg kg-1 and 15.66 mg kg-1 of P, respectively, while the consortium composed of strains MGB3314 + MGB289 + RZ2MS13 (CX) made available 21.03 mg kg-1 of P, compared to the control (0.74 mg kg-1). These were identified as MGB289 (*Priestia aryabhattai*), MGB3314 (*Priestia megaterium*), and RZ2MS13 (*Bacillus* toyonensis). The screening of elite microorganisms for phosphate rock solubilization represents an advance in sustainable strategies to increase the efficiency of phosphorus use in tropical soils.

KEYWORDS: Phosphorus; Microorganisms; Screening; Consortium.

Effects of Archaea-Bacteria Consortium on Maize under salinity stress

J.P Ventura, G. V. Lacerda Júnior, P. I. Fernandes Júnior, I. S. Melo*

1 Embrapa Environment, Jaguariúna, SP, Brazil
2 College of Agriculture "Luiz de Queiroz", University of São Paulo, Piracicaba, SP, Brazil
3 Embrapa Semi-arid Region, Petrolina, PE, Brazil
*itamar.melo@embrapa.br

Soil salinization is a major cause of abiotic stress worldwide, severely affecting crop productivity and leading to desertification and reduced arable land. To tackle this issue, innovative methods are required to increase plant resilience and strengthen agricultural production, which is vital for global food security. One promising approach involves studying the plant microbiome and identifying microorganisms that can increase salt tolerance and promote growth. Adopting sustainable techniques to enhance crop resistance against abiotic stress is crucial for future food security on a global scale.ing sustainable techniques to strengthen crop resistance against abiotic stress is vital for future food security. Our study aimed to identify key microorganisms associated with *Atriplex nummularia* under saline irrigation in the Caatinga biome. Using microbiome-based 16S rRNA sequencing, we identified two primary microorganisms: *Kushneria* sp. (CMAA 1923), and *Haladaptatus* sp. (CMAA 1911). Greenhouse experiments with saline irrigation demonstrated that these strains, individually and combined, positively influenced maize tolerance. Notably, maize response significantly varied when the archaeon was co-inoculated with the bacterium. Both microorganisms effectively reduced Abscisic acid levels and increased Indole-3-acetic acid. Nutrient uptake was notably higher in treatments with *Haladaptatus*, even when both archaea and bacteria were present. *Haladaptatus* showed potential in enhancing potassium uptake. We also assessed gene expression responses, observing distinct reactions to inoculation. Bacteria notably increased PIP gene response (zmPIP1-1), while archaea significantly boosted ZmLea3 gene expression. This study underscores the potential of archaea and bacteria to modulate hormonal and genetic responses uniquely, offering a promising avenue for enhancing maize salt stress resilience.

KEYWORDS: *Haladapatatus* sp., Salinity, Consotium, Gene expression

Assessing enzyme activity in soils amended with fresh and industrially composted sewage sludge

MENDONÇA, RAFAEL SANTANA[1], OLIVEIRA, L.P.C. [1], REGITANO, J.B.[1]*

[1] Department of Soil Science, University of São Paulo (USP) – Luiz de Queiroz College of Agriculture (ESALQ) (P.O BOX 9, 13418-900, Piracicaba – São Paulo State, Brazil
* Correspondent author: regitano@usp.br

Sewage sludge (SS) is a byproduct from Wastewater Treatment Plants and holds potential for agricultural use due to its rich organic matter source for soils. However, the proper disposal of SS is challenging due to the high volumes generated and the potential contaminants in its composition. Thermophilic composting appears to be an effective approach to manage SS, reduce contaminants, and generate a stable material, but the effectiveness of this treatment on an industrial scale is still lacking. Measuring enzyme activity of SS-amended soils is a sensitive form to determine whether composting was beneficial to microbial activity and to nutrient cycling enhancement in soils. Therefore, this study aimed to evaluate enzyme activity in soils amended with fresh and industrially composted SS. For this, the amendments were placed in a sandy soil alongside a control group with no soil amendments, and betaglucosidase (βG) and dehydrogenase (DHA) activities were evaluated. βG showed higher activity when fresh SS were applied within the soil due to its higher labile-C content, however, composted SS-amended soils also enhanced βG compared to the control group, even with more stable-C content due to the composting process. DHA activity is related to intact bacterial cells, and it was enhanced by composted SS amendments (600 µg TPF g-1 soil 24h-1), whereas fresh SS-amended and no-amended soils showed lower values (480 and 370 µg TPF g-1 soil 24h-1, respectively). In conclusion, the stabilization promoted by industrial thermophilic composting can enhance microbial activity in amended soils.

KEYWORDS: Biosolid, organic matter, microbial activity, soil functions.

FUNDING: CNPQ AND FAPESP

Industrial composting of sewage sludge mitigates mobile genetic elements genes in amended soils

MENDONÇA, RAFAEL SANTANA[1], OSTI, J.F.[1], LEAL, R.M.P.[2], REGITANO, J.B.[1]*

1 Department of Soil Science, University of São Paulo (USP) – Luiz de Queiroz College of Agriculture (ESALQ) (P.O BOX 9, 13418-900, Piracicaba – São Paulo State, Brazil
2 Goiano Federal Institute of Education, Science and Technology, Campus Rio Verde (P.O BOX 66, 75901-970, Rio Verde – Goiás State, Brazil
* Correspondent author: regitano@usp.br

Sewage sludge (SS) is an urban waste generated in massive quantities and holds potential for agricultural use, however, SS must be adequately treated before soil application due to its pollution potential. Amongst the emerging contaminants, the mobile genetic elements genes (MGEs) are primary facilitators in the global spread of antimicrobial resistance and are commonly found in SS. MGEs from Class-I-Integron are anthropogenic pollution indicators and are considered a priority for environmental monitoring, especially in soils. Thermophilic composting emerges as a promising approach to decrease MGEs, but the effectiveness of this treatment on an industrial scale is still lacking. Therefore, this study aimed to assess the relative abundance of Class-I-Integron genes in different textures soils amended with fresh and industrially composted SS. For this, the amendments were placed in a sandy and clay soils alongside a control group with no soil amendments. MGEs were identified using high-throughput quantitative PCR, and their relative abundances were assessed in relation to bacterial 16SrRNA genes. Class-I-Integron genes were identified in all treatments and soils, however, fresh SS-amended soils showed a relative abundance 260 and 390 times greater for the clay and sandy soil compared to the control group, respectively. Moreover, composted SS amendments did not significantly affect Class-I-Integron genes within both soils. Therefore, fresh SS amendments can lead to a notable increase in Class-I-Integron genes within the soil. However, composting showed to be an effective way to reduce that type of MGEs, indicating that industrial-scale thermophilic composting is effective in reducing risks of antimicrobial resistance.

KEYWORDS: Soil pollution, biosolid, resistome, horizontal gene transfer.

FUNDING: CNPQ AND FAPESP

Impacts of the bacteriophage application on nitrifying sludge

L. Medina[1]*, M. Souza[2], J. Soares[1], S. Paula[1], C. Silva[1].

1 Department of Microbiology, Universidade Federal de Vicosa, Vicosa, Minas Gerais, Brazil
2 CENPES/ Petrobras, Ilha do Fundão, Rio de Janeiro, RJ, Brazil
* lutecia.medina@ufv.br

Biological processes are widely used to remove ammonia in industrial wastewater, in which the ammonia is transformed into nitrogen gas. However, a significant challenge for the oil industry is the efficient biological ammonia removal from high salinity effluents generated during oil extraction from the pre-salt layer. Then, in this study, we used a nitrifying sludge adapted to high salinities to evaluate the impact of phage cocktails on the nitrification process. It worked with two nitrifying sludge, one in the 55 g/L of NaCl and another with 125 g/L of NaCl 25 mL of each sludge was added in 500 mL flasks containing 250 mL of culture medium or produced water; in each flask was added the phage cocktail dosed at the contraction of 107 PFU/ml and under agitation in a shaker (150 rpm) at 30 °C for 35 days. Flasks containing culture medium or produced water added with nitrifying sludge were used as experiment controls. All experiment was done in triplicate. The results demonstrated that the use of these in this phage cocktail did not inhibit the nitrification process, but sludge continued to remove ammonium after the presence of phages. Therefore, the presence of this phage cocktail in hypersaline effluent treatment systems will not pose a risk to the biological ammonia removal process.

KEYWORDS: biological ammonia removal, high salinity, phage cocktail, production water.

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Nitrogen Regulation Pathway Rewires to Flagella Biosynthesis in Aflagellated Mutant Strains of *Pseudomonas fluorescens* SBW25

Astrid Eleanor Altamirano-Junqueira[1]*

1School of Biological Sciences, University of Reading, Whiteknights, PO Box 217, Reading RG6 6AH, United Kingdom
*Email: AstridAltamirano-Junqueira@my.unt.edu

Plant Growth Promoting Bacteria (PGPB) such as *Pseudomonas fluorescens* SBW25 (i.e. gram-negative endophyte) enhance plant growth, increase productivity and confer protection against abiotic stress and pathogens. Therefore, the application of inoculants containing PGPB on crops enhances agricultural efficiency for increasing yield, decreasing environmental pollutants (e.g. chemical fertilizers, pesticides) and lowering food cost production (i.e. reducing the volume of fertilizers applied). Swimming motility is an important trait for a successful plant-host colonization, underscoring the importance of studying flagella regulation to gain insights into its impact on effective plant colonization. Flagella biosynthesis is controlled by the master flagella regulator FleQ, which activates the expression of the flagella operon. In the absence of FleQ, bacteria become aflagellated, resulting in colonies that slide on soft agar. The NtrBC two-component system regulates the expression of genes involved in stress responses and nitrogen depletion. Under nitrogen starvation (i.e. inoculating SBW25ΔfleQ into soft agar M9 modified medium: 10 mM ammonium, glucose 20 % w/v). The parent strain SBW25ΔfleQ (Fla-, Visc+), restored swimming motility. Whole Genome Sequencing (WGS) and targeted sequencing of segments of ntrB, ntrC showed primarily a T97P mutation within the PAS domain of NtrB. Hence, the mutant NtrB assumes the function of FleQ, impacting nitrogen metabolism. Consequently, an evolved strain, termed FleQS4, underwent a mutation in a predicted ammonium transporter PFLU_RS08590, thereby enhancing ammonia metabolism as evidenced by its growth curve (i.e., M9 modified media: 10 mM Ammonium, glucose 20 % w/v). In this research, 12 evolved FleQS strains were obtained.

KEYWORDS: FleQ, NtrBC, PGPB, *Pseudomonas fluorescens*, flagella, experimental evolution, bacterial motility

Exploiting coffee industry by-products for solid-state enzyme production by *Kluyveromyces lactis* B10: a response surface tool.

D. J. M. Abreu[1]*, A. A. D. Silva[1], F. L. J. de Paula[1], S. N. M. Alencar[1], W. F. Duarte[1].

1Department of Biology, Institute of Natural Sciences, Federal University of Lavras, MG, Brazil.
*danilo.abreu@ufla.br

The valorization of coffee by-products as a source of energy and chemical products, via biorefinery or biotechnology, contributes to the circular bioeconomy. The pressing need for sustainable development and the efficient use of resources led to the aim of this work, which was to optimize the production of enzymes through solid-state fermentation using a central composite rotational design (CCRD). Time (3-9 days) and humidity (60-70%) were independent variables. The response variables were total cellulases (Fpase), b-galactosidase and b-glucosidase. Fermentation was carried out using the coffee silver skin and conducted at 28 ºC. The enzyme extract was obtained with 100 mL of distilled water at 250 rpm/25 ºC. Fpase production has shown time and humidity were significant for the linear and quadratic models, which optimization showed that 10 days and 5 hours and 61% humidity obtained 46,66 U/g. For □-galactosidase, only the linear model for time was significant, so the maximum value of 0.2 U/g can be obtained under the same conditions as Fpase. For b-glucosidase, although the enzyme production values increased over time, the independent variables did not influence the significance level applied ($p>0.05$). With this, the maximum optimization of 5.8 U/g of b-glucosidase, the conditions should be 1.75 days/42 hours and 72% humidity. Given these results, it was observed that it was possible to optimize the production of these enzymes and allocate the use of this coffee by-product.

KEYWORDS: Coffee silver skin, Bioeconomy, Hydrolases, Biorefinery.

FUNDING: CAPES AND FAPEMIG

Detection of volatiles and organic acids in sterilized wet fermentations inoculated with yeasts and bacteria

S. J. Martinez[1]*, A. P. P. Bressani[2], D. R. Dias[2], R. F. Schwan[1]

1Biology Department, Federal University of Lavras, Trevo Rotatório Professor Edmir Sá Santos, 37203-202, Lavras, Minas Gerais, Brazil
2Food Science Department, Federal University of Lavras, Trevo Rotatório Professor Edmir Sá Santos, 37203-202, Lavras, Minas Gerais, Brazil
*Corresponding author: silvia292004@gmail.com

Wet fermentations use depulped beans which are fermented with water, several microbial groups take part in this process including bacteria, yeasts, and filamentous fungi. The aimed of this work was to detect volatiles and organics acids produced in sterilized beans fermented under water when inoculated with bacteria and yeasts. Beans (150 g) were autoclaved for 5 min in sealed glass flasks, following by addition of sterilized water (150 g), and starters (CCMA0543 *Saccharomyces cerevisiae*, CCMA0544 *Candida parapsilosis*, CCMA0684 *Torulaspora delbrueckii*, CCMA1203 *Pantoea dispersa*, CCMA1236/ CCMA1238/ CCMA1239/ CCMA1242 Bacillus subtilis, and CCMA1279 *Arthrobacter koreensis*). A GC-MS equipment was used to detect volatiles and a HPLC was used for organic acids. Only yeast CCMA0543 showed a significant quantity of citric acid (2.90 mg/g) and bacteria CCMA1279 (6.52 mg/g) at the end of fermentation. Malic, lactic, and acetic acid were only detected in the inoculation with bacteria. All bacteria and yeasts CCMA0544 and 0684 produced succinic acid expect in the control. The compound 7-methyl-4-octanol was only detected in the bacterial treatments, showing a potential as a marker. Heptadecanol and 4-hydroxy-2-methylacetophenone were detected in the three yeast species starters, having this last important antioxidant properties. Two compounds (guaiacol and ester methyl salicylate) were only detected in the bacterial treatments, the first only found in those inoculated with *B. subtilis* in agreement with the literature. Yeasts as inoculants in wet fermentation were better for volatiles production and in contrast bacteria for acid production.

KEYWORDS: Coffee wet fermentation, volatiles, bacteria, yeasts, organic acids

FUNDING: CAPES, CNPQ, FAPEMIG

Characterization of *Streptomyces* spp. as tolerant to glyphosate-based herbicide and their potential to promote plant growth maize plants (*Zea mays* L.)

Luísa M. Ramos[1]*, Eliane R. Santarém1

1Pontifical Catholic University of Rio Grande do Sul, PUCRS, School of Health and Life Sciences, Plant Biotechnology Laboratory, Porto Alegre, RS,
*E-mail (corresponding author): luisa.ramos99@edu.pucrs.br

The glyphosate, N-(phosphonomethyl)glycine, affects microorganisms by inhibiting the shikimic acid pathway, leading to consequences for the soil microbiome. In addition to possessing characteristics as plant growth promoters, bacteria of the *Streptomyces* genus are recognized for their potential in the biodegradation of agrochemicals. The *Streptomyces* CLV322, CLV337, CLV340, and CLV346, obtained from the rhizosphere with and without the use of glyphosate-based herbicide (Roundup® Original DI), were evaluated for cell viability and metabolite production under herbicide stress at concentrations ranging from 0.002 to 7.2 mg mL-1. Culture medium without the addition of agrochemical was used as the control. The production of siderophores, indole-3-acetic acid (IAA), and phenazines as well as the phosphate solubilization capacity, were the metabolic parameters analyzed. The isolates produced indolic compounds, siderophores, phenazines, and solubilized phosphate at different concentrations of glyphosate, although the addition of glyphosate to the medium was mainly detrimental to the growth of *Streptomyces* isolates. However, CLV322 and CLV337 showed the highest tolerance among the isolates, maintaining its characteristics as plant growth-promoting rhizobacteria (PGPR) even under high stress. The responses of maize plant growth treated with Streptomyces were also evaluated. The *Streptomyces* CLV346 promote root growth in plants under glyphosate culture management. Our results also suggest a potential use of Streptomyces for the biodegradation of glyphosate in contaminated soils.

KEYWORDS: Abiotic stress; Agrochemicals; PGPR; Tolerance to xenobiotics

FUNDING: CNPQ

Efficiency of the PGPR *Streptomyces* CLV179 on promoting the growth of maize plants (*Zea mays* L.) under water deficit

Luísa M. Ramos[1]*, Eliane R. Santarém[1]

[1]Pontifical Catholic University of Rio Grande do Sul, PUCRS, School of Health and Life Sciences, Plant Biotechnology Laboratory, Porto Alegre, RS, Brazil
*E-mail (corresponding author): luisa.ramos99@edu.pucrs.br

The current agricultural practices face several challenges, including abiotic stresses such as drought, especially in arid and semi-arid regions. Rhizobacteria *Streptomyces* spp. are known for their ability to promote plant growth (PGPR) through nutrient mobilization and metabolite production. This study aimed to evaluate the tolerance of the PGPR Streptomyces CLV179 to water stress and its effect on the growth of maize plants cultivated under drought conditions. We assessed the tolerance of CLV179 to water deficit by cultivating it at water potentials of -0.6, -1.0, and -1.7 MPa and by evaluating the production of indole compounds and siderophores. Streptomyces ability to mitigate the deleterious effects of drought on the growth of maize plants was evaluated by cultivating plants under 100% (no stress) and 30% (water stress) of field capacity. The isolate proved viable under a water deficit of -1,0 MPa. Siderophore synthesis increased at -0.6 MPa, whereas the production of indolic compounds was negatively affected by the stress. The response of *Streptomyces*-treated plants to water deficit showed that root colonization by CLV179 increased the root biomass of plants by 30% even under stress, although there was no effect on the root length. Moreover, the bacterium did not affect growth and biomass accumulation of maize leaves. Preliminary results suggest that CLV179 can stimulate the growth of the root system of *Z. mays* under drought stress, potentially by enhancing water and nutrient acquisition.

KEYWORDS: Abiotic stress; Actinobacteria; Drought stress; PGPR

FUNDING: CAPES; PRO-STRICTO (PUCRS)

Diversity of root endophytic fungi from banana plants in Brazil

J. S. Santana[1], J. A. Oliveira[1], F. A. Custódio[2], O. L. Pereira[2]*

1Departamento de Microbiologia, Universidade Federal de Viçosa, Avenida PH Rolfs, s/n, Viçosa – MG
2Departamento de Fitopatologia, Universidade Federal de Viçosa, Avenida PH Rolfs, s/n, Viçosa – MG
*oliparini@ufv.br

The banana is one of the main agricultural crops produced worldwide. This plant harbors an associated microbial diversity that is still little explored. Many studies report that native microbiota can contribute to plant health and development. This study aimed to identify endophytic fungi isolated from healthy banana roots. The fungi were isolated from the roots of healthy banana trees (Prata and Nanica varieties) from an area with a high incidence of Panama disease. The dilution-to-extinction technique was utilized as the isolation method. The isolates were submitted to morphological analysis, using the culture media Potato Dextrose Agar (PDA) and Corn Meal Agar (CMA), and phylogenetic analysis using the Internal Transcribed Space (ITS) region. Phylogenetic analysis was performed by Maximum Likelihood using the IQTree platform on the CIPRES Portal. Approximately 600 endophytic isolates were obtained from healthy banana roots, of which 10 were characterized and identified to the genus level. According to the phylogenetic analysis, among the 10 isolates identified, there are representatives of Orders: Botriosphaeriales, Chaetosphaeriales, Pleosporales, Sordariales, Xylariales, and the genera Acrocalymma, Chaetomium, Codinaea, Cyphellophora, Dichotomopillus, Endomelancaniopsis and Rhexoacrodictys. many of these fungi produces a dark pigmentation, septate hyphae and microsclerotia, the so-called Dark Septate Endophytes (DSE). This study reports for the first time fungal genera that had not been yet reported in association to banana roots worldwide. This study contribues to expand knowledge regarding the endophytic fungal diversity associated with banana roots.

KEYWORDS: Dark septate endophytes; Dothideomycetes; Musa; Sordariomycetes.

FUNDING: CNPq, CAPES, FAPEMIG, Sítio Barreiras.

Temporal changes in symbiotic mycorrhizal traits of Claroideoglomeraceae (Glomeromycota)

L. K. Laurindo[1]*, P. M. Antunes[2], S. L. Stürmer[3]

1Programa de Pós-Graduação em Biodiversidade, FURB, Blumenau, SC 89030-903, Brazil
2Department of Biology, Algoma University,1520 Queen St. East, Sault Ste. Marie, ON P6A 2G4, Canada
3Departamento de Ciências Naturais, Universidade Regional de Blumenau, Blumenau, SC 89030-903, Brazil
*lidia.klestadt@gmail.com

Traits are specific characteristics (e.g., morphological, physiological, phenological) of an organism that can be measured at the individual level. Trait quantification is important to understand the environmental adaptations of the organism and to predict ecological responses to its presence. Phenological trait variation in mycorrhizal symbioses is not well studied. The aim of this study was to evaluate mycorrhizal root colonization in different species of Claroideoglomeraceae over time, and compare it with fungi in the Acaulosporaceae, Gigasporaceae, and Glomeraceae families. An experiment was set up with five species and eight isolates of Claroideoglomeraceae, and two species for each of the other families. Measurements of mycorrhizal colonization were made 21, 28, 42, 56, and 84 days after the emergence of Sorghum bicolor seedlings. We found that root colonization was detected after 21 days for all mycorrhizal families. Maximum levels of colonization were detected at 42 days for Gigasporaceae (30.5%) and Claroideoglomeraceae (55.1%), and at 56 days for Acaulosporaceae (24.2%) and Glomeraceae (80.8%). The abundance of arbuscules tended to increase from 21 to 42 days and then decrease, while that of vesicles peaked at 28 days for Claroideoglomeraceae. In conclusion, there is wide phenological variation in root colonization among AM fungal taxa, with members of Claroideoglomeraceae being more similar to Glomeraceae than to the other families.

KEYWORDS: arbuscular mycorrhizal fungi, Claroideoglomeraceae, life-history traits, root colonization

FUNDING: CNPQ

Identification of pentose transporters in the oleaginous yeast *Papiliotrema laurentii* UFV-1

M. E. Romanizio[1]*, E. L. M. Almeida[1], J. V. M. G. Assis[1], W. B. Silveira[1]

1 Department of Microbiology, Universidade Federal de Viçosa, Minas Gerais, Brazil
*miguel.romanizio@ufv.br

Papiliotrema laurentii is an oleaginous yeast capable of accumulating high amounts of lipids using sugars from lignocellulosic hydrolysates, mainly xylose. Hence, it is pivotal to identify the pentose transporters of *P. laurentii* to develop future metabolic engineering strategies to improve lipid production. Here, we first curated a list of 74 known pentose transporters from Fungi and Bacteria. Then, we conducted a BLASTp of these transporters against the predicted proteome of *P. laurentii* UFV-1 and used an HMM predictor, obtaining 73 possible candidates. Next, we used DeepLoc 2.0 to predict the subcellular localization. To identify which transporters belong to the Major Facilitator Superfamily (MFS), we used CCTOP to analyze the membrane topology and selected proteins with 12 transmembrane domains and cytosolic C- and N-terminal ends. Since transporter sugar affinity is difficult to determine based only on sequence and structure, we aligned these candidates with a list of known sugar transporters using MUSCLE and did a phylogenetic reconstruction on MEGA X. Based on the phylogenetic analysis, five proteins were grouped as xylose transporters. Next, to evaluate which genes were structurally clustered with these transporters, we visualized the linear genome on Jbrowse2 and conducted a BLASTp and an InterPro scanning. We found that the five best candidates were mainly clustered with transcriptional factors, membrane bound proteins and other transporters. In further studies, we will evaluate in vivo the expression of the best candidates on xylose and glucose to validate our in-silico results.

KEYWORDS: xylose transport, lignocellulosic hydrolysate, MFS transporter, non-Saccharomyces

FUNDING: FAPEMIG

Transcriptomics on *Azospirillum brasilense* Ab-V5 reveals role of MBOA in early plant-microbe interactions.

Baatsen J.[1], Hosaka G. K.[1], Mondin M.[2], Kitajima E. W.[3], Azevedo J. L.[1], Quecine M. C.[1]

1Laboratory of Genetics of Microorganisms "Prof. Joao Lucio de Azevedo", Department of Genetics, Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba, Brazil
2Laboratory of Cytogenetics, Department of Genetics, Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba, Brazil
3Laboratory of Electron Microscopy, Department of Phytopathology and Nematology, Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba, Brazil

Root colonization by plant growth-promoting bacteria (PGPB) involves recruiting symbiotic partners from a diverse biosphere. Among PGPB, *Azospirillum brazilense* Ab-V5 and *Psuedomonas protegens* Pf-5 are two potent inoculants renowned for their growth enhancing capacity through production of phytohormones and nitrogen fixation, and by disease suppression respectively. Benzoxazinoids (BXs) have a strong impact on microbiome dynamics in the rhizosphere, therefore we studied the transcriptome of two well characterized PGPB, Ab-V5 and Pf-5 in presence of the relatively stable lactam BX derivative MBOA. We performed RNA sequencing of the total RNA extracts from Ab-V5 and Pf-5, grown statically in liquid cultures for 72 hours in three MBOA concentrations. In Ab-V5, we could reveal the upregulation of a chemotaxis regulatory gene in response to 0.05 mM MBOA while 0.05 mM and 0.50 mM substantially impacted cellular respiration and energy metabolism. The absence of upregulated chemotaxis genes at 0.50 mM and large alterations in expression profiles of primary metabolism related genes, suggested surplus energy was directed towards metabolic adaptation. Interestingly, symbiosis-related gene downregulation occurred in both treatments, leading to reduced biofilm formation, impaired auxin efflux carriers, and varied nitrogen homeostasis. In contrast, Pf-5 showed very little alterations of gene expression. Biofilm formation and chemotaxis were validated by in vitro assays, which additionally uncovered a time dependent-effect of MBOA on Ab-V5 biofilm. Surprisingly, from microscopic analysis we inferred that biofilm on Ab-V5 infected Arabidopsis roots was augmented in MBOA treatment. This study provides insights into how MBOA influences early plant-microbe interactions.

KEYWORDS: PGPB, Benzoxazinoids, chemotaxis, biofilm, symbiosis, transcriptomics, fluorescent microscopy, SEM, root colonization

Root exudation of maize (*Zea mays* L.) is modulated by salinity and colonization by halotolerant *Streptomyces* spp.

Emanuelle P. Gattino[1]*, Felipe D. B. Berleze[1], Eliane R. Santarém[1]

1Pontifícal Catholic University of Rio Grande do Sul, School of Health and Life Sciences, Plant Biotechnology Laboratory, Porto Alegre, RS, Brazil
*Email (corresponding author): emanuelle.g@edu.pucrs.br

Salinity poses a significant threat to global crops, such as maize. Plant-growth promoting rhizobacteria (PGPR) can mitigate salt stress by modulating root exudation, thereby enhancing plant salt tolerance. In this work, we evaluated the efficiency of root colonization by *Streptomyces* spp. and their ability to modulate the profile of root exudates of maize plants (*Zea mays* L.) under saline conditions. Maize seeds were bacterized with isolates CLV45 and CLV179 and subjected to 50 and 100 mM NaCl. Root colonization was confirmed by scanning electron microscopy and re-isolation. Bacterial growth, cell viability, metabolite production, and biofilm formation under stress conditions was also examined. Root exudates from 20-day-old plants were collected in 100 mL of sterile filtered water for 12h under continuous light, and the concentrations of amino acids, reducing sugars, phenolic compounds and flavonoids were quantified. Both isolates proved viable and exhibited production of indolic compounds, siderophores, ammonia, phosphate solubilization, and ACC deaminase under normal and saline conditions. High biofilm production was confirmed in CLV179 under NaCl concentrations, although CLV45 exhibited higher EPS production. Analyses of maize root exudates revealed that salt stress affected the exudation of total phenolic compounds and reducing sugars. Interaction between maize roots-Streptomyces showed that CLV179 modulates the exudation metabolism of total phenolic compounds and amino acids, whereas CLV45 modifies the profile of secondary metabolites, phenolic compounds and flavonoids. Our results suggest the potential of Streptomyces as bioinoculants in agronomic crops under salinity.

KEYWORDS: PGPR, Rhizodeposition, Salt stress, Secondary metabolites

FUNDING: CAPES; PRO-STRICTO (PUCRS)

Acid Phosphatase Activity in Sandy Soil with Different Granitic Powder Dosages

R. L. Oliveira[1]*, N. Andrade[1], C. C. G. Freitas[1], A. M. S. Oliveira[1], D. F. Silva[1], M. S. Vieira[1], F. D. Andreote[1]

[1]Luiz de Queiroz Higher School of Agriculture. University of Sao Paulo
*rl.oliveira@usp.br

Rock powder plays a crucial role in the sustainable management of mineral waste, offering a simultaneous approach to improve soil fertility and reduce agricultural production costs. In this study, we investigated the activity of acid phosphatase at different doses of granite dust, using a Typical Dystrophic Red Yellow Latosol with 15% clay. Treatments consisted of a control (T1) and doses of 1.50 t.ha-1 (T2), 3.00 t.ha-1 (T3), and 5.00 t.ha-1 (T4) of granite dust. Over a period of 300 days, Urochloa brizantha plants were grown in a greenhouse, with collections made every 100 days. We observed a negative correlation between acid phosphatase enzymatic activity, soil phosphorus content, and proteins related to glomalin. Doses of 1.5 and 5.0 t.ha-1 showed average enzymatic activity of 553 and 436 µg p-nitrophenol h-1 g-1 of soil, respectively, while the control registered 459 µg. The dose of 3.00 t.h-1 did not differ significantly from any of the treatments. PCA analysis revealed that acid phosphatase primarily grouped with the 5.0 t.ha-1 doses, while the control treatment distanced itself from acid phosphatase, glomalin, and soil phosphorus. Although all doses positively influenced the accumulated fresh matter of the plants compared to the control, there was no significant difference between them, suggesting that higher doses may not provide additional benefits in fresh matter, despite increasing acid phosphatase activity.

KEYWORDS: Enzymatic activity, Soil Management, Agricultural production

FUNDING: FAPESP

Metagenomic Analysis Fungi Diversity in Experimental Soil

José Alyson Rocha Pismel[1]*, Carlos Henrique Barbosa Santos[2], Luana Alves de Andrade[1] and Everlon Cid Rigobelo[1].

1Agricultural and Livestock Microbiology Graduate Program, School of Agricultural and Veterinarian Sciences, São Paulo State University (UNESP), São Paulo 14884-900, Brazil.
2 Laboratory Agricultural Microbiology, Department of Plant Production, Faculty of Sciences Agrarian and Veterinary, UNESP/FCAV, Jaboticabal, SP, Brazil.
*alysonpismel@yahoo.com

The advent of metagenomic techniques has enabled researchers to investigate microorganisms through culture-independent methodologies utilizing DNA sequencing in various microbial environments. Analysis of sequencing data is crucial for accurately characterizing the entire microbial community, including non-cultivable microscopic organisms. The aim of this study was to employ state-of-the-art DNA sequencing technologies to identify fungi present in soil samples. To achieve this objective, soil samples were manually homogenized, and an aliquot of 1mL or 250 mg was used to extract total DNA. Subsequently, the ITS genes (for fungal analysis) were amplified from the DNA. The amplified fragments were then sequenced on the Illumina MiSeq platform and the sequences were analyzed using Qiime software to identify the microorganisms present in the samples and their respective percentages. These results were compared with those reported in literature to determine the phenotypic characteristics of the identified microorganisms. The results of this study demonstrated that it is possible to identify fungi from nine phyla and organize them taxonomically into 20 different classes, orders, families, and genera. This finding highlights the abundant diversity of these microorganisms in the soil, which are responsible for nutrient cycling, plant defense, and organic matter breakdown. In conclusion, this study underscores the need for more in-depth research on the diversity of fungi in soil, as the metagenomes of these organisms are rich resources for new enzyme genes. These genes may reveal new routes of biosynthesis of secondary metabolites of biotechnological interest by non-isolated species.

KEYWORDS: Fungi, Biotechnology, Metagenomics, Microbiome.

FUNDING: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

Impact of overgrazing-induced desertification on mycorrhizal community in the Caatinga biome of Brazil

Danilo Ferreira da Silva[1]*, Nariane de Andrade[1], Rafael Lima Oliveira[1], Antonio Marcos Miranda Silva[1], Arthur Prudêncio de Araujo Pereira[2], Elke Jurandy Bran Nogueira Cardoso[1]

1Luiz de Queiroz College of Agriculture, Av. Pádua Dias, 11 | Piracicaba/SP | Brazil
2Federal University of Ceará, Av. Mister Hull, Escola de Agronomia – Fortaleza/CE | Brazil
*danilo_ferreira@usp.br

Soil desertification poses a critical ecological challenge in arid and semiarid climates worldwide, leading to decreased soil productivity due to the disruption of essential microbial community processes. Fungi, as one of the most important soil microbial communities, play a crucial role in enhancing nutrient and water uptake by plants through mycorrhizal associations. This study investigated the impact of overgrazing-induced desertification on fungal communities in the Caatinga biome of Brazil. Our assessment was conducted during both the dry and rainy seasons in Irauçuba, Ceará, across three scenarios: 1. Natural vegetation (native), 2. Grazing exclusion (20 years) (restored), and 3. affected by overgrazing-induced degradation (degraded), which were analyzed using genomic DNA sequencing. AMF spores were extracted through wet sieving and decanting of 50 g soil, and taxonomically classified based on the International Collection of Arbuscular and Vesicular Mycorrhizal Fungi (INVAM). Results showed higher arbuscular mycorrhizal fungal (AMF) diversity and abundance in restored soils compared to native soils. Seasonal fluctuations in AMF composition highlighted the dynamic nature of these communities. Restored areas exhibited intermediate clustering between native and degraded sites, indicating transitional community states. Key AMF families like Acaulosporaceae and Glomeraceae were identified as pivotal in mitigating climate change impacts. Grazing exclusion demonstrated potential in restoring fungal communities and improving soil properties. Integration of spore quantification and DNA sequencing provided a comprehensive understanding of AMF community dynamics. This study enhances knowledge of fungal ecology in semiarid ecosystems and underscores the importance of microbial conservation for ecosystem resilience against desertification.

KEYWORDS: Soil desertification, mycorrhizal associations, drylands, climate change.

FUNDING: SÃO PAULO RESEARCH FOUNDATION (GRANTS 2021/14418-3, 2022/07117-0, AND 2016/18944-3)

Application of *Bacillus subtilis* and *Bacillus cereus* in compost

Gabriel Marafão dos Santos[1]*, Carlos Henrique Barbosa Santos[2], Edvan Teciano Frezarin[2], Luana Alves de Andrade[2], Everlon Cid Rigobelo[3]

1Undergraduate student in Agronomic Engineering, State University of Minas Gerais, UEMG, Ituiutaba, MG, Brazil
2Undergraduate student in the agricultural microbiology program, São Paulo State University – Faculty of Agrarian and Veterinary Sciences, UNESP/FCAV, Jaboticabal, SP, Brazil
3Laboratory of Agricultural Microbiology, Department of Plant Production, Faculty of Agricultural and Veterinary Sciences, UNESP/FCAV, Jaboticabal, SP, Brazil
*gmarafao@yahoo.com.br

Composting, a widely practiced technique in modern times, involves the decomposition of waste, whether urban, agricultural, or industrial, by microorganisms found in the environment. This process occurs in two distinct stages: the thermophilic and maturation phases. Considering the benefits that composting can offer to modern agriculture, numerous studies have been conducted to find ways to improve the results of this technique. A study carried out by researchers at the Reunidas da Baggage Group farm applied two species of *Bacillus*, *subtilis* and *cereus*, in a compost mixture that was later used in agriculture. The researchers evaluated the results in terms of organic matter, phosphorus, sulfur, calcium, potassium, and the sum of the bases. The findings revealed that the addition of compost led to a reduction in organic matter content, which could indicate an adverse interaction between the compost adjunct bacteria and the soil organic matter. Moreover, the soil fertility values, including phosphorus, K, Ca, and sulfur, were lower in the compost-treated soil than in the soil without compost and the sugarcane area without bacteria. This suggests that the bacteria may have mineralized the organic matter, reducing its value and making the nutrients more available to plants. However, there is a possibility that the nutrients may have been leached.

KEYWORDS: Growth-Promoting Microorganisms, Increased Productivity, Mineralization, Soil Fertility.

FUNDING: LABORATORY OF SOIL MICROBIOLOGY, FACULTY OF AGRICULTURAL AND VETERINARY SCIENCES, UNESP/FCAV, JABOTICABAL, SP, BRAZIL

Cover crop management affects bacterial diversity and richness in soils from the Brazilian Savanna

G. S. Sousa[1], L. S. Bortolo[1], R. S. Mendonça[1], M. R. Cherubin[1], J. B. Regitano*[1]

1"Luiz de Queiroz" College of Agriculture (ESALQ), University of São Paulo, Av. Pádua Dias,11, 13418-900, Piracicaba, São Paulo, Brazil
*Contact of the correspondent author : regitano@usp.br

Agricultural management in the Brazilian Savanna is challenging due to its naturally acidic and less fertile soils, but significant efforts have allowed for successful agriculture in these areas. However, resource scarcity can lead to losses in soil biological attributes, and cover crop management appears promising as it can increase organic matter and promote element cycling in soil. Nevertheless, its actual contribution as an activator and promoter of soil microbiota diversity is still lacking, as each plant can stimulate the growth of specific populations, enhancing directly or indirectly interconnected ecosystem functions within the soil. Therefore, this study aims to evaluate the soil microbiota of the Brazilian Savanna (Tocantins State) cultivated for 10 years with soybean in consortium with cover crops (6) and pasture as the control group. For this, total soil DNA was extracted, and the 16S rRNA gene sequencing allowed the bacterial community at the genus level assessment. Soil management with cover crops and soybeans increased the number of observed bacterial genera (richness) in the soils compared to the control group (pasture), with averages of 230 and 180, respectively. However, bacterial diversity in the soils was differently affected depending on the cover crop used. The lowest diversity of genera was observed in the control group cultivated only with pasture (3.7), while Marandu (4.1) and Massai (4.2) were able to promote greater diversity in the soils. In conclusion, cover crop management in the Brazilian Savanna can enhance soil diversity and richness; however, the choice of plant species can potentialize such benefits.

KEYWORDS: Cover crop; microbiota; soil.

FUNDING: CAPES

Cover crop management and its effects on Atrazine and Glyphosate mineralization in soils

SOUSA, G.S.[1]; BORTOLO, L.S. [1]; TORNISIELO, V.L.[2] ; CHERUBIN, M.R. [1]; REGITANO, J.B.[1]*

1"Luiz de Queiroz" College of Agriculture (ESALQ), University of São Paulo, Av. Pádua Dias,11, 13418-900, Piracicaba, São Paulo, Brazil
2Centre for Nuclear Energy in Agriculture (CENA), University of São Paulo, Av. Centenário 303, 13416-000, Piracicaba, São Paulo, Brazil
*Contact of the correspondent author : regitano@usp.br

The population growth enhances the demand for food and intensifies land use. Chemical soil management makes agricultural production more effective; however, agrochemicals such as herbicides, depending on their dynamics, can cause environmental damage as they may undergo processes like leaching, surface runoff, adsorption, among others. Remediating these damages is costly and challenging to implement on a large scale, and soil management with cover crops seems a promising alternative since they can accelerate the biotransformation of agrochemicals in the soil. Therefore, the study aims to understand whether the use of cover crops affects the soil microbiota responsible for the ecosystem function of xenobiotic biodegradation. For this purpose, soils managed for 10 years with a mixture of cover crops (5) in association with soybean cultivation were collected in Tocantins State, Brazil. Mineralization function was assessed by the biodegradation of radiolabeled atrazine and glyphosate molecules (14C) over 70 days. It was observed that cover crop management in soil influenced the biodegradation process of the molecules compared to soybean in cover crop consortium, sole soybean, and pasture. However, the accumulation of 14C-CO2 from the atrazine molecule remained <10%, due to its recalcitrant molecule characteristic. Therefore, the difference between treatments was more evident for the glyphosate molecule, where Massai (32%) and Marandu (28.5%) showed positive distinctions. In conclusion, it was evident that soil microbiota plays a secondary role in the dynamics of these agricultural pesticides, with the characteristics of the molecule predominating in its dissipation process.

KEYWORDS: biodegradation; cover crop; radiolabeled; xenobiotics.

FUNDING: CAPES

Optimization of Sugarcane Productivity by Reducing Fertilization and Use of Microorganisms

Dalilla Berlanda Gonilha de Lima[1]*, Edvan Teciano Frezarin[2] Carlos Henrique Barbosa Santos[2], Luana Beatriz Gonçalves[2], Josiane Soares Siqueira[2], Everlon Cid Rigobelo[3],

[1,2] Graduate Program in Agricultural Microbiology, Faculty of Agricultural Sciences and Veterinarians, UNESP/FCAV, Jaboticabal, SP, Brazil
3 Laboratory Agricultural Microbiology, Department of Plant Production, Faculty of Sciences Agrarian and Veterinary, UNESP/FCAV, Jaboticabal, SP, Brazil.
*dalilla.berlanda@unesp.br

The introduction of sugarcane to Brazil by the Portuguese during the 16th century had a profound impact on the country's economy. Today, Brazil is the world's largest producer of sugarcane, which is not only used to produce sugar, but also to generate ethanol. However, excessive use of pesticides and fertilizers has led to the degradation of soil biodiversity. To address this issue, a sustainable alternative is to use biofertilizers. This study aimed to evaluate the effectiveness and appropriate dosage of fertilization combined with the inoculation of a mix of microorganisms, including *Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Herbaspirillum seropedica*, and *Bacillus pumilis*. This study also aimed to propose a reduction in the use of chemical fertilizers to minimize environmental impacts without compromising crop productivity. The study analyzed three results: root and shoot dry matter, and tillers. The study utilized five treatments, with five replicates each, the first of which was 100% fertilization (control), and the others were 100% fertilization + mix, 90% fertilization + mix, 80% fertilization + mix, and 70% fertilization + mix. The results suggest that it is possible to reduce nitrogen fertilization in sugarcane using a mix of microorganisms without causing any damage to the crop in terms of plant height, dry mass, and tiller development at the appropriate time.

KEYWORDS: *Bacillus subtilis, Bacillus amyloliquefaciens, Herbaspirillum seropedica e Bacillus pumilis.*

FUNDING: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001. To the Graduate Program of Agricultural Microbiology of the Faculty of Agrarian Sciences and to the Laboratory of Agricultural Microbiology of the Faculty of Agrarian and Veterinary Sciences of Jaboticabal – UNESP/FCAV.

Edaphic interconnections: mycorrhizae, soil, and fertilization managements in coffee plantations

T.L. Entringer[1]*, G.C. Públio[1]1, T.G.R. Veloso[1], K.M.S. Menezes[1], M.C.S. Silva[1]

1 Department of Microbiology, Universidade Federal de Viçosa, Minas Gerais, Brazil
*thaynara.entringer@ufv.br

Coffee, cultivated in more than 50 countries, ranks the world's second largest commodity in terms of market value. Its cultivation is influenced by factors such as technology, production costs, climate, and diseases. In this context, soil microbial communities, especially arbuscular mycorrhizal fungi (AMF) are crucial in biogeochemical cycles, significantly contributing to both soil and coffee plant health. Leaf rust is one the main threats to coffee plants; many producers treat this disease with fungicides based on copper, which have a broad activity spectrum and less environmental impact. However, high levels of this micronutrient can negatively affect the AMF community. Herein, to assess the influence of macro and micronutrients, including copper, on AMF colonization, chemical analyses were carried out on soils from conventionally and organically managed coffee plantations in Araponga - MG. Data was analyzed using Pearson's correlation tests in the R program. Of all correlations evaluated, mycorrhizal colonization was significantly correlated with the following edaphic variables: pH (rPearson = 0.42; p-value < 0.05), Zn (rPearson = 0.44; p-value < 0.05), Cu (rPearson = -0.46; p-value < 0.05) and S (rPearson = 0.41; p-value < 0.05). Copper levels in the soil were negatively correlated with mycorrhizal colonization. These results highlight the importance of research to develop sustainable practices in coffee production due to the complex interaction between nutrients, microorganisms, and soil health. Organic management might be a sustainable alternative since it provides better soil conditions for the development of a microbiota that is beneficial to the plant.

KEYWORDS: arbuscular mycorrhizal fungi; organic management; micronutrients; macronutrients

FUNDING: FAPEMIG, CAPES, CNPQ

Bacterial diversity in agricultural soil and its potential in maintaining the environment

Edvan Teciano Frezarin[1]*, Carlos Henrique Barbosa Santos[2], Dalilla Berlanda Gonilha de Lima[2], Josiane Soares Siqueira[2], Luana Beatriz Gonçalves[2], Everlon Cid Rigobelo[3]

[1],[2]Graduate Program in Agricultural Microbiology, Faculty of Agricultural Sciences and Veterinarians, UNESP/FCAV, Jaboticabal, SP, Brazil
[3]Laboratory Agricultural Microbiology, Department of Plant Production, Faculty of Sciences Agrarian and Veterinary, UNESP/FCAV, Jaboticabal, SP, Brazil.
*edvan.frezarin@unesp.br

Utilizing metagenomics to understand the genetic and functional diversity of soil microorganisms can provide valuable knowledge for various applications such as sustainable agriculture, remediation of contaminated soils, and the development of biofertilizers, as well as the discovery of new enzymes or compounds of biotechnological interest. Metagenomics can also be employed to monitor modifications in microbial communities in response to environmental disturbances, including climate change or agricultural practices, which can aid in the conservation and management of natural resources. The objective of this study was to employ metagenomics to identify bacteria present in soil samples from an agricultural area in Minas Gerais, Brazil, and to determine their potential in this environment based on the existing literature. Eleven composite soil samples were collected from an agricultural area in Minas Gerais, Brazil, at depths of up to 20 cm. The samples were homogenized, and a 1-milliliter aliquot was used to extract the total DNA. The 16S gene was amplified, and the fragments were sequenced on an Illumina MiSeq platform. The sequences were analyzed using Qiime software to identify and quantify the bacteria present in the samples. This analysis revealed the presence of various bacterial phyla in the soil samples, with Actinobacteriota, Proteobacteria, Acidobacteriota and Firmicutes being the most abundant. Additionally, a wide variety of genera have been observed, including Bacillus, Gaiella, Streptomyces, and Bradyrhizobium, which, according to the existing literature, are commonly found in different environments, including soil, and can play important roles in nutrient cycling, ecological relationships, and soil bioremediation.

KEYWORDS: Ecological Relations, Metagenomics, Soil Microorganisms, Sustainable Agriculture.

FUNDING: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

Effect of Rhizobacteria on Germination and Initial Growth of Tomato Seedlings cv. Micro-Tom (*Solanum lycopersicum* L. cv. Micro-Tom)

Jéssica Maria Israel de Jesus[1]*; Mísia Souza Vieira [1]; Luis Eduardo Aranha Camargo[1]

[1]Universidade de São Paulo, Escola Superior de Agricultura "Luiz de Queiroz", Piracicaba, São Paulo, 13400-970, Brazil
*E-mail:jmijesus@usp.br

Rhizobacteria have great potential for use in agriculture as plant growth-promoting rhizobacteria (PGPR) and in the biological control of plant diseases, thus contributing to increased crop productivity. In this context, the objective was to evaluate the effect of rhizobacteria on the germination and initial growth of Micro-Tom tomato seeds (*Solanum lycopersicum* L. cv. Micro-Tom). The experimental design consisted of randomized blocks composed of five treatments and twenty replications. The treatments consisted of: T0 – Control (Micro-Tom tomato seeds); T1 – *Azospirillum* sp.; T2 - *Bradyrhizobium* sp.; T3 – LGM1 (*Bacillus* sp.); T4 – LGM3 (*Bacillus* sp.). To this end, Micro-Tom tomato seeds were superficially disinfested with 70% ethanol (3 min), followed by a 2% hypochlorite solution (1 min) and 70% ethanol (1 min), and washed three times with sterile distilled water. After disinfection, the seeds were inoculated with each rhizobacteria isolate, mixing for 30 minutes with a one-day inoculum (108 CFU/mL [OD 550 = 0.1]), adjusted with saline solution (0.85%). In the control treatment, the seeds were immersed for 30 minutes in sterilized saline solution (0.85%). After inoculation, the seeds were placed in Petri dishes containing agar-water medium and incubated in a B.O.D at 28°C, with a 10h/light photoperiod, for ten days. The highest germination rates were observed in seeds inoculated with *Bradyrhizobium* sp. and the *Bacillus* sp. isolate (LGM1).

KEYWORDS: *Bacillus* sp.; growth promotion; *Bradyrhizobium* sp.; seed treatment.

Grazing exclusion on functional genes related to nitrogen cycling in the Brazilian Caatinga biome

Danilo Ferreira da Silva[1]*, Amanda Manuelly da Silva Oliveira[1], Mísia Souza Vieira[1], Victor Lucas Vieira Prudêncio de Araujo[1], Arthur Prudêncio de Araujo Pereira[2], Elke Jurandy Bran Nogueira Cardoso[1]

1Luiz de Queiroz College of Agriculture, Av. Pádua Dias, 11 | Piracicaba/SP | Brazil
2Federal University of Ceará, Av. Mister Hull, Escola de Agronomia – Fortaleza/CE | Brazil
*danilo_ferreira@usp.br

Grazing exclusion has been widely employed for soil conservation, especially in semiarid regions. Nevertheless, its effectiveness in alleviating the adverse impacts of overgrazing on essential nutrient cycles, such as nitrogen (N) and phosphorus (P), remains unclear in the Caatinga biome. This study investigates the influence of soil degradation and restoration on microbial functional genes associated with nitrogen cycling in the Brazilian Caatinga biome. Soil samples were collected from native, degraded (attributed to overgrazing), and restored (twenty years of grazing exclusion) areas during both dry and rainy seasons in Irauçuba, Ceará, Brazil. The analysis focused on the abundance of functional genes involved in N cycling, encompassing ammonia oxidation (AmoA), biological nitrogen fixation (NifH), nitrate reduction (NirS, NirK), and nitrous oxide reduction (NosZ). Additionally, concentrations of NH4+, NO3-, and total N were measured. The results revealed significant differences in gene abundance and nutrient levels among the studied areas. Degraded soils exhibited lower gene abundance and N concentrations compared to native and restored soils. Conversely, restoration efforts resulted in increased gene abundance and N concentrations, approaching levels observed in native soils. Notably, the abundance of denitrification-related genes (NirS, NosZ) was elevated in degraded soils, indicating intensified denitrification processes and potential N losses. This study underscores the significance of soil management strategies, such as grazing exclusion, in mitigating soil degradation and preserving microbial functions crucial for nutrient cycling in semiarid ecosystems like the Caatinga biome.

KEYWORDS: Soil desertification, functional genes, drylands, climate change.

FUNDING: SÃO PAULO RESEARCH FOUNDATION (GRANTS 2021/14418-3, 2022/07117-0, AND 2016/18944-3)

Genotoxicity assessment in Allium cepa cells by water and sewage treatment plant sludge, before and after bioaugmentation

A.C.Z. Souza[1]*, M.G.F. Lima[2], M.B.T. Zanatta[3], A. A. Menegário[3], D.E.C. Mazzeo[4], M.A. Marin-Morales[1].

1Department of General and Applied Biology, Institute of Biosciences, São Paulo State University, Avenue 24-A, 1515, Rio Claro, SP 13506-900, Brazil.
2Department of Biology, Federal University of Uberlândia, Ceará street, Uberlândia, MG 38405-302, Brazil.
3Environmental Studies Center, São Paulo State University, Avenida 24-A, 1515, Rio Claro, SP 13506-900, Brazil.
4Department of Biotechnology and Plant and Animal Production, Center for Agricultural Sciences, Federal University of São Carlos, Anhanguera highway, km 174 - SP-330, Araras, SP, CEP: 13600-970, Brazil.
*ana.zullo@unesp.br

During water and sewage treatment, by-products such as water treatment plant sludge (WTS) and sewage sludge (SS) are formed, which can be toxic. The aim of this study was to evaluate the bioremediation of these sludges, associated with the bio-stimulant agents coconut fiber (CF) and spent mushroom substrate (SMS) from Pleurotus ostreatus, using genotoxicity bioassays. The bioremediation experiment was conducted in greenhouses, where samples WTS, SS, WTS+CF, WTS+CF+SMS, WTS+CF+SS, WTS+CF+SS+SMS were evaluated in 2 periods (T1-T2). The genotoxicity bioassay was conducted in triplicate, using Allium cepa seeds exposed to the aqueous extract of the samples, in a BOD incubator, at 22°C, for 120 hours. Reverse osmosis water was used as the negative control (NC), and the herbicide trifluralin was used as the positive control (PC). After this period, slides were prepared with the meristematic region for analysis. The genotoxic potential in A. cepa was estimated by the presence of chromosomal and nuclear abnormalities. In T1, genotoxic effects were observed in WTS and WTS+CF+SMS, while SS, WTS+CF+SS, and WTS+CF+SS+SMS showed no genotoxicity, possibly due to the decrease in mitotic indices. In T2, statistically significant differences were no longer observed, and WTS+CF showed a reduction in genotoxicity. This result may be associated with increased aeration induced by CF and SMS. It is concluded that the addition of CF and SMS was beneficial for bioremediation. However, it is recommended to conduct further monitoring over a longer period to ensure the toxicological safety of the bioremediated compound.

KEYWORDS: Ecotoxicology; Bioremediation; Coconut fiber; *Pleurotus ostreatus*.

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In vitro biological control of *Fusarium* sp. by isolates of *Bacillus* spp.

Maria Eduarda de Carvalho Rezende[1]; Katherine Anders Lins de Carvalho[1]; Jéssica Maria Israel de Jesus[1]; Luís Eduardo Aranha Camargo[1]

[1]Universidade de São Paulo, Escola Superior de Agricultura "Luiz de Queiroz", Piracicaba, São Paulo, 13400-970, Brasil
meduardarezende@usp.br

Several species of fungi from the Fusarium genus cause diseases in plants of economic interest, such as tomatoes (*Solanum lycopersicum*). Fungi of this genus are found in the soil, have saprophytic activity and produce resistance structures, making it difficult to control diseases caused by these phytopathogens. The use of biological control of plant diseases has great potential for application in various pathosystems. In this study, using the in vitro culture pairing technique, the potential to inhibit the mycelium growth of *Fusarium* sp. by two isolates of bacteria from the genus *Bacillus* (LGM1 and LGM3) was evaluated. The bacterial isolates were obtained from tomato plants under field conditions. The experiment was conducted using a Completely Randomized Design (CID) and carried out twice. Thirty Petri dishes were used with PDA (Potato Dextrose Agar) culture medium, where: 10 dishes were used as control treatment (T0), receiving only one mycelium disk (1cm) of the fungus; another 10 dishes to analyze the antagonistic activity of the "LGM" isolate on Fusarium (T1), and finally, 10 dishes for the pairing between the fungus and the "LGM3" isolate (T2). The plates were placed in a B.O.D. at 28°C for 15 days under a 12h/light photoperiod. The mycelial growth of the fungus was measured daily. It was observed that both isolates of *Bacillus* spp. had a fungistatic effect, but the "LGM1" isolate showed a positive effect on the fungus. The results indicate potential for possible studies in the field of biological control.

KEYWORDS: Tomato; Fusariosis; Antagonism; Sustainable management.

Utilization of *Bacillus* ssp. in soil-borne disease control

I.A.S. Pinto[1]*, M.M.S. Guedes[1], C.R. Polaquini[1]

1Biotrop Soluções em Tecnologia Biológica, Santo Antônio de Posse, São Paulo, Brasil
*isadora.amalfi@biotrop.com.br

Phytopathogenic fungi can harm various agricultural crops, causing diseases that affect from roots to storage organs. Research indicates that bacteria of the *Bacillus* genus produce antifungal secondary metabolites, offering a promising alternative to protect cultivated areas. The study aimed to evaluate the in vitro effects of using two different Bacillus-based products in controlling two phytopathogenic fungi, *Ceratocystis* sp. and *Rosellinia necatrix*. The experiment involved three treatments: 1- Control, 2- *Bacillus amyloliquefaciens* (CNPSo3202), *B. velezensis* (CNPSo3602), and *B. thuringiensis* (CNPSo3915), at a dose of 400 mL/ha; 3- *B. pumilus* (CCTB05), B. subtilis (CCTB04), and B. amyloliquefaciens (CCTB09), at a dose of 300 mL/ha. The methodology consisted of placing a mycelium disk in the center of plates containing PDA medium (Potato-Dextrose-Agar), and adding the treatment solution to four paper disks positioned in a cross pattern on the edges of the plates. These plates were kept at 27°C for 10 days. The evaluation measured the mycelial growth radius on the fourth and tenth days after incubation. The experiment had three repetitions per treatment, subjected to ANOVA and Tukey's test, and the control efficiency was calculated using Abbott. As a result, treatment 2 controlled 39.7% and 61.4% of the growth of *Ceratocystis* sp. and *R. necatrix*, respectively. Treatment 3 showed lower control levels, with 22.7% and 26.8%, respectively, indicating that the product in treatment 2 exhibits superior antifungal effects compared to the product in treatment 3, especially against R. necatrix.

KEYWORDS: *Bacillus*, Biological control, Plant diseases, *Ceratocystis* sp., *Rosellinia necatrix*, Phytopathology.

Isolation and characterization of phosphorus-solubilizing, nitrogen-fixing bacteria end AIA production in *Tillandsia* L. (Bromeliaceae)

Josiane Soare Siqueira[1]*, Carlos Henrique Barbosa2, Edvan Teciano Frezarin2, Luana Alve de Andrade[2], Luziane Sales[2], Everlon Cid Rigobelo3.

[1] Graduate Program in Agricultural Microbiology, Faculty of Agricultural Sciences and Veterinarians, UNESP/FCAV, Jaboticabal, SP, Brazil
[2] Laboratory Agricultural Microbiology, Department of Plant Production, Faculty of Sciences Agrarian and Veterinary, UNESP/FCAV, Jaboticabal, SP, Brazil.
*josiane.siqueira@uneps.br

Epiphytic plants are characterized by their ability to grow on trees without any contact with soil and limited access to water and nutrients. Despite the challenging environmental conditions, these plants have unique anatomical, morphological, and physiological characteristics and adaptations that enable their growth in these areas. Furthermore, many studies have demonstrated the role of endophytic microbiota in plant growth-promoting bacteria. These bacteria, which were initially found in the rhizosphere, have various abilities that promote growth and increase productivity in agricultural crops, including phosphorus solubilization, biological nitrogen fixation, and phytohormone production. The objective of this study was to characterize bacteria with growth-promoting abilities associated with plants of the Tillandsia genus that have agricultural potential. To achieve this, 20 plants of the genus *Tillandsia* were collected and disinfected to remove epiphytic microbial communities. Subsequently, the leaves and roots were macerated in saline solution, and the liquid material was diluted and plated on Tryptic Soy Agar (TSA) plates. After five days of growth at 37°C in an oven, the microorganisms were morphologically characterized, isolated, and evaluated for their ability to solubilize phosphorus, fix nitrogen, and produce indole acetic acid (IAA). Among the isolates, three were efficient in all tests and were considered potential plant growth promoters.

KEYWORDS: Phosphorus solubilization, nitrogen fixation, bromeliads, AIA production and growth-promoting bacteria.

FUNDING: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

Lipid production by *Papiliotrema laurentii* ufv-1 from pre-treated sugarcane bagasse hydrolysate

A.C.N. Souza[1], E.L.M. Almeida[1]*, R.Z. Ventorim[1], W.B. Silveira[1]

1 Department of Microbiology, Universidade Federal de Viçosa, Minas Gerais, Brazil
*eduardo.menezes@ufv.br

The environmental crisis caused, in part, by fossil fuels has stimulated the search for sustainable sources such as biofuels. Lignocellulosic biomasses, abundant agricultural by-products, can be used as feedstock for biofuel production. The use of bagasse in bioprocesses requires physical and/or chemical pre-treatment to make the polysaccharides more accessible for enzymatic hydrolysis. The oleaginous yeast *Papiliotrema laurentii* UFV-1 is capable of accumulating lipids from sugars present in lignocellulosic biomasses, such as glucose and xylose. In the present study, we evaluated the lipid production of *P. laurentii* UFV-1 using enzymatic hydrolysate from pre-treated sugarcane bagasse as a carbon source. The sugarcane bagasse was subjected to acid and alkaline pretreatments, with 0.5% (w/v) sulfuric acid and then 1% (w/v) NaOH. The final yield after pre-treatment was 30.2%. Then, we hydrolyzed the bagasse in citrate buffer pH 5.0 with commercial cellulase. The hydrolysate recovered contained 32.16 g/L of total reducing sugars, representing a yield of 26.93%. To assess lipid production, we cultured the yeast in a medium containing 1.0 g/L yeast extract; 0.1 g/L $(NH_4)_2SO_4$; 0.1 g/L NaCl; 0.1 g/L CaCl2; 0.5 g/L $MgSO_4$, and the hydrolysate adjusted to 20 g/L of sugar. After 72 h, the biomass, lipid content and lipid titer were 10.68 g/L, 29.84% (w/w) and 3.19 g/L, respectively. Therefore, *P. laurentii* UFV-1 displays potential to be used in bioprocesses to produce lipids from pre-treated sugarcane bagasse hydrolysate as a carbon source.

KEYWORDS: lignocellulosic hydrolysate; oleaginous yeast; biofuel; microbial oil

FUNDING: FAPEMIG, CAPES, CNPQ

Utilization of *Bacillus* spp. in the in vitro control of foliar diseases in soybean (*Glycine max*)

I.A.S. Pinto[1], M.M.S. Guedes[1]*, C.R. Polaquini[1]

1Biotrop Soluções em Tecnologia Biológica, Santo Antônio de Posse, São Paulo, Brasil
*meronys.guedes@biotrop.com.br

Soybean (*Glycine max*) plays a crucial role in agriculture due to its versatile applications; however, its production is often compromised by diseases resulting in significant losses. A distinctive solution involves the application of *Bacillus* genus bacteria-based products to control these pathogens. These bacteria synthesize antifungal metabolites, providing a promising alternative for use in cultivated areas. The study aimed to assess the control percentages of a Bacillus-based product against different phytopathogens affecting the soybean aerial parts, *Corynespora cassiicola*, *Septoria glycines*, and *Colletotrichum* sp. The experiment comprised two treatments: 1- Control, 2- *Bacillus* pumilis CNPSo3203, *Bacillus* velezensis CNPSo3602, and *Bacillus subtilis* CNPSo2720, at a dosage of 300 mL/ha. The methodological approach involves placing a mycelium disk in the center of plates containing PDA medium (Potato-Dextrose-Agar) and applying the treatment to four paper disks arranged in a cross pattern on the plate peripheries. The plates were incubated in a B.O.D. at 27°C for 14 days. Evaluation measured the mycelial growth radius on the seventh- and fourteenth-days post-incubation. The experiment comprised 5 repetitions per treatment, subjected to ANOVA and Tukey's test, and control efficiency was assessed using Abbott's formula. As a result, Treatment 2 controlled 47.7%, 49.2%, and 59.5% of the plate growth of fungi *C. cassiicola*, *S. glycines*, and *Colletotrichum* sp., respectively. The treatment demonstrated good control levels when assessed in vitro, particularly notable for the control of *Colletotrichum* sp.

KEYWORDS: Bacillus, Biological control, Plant diseases, *Corynespora cassiicola*, *Septoria glycines*, *Colletotrichum*.

Co-inoculation of different *Bacillus* species in seed treatment and its potential to mitigate the negative effects of drought in soybean

Carlos Eduardo Oliveira da Silva[12]*, Itamar Soares de Melo[3]

1PhD student of Agricultural Microbiology - Escola Superior de Agricultura 'Luiz de Queiroz'- ESALQ/USP – Av. Pádua Dias, 11, Piracicaba-SP
2Lead Researcher - Incotec América do Sul Tecnologia em Sementes LTDA. – Rua das Sementes, 291, Holambra-SP
3Researcher - Embrapa Meio Ambiente – Rod. SP-340, Km127,5, Jaguariúna-SP
* carlos.oliveira@incotec.com /carlos.oliveirasilva@usp.br

Seed treatment with exogenous microorganisms is an important tool in integrated management program. This adoption can increase crop yield, protect against soilborne pathogens and improve abiotic stress tolerance, mainly related to water deficience in soybean. Among all the microorganisms which can be applied in soybean seed treatment to mitigate the negative effects of water scarcity, the bacteria of *Bacillus* genera emerges with interesting potential. In this regard, the objetive of this study was to evaluate the effects of *Bacillus velezensis*, *B. siamensis* and *B. pseudomycoides* when applied during seed treatment combined with commercial Bradyrhizobium-based product and its potential to mitigate negative effects of drought in soybean. For this, soybean seeds were treated with three *Bacillus* species combined or not with commercial *Bradyrhizobium* product. Under greenhouse conditions, seeds were sowed in pots with sand and conventional soil with adjusted field capacity of 40% during the entire test. The experiment was conducted for 35 days and at the end of this period, it was evaluated the dry and fresh mass of root and shoot, lenght of root and shoot, root volume and photosynthetic pigments. Based on the results obtained, it was possible to conclude that application of *Bacillus pseudomycoides* combined with commercial Bradyrhizobium-based product has potential to be used in soybean seed treatment to mitigate the negative effect of drought. However, more studies must be performed to clarify this hipothesis.

KEYWORDS: Soybean, *Bacillus*, Seed, Inoculation

Bacterial strains producing lignin peroxidase and with potential for SEQUENCE© bioremediation

Evelyn Tavares Ruas [1], Natália Sarmanho Monteiro Lima[2], Eliana G. Macedo Lemos[3]

[1]Undergraduate student in Biological Sciences at Unesp/FCAV, email: evelyn.ruas@unesp.br;
[2]Postdoctoral researcher in Agricultural Microbiology at Unesp/FCAV, email: natalia.sarmanho@unesp.br;
[3] Professor. Department of Agricultural and Environmental Biotechnology, Unesp/FCAV, email: eliana.lemos@unesp.br

The use of agricultural pesticides is one of the main ways to maintain crop health, but due to various factors such as dosage errors in application, soil type, and rainfall density, these compounds end up becoming persistent in the environment. Lignin peroxidase (LiP) is an enzyme that degrades lignin and also has the potential to degrade xenobiotics. This study used the laboratory's bacteria bank to find strains with LiP enzymatic activities and potential for bioremediation of the herbicide SEQUENCE©, composed of S-Metolachlor and Glyphosate, a systemic herbicide for post-emergence application on weeds. In a previous assay, we analyzed the growth of 34 isolates at concentrations of 0.5, 1.0, 2.0, and 5.0% (v/v) and selected 11 bacteria that grew at all herbicide doses. These bacteria were inoculated at different doses to get a more precise idea of their resistance, namely: 1, 2, 6, and 12 L/ha. The identification of LiP activity was performed on solid LB medium supplemented with 0.025% methylene blue. Only isolate eight showed an inhibition halo at the concentration of 12 L/ha. For LiP activity, the 11 bacteria showed transparent halos, indicating potential for lignin peroxidase production. Therefore, the resistance of these isolates to the herbicide and the presence of this enzyme activity suggest that LiP may be one of the agents of this resistance.

KEYWORDS: Glyphosate, S-Metolachlor, lignin peroxidase, bioremediation

Exploring Microbial Diversity in Iron Caves of Southeastern Pará

Amanda Manuelly da S. Oliveira[1]*, José Augusto P. Bitencourt[1], Gisele Lopes Nunes[1]

1Vale Institute of Technology, Belém, Pará, Brazil
*amandamanullyoliveira@gmail.com

The southeast region of Pará stands out for possessing a unique ecosystem due to its vast biodiversity and the abundant presence of natural underground cavities in iron formations within its territory. These cavities exhibit special characteristics such as annual thermal stability, low air circulation, high relative humidity, considerable concentrations of guano, high metal content, and are classified as oligotrophic. These attributes significantly influence the structure and dynamics of microbial communities associated with these environments. Thus, this study conducted a survey of taxonomic diversity and investigated the structure of microbial communities present in iron caves of the Carajás FLONA, using a 16S rRNA approach. Environmental DNA was extracted from soil samples from nine cavities; DNA libraries were constructed for the 16S rRNA gene and sequenced using the Illumina platform. According to the results, approximately 50% of the generated OTUs were taxonomically identified at the genus level, where possible. The prominent phyla were Actinobacteria, Proteobacteria, and Acidobacteria. It was observed that one cavity exhibited a low alpha diversity index compared to the other cavities studied in this work, which may have been influenced by the absence of animals (bats, pollinators, and seed dispersers) affecting the flow of organic matter within the caves.

KEYWORDS: microbiology, caves, carajás, metabarcoding.

Selection of Antagonistic Actinobacteria to Soybean Phytopathogenic Fungi

R. C. A. Marçal[1]*, J. L. Teixeira[1], S. P. Lira[1]

1ESALQ-USP, Av. Pádua Dias, 11 - Agronomia, Piracicaba - SP
* rafaelamarcal@usp.br

Soybean phytopathogens cause more productivity losses every year, also, the diseases chemical control resistance being increasingly common. Moreover, there is a growing demand for biological inputs, such as microorganisms and their metabolic by-products, which demonstrate effectiveness in combating phytopathogens, especially for large crops, such as soybeans. Therefore, the objective of this work is to select isolates of actinobacteria with antagonistic potential to fungi that cause disease in soybeans *Macrophominia phaseolina* and *Cercospora kikuchii*. For this, 20 strains of actinobacteria were tested against two phytopathogens. In 90x15 Petri dishes containing PDA medium, actinobacteria, from pure culture, were inoculated with a 1 cm streak. After 48 hours, the phytopathogens were inoculated at the opposite end with 1 cm ø discs. The evaluation was carried out when the control treatment colonized the entire plaque. The inhibition area was measured using the IMAGEJ program and the percentage of inhibition was measured by dividing the inhibition area by the total area of the plate. Among the 20 actinobacteria strains, A5.1, A15A, and A15B showed inhibition of 94%, 91%, and 87%, respectively, for *C. kikuchii* and 82%, 88%, and 89% for *M. phaseolina*. For inhibition between 50 and 80%, 8 actinobacteria stood out for *C.kikuchii* and 7 strains against *M.faseolina*. Therefore, strains A5.1, A15A, and A15B were selected for tests with secondary metabolites and in vivo tests.

KEYWORDS: *Streptomyces* spp., biological control, *Glycine max*, secondary metabolites

Microbial Growth of Soil Isolates from the Cerrado Mineiro: A Novel Classification Approach

A. Fernandes[1], K. Paiva[1], S. Fernandes[1], A. Castro[1], P. Fontes[1] and M. Santana1*

1 Department of Agricultural Microbiology, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil.
*mateus.santana@ufv.br

Microbial communities exhibit diverse growth capacities, yet a clear method for distinguishing fast, moderate, or slow-growing microorganisms is currently lacking. Here, we propose a methodology capable of classify bacteria according to growth speed. Bacteria (n=244), previously isolated from the Brazilian Savana in on oligotrophic medium (VL55), were inoculated into an R2A medium at pH 5.5 using the stab inoculation method, with four replicates per plate. The plates were incubated for five days at three different temperatures (25°C, 28°C, and 37°C) and evaluated every 3 hours. Escherichia coli strain D45 was used as a growth reference, where isolates exhibiting growth equal to or faster than E. coli in at least three replicates were considered fast-growing organisms; those with growth equivalent to or slower than E. coli after 12 hours were considered moderate; and 24 hours later, slow growth was observed. Isolate diameters were measured to assess the correlation between development and incubation temperature. In this study, 190 isolates showed fast growth, 40 moderate, and 14 slow. Furthermore, out of the 45 genera analyzed, only 6 had all isolates displaying the same growth pattern, namely Moraxella, Pandoraea, Calidifontibacillus, Prescottella, Sphingobium, and Streptomyces. Despite these microorganisms being isolated in oligotrophic medium at 28°C after a long incubation period when exposed to different temperatures and extremely nutrient-rich sources, these isolates were able to exhibit faster growth. Thus, we propose an approach for the classification of the growth of extensive collections of microorganisms.

KEYWORDS: Bacterial isolation, Soil microorganisms, Growth rate, Isolate characterization.

FUNDING: FAPEMIG, CAPES AND DMB.

Unraveling the impact of land-use change on soil fungal microbial communities in the Eastern Amazon

J.A.Mandro1*, A. M. Venturini2, J. B. Gontijo3, S. M. Tsai1

1Cell and Molecular Biology Laboratory, Center for Nuclear Energy in Agriculture, University of São Paulo, Piracicaba, SP, Brazil.
2Department of Biology, Stanford University. Stanford, CA 94305, USA.
3Department of Land, Air and Water Resources, University of California. Davis, CA, USA.
*email: jessica.mandro@usp.br

Previous studies in the Amazon have shown that anthropogenic land-use changes alter the abiotic and biotic properties of the soil, including bacterial and archaeal communities. However, little is known about the effects on other taxonomic groups, such as soil fungi. Thus, we evaluated the impacts of land-use change on the fungal communities in soils of primary forest, pasture, and secondary forest during the dry and rainy seasons in the Eastern Amazon. Soil samples were DNA-extracted and evaluated through ITS sequencing. The sequences were analyzed in R Studio using the DADA2 and UNITE database, followed by FUNGuild for functional prediction. We obtained 1530 amplicon sequence variants (ASVs) from fungi and the total community was impacted by land use and season. Although most ASVs were assigned as unclassified, we found seven trophic modes and 63 distinct life modes among them, which were also impacted by environmental factors. Saprotrophic ASVs were the most representative, especially in the pasture, as a result of land-use changes. We detected 15 different phyla of fungi, in which the most abundant (above 1%) in all land uses and seasonal periods were Ascomycota, Basidiomycota, and Rozellomycota. Interestingly, diversity indexes of the ASVs increased after the conversion, except for richness, which was affected by the interaction between land use and season. However, the lower values in the secondary forest indicated that the fungal structure may not have been recovered together with forest restoration. Thus, the results showed that land-use change is a preponderant factor influencing soil fungal communities.

KEYWORDS: Amazon rainforest, Forest-to-pasture conversion, Forest recovery, Soil fungal communities, ITS sequencing.

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Increasing quality of wet coffee by lactic acid bacteria and yeast co-inoculation

N.N. Batista[1]*, D.M.J. Cassimiro[1], H.C. Fonseca[2], J.A.O. Naves, D.R. Dias[1], R.F. Schwan[1]

1 Federal University of Lavras, Trevo Rotatório Professor Edmir Sá Santos, CEP 37200-900, Lavras, MG, Brazil
2Institute of Agricultural Sciences, Federal University of Minas Gerais, Avenida Universitária, CEP 39404-547, Montes Claros, MG, Brazil
3GN Trading Company Ltda, Varginha, MG, Brazil
*ndianara.batista@gmail.com

The post-harvest processing of coffee fermentation has a role in forming flavor, aroma, and other attributes related to the final product's quality. This study aimed to evaluate inoculating the lactic acid bacteria Lactiplanti*Bacillus* plantarum CCMA 1065 and the yeasts Saccharomyces cerevisiae as starter cultures on wet coffee fermentation using the SIAF method (self-induced anaerobiosis fermentation). Population of L. plantarum, and S. cerevisiae were monitored by qPCR. Malic, citric, acetic, lactic, and succinic acids contents were evaluated during the different fermentations by high-performance liquid chromatography. Sensory analysis was evaluated with the Specialty Coffee Association protocol. In spontaneous fermentation, higher concentrations of S. cerevisiae (approximately 6 log10 cells/mL) was detected at beginning fermentation. L. plantarum showed the best growth with 36 h of fermentation with/out inoculation. Lactic acid concentration increased during fermentation and higher concentration were detected after 72 h of fermentation (Control :11.42 g/kg and Co-inoculation: 11.84 g/kg). The spontaneous fermentation coffees were not classified as special (79 points). The inoculated fermentation enhanced coffee quality that reached a score above 80, intensifying sweetness, acidity, and body. Co-inoculation showed high acidity (6.75) and body (7.5). Furthermore, some sensory descriptors stood out in the aroma of the coffee drink, such as wine and red fruits. Honey was the most intense flavor. Therefore, whole coffee fruit processed via wet using SIAF method and co-inoculated with yeast and lactic acid bacteria is an alternative for improving wet fermented coffee quality and obtaining coffee beverages with a different sensory profile.

KEYWORDS: SIAF method, Coffee fermentation, Sensory descriptors, Co-inoculation

FUNDING: CNPQ, FAPEMIG AND CAPES

Production of lignocellulolytic enzymes by *Bacillus subtilis* CCMA 0085 in solid fermentation

T. Torres[1]*, N. Ribeiro[1], C. Castro[1], C. Silva[1]

1 Universidade Federal de Lavras (UFLA) -Instituto de Ciências Naturais– Departamento de Biologia- Setor microbiologia Agrícola — Caixa Postal 3037-37200-000 – Lavras, MG -Brazil.
*tainatorres1@gmail.com/taina.torres@estudante.ufla.br

The production of enzymes from microorganisms has extensive application in various industrial sectors, due to its versatility and natural biological and biodegradable production process. The objective of this work was to perform an optimization of *Bacillus subtilis* CCMA 0085 for the production of three lignocellulolytic enzymes, such as carboxymethylcellulase; manganese peroxidase and xylanase through solid state fermentation using sugarcane bagasse as substrate. A central composite rotational design (DCCR) was carried out to optimize the enzyme production using pH and temperature as variables, totaling 11 expggerimental tests. The inoculum was standardized at (colocar concentração) and the incubation occured for 120 hours. Subsequently, the crude extract was obtained, enzyme activity and total proteins were quantified. The best results for each specific enzyme activity and culture condition were, respectively: CMCase (Assay 1: pH 6 at 42°C, specific activity 9.99); MnPase (Assay 4: pH 3.9 at 35°C, specific activity 165.31); Xylanase (Trials 3, 10 and 11: pH 8.1; 7.5; 6 to 35; 30; 35°C; enzymatic activity 0.79 U/mL). Therefore, it is concluded that in this study, the best conditions to produce CMCase are pH 6 at 42°C; MnPase is 3.9 at 35°C; Xylanase pHs were 8.1; 7.5 and 6 at 30 and 35°C.

KEYWORDS: Enzyme. *Bacillus* subtilis. Fermentation. Design

FUNDING: CAPES

Acaricidal Activity from *Streptomyces* sp against *Tetranychus urticae*

B. Souza[1], P. Fontes[12], M. Assis[1], G. Feletto[1], P. Medrado[1], D. Bazzolli[12] *

1 Programa de Pós-graduação em Microbiologia Agrícola, Laboratório de Genética Molecular de Bactérias – LGMB - Instituto de Biotecnologia Aplicada à Agropecuária -Bioagro, Universidade Federal de Viçosa, Viçosa Minas Gerais, Brasil.
2 Departamento de Microbiologia, Universidade Federal de Viçosa, Viçosa Minas Gerais, Brasil.
*dbazzolli@ufv.br

Tetranychus urticae Koch is an agricultural pest that inflicts significant economic damage on crops, including soybeans. Streptomyces spp is known for production of secondary metabolites production of secondary metabolites of wide biotechnological application, which includes agricultural practices. This study investigated the use of Streptomyces sp as alternative to conventional chemical pesticides for controlling the T. urticae Kock. Soybean leaves infested by T. urticae were collected, fragmented, and distributed on Petri dishes, each one representing one of the treatments: 1. Streptomyces suspension (cells + supernatant – 14 days of cultivation); 2. Streptomyces sp free broth – Sfb (supernatant – 14 days of cultivation) and 2. spores suspension 2.5x106 CFU.mL-1 in saline solution. After the inoculation with treatments mite counts were conducted over a 120 -hour period. The results revealed a significant reduction in mite populations following treatment application, with the cell-free broth and spore suspension treatments proving effective, achieving reductions of 90% and 98%, respectively, compared to the control. The Streptomyces suspension (1) resulted in a complete eradication of mites from the plant tissue. The efficacy may be attributed to the production of primary and secondary metabolites such as chitinases in the cell-free broth and produced during the interaction. All treatments exhibited statistically significant differences compared to the control, underscoring the potential of Streptomyces sp as an effective agent for controlling T. urticae. These findings underscore the importance of conducting further research on the chitinase production profile of Streptomyces sp to develop integrated pest management strategies in agricultural systems.

KEYWORDS: Actinobacteria; two-spotted spider mites; biological control.

FUNDING: MAI DAI CNPQ, SITIO BARREIRAS FRUTICULTURA LTDA; CAPES PROEX AND FAPEMIG.

Streptomyces sp free broth against *Fusarium triseptatum* JSM15: antagonistic activity and stability

M. Assis[1], P. Fontes[12], B. Souza[1], P. Medrado[1], M. Melo[1], D. Bazzolli[12]*

1 Programa de Pós-graduação em Microbiologia Agrícola, Laboratório de Genética Molecular de Bactérias – LGMB - Instituto de Biotecnologia Aplicada à Agropecuária -Bioagro, Universidade Federal de Viçosa, Viçosa Minas Gerais, Brasil
2 Departamento de Microbiologia, Universidade Federal de Viçosa, Viçosa Minas Gerais, Brasil
* dbazzolli@ufv.br

The use of microorganisms as bioinputs has become increasingly relevant in the agro-industrial sector, particularly in banana production, where fusariosis represents a significant challenge. This study aimed to evaluate the antagonistic activity and stability of Streptomyces sp free broth– Sfb (supernatant) against Fusarium triseptatum JSM15. Streptomyces sp was inoculated in ISP-2 broth for 5, 7 and 14 days at 28°C and 150 rpm. Subsequently, the cultures were subjected to centrifugation and filtration (0.22 μm) to obtain cell-free broth. Antagonistic activity against F. triseptatum JSM15 was investigated using Sfb (25% v/v) on PDA agar. The plates were then incubated at 28°C for seven days, followed by a growth inhibition analysis. The stability of Sfb with greater activity against F. triseptatum JSM15 was evaluated at specific temperatures (-20°C, 4°C, 25°C and 37°C) for 120 days. The results indicated that Sfb from Streptomyces sp growth over 14 days inhibited 75% of fungal growth. The most effective storage temperatures were -20°C and 4°C, resulting in 69% and 61% activity, respectively. This research demonstrates that Streptomyces sp growth is stable and has the potential to control F. triseptatum JSM15 in vitro, providing a cost-effective alternative. New studies will be carried out to identify the bioactive compounds produced by Streptomyces sp.

KEYWORDS: Actinobacteria; fusariosis; biological control.

FUNDING: MAI DAI CNPQ, SITIO BARREIRAS FRUTICULTURA LTDA; CAPEX PROEX AND FAPEMIG.

Isolation and characterization of endophytic fungi from Paspalum: Biotechnological potential for plant growth and biocontrol

Isadora Camargo Pedrino[1]*; Jonatas Campanella[2]; Iran Malavazi[2]; Sônia Regina Stephan[3]; Alessandra Pereira Fávero[3]; Paulo Teixeira Lacava[1].

1Federal University of São Carlos (UFSCar), Center for Biological and Health Sciences, Department of Morphology and Pathology, São Carlos, SP, Brazil.
2Federal University of São Carlos (UFSCar), Center for Biological and Health Sciences, Department of Genetics and Evolution, São Carlos, SP, Brazil.
3Embrapa Southeast Livestock, São Carlos, SP, Brazil.
*Contact of the correspondent author: isadorapedrino@gmail.com

The genus Paspalum sp. is found in pastures throughout the Americas, and some species can be used as an alternative to the Urochloa in specific conditions. Paspalum atratum stands out for its adaptation to acidic soils, low fertility, and periodically waterlogging conditions, while P. notatum demonstrates adaptability to dry, flooded, and low-fertility soils. The symbiosis with endophytic fungi plays a crucial role in maintaining these plants, providing them with resistance to both abiotic and biotic stresses. In this way, to decrease the dependence on chemicals associated with promoting plant growth and controlling phytopathogens, strategies involve the use of endophytic fungi to stimulate growth and perform biocontrol. This study aims the isolation and characterization of endophytic fungi associated with P. atratum and P. notatum, the evaluation of their potential for inorganic phosphate solubilization (IPS) and the antagonism against phytopathogens. Out 385 fungal isolates, 213 exhibited IPS, with 143 showing high solubilization potential. Among these, 36 demonstrated significant results about controling Colletotrichum sp. and Bipolaris sp. mycelium growth, with notable inhibition from 90% to 70%. The genus identification of the isolates was performed by ITS region sequencing revealing genera such as Aspergillus sp., Ceratobasidium sp., Colletotrichum sp., Curvularia sp., Fusarium sp., Microdochium sp., Neosartorya sp., Penicillium sp., Phoma sp., and Talaromyces sp. This pioneering study describes specific fungi genera as endophytes of the Paspalum sp. genus, unveiling their potential for inorganic phosphate solubilization, as well as their antagonistic capacity against the phytopathogens Colletotrichum sp. and Bipolaris sp.

KEYWORDS: Endophytic fungi; Biological control; Plant growth promotion; Inorganic phosphate solubilization.

FUNDING: FAPESP (Grant No. 2020/11315-6) and Embrapa Southeast Livestock.

Analysis of the effectiveness of azo dye degradation by the fungus *Pyricularia oryzae*

L. Peres[1]*, R. Montagnolli[1].

1Federal University of São Carlos; Rodovia Anhanguera km 174 - SP-330 - Araras - SP
*lauramunhoz@estudante.ufscar.br

Azo dyes, prevalent in textile industry processes, pose significant environmental and health risks due to their toxicity and persistence. Their complex synthetic structure renders them resistant to conventional degradation methods, necessitating specialized treatment of effluents and contaminated soils. This study investigates the efficacy of biodegradation and biosorption of Congo Red dye by various strains of *Pyricularia oryzae*, a fungus known as phytopathogenic in agriculture, yet with potential for environmental remediation. Through a series of experiments conducted in both liquid and solid media, the decolorization of Congo Red dye at different concentrations was assessed. While visualizing halos indicative of dye degradation proved challenging in solid medium due to the rapid growth and intense pigmentation of the fungus, discernible halos were observed at a concentration of 10 mg/L. In both solid and liquid media, substantial decolorization was achieved at concentrations of 10 and 50 mg/L, with diminished activity observed at 100 mg/L, suggesting a concentration-dependent response. Furthermore, the study elucidated the fungus's ability to function effectively across a range of dye concentrations, exhibiting heightened efficiency at lower concentrations. This finding underscores the potential of *Pyricularia oryzae* in mitigating azo dye contamination, with implications for sustainable soil and effluent treatment practices, whereas its prospection could be viable in agricultural biodiversity settings. In conclusion, *Pyricularia oryzae* emerges as a promising candidate for the degradation and adsorption of azo dyes, offering a viable solution for the remediation of contaminated environments.

KEYWORDS: biodegradation; synthetic dyes; discoloration; bioremediation.

FUNDING: CNPQ - NATIONAL COUNCIL FOR SCIENTIFIC AND TECHNOLOGICAL DEVELOPMENT (PIBITI).

Production of the acaricidal fungus *Hirsutella thompsonii* in submerged fermentation

L. A. Moreira[1]*, A. B. Neto[1]

1São Paulo State University, faculty of pharmaceutical sciences, Araraquara - SP
*lucas@solatusbio.com.br

Some insects and arachnids can negatively affect global agricultural production, and one of the most used measures to mitigate them is the adoption of chemical pesticides in crop management. However, the use of these substances can cause enormous damage to the environment and human health, which has encouraged a gradual replacement by biological-based products. These have a relevant property, which is entomopathogenic activity, that is, they are capable of causing diseases and/or death of arthropods and insects via their colonization. One of these microorganisms that has gained prominence is the fungus *Hirsutella thompsonii* (Fisher) (Hypocreales: Ophiocordycipitaceae) which has high efficiency against a variety of mites, but its multiplication is challenging as it is a sensitive and slow-growing fungus. Thus, the objective of this research is to evaluate the best cultivation parameters for the filamentous fungus H. thompsonii, aiming at greater production of viable cells through submerged cultivation. For this, the fungus was cultivated in different treatments over the course of 720 hours, evaluating the best sources of carbon, nitrogen, the combination between them and the presence of inorganic salts, using the serial dilution methodology to quantify viable cells and the result expressed in CFU/mL. The highest production ($5.5x10^8$ ± $2.12x10^8$ CFU/mL) was found after 360 hours of cultivation, when using sucrose, soy extract, KCl, $MgSO_4$, K_2HPO_4, remaining stable for up to 720 hours. In this way, the selected formulation presents the nutrients necessary for the growth and stability of this microorganism.

KEYWORDS: Biocontrol, Hirsutella, culture medium, formulation.

FUNDING: SOLATUS BIOINSUMOS

From Industrial Byproducts to Valuable Metabolites: Colored Yeasts as Efficient Producers of Lipids and Carotenoids

A. T. de Castro[1]*, A. C. de Souza[1], R. F. Schwan[1], D. R. Dias[1]

1Federal University of Lavras, Aquenta Sol, CEP 37200-900, LAVRAS-MG, BRAZIL
*andreisa_tc@hotmail.com

Colored yeast species have the capacity to synthesize lipids and carotenoids, offering significant biotechnological advantages for the production of these industrially valuable compounds. However, the high costs associated with microbial production have constrained its widespread application. Utilizing industrial byproducts as carbon sources emerges as a viable strategy to mitigate these challenges. This study aimed to assess and compare the production of lipids and carotenoids by *Rhodotorula dairenensis* CCMA 945, *Rhodosporidium toruloides* CCMA 2032, and *Cystofilobasidium ferigula* CCMA 1623. The evaluation encompassed conventional media (glucose and pure glycerol) as well as alternative media using sugarcane molasses and crude glycerol. Submerged cultures were conducted in flasks with 150 mL of fermentation medium, incubated on an orbital shaker for 96 hours. Lipid content was determined through chloroform and methanol extraction, expressed as a percentage of dry biomass weight. Carotenoids were extracted using acetone and methanol, with total carotenoid concentration estimated through a spectrophotometric colorimetric assay calibrated against the β-carotene standard. Remarkably, the strains exhibited higher average lipid accumulation (21.2 - 77.98%) and total carotenoid concentration (2.31 - 3.3 µg/mL) when cultivated in crude glycerol. *Rhodosporidium toruloides* demonstrated superior efficiency in the coproduction of both metabolites. These findings underscore the potential of colored yeast species for sustainable and simultaneous lipid and carotenogenic accumulation, particularly when cultivated in crude glycerol, thereby offering promising prospects for their integration into industrial bioprocesses.

KEYWORDS: By- products; metabolites; pigments; yeasts

FUNDING: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) , Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

Contribution of the Biological Institute to the use of bacteria-based bioinputs in agriculture

R.A.S. Satochi[1]*, F.B. Baldo [1], J.G Chacon-Orozco[1], L.G. Leite [1]

1Instituto Biológico
*Raphaelsatochi@gmail.com

Commercial bioinputs often come with high costs and frequently low viability due to challenges in formulation, storage, and transportation. The proliferation of microorganisms on rural properties is increasing due to input scarcity and the pursuit of sustainable practices, offering advantages such as improved field efficacy and sustainable management in agriculture. However, the lack of quality control in the multiplication of bioinputs poses a significant challenge, resulting in contaminated products and/or low concentration. ULRCB/IB plays a crucial role by offering services beyond research, including analysis and production of bacterial inoculants with guaranteed purity and concentration. These inoculants, intended for subsequent scaling, can be requested by companies or rural properties, contributing to good practices in On-Farm production of microorganisms. As a reference in microbiological analyses, ULRCB/IB is gaining increasing interest, evidenced by a 600% increase in inoculants produced between 2021 and 2023. In 2023, 53% of the inoculants were allocated to sugarcane cultivation, 41.5% to soybeans and corn, with the remainder distributed among other crops. Goiás stands out as the main recipient (51.9%) of the produced inoculants, followed by São Paulo (13.9%) and Tocantins (12.6%). The bacterial species in the inoculants varied, with *Azospirillum brasilense* and *Priestia aryabhattai* being more commonly used in sugarcane, while *Bacillus subtilis* and *Bacillus amyloliquefaciens* were used in soybeans and corn. Despite the crucial role of IB in disseminating good practices and ensuring the quality of inoculants, the expansion of bioinput self-production in Brazil requires more research and involvement from other institutions to strengthen the practice and enhance the processes.

KEYWORDS: on-farm, multiplication of bacteria, sugarcane, soybeans.

FUNDING: FUNDAG - Fundação de Apoio a Pesquisa Agrícola

Co-encapsulation of *Azospirillum* spp. and *Bradyrhizobium* spp. in alginate hydrogels

A. A. L. Ferrando[1]* E. Guerlinguer1, E. T. Tenório-Neto[2], M. K. Lima-Tenório[2], C. W. Galvão[1], R. M. Etto[1].

1Microbial Molecular Biology Laboratory, State University of Ponta Grossa (UEPG), Ponta Grossa, PR, Brazil
2Laboratory of Multifunctional Polymeric Materials, State University of Ponta Grossa (UEPG), Ponta Grossa, PR, Brazil
*angeloferrando4@gmail.com

In soybean co-inoculation, *Bradyrhizobium* spp. performs the formation of nodules that carry out Biological Nitrogen Fixation and *Azospirillum* spp. stimulates root growth and therefore improve grain productivity. Since plant-beneficial microbial inoculants are often outcompeted by native soil microbes in the field and drought stress is an alarming constraint to plant growth, in the present work we developed an inoculant formulation based on hydrogel (HG) aiming to protect bacteria and reserve water. HG has the ability to retain fluids and control the release of substances. *Bradyrhizobium diazoefficiens* CPAC 7 (SEMIA 5080) and *Azospirillum brasilense* AbV5 were encapsulated separately and combined at the concentration of 1011 CFU/g of HG based on alginate. Then the effect of HG drying and storage time on bacteria viability were evaluated by microdrop using semi-selective media and selective antibiotics (YMA and tetracycline for CPAC 7 and RC and streptomycin and nalidixic acid for AbV5). Drying the HG at 30°C affected differently both genera: when AbV5 was alone or together with CPAC 7, the population decreased by 102 CFU/g. CPAC 7 decreased by 102 CFU/g alone and decreased by 101 CFU/g when together with AbV5. Furthermore, Scanning Electron Microscopy demonstrated the presence of the bacteria on the HG and a study on the swelling capacity in water showed that the alginate HG is superabsorbent; it absorbs 600 times its weight. Our results showed that the developed inoculant formulation guaranteed bacteria viability and water reserve and therefore may provide a better crop performance.

KEYWORDS: Biological Nitrogen Fixation, Biotechnology, Sustainable Agriculture.

FUNDING: CNPq, Fundação Araucária and CAPES

Use of molasses in UASB reactors during the off-season of sugarcane

Karen Cristina Rocha de Oliveira Silva[1]*, Roberto Alves de Oliveira[2], Rose Maria Duda[3]

1Post-Graduate Program in Agricultural and Livestock Microbiology, São Paulo State University (UNESP), School of Agricultural and Veterinarian Sciences, Jaboticabal,SP, Brazil.
2Laboratory of Environmental Sanitation, Department of Rural Engineering, São Paulo State University (UNESP), School of Agricultural and Veterinarian Sciences,Jaboticabal, SP, Brazil
3Faculty of Technology "Nilo de Stéfani", Jaboticabal, SP, Brazil.
* kcr.oliveira@unesp.br / rose.duda@unesp.br

Brazil is the world's largest producer of sugarcane and one of the largest in global ethanol production. Brazilian sugar-energy industries have been using UASB reactors for the processing of sugarcane vinasse, resulting from ethanol production, for the production the effluent and biogas, can be used in fertigation and energy source, respectivily. However, there is a need for studies on the maintenance of anaerobic reactors in the off-season of sugarcane due to the difficulty in restarting the reactors at the beginning of the next harvest. This makes the use of sugarcane molasses an alternative to keep the reactor running and producing biogas. Combined with supplementation with filter cake, these two by-products have great potential for increasing biogas production, being a possible alternative for the sugar-energy sector, as an energy source, in addition to offering an option for waste disposal. In this study, a treatment system composed of two UASB reactors (R1 - 12.0 L and R2 - 5.6 L) was used, operated in the mesophilic temperature range, with a hydraulic detention time of 24 h and an organic load rate of 8.1g COD (L d)-1 in R1. In the system, a maximum removal efficiency of 65% was observed, and biogas production of 36.6 L d-1. In the R1 and R2, the average ratio of intermediate alkalinity to partial alkalinity for 0.16 and the volatile acid values of 282 mg L-1. This indicates a high potential of molasses to maintain the stability of UASB reactor operation in the off-season.

KEYWORDS: Molasses, UASB reactor, Residue, Bioenergy.

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Genetic Nanoswitch in post-transcriptional modulation of arginine biosynthesis for lipid production in microalgae

I. Sandoval-Salazar[1]*, K. Araki[2], F. Vischi Winck[1]*

1Lab. of Regulatory Systems Biology, Center for Nuclear Energy in Agriculture, University of São Paulo, Brazil
2 Lab. Supramolecular Chem. & Nanotech., Institute of Chemistry, University of São Paulo, São Paulo, Brazi.
*isandoval@usp.br; winck@cena.usp.br

The negative control of post-transcriptional regulation shows promise for increasing lipid production efficiency in microalgae; however, conventional techniques offer limited knowledge or require permanent genetic changes. Antisense DNA allows for the transient silencing of genes, enabling more dynamic and precise metabolic investigations. In this study, gold nanoparticles were synthesized and functionalized with sense and antisense DNA oligonucleotides targeting the N-acetylglutamate synthase gene (NAGS). Intracellular uptake of the nanostructures and production of reactive oxygen species (ROS) were monitored during a 12-hour incubation period using ICP-MS and fluorescence, respectively. Real-time gene expression was evaluated via qRT-PCR, while intracellular lipid accumulation was analyzed by confocal microscopy using Nile red dye two hours after induction of gene silencing. Results revealed peak gold uptake at 8 hours, with 155.82 ± 21.66 μg/L. ROS production peaked within the initial 2 hours but stabilized after 4 hours, likely due to the activity of antioxidant enzymes. Gene expression analysis indicated that the control showed a Cq of 20.57 ± 0.09 and the two-hour silencing treatment with Cq of 24.33 ± 1.25, showing 80% gene knockdown. The effect of gene silencing in inducing the transient phenotype, characterized by the formation of intracellular lipid bodies, was observed after 2 hours. In conclusion, antisense DNA emerges as an effective tool for temporally modulating NAGS gene expression, facilitating transient lipid production induction and offering a promising avenue for investigating diverse metabolic pathways of biotechnological and industrial relevance.

KEYWORDS: Post-transcriptional regulation, Antisense DNA, Silence gene, microalgae.

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Endophytic fungi from the São Paulo Coast as potential biocontrol agents of *Colletotrichum falcatum*, the causal agent of red rot.

Ana Gabriela Volpato[1], Dr. Jairo I. Quintana-Bulla[1], Gabriel Luiz Padoan Gonçalves[1], Simone Possedente de Lira[1]*

1Department of Math, Chemistry and Statistics. Luiz de Queiroz College of Agriculture. University of São Paulo – CEP 13418-900, Piracicaba, SP, Brazil
*Contact: splira@usp.r

Sugarcane is a very important crop used to produce sugar and ethanol, being Brazil the largest producer worldwide. Among the diseases that affect it most, is red rot caused by the phytopathogenic fungus *Colletotrichum falcatum*, which may be present since the beginning of cultivation and result in losses of 50-70% of sucrose in the affected stalks. Chemical pesticides are the most common method used in agriculture to control phytopathogens. However, the use of endophytic fungi as biological control agents has gained notoriety due to their ability to produce specialized metabolites with antimicrobial activities. In this context, the aim of this study was to evaluate the growing inhibitory activity of six endophytic fungi strains from the São Paulo Coast, against *C. falcatum*, using the paired-culture antagonist bioassay. All of the fungi tested showed activity against the phytopathogen, with fungi F6 and F9 exhibiting the most potent inhibition. Small scale cultivation on three different media of fungi F2 and F3 was carried out in order to obtain crude organic extracts and their chemical profiles were evaluated by HPLC-UV. We hypothesize that inhibitory activity displayed by the fungi may be due to their specialized metabolites produced, which will be tested in further in vitro bioassays. These initial results showed that endophytic fungi possess promising activity for the biocontrol of *C. falcatum*, indicating that they may become a source of novel biocontrol agents against economically relevant phytopathogens.

KEYWORDS: *Colletotrichum falcatum*, biological control, endophytic fungi, specialized metabolites.

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Use of coffee waste as substrate for the cultivation of *Pleurotus ostreatus* in blocks

A.C.E. Medici[1]*, M.S.B. Paula[1], C.G. Abreu[1], A.A.R. Oliveira[1], E.S. Dias[1]

1Departamento de Biologia, Instituto de Ciências Naturais, Universidade Federal de Lavras, MG-Brazil
*annacarolinamedici@gmail.com

Brazil is one of the world's largest coffee producers, being responsible for around 1/3 of world production. Minas Gerais accounts for around 50% of national production, with a huge production of by-products from coffee production, especially coffee husk (CH) and parchment. Considering the constant growth of mushroom production in Brazil, the use of these by-products as ingredients in the formulations of mushroom cultivation substrates is studied. Knowing the antinutritional or inhibitory properties of the mycelial growth of many fungi that CH presents, authors propose that CH should be boiled, inactivating or diluting these compounds. However, this strategy is not viable for large-scale production systems. As a result, this work aimed to evaluate different procedures for using waste from coffee farming for the production of *Pleurotus mushrooms*. Different approaches were used, from axenic cultivation to cultivation on composted substrates, in different proportions of CH and parchment. For control, substrate formulations known to present excellent production yields were used. The results demonstrated that, for axenic substrates, up to 50% of raw CH can be used and, for composted substrates, the best results were obtained with a maximum of 20% CH. With parchment, it was observed that this by-product can be used pure, with excellent mushroom production. However, pure parchment has low compaction, causing the substrate to disintegrate quickly after the first production flow.

KEYWORDS: coffee husk, parchment, Pleurotus ostreatus, by-products

FUNDING: Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-CAPES

Antagonistic effect of *Chromobacterium* spp. against *Corynespora cassiicola* and *Colletotrichum truncatum* under in vitro conditions

Zumpano, L.F[1]*, Zanotto, A.W[1], Iwanicki. N.S.A[1], Delalibera Jr. I.[1]

1 University of São Paulo, "Luiz de Queiroz" College of Agriculture (ESALQ) Av. Pádua Dias, 11 – P.O. Box 9 – CEP, 13418-900 Piracicaba, São Paulo, Brazil
* luizfelipezumpano@gmail.com

Chromobacterium, a gram-negative, beta-proteobacteria, can produce various metabolites whose effects on phytopathogenic fungi remain largely unknown. This study aimed to evaluate the antagonistic potential of five *Chromobacterium* species against the economically significant phytopathogenic fungi, *Corynespora cassiicola* and *Colletotrichum truncatum*, on TSA plates. The strains C01 (*Chromobacterium paludis*), C02 (*Chromobacterium alticaptis*), 599 (*Chromobacterium sphagni*), 310 (Chromobacterium amazonense), and 1092 (*Chromobacterium subtsugae*) were individually confronted with the phytopathogens on TSA plates, with the application regions standardized using a 3D print mold. Incubation occurred for ten days at 28 °C, and the resulting inhibition zones were measured using ImageJ software. The experiment was conducted twice in time. All strains exhibited the ability to suppress the growth of both phytopathogenic fungi. Significant reductions in fungal growth were observed, reaching up to 53% and 51% for *C. cassiicola* by strains 1092 and C02, respectively, and up to 41% for *C. truncatum* by strain 1092. It is hypothesized that inhibition may occur through the diffusion of metabolites within the agar medium or via volatiles produced by the strains. These findings underscore the potential of *Chromobacterium* strains as a promising alternative for controlling economically significant fungal diseases.

KEYWORDS: biological control, non-sporulating bacteria, phytopathogenic fungi, Gram- negative.

Mycorrhizal colonization in maize roots inoculated with ACC deaminase-producing PGPB under saline stress

D. Gomes[1]*, G. Fracetto[1], M. Lidelias1, C. da Silva1, G. Galvão[1], M. Massena[1]

1 UFRPE, Rua Dom Manuel de Medeiros, s/n - Dois Irmãos, Recife - PE, 52171-900
*diogo.gomes@ufrpe.br

Soil salinity can jeopardize agriculture crop development worldwide. Under salt stress, the plants accumulate ethylene in their tissues, impairing their physiology and growth. This research aimed to analyze the effects of co-inoculation of Plant Growth Promotion Bacteria (PGPB) that produces ACC deaminase inoculated with Arbuscular Mycorrhizal Fungi (AMF) on mycorrhizal colonization in maize under saline stress conditions. The experiment was performed in randomized blocks at 0, 40, and 80 mM contents of NaCl with 5 replications and 12 treatments being: a) co-inoculation of PGPB obtained from the rhizospheric and bulk soil of *Mimosa bimucronata* from a temporary pond in Pernambuco State, Brazil (i.e. tropical semiarid region) and the AMF; b) two controls being one without inoculation and the other with AMF alone, both totalizing 180 experimental units. After 52 days, the roots were collected, washed, and stained and the mycorrhizal colonization was analyzed. Regarding mycorrhizal colonization, the co-inoculations with 28-10+AMF, 43+AMF, 52+AMF, 70+AMF, and 85+AMF were significantly different ($p<0.05$). Altought the colonization rate decreased at 80 mM, there was a increased by 34% and 24% when used the bacteria 43 and 85, respectively. There was an increase of 13% in the colonization rate compared to all isolates versus AMF control. Therefore, ACC deaminase-producing PGPB enhanced the mycorrhizal colonization in maize under saline stress and can be considered a mycorrhizal helper.

KEYWORDS: Zea mays, Ethylene, Rhizophagus clarus, NaCl

FUNDING: FACEPE, CNPq

Exploration and identification of agriculturally relevant bacteria with multifunctional potential

J. Huanca-Juarez[1]*, ME. Guazzaroni[2]

1Department of Cell and Molecular Biology, Ribeirão Preto School of Medicine (FMRP) - University of São Paulo (USP) - Ribeirão Preto, SP, Brazil
2Department of Biology, Faculty of Philosophy, Sciences and Letters of Ribeirão Preto (FFCLRP) - University of São Paulo (USP) - Ribeirão Preto, SP, Brazil.
*meguazzaroni@ffclrp.usp.br

The utilization of bioinputs presents a dual advantage in agriculture, fostering plant growth while simultaneously curbing diseases. This approach stands as a promising avenue to reduce reliance on agrochemicals, thereby enhancing soil health and bolstering crop yields. However, despite their promise, certain strains exhibit variable efficacy due to limited field experimentation. To address this, we selected potential multifunctional microorganisms sourced from a soil bacterial isolate bank. Due to their biotechnological potential, we sequenced and assembled the whole genomes of bacterial isolates. Taxonomic classification based on sequence data was performed employing tools such as Centrifuge, MiGA (Microbial Genome Atlas Online), and TYGS (Type Strain Genome Server). Furthermore, we characterized plant growth promoter mechanisms including biofilm formation and phosphate solubilization, and greenhouse experiments assessing their impact on Brachiaria growth under inoculation conditions. In total, 34 isolated bacteria from regions within São Paulo were selected based on the criteria of (i) non-pathogenicity, (ii) absence of reported growth problems, (iii) proven biocontrol activity (previously attested in unpublished work), and (iv) multifunctional potential described in the literature. Of all the selected strains, approximately 70% are potential new species. Overall, plant growth-promoting rhizobacteria are found in various bacterial phyla, such as Actinomycetota, Pseudomonadota, and Bacillota. In this context, it was found that the selected microorganisms belong to these bacterial phyla. Additionally, out of 10 bacterial isolates tested, 3 demonstrated biofilm-forming capacity and at least 1 exhibited phosphate solubilization ability. Therefore, this research highlights the significant potential of bioinputs, particularly multifunctional microorganisms, in enhancing agricultural practices.

KEYWORDS: Bioinputs, multifunctional microorganisms, sustainable agriculture, agrochemicals

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Exploring adaptive responses of a bacterial consortium to lignocellulosic biomass degradation through RNA-Seq

Lucas Amoroso Lopes de Carvalho[12]*, Lúcia Maria Carareto Alves[2], Anna Carolina De Oliveira Souza[2]2, Eliana Gertrudes de Macedo Lemos[2], Camila Fernandes[3], Daniel Guariz Pinheiro[2]

1Programa de Pós-Graduação em Microbiologia Agropecuária, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista (UNESP), Jaboticabal, SP, Brasil
2Departamento de Tecnologia, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista (UNESP), Jaboticabal, SP, Brasil
3Laboratório Multiusuário Centralizado para Sequenciamento de DNA em Larga Escala e Análise de Expressão Gênica, Faculdade de Ciências Agrárias e Veterinárias, LMSeq Universidade Estadual Paulista (UNESP) Jaboticabal, SP, Brasil
*lucas.amoroso@unesp.br

Efficient degradation of lignocellulosic biomass is essential for sustainable bioenergy, reducing reliance on fossil fuels and mitigating climate change. RNA-Seq analysis of bacterial consortia shows how microbes interact with and decompose biomass, identifying efficient natural processors and enzymes. This work aimed to explore a microbial consortium capable of degrading lignocellulosic biomass, highlighting its potential. The consortium was obtained from soil covered with sugarcane bagasse. For targeted-microbial enrichment and adaptation, the initial community were inoculated into BHB medium with sugarcane bagasse, weekly renewed. Aliquots from weeks 2 and 20 were selected for exposition to lignocellulosic source, from which samples were collected on days 5 and 10 for RNA sequencing. The RNA-Seq data processing included quality control, de novo metatranscriptome assembly, and differential gene expression analysis, providing insights into active metabolic pathways. Functional annotation, PCA, and heatmaps were made to enable a comprehensive understanding of transcriptional dynamics across the conditions. The results revealed a significant shift in transcript profiles between weeks, with a decrease in overall transcript expression over time. Key findings include a higher diversity in functional categories in the early stages, particularly in amino acid transport and metabolism, and energy production and conversion. By week 20, there is a notable increase in signal transduction and carbohydrate metabolism, suggesting an adaptation to the biomass degradation process. The study highlights the potential of specific microbial functions in enhancing bioenergy production, emphasizing the role of glycoside hydrolases and carbohydrate metabolism in the breakdown of lignocellulosic biomass.

KEYWORDS: RNA-Seq, Lignocellulosic biomass degradation, Microbial adaptation, Sugarcane.
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Comparative Analysis of Microbial Communities in the Rhizosphere under Different Agricultural Managements

N. Mancine[12]*, G. B. Peruchi[12], H. S. Lima[1], G. T. Santos[13], L. M. N. Massone[12], H. D. Coletta1

[1] Centro de Citricultura Sylvio Moreira, IAC - Cordeirópolis, SP, Brasil
[2] Universidade Federal de São Carlos, Araras, SP, Brasil
[3] Fundação Hermínio Ometto – FHO, Araras, SP, Brasil
*nathaliamancine@gmail.com

Rhizosphere microorganisms intricately interact with host plants, contributing to enhanced plant resilience. Citrus, vital for economy and culture, faces challenges like nutrient scarcity and phytopathologies, prompting a global exploration of its microbiome. Soil microbial community resilience is tied to agricultural management practices. Conventional systems, reliant on agrochemicals, impact microbial communities over time. Conversely, organic management is vital for soil resilience amid rapid environmental changes, displaying greater community heterogeneity. Understanding the interplay between agricultural management and climatic conditions is pivotal for leveraging microbial technologies to enhance productivity in agroecosystems. This study specifically aims to compare rhizosphere microbial communities in Citrus lemon orchards with citrumelo Swingle rootstock in Rio Claro - SP, under both organic and conventional management. Rhizosphere soil samples were seasonally collected, followed by DNA extraction and Illumina NovaSeq 250x250bp sequencing of the 16S rRNA V4-V5 region. Raw data processing and statistical analyses were conducted using the R software, considering two variables: management (organic or conventional) and treatments (summer or winter), corresponding to the collection seasons. α-diversity indicates significance between treatments for each management (Shannon index, $p < 0.05$). In β-diversity, both factors significantly impact community structure (Management: $R^2=0.089$, $P=0.03$; Treatments: $R^2=0.36$, $P=0.001$). In the core microbiome, 39% of genera are shared considering both variables. When focusing exclusively on management, 75% sharing rate is observed, with a prevalence of genera in organic cultivation. This highlights the seasonal influence on the overall composition of the microbiome. These results pave the way for future research, deepening the understanding of microbial dynamics in agroecosystems.

KEYWORDS: Soil Microbiome ; Citrus ; Microbial diversity ; Agricultural management
FUNDING: FAPESP – FUNDAÇÃO DE AMPARO À PESQUISA DO ESTADO DE SÃO PAULO.

Antifungal activity of the *Bacillus velezensis* CNPMS22 volatile organic compounds against the bean pathogen *Fusarium solani*

M. S. Marins[12]*, J. E. F. Figueiredo[2], J. M. Sousa[1], L.H. Pfenning[1], L.V. Cota[2], C.A. Oliveira-Paiva[2].

1Uniiversidade Federal de Lavras (Trevo Rotatório Professor Edmir Sá Santos Universidade Federal de, Lavras - MG, 37203-202).
2 Embrapa Milho e Sorgo (Rod MG 424 Km 45, Zona Rural - Sete Lagoas, MG, 35701-970)
*mikaelysousam@hotmail.com

The dry root rot disease caused by *Fusarium solani* species complex (FFSC) causes yield losses of common beans (*Phaseolus vulgaris* L.). Using fungicides, the primary strategy for disease control generates environmental risks and high costs. Some microbial volatile organic compounds (VOCs) can act as antagonistic weapons against plant pathogens. This study tested the effect of VOCs produced by *Bacillus velezensis* (CNPMS22) to inhibit the *F. solani* (CML4255) growth in vitro. CNPMS22 was grown in TSB at 28 °C for 48 h and agitated at 120 RPM. Subsequently, 20 µL of cell suspension adjusted to 108 CFU mL-1 was evenly spread in the PDA medium and incubated at 28 °C for 48 h. Five millimeter-diameter discs of CML4255 were removed from the edge of colonies of 7-day-old cultures and placed in the center of another plate. The plates containing the fungus were inverted over the antagonist plates, sealed with parafilm, and incubated at 28 °C for ten days. Control samples were prepared with a non-inoculated plate. The mycelial area was measured using ImageJ software, and the percentage reduction was calculated. The pathogen F. solani (CML4255) exhibited a growth area of 9.86 cm2 in control samples, while in plates exposed to VOCs, the mycelial growth was 0.73 cm2, representing a growth reduction of 92.56%. Previous results also demonstrated the ability of CNPMS22 to antagonize CML4255 in a double-paired culture. The result revealed the potential of CNPMS22 as a biocontrol agent against F. solani, an important phytopathogen of common bean and other crops.

KEYWORDS: biological control, fungal disease, FFSC.

FUNDING: Capes. CNPq. Embrapa Milho e Sorgo. Finep.

Non-lactic probiotic beverage enriched with microencapsulated red propolis: microorganism viability and physicochemical characteristics

Dirceu de Sousa Melo[1], Iara Ferreira[1], Marly Silveira Santos[2], Disney Ribeiro Dias[3], Carolina Oliveira de Souza[2], Carmen Sílvia Favaro-Trindade[4], Lorena Silva Pinho[4], Rogeria Comastri de Castro Almeida[2], Karina Teixeira Magalhães-Guedes[2] and Rosane Freitas Schwan[1]

1Post-Graduate Program in Agricultural Microbiology, Department of Biology, Federal University of Lavras, Lavras 37200-900, MG, Brazil
2Post-Graduate Program in Food Science, Federal University of Bahia (UFBA), Rua Barão de Jeremoabo, 147–Campus Ondina, Salvador 40170-115, BA, Brazil
3Post Graduate Program in Food Science, Department of Food Science, Federal University of Lavras, Lavras 37200-900, MG, Brazil
4Faculty of Animal Science and Food Engineering, University of São Paulo, Av. Duque de Caxias Norte, 225, CP 23, Pirassununga 13535-900, SP, Brazil

This work aimed to develop a non-dairy functional beverage fermented with probiotic strains and fortified with Brazilian red propolis (microencapsulated and extracted). The non-dairy matrix consisted of oats (75 g), sunflower seeds (175 g), and almonds (75 g). It was fermented by a starter co-culture composed of *Lactiplantibacillus plantarum* CCMA 0743 and *Debaryomyces hansenii* CCMA 176. Scanning electron microscopy analysis was initially performed to verify the integrity of the microcapsules. The viability of the microorganisms after fermentation and storage, chemical composition (high performance liquid chromatography (HPLC) and gas chromatography coupled to mass spectrometry (GC-MS) analyses), rheology, antioxidant activity, and sensory profile of the beverages were determined. After fermentation and storage, the starter cultures were well adapted to the substrate, reducing the pH (6.50 to 4) and cell count above 7.0 log CFU/mL. Lactic acid was the main organic acid produced during fermentation and storage. In addition, 39 volatile compounds were detected by gas chromatography coupled to mass spectrometry (GC-MS), including acids, alcohols, aldehydes, alkanes, alkenes, esters, ethers, phenols, terpenes, and others. The addition of propolis extract increased the antioxidant and phenolic activity and the presence of volatile esters but reduced the beverage's acceptability. The addition of microencapsulated propolis was more associated with the presence of higher alcohols and had similar acceptance to the control beverage. The combination of a non-dairy substrate, a starter co-culture, and the addition of propolis culminated in a probiotic beverage with considerable potential for health benefits.

FUNDING: CAPES.

Relationship between productivity and nodulation of cowpea irrigated with treated domestic sewage and fertilized with biosolid

Maria Tatiane Leonardo Chaves[1]*, Átala Rebeca da Silva Ávila[1], Douglas Monteiro Cavalcante[2], Kenia Kelly Barros[3], Mario Takayuki Kato[1].

1Federal University of Pernambuco, UFPE, Recife Campus. Pernambuco, Brazil
2Campinas State University, UNICAMP, Campinas, São Paulo, Brazil
3Federal University of PernambucoUFPE, Agreste Campus. Pernambuco, Brazil
*maria.tatiane@ufpe.br

The need to increase food productivity under conventional cultivation practices has contributed to increased water consumption for irrigation and decreased soil microbiota. The use of by-products from sewage treatment in agriculture is a sustainable alternative to reduce this problem. In this experiment, reclaimed water and biosolids were added to cowpea cultivation. The plants were grown in a greenhouse in pots arranged in randomized blocks, with eight treatments and four replications: T1 (W) water; T2 (NPK); T3 (Fe/Zn); T4 (biosolids) composted sludge; T5 (TDS) treated domestic sewage; T6 (TDS+NPK); T7 (TDS + Fe/Zn); T8 (TDS + biosolids). Productivity and the presence of nodules on plant roots were evaluated. T8 stood out in the main evaluated parameters: the grain productivity of 2323.4 ± 1.4 kg ha-1 represented an increase of 96.1% in relation to T1 and 47.8% to T2, and it was statistically different from those of the lowest treatments, T1 and T3; the fresh and dry mass of the leaves was 97 ± 1.22 and 12.9 ± 0.29g, respectively, differing statistically from those of all other treatments, being 59.2% greater than that of T2 and 96% than that of T1. The treatments with the lowest productivities T1 and T3 were the only ones that did not present nodules in their root system, whereas the treatments that used sewage and/or biosolids presented nodules in all plants. It is concluded that the use of TDS and biosolids favored the formation of nodules of nitrogen-fixing bacteria in the roots and increased cowpea productivity.

KEYWORDS: Sewage treatment, composted sludge, nitrogen-fixing bacteria, root system.

Strategies to enhance cell concentration of lactic acid bacteria in batch fermentation

Silva-Neto, J. M.[1], Jesus, G. S.[2], Ceccato-Antonini, S. R.[3]*

1PhD student in Food Science and Technology - PPGCTA - Esalq. Email : josemachadoneto@usp.br
2Master's student in Plant Production and Associated Bioprocesses - PPGPVBA - UFSCar/Araras Campus.. E-mail : gabriel.scanavachi@estudante.ufscar.br
3Faculty at DTAISER/UFSCar/Araras Campus. Email : antonini@ufscar.br
*antonini@ufscar.br

Some lactic acid bacteria are considered probiotics, that is, live microorganisms that, when ingested in correct quantities, provide health benefits. Furthermore, they are important in several areas, especially in silage, providing acidity to the environment and preventing the proliferation of mainly mycotoxigenic fungi. One of the challenges in this context is the production of a concentrated cell culture for commercial purposes. The objective of this study was to evaluate whether the application of two strategies, feeding medium with pH adjustment or cell recycling, after a simple batch cycle, would lead to an increase in the number of cells of three lactic acid bacteria, *Lactiplantibacillus plantarum* (LP), *Lactobacillus buchneri* (LB) and *Pediococcus acidilactici* (PA), in de Man-Rogosa-Sharpe (MRS) medium (pH 7.0), at 35oC, initial inoculum of 108 CFU/mL. For LP, it was possible to increase the optical density (OD) with the medium feeding system with pH adjustment or recycling with new medium from 5 to 11. For LB and PA bacteria, the best system was recycling with new medium, with the OD changing from 5 to 10, or from 4 to 6, respectively. Adjusting the pH of the medium after growth in the feeding system was essential due to the acidity generated in the first growth cycle (final pH ~ 4.5). The increase in bacterial numbers using the feeding or recycling strategies averaged 2 log cycles relative to the initial inoculum. The strategies utilized here showed more significant results with LP and LB.

KEYWORDS: cell concentration, fermentation, microbial growth, probiotics.

Assessment of soil health-linked enzyme activity in three distinct soil management practices

M.B Oliveira, N. Andrade, A.M.S Oliveira, D.F. Silva, N.B Perez, F.D Andreote

[1] Luiz de Queiroz College of Agriculture and Av. Paduá Dias, 11
moisesbarbosa@usp.br

Understanding the relationship between soil use and its biota is crucial for assessing its health. Three types of soil use were analyzed in areas managed by Embrapa Pecuária Sul, in Bagé, RS, belonging to the order of Luvissols in a Cfa climate region. The uses were: (1) Degraded Native Field (CND), (2) Crop-Livestock Integration (ILP), practiced since 2009, and (3) Livestock-Forest Integration (IPF), implemented since 2013. Enzymatic analyses were conducted to observe the influence of these management practices on soil biota. ß-glucosidase enzyme activity in the 0-5 cm layer was lower in CND and different from the other soil uses. In the 5-10 cm layer, IPF was superior and did not differ from ILP. At depths of 10-20 cm and 20-30 cm, there was no statistical difference between soil uses. Between 5 and 10 cm, CND was higher, without differing from IPF. Between 10 and 20 cm, CND was superior and differed from the other uses, which did not differ from each other. Arilsulfatase enzyme activity showed no statistical difference between soil uses in the 0-5 cm and 10-20 cm layers. In the 5-10 cm and 20-30 cm layers, IPF was higher, without differing from CND. PSRG concentration did not differ between soil uses in the first and second layers evaluated. Between 10 and 20 cm, ILP was superior and differed from IPF and CND. In the last layer (20-30 cm), IPF had a lower result and differed statistically from ILP and CND, which were superior.

KEYWORDS: Soil Enzymes, soil Health, soil Management, enzyme Activity.

Evaluation of the development of *Beauveria bassiana* in a culture medium containing chlorine neutralized with sodium thiosulfate

H. O. Ribeiro[1]*, A. B. Q. Aguiar[1], V. A. R. Moreira[2], M. P. Bagagli[1]

1Instituto Federal de Educação, Ciência e Tecnologia de São Paulo, Campus Avaré, Av. Prof. Célso Ferreira da Silva, 1333 - Jardim Europa II, Avaré - SP, 18707-150
2Escola Superior de Agricultura Luiz de Queiroz da Universidade de São Paulo – USP. Av. Pádua Dias, 11 - Agronomia, Piracicaba - SP, 13418-900.
* huany.o@aluno.ifsp.edu.br

The production of bioinputs for on-farm use raises concerns regarding the quality of the products, which is influenced by the use of equipment sanitation techniques, water quality, and the ingredients of the culture media. In order to establish techniques that simplify submerged fermentations and enable the production of efficient and contaminant-free products, specifically targeting small rural producers, this study evaluated the simultaneous sanitization of bioreactors and the treatment of water used for preparing the culture medium with 200 ppm sodium hypochlorite for 15 minutes, with residual chlorine neutralized using sodium thiosulfate. The model microorganism chosen for this study was *Beauveria bassiana*. The culture medium components were prepared in concentrated form and autoclaved, then mixed with the treated water. A control test using the same culture medium but prepared with sterile water was conducted. Mold counts on PDA agar and pH were monitored. The results demonstrate that the pH did not undergo significant variations, remaining between 6.0 and 7.0 throughout the fermentations. The microorganism cultivated in the control medium exhibited typical development, reaching 109 CFU/mL after 72 hours of fermentation. However, it was observed that Beauveria bassiana did not develop properly in the culture medium prepared with chlorinated water and sodium thiosulfate. After 48 hours of the process, no typical colonies of the microorganism were observed on PDA agar. Instead, the presence of contaminants was noted, indicating that, for this microorganism, this sanitation technique was not suitable.

KEYWORDS: Bioinputs, Sanitization, Beauveria bassiana, On-farm

Production of Fpase, β-galactosidase and β-glucosidase by the yeast *Kluyveromyces lactis* using coffee by-product as substrate

A. Silva[1]*, F. de Paula[1], S. Alencar[1], D. de Abreu[1], W. Duarte[1]

1'Federal University of Lavras. Institute of Natural Sciences. Agricultural Microbiology Sector.
* adriele.silva1@estudante.ufla.br

Lignocellulosic substrates of agro-industrial origin have increasingly become the target of biotechnological interest. They are rich in precursors necessary for the formation of microbial biomolecules. Therefore, there is a growing need for research into the use of these substrates as alternatives to conventional substrates. The objective of this work was to use coffee by-product (PVA - defective beans) as substrate for the synthesis of enzymes and microbial biomass by *Kluyveromyces lactis*. To evaluate the production of Fpase, β-galactosidase and β-glucosidase through solid state fermentation, a DCCR (rotational central composite design) 23 was carried out, where the independent variables were humidity and fermentation time. Fermentation was carried out in solid state at 27 ºC. At the end of fermentation, the yeast maintained a population of 7.66 Log CFU/mL. The combination of 3 days of fermentation and 70% humidity was where the best enzymatic activity of the extracts evaluated for Fpase (7.260 U/g) and beta galactosidase (0.0174 U/g) was observed. For beta glycosidases, the combination of 10.24 days and 65% humidity was where the highest enzymatic activity was observed (0.6503 U/g). Furthermore, the analysis of regression coefficients showed that despite an increase in enzyme yield with increasing fermentation days, no variable had a significant effect at the 95% significance level ($p<0.05$). However, the results of microbial growth and enzyme production show that this substrate has the potential to be used in future research.

KEYWORDS: yeasts, lignocellulosic substrates, fermentation, biomolecules

FUNDING: Capes, CNPQ and FAPEMIG

Activity of the enzymes Arylsulfatase and Acid Phosphatase in Brachiaria plants with two types of rock powder inoculated with *Bacillus subtilis*

E.H. Rocha[1], G.M. Castilho1[1], M.O. Barbosa[1], N.T. Komori[1], D.M. Silva[1], F.D. Andreote[1]

[1] Luiz de Queiroz College of Agriculture and Av. Paduá Dias, 11
eloisarocha@usp.com

The use of rock powder as an alternative to fertilizers proves to be a beneficial technique, as it provides nutrients slowly to plants. *Bacillus subtilis* can solubilize rock powders, making potassium available to the plant and can increase the enzymatic activity of the soil. The objective of this study was to evaluate the enzymes Arylsulfatase and Acid Phosphatase activity in Brachiaria plants inoculated with *Bacillus subtilis* and using two types of rock powder. The experimental design was a randomized with five replications in a 3x2 factorial scheme. In the, Brachiaria B. cv. Marandu were allocated em vasos de 5 kg com the following treatments: 1 – Soil only (T1); 2 - Soil without B. subtilis (T2); 3 - Soil with B. subtilis (T3); 4 - Soil + Granite (T4); 5 - Soil + Phonolite (T5); 6 - Soil + B. subtilis + Granite (T6); 7 - Soil + B. subtilis + Phonolite (T7). The first collection was carried out at 37 days after emergence (DAE) and the second collection at 92 DAE. The activity of Acid Phosphatase and Arylsulfatase enzymes did not differ between types of rock powder and control, regardless of whether they were inoculated or not. The correlation of Acid Phosphatase with Arylsulfatase in the second collection expressed positive values in treatment T2 and negative in T5, with R^2 of 0.7 and 0.9, respectively.

KEYWORDS: Microorganisms, solubilizers, potassium, Arylsulfatase.

Solubilization of rock phosphate by yeast strains as an alternative to plant nutrition

R. Silva[1]*, M. M. Rosa-Magri[1]

1 Federal University of São Carlos (UFSCar), Department of Natural Resources and Environmental Protection, Araras, SP, Brazil.*rafaelagomide@estudante.ufscar.br

Recent studies highlight the ability of yeast to benefit plants by solubilizing inorganic phosphates, an important mechanism for improving the availability of this essential nutrient. This study seeks to expand knowledge about the ability of yeast strains to solubilize sources of mineral phosphorus, investigating the potential of yeast strains, specifically *Torulaspora* sp. 5S55 and MCP22, in solubilizing inorganic phosphates, aiming to improve the availability of phosphorus for plants. Carried out in vitro, the first experiment evaluated the ability of these strains to solubilize tricalcium phosphate in medium (NBRIP), through three treatments (T1: medium + phosphate, T2: medium + phosphate + yeast and T3: medium + yeast), in triplicate and at different incubation times (0h, 24h, 48h, 72h). The molybdenum blue colorimetric method was used to determine phosphorus, and the absorbance and pH values were analyzed. Preliminary results indicate that both strains solubilize tricalcium phosphate with similar performance. Treatments with the strains demonstrated higher concentrations of soluble phosphorus with increasing incubation time, exceeding 2,000 µg/mL after 24 hours, while treatments without yeast did not show an increase in the concentration of the nutrient. The reduction in pH in treatments with yeast indicates the production of organic acids, while treatments without yeast did not show a significant reduction in pH. These results indicate the potential of both strains to solubilize phosphorus, therefore future tests will evaluate the concentration of soluble phosphorus and the performance of these strains in vivo, as well as their applicability in experiments with corn plants in a greenhouse.

KEYWORDS: Microorganisms, yeasts, *Torulaspora* sp., Phosphate solubilization.

Is it possible to produce hydrolases by solid-state fermentation using coffee by-products and *Torulaspora delbrueckii*?

F. de Paula[1]*, A. Silva[1], D. de Abreu[1], S. Alencar[1], W. Duarte[1]

1Federal University of Lavras. Institute of Natural Science. Agricultural Microbiology Sector
*E-mail (correspondent author): francielle.paula1@estudante.ufla.br

The agro-industrial residue generated during coffee production can cause environmental damage when improperly disposed of. Therefore, sustainable alternatives for utilizing these by-products are essential. Green coffee grain residue (GCSR) affects beverage quality, and the silver skin obtained during the coffee roasting process is a by-product generated during fruit processing. This study aimed to evaluate the efficiency of these by-products as a carbon source for the synthesis of β-glucosidase and β-galactosidase through solid-state fermentation using strains of *Torulaspora delbrueckii* B14. To assess the production of these enzymes, solid-state fermentation was conducted at 27°C, and statistical differences between treatments were analyzed by a completely randomized design (CRD). Parameters such as residue and fermentation time (1, 3, and 6 days) were evaluated. Statistical differences between residues and fermentation time were observed at the 95% significance level ($p < 0.05$). β-glucosidase enzyme showed higher activity in GCSR, with the highest production on the third day of fermentation (0.43 U/g), while in the silver skin, production remained the same regardless of time. The highest production of β-galactosidase was also observed in GCSR, with a maximum activity of 0.0098 U/g. In the silver skin, there was higher production only on days 1 and 6. However, both coffee industry residues proved to be excellent carbon sources for the synthesis of β-glucosidase and β-galactosidase using *Torulaspora delbrueckii* B14.

KEYWORDS: β-glucosidase, β-galactosidase, sustainability, yeasts.

FUNDING: CAPES, CNPQ AND FAPEMIG

Unraveling the Effects of Natural Seasonal Precipitation on Citrus Rhizosphere Microbial Communities

H. S. Lima[1]*, G. B. Peruchi[1,2], N. Mancine[1,2], G. T. Santos[1,3], L. M. N. Massone[1,2], H. D. Coletta-Filho[1]

[1] Centro de Citricultura Sylvio Moreira, IAC - Cordeirópolis, SP, Brasil
[2] Universidade Federal de São Carlos, Araras, SP, Brasil
3 Fundação Hermínio Ometto-FHO
*hsantiagolima@gmail.com

The rhizosphere represents a deterministic environment driven by plant-microorganism interactions. Microbial communities play a crucial role in regulating several ecosystem services. Consequently, the potential impact of climate change on the structure and function of soil microbial communities could significantly influence agricultural production systems. Understanding the response of microbial communities to seasonal changes is paramount in mitigating these processes. This study aims to evaluate the impact of seasonality on the citrus rhizosphere microbiome. Thus, rhizosphere soil samples were collected from 40 healthy citrus trees across four distinct regions of São Paulo State-Brazil, during both the dry and rainy seasons. The DNA was extracted from each sample and sequenced for 16SrRNA(V4-V5) and ITS1-5F (Illumina/NovaSeq-250bp). Data processing, statistical analyses, and the construction of a co-occurrence network were executed utilizing R and FlashWeave. The average precipitation varied between collections ($P<0.05$), being 16.2 (dry) and 272.7 (rainy) mm/month. Bacterial diversity (6.1±0.8 and 6.0±1.1) was greater than fungal diversity (3.7±0.3 and 3.4±0.6) in both seasons ($P<0.05$), dry and rainy respectively. No significant diversity difference was observed within Bacteria and Fungi domains between seasons. The inter-kingdom co-occurrence network encompassed 8665 and 8913 nodes and 12916 and 12746 edges, being represented by 78.7% and 75.8% positive interactions, dry and rainy respectively. Biomarker analysis identified 26 and 38 indicator species for the dry and rainy seasons, respectively ($P<0.05$, LDA>2). These findings enhance understanding of how natural seasonality impacts rhizosphere microbiomes. Precipitation variation due to seasonality influences microbial interactions, thereby enabling the identification of biomarkers for these distinct environmental conditions.

KEYWORDS: Sasonality, Soil microbiome, Microbial interactions, Dry, Rainy
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Macaw palm roots harbor new taxa of dark septate endophytes

Jaqueline Aparecida de Oliveira[1]*, Fábio Alex Custódio[2], Olinto Liparini Pereira[3]*

1Departamento de Microbiologia, Universidade Federal de Viçosa, Viçosa, Minas Gerais 36570-900, Brazil
2Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa, Minas Gerais 36570-900, Brazil
*jaqueline.a.oliveira@ufv.br; oliparini@ufv.br.

Dark septate endophytes (DSE) are a group of fungi associated with plant roots, known by producing melanized microsclerotia and dematiaceous hyphae. DSEs can improve plant performance through different mechanisms. Despite their importance, DSE were never reported associated to *Acrocomia aculeata* (macaw palm), which is a native Brazilian palm that produces high oil content with various industrial applications. Dark septate endophytes associated with Macaw palm roots were isolated and identified. The dilution-to-extinction technique was utilized for isolation. The DSE isolates were grown on potato dextrose agar and corn meal agar, and specific gene regions were selected for amplification. After sequencing, phylogenetic analysis was performed by Bayesian inference, and some possible new taxa were characterized. A total of 51 DSE isolates were obtained. Our analysis found two possible new species of the genus *Pseudophialophora* (Magnaporthaceae) and a possible new genus belong to the Latoruaceae. The *Pseudophialophora* isolates are distinct species based on phylogenetic analysis, a isolate produces phialidic conidiogenous cells and conidia oblong and smoot. However, it was not possible to observe sporulation of the other Pseudophialophora isolate in the different culture media utilized, as well as the possible new genus. Furthermore, the generated sequences of DSE compared to the sequences available in Genbank database by MegaBLAST tool indicates other possible new taxa belonged to the order Chaetothyriales, due to the percentage of identity of the ITS region being close to 90.0%. This study marks the beginning of access to a hidden taxonomic diversity of DSE on Macaw palm.

KEYWORDS: Acrocomia, Diversity, Funga, Magnapothaceae, Latoruaceae.

FUNDING: COORDENAÇÃO DE APERFEIÇOAMENTO DE PESSOAL DE NIVEL SUPERIOR, BRAZIL (CAPES), FINANCE CODE 001, CONSELHO NACIONAL DE DESENVOLVIMENTO CIENTIFICO E TECNOLOGICO (CNPQ), FUNDAÇÃO DE AMPARO A PESQUISA DO ESTADO DE MINAS GERAIS (FAPEMIG), AND S. OLEUM COMPANY.

Selection of *Papiliotrema laurentii* strains with improved glycerol uptake by adaptive laboratory evolution

A.C.N. Souza[1], J. Soares[1], R.G. Dias[1]*, E.L.M. Almeida1*, W.B. Silveira[1]

1Department of Microbiology, Universidade Federal de Viçosa, Minas Gerais, Brazil
* rodrigo.dias@ufv.br

Glycerol is a by-product of the biodiesel industries; thus, glycerol is a promising carbon source for yeasts in bioprocesses. The oleaginous yeast Papiliotrema laurentii UFV-1 can accumulate high lipid content using sugars as carbon sources; however, it does not grow in media with glycerol as the sole carbon source. Therefore, we selected herein P. laurentii strains able to assimilate glycerol by Adaptive Laboratory Evolution (ALE). The ALE strategy was carried out in two stages: i) in the modified SS2 medium containing glycerol for 102 days, and ii) in Yeast Nitrogen Base (YNB) minimal medium with glycerol as the sole carbon source for 48 days. Prior to ALE, the specific growth rate and final biomass recorded in modified SS2 medium were 0.1633(h-1) and 0.87 (g/L), respectively. After 150 days, the evolved strains grew better than the parental strain. The specific growth rates and final biomasses recorded by ALE1, ALE2 and ALE3 were 0.2786; 0.2536 and 0.2918(h-1) and 3.40; 3.03 and 2.95 (g/L), respectively. Next, we evaluated the oleaginous phenotype in cultivations with glycerol or glucose as carbon sources in SS2 medium with a high C:N ratio (100:1). In glucose, the parental strain had a lipid content of 42.46 ± 0.62% (m/m); meanwhile, the evolved strains lost the oleaginous phenotype regardless of the carbon source. Even though this trade-off, the evolved strains, contrary to parental strain, display the remarkable feature to metabolize glycerol as carbon source. Hence, ALE was a suitable strategy to select glycerol-growing strains. New strategies of metabolic engineering are required to recovery the oleaginous phenotype in the evolved strains.

KEYWORDS: biodiesel industries, oleaginous yeast, oleaginous phonotype, metabolic engineering

FUNDING: CAPES, CNPq, FAPEMIG

Study of the optimization of mineral fertilizers in substrate for the cultivation of *Pleurotus ostreatus*

M.S.B. Paula[1]*, A.C.E. Medici[1], C.G. Abreu[1], A.A.R. Oliveira[1], E.S. Dias1

1Departamento de Biologia, Instituto de Ciências Naturais, Universidade Federal de Lavras, MG-Brazil
*mariliabsantiago@gmail.com

Worldwide, oyster mushrooms rank second among commercially cultivated mushrooms and are responsible for ¼ of the total production of these foods. Belonging to the genus Pleurotus spp, these mushrooms are also classified as "white rot mushrooms" due to their lignocellulolytic capabilities, converting agricultural residues into protein sources. The purpose of this work was to evaluate the effectiveness of mineral supplementation of the Pleurotus ostreatus mushroom cultivation substrate using Plackett - Burman and Central Composite Rotational Design (DCCR) tests. The effect of five variables (dolomitic limestone, gypsum, simple superphosphate, ammonium sulfate and KCl) on the mycelial development of Pleurotus ostreatus were analyzed using the Plackett-Burman statistical design. Based on the results, gypsum, simple superphosphate and ammonium sulfate were selected for the DCCR tests. 13 experiments were carried out with different combinations of selected factors and the results of the linear and quadratic model, however, the results were not significant. The use of mineral fertilizers for macronutrient supplementation requires further studies for the results to be confirmed as there was disagreement between the Plackett-Burman and DCCR results.

KEYWORDS: mineral fertilizers; optimization; Pleurotus ostreatus; Plackett-Burman

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Evaluating the phytostimulating capacity of Brazilian Cyanobacteria on Lactuca sativa L.

Laura C.C. Astudillo[12]*, Mauricio J. Machado[2] Natalia B. Botero[3], Marlon S. Azevedo[2], Timotio N Jesus[2]; Marli F. Fiore[2]

1 Faculty of Agricultural Sciences, National University of Colombia, Palmira, Colombia;
2Center for Nuclear Energy in Agriculture, University of São Paulo, Piracicaba, SP, Brazil;
3School of Applied Sciences and Engineering, EAFIT University, Medellin 050022, Colombia
*lcardonaas@unal.edu.co

The growing urgency of sustainable agriculture is encouraging the exploration of innovative technologies in food production, particularly the utilization of microorganisms to enhance plant growth. In this context, Cyanobacteria emerge as promising organisms due to their photosynthetic capacity and synthesis of specialized metabolites. This study aimed to select cyanobacterial strains capable of stimulating lettuce (Lactuca sativa) growth. Extracts from selected Brazilian cyanobacterial strains were tested for their efficacy in stimulating seed germination, biomass, and root and stem elongation. Additionally, auxin production was assessed using the Salkowski test, both with and without supplementation of L-tryptophan (100 µM). Results from initial screening of 37 strains revealed several beneficial effects on plant growth, with notable increases in biomass and enhancement of root and shoot growth, particularly with strains CENA281, CENA357, CENA367, CENA567, and CENA621 exhibiting higher means. However, strains CCIBT3148, CENA516, CENA520, and particularly CENA597 exhibited significant phytotoxicity, suggesting a potential risk for crop development. Further evaluation of 60 strains identified two that produced low concentrations of indole compounds, which include auxins, without exogenous L-tryptophan supplementation (CCIBt3148 and CENA597), while twelve produced indole compounds when supplemented, including strains CENA533, CENA516 and CENA112 that presented a higher level of these compounds in the qualitative test. This study significantly contributes to the understanding of the potential of Brazilian Cyanobacteria strains as bio-stimulant agents. Nonetheless, continued research is important to elucidate mechanisms and optimize their practical applicability in agriculture.

KEYWORDS: Biostimulants, Phytotoxicity, Seed Germination, Sustainable Agriculture.

Effects of Cultivation Conditions on the Biomass and Lipid Production of *Papiliotrema laurentii*

S.L. Barbosa[1]*, E.L.M. Almeida[1], J. V. M. G. Assis[1], W.B. Silveira[1]

1Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brasil.
*Samuel.lessa@ufv.br

Papiliotrema laurentii is an oleaginous yeast capable of assimilating various carbon sources and accumulating high lipid contents (up to 63.5% of its dry weight) with a fatty acid profile suitable for oleochemical and biofuel industries. Recently, our research group identified nutritional conditions that favor its biomass and lipid production. Herein, to enhance these fermentative parameters, we optimized the cultivation conditions using a rotational central composite design considering the carbon-to-nitrogen ratio (C:N); initial optical density (iOD600); and aeration, represented by the ratio between the volume of the culture medium and the flask (VM:VF). All parameters influenced the biomass production, with increases in OD600 and aeration promoting higher biomass production, whereas increases in the C:N ratio have the opposite effect. Only aeration affected the lipid production (content and titer), with higher aeration leading to higher lipid production. Aeration was the most relevant factor for all response variables. The biomass concentration increased 27% under optimized conditions (iOD600 = 0.45; C:N = 80; VM:VF = 0.1). Aeration was the major limiting factor for biomass and lipid production by P. laurentii. Further cultivations in bench bioreactors can provide a better understanding of the effect of aeration on the growth and lipid production by P. laurentii.

KEYWORDS: carbon-to-nitrogen ratio, initial optical density, aeration, response surface analysis.

FUNDING: CAPES, CNPq, FAPEMIG.

Influence of aeration on the cultivation of *Metarhizium anisopliae* through solid-state fermentation in plastic reactors

G.L. Maciel[1], I. Lima[1]*, R. A. F. Moreira[1], M. P. Bagagli[1].

1 Instituto Federal de Educação Ciência e Tecnologia de São Paulo- Campus Avaré. Av. Prof. Célso Ferreira da Silva, 1333 - Jardim Europa II, Avaré - SP, 18707-150.
*lima.iris@aluno.ifsp.edu.br

The on-farm multiplication of microorganisms for the control of agricultural pests and diseases demands caution and attention to practices that prevent the proliferation of contaminants while ensuring the efficient performance of the product. This study assessed the propagation through solid-state fermentation of *Metarhizium anisopliae* using rice as a substrate and 5 L HDPE containers with different aeration conditions. These conditions were determined by the use of cotton plugs, 2 and 4 hydrophobic adhesive air filters (0.2 µm) in openings made in the containers. The containers had a rubber seal attached for inoculation and were autoclaved for 15 minutes at 121°C. Under laminar flow, 2 mL of spore solution (5x108 CFU/mL) of the microrganism were added through the seal using a syringe. The containers were kept horizontally in a dry environment without direct sunlight, but without temperature control. After 7 days, spore extraction was performed in a sterile environment with 800 mL of saline solution containing 0.05% polysorbate 80, and the propagules were indirectly quantified on PDA agar. On the plates, homogeneity of colonies was observed, with no atypical growth. The average counts were 2x108 CFU/mL for the use of 4 filters, 2x107 CFU/mL for the cotton plug, and 8x106 CFU/mL for the use of 2 filters, with the first result being significantly superior to the others (p-value <0.05). Thus, it is concluded that the better the gas exchange condition in the reactors, the better the development of the fungus *M. anisopliae*.

KEYWORDS: Metarhizium anisopliae, on-farm, aeration, HDPE

Exploring *Burkholderia* JBC02: A Versatile Strategy to Combat Phytopathogenic Fungi with Bioactive Compounds

L. A. Rojas[1]*, P. K. Rodrigues[1], M. V. Lemos[2], E. G. de Macedo Lemos[1]

1 Department of Agricultural, Livestock and Environmental Biotechnology, São Paulo State University (UNESP), School of Agricultural and Veterinary Sciences, Jaboticabal, 14884-900, SP, Brazil.
2 Department of Biology, São Paulo State University (UNESP), School of Agricultural and Veterinary Sciences, Jaboticabal, 14884-900, SP, Brazil.
*luis.chicoma@unesp.br

Plant pathogens pose significant threats to crop production worldwide, prompting the need for sustainable disease management strategies. This study evaluates the efficacy of Burkholderia JBC02, a bacterial isolate, in controlling phytopathogenic fungi through the release of diffusible substances (DSs) and volatile organic compounds (VOCs). Antagonistic activity was assessed using dual culture techniques, demonstrating over 50% inhibition of radial growth in eight phytopathogenic fungi. Scanning electron microscopy (SEM) revealed distorted mycelial structures, indicative of bacterial antagonism. Additionally, VOC bioassays exhibited effective fungal control, with treated soybean leaves showing no symptoms compared to controls. Notably, *Burkholderia* JBC02 displayed robust inhibition, particularly against Fusarium oxysporum and Sclerotinia sclerotiorum, with SEM confirming significant mycelial damage. The results underscore the strain's multifunctional activity and potential as a biocontrol agent, with dual mechanisms of action: DS production and VOC release. This promising efficacy suggests *Burkholderia* JBC02 as a viable candidate for bioinput development, offering novel avenues for sustainable plant health management and agricultural advancement.

KEYWORDS: Biocontrol agent, Antagonistic activity, Phytopathogenic fungi, Sustainable Disease Management

FUNDING: CAPES, FAPESP

Molecular characterization of microbial consortia tolerant to the herbicide glyphosate

C.G. Abreu[1]*, M.P.O. Paula[1] , V.S. Pylro[3]

1Universidade Federal de Lavras, carlosgodinhoab@gmail.com
1Universidade Federal de Lavras, mariana.paula3@estudante.ufla.br
2Universidade Federal de Lavras, victor.pylro@gmail.com
*carlosgodinhoab@gmail.com

Glyphosate is a non-selective herbicide widely used around the world. Due to the environmental and human health impacts attributed to this product, the need to remediate environments contaminated by its residues is evident. Among the technologies developed to remove glyphosate, degradation through microbial enzymatic catalysis appears to be more ecological and effective. This work aims to establish a microbial enrichment culture from soil samples collected from coffee plantations and characterize consortia of glyphosate-degrading microorganisms. Soil collection was carried out on Conilon and Arabica coffee plantations in the state of Espírito Santo, subject to different weed management practices. After the enrichment process, DNA from soil samples and microbial biomass was analyzed using a metataxonomic approach using a massive DNA sequencing platform. Two consortium profiles were identified through metataxonomic analyses, using 16S rDNA as a marker. One of these profiles was predominantly made up of the genus Labrys, while the other was mainly made up of the genus Serratia. There was no amplification of the internal transcribed spacer (ITS) region in DNA samples from microbial consortia, suggesting that these are composed of bacteria. This work identifies microorganisms with great potential for glyphosate degradation, which can be explored for the design of bioremediation processes.

KEYWORDS: soil microbiota, coffee farming, pesticide, biodegradation.

FUNDING: CAPES, CNPQ, FAPEMIG

Potential of *Chromobacterium* spp. in controlling *Diaphorina citri* (Hemiptera: Psyllidae)

Veloso. L.F.[1]*, Zanotto. A.W. [1], Delalibera Jr. I.[1]

1 University of São Paulo, "Luiz de Queiroz" College of Agriculture (ESALQ) Av. Pádua Dias, 11 – P.O. Box 9 – CEP, 13418-900 Piracicaba, São Paulo, Brazil
*leonardo.veloso@usp.br

Diaphorina citri is the primary vector of Huanglongbing (HLB), also known as citrus greening disease. This disease significantly threatens the global Citrus industry, leading to substantial economic losses. In light of this challenge, recent research has explored the efficacy of biological control agents as an environmentally friendly alternative to chemical pesticides. This study aimed to evaluate the potential of strains CO1 (*Chromobacterium paludis*), CO2 (*Chromobacterium alticaptis*), CO4 (*Chromobacterium subtsugae*) 599 (*Chromobacterium sphagni*), 310 (*Chromobacterium amazonense*), 890 (*Chromobacterium subtsugae*) and 1092 (*Chromobacterium subtsugae*) to control *Diaphorina citri*. The isolates were grown through liquid fermentation in LB medium for 48 hours at 28 °C±2, reaching a final 1010 CFU/ml concentration. They were then applied by spraying on Citrus plants infested with 15 insects each, with five replicates. The mortality of D. citri was assessed over ten days under controlled temperature (27 ºC±2) and relative humidity (70%). The isolates, CO4, 890 and 1092 demonstrated a significantly higher mortality rate than the other isolates and the control group (CO1 = 21,62% ± 0, 26; CO2 = 22,76% ± 0,67; CO4 = 29,06% ± 0,42; 310 = 17,78% ± 48; 599 = 7,22% ± 0,21; 890 = 26,24 ± 0,35; 1092 = 27,15% ± 0,62; Control group = 4,44% ± 0,34). These results indicate that *Chromobacterium* has potential as a biological control agent against D. citri, which could be used in developing more sustainable and efficient pest management strategies in Citrus crops, to mitigate HLB the spread.

KEYWORDS: *Chromobacterium*, *Diaphorina citri*, Biological control

Influence of soil properties on the differential abundance of prokaryotes in silvopastoral systems in the western Amazon

H.N. Cipriani[1]*, T.A. Pellegrinetti[2], A.K.D. Salman[3], E.C. Souza[4], P.G. Cruz[5], S.M. Tsai[2]

1Embrapa Florestas, Colombo, Paraná, Brazil
2USP/CENA, Piracicaba, São Paulo, Brazil
3Embrapa Rondônia, Porto Velho, Rondônia, Brazil
4UNIR, Porto Velho, Rondônia, Brazil
5Embrapa Café, Vitória, Espírito Santo, Brazil
*Correspondent author, henrique.cipriani@embrapa.br

Livestock-forestry systems (ILFSs) may improve soil health by promoting diverse microbial communities. The soil microbiome is influenced by soil chemical and physical properties. Understanding this influence is important in explaining differential abundance and in defining management practices. The objective of this study was to analyze the correlation between soil environmental variables and prokaryote community structure in two ILFSs and a native forest fragment (NF) located in Porto Velho, Rondônia State, Brazil. Soil samples were taken from the 0-10 cm layer from two ILFSs (0.00 and 5.25 m from the trees) and a NF fragment (Am climate, Udox soil, clay texture), with five replicates, totaling 25 samples. Both ILFSs consisted of long-established pastures in which Samanea tubulosa (ILFS-ST) and Eucalyptus pellita (ILFS-EP) strips were planted. The soil DNA was extracted, and the 16S rRNA gene was sequenced. The sequences were grouped into OTUs and classified taxonomically. The influence of sixteen soil chemical and physical properties on the microbial community was analyzed using the dbRDA function of the microeco package for R, with Bray distance and grouping by area. The soil properties accounted for 83.43% of the variance in the community composition among areas, excluding moisture, available P and K ($p>0.05$). Aluminum, exchangeable Ca and Mg, clay content, and pH were the soil properties with the strongest influence. The native forest had the most distinct microbial community, likely due to its higher acidity. This study suggested that ILFS practices can influence soil microbial communities, potentially impacting soil health, especially by reducing acidity.

KEYWORDS: 16S rRNA gene sequencing, ICLFS, Soil microbiome, Soil quality

FUNDING: BNDES, CAPES, FAPERO

Assessment of microbiological activity in soil macro and microaggregates in different soil uses

Estela Rodrigues Camargo[1], Glaciela Kaschuk[1], Karina Maria Vieira Cavalieri Polizeli[1]

[1]Department of Soil and Agricultural Engineering, Federal University of Paraná, Rua dos Funcionários, 1540, PR, Curitiba, CEP 80035-050, Brazil.
Email: estelacamargo@ufpr.br

Livestock-forestry integration represents a viable alternative for the expansion of livestock activities, mitigating the negative impacts associated with large-scale animal husbandry. The objective of this study was to evaluate and compare the soil microbiological activity in different soil aggregate sizes in a livestock-forestry integration area in Pinhais, PR, Brazil. The experiment was conducted under randomized blocks design, with three land use or treatments (native forest, livestock, and livestock-forestry integration). Soil monoliths were collected with dimensions of 0.10 m depth, 0.15 m width, and 0.10 m length, obtained from the NITA (Núcleo Inovação e Tecnologia Agropecuária) at the Canguiri Experimental Farm. Samples were collected in duplicates at three trenches in each land use, wrapped in plastic film, and transported to the laboratory. The samples were air-dried, manually disaggregated, and subdivided in soil macroaggregates and microaggregates using sieve with an opening of 0.25mm., then subjected to the following analyses: aggregates stability, by dry sieving using an electromagnetic shaker, obtained the weighted mean diameter through a set of sieves with mesh sizes of 4.00, 2.00, 1.00, 0.50, and 0.25 mm., soil microbial biomass carbon, by fumigation-extraction methods, and β-glucosidase enzyme activity. The analyses revealed that microbial biomass was not affected by the treatments and soil aggregate sizes. However, the β-glucosidase enzyme activity, an indicator of soil health, was higher in microaggregates than in macroaggregates, in all treatments. Thus, further studies should focus on the role of soil aggregation in improving soil health.

KEYWORDS: Aggregate size distribution, β-Glucosidase Enzyme Activity, field experiment, soil health indicator.

Bacterial alpha diversity and fractions of soil organic matter in integrated agricultural production systems (IAPS)

N. de Andrade[1]*, R. L. Oliveira1, C. C. G. Freitas[1], D.A. de Borba[1], M. B. Oliveira[1], F.D. Andreote[1]

1Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo
*narianedeandrade@usp.br

Integrated agricultural production systems (IAPS) have the potential to increase carbon (C) and modify the composition of the soil bacterial community. The objective of this study was to understand how IAPS alter the fractions of organic matter and bacterial diversity in the soil. For this, soil samples were collected in Livestock-forest (ILF), Crop-livestock (ICL), Native Forest (NF) and Pasture (PP), in layers 0-5, 5-10, 10-20 and 20-30 cm. Soil C was determined by dry combustion and expressed as "Mg ha-1" in two fractions: particulate (POM) and associated mineral (MAOM).The shannon bacterial diversity index was calculated (0-5 and 5-10cm) from 16S amplicon sequencing. All data were compared using the t-test (comparing two by two). POM was higher in NF (1.43 ± 0.25) in the first layer, however, it did not differ at other depths. MAOM was superior in ILF between 0 – 5 cm (6.39 ± 1.44) and did not differ from NF (5.24 ± 0.86) and PP (5.34 ± 0.48). In the 5 – 10 cm layer ILF (5.27 ± 1.29) was equal only to NF (4.14 ± 0.47) and between 10 and 30 cm ILF was equal to ICL. Contrary, the lowest alpha diversity values were found in NF in the two layers evaluated, not differing from ILF between 0 – 5 cm. In layer 5 - 10, NF was lower and did not resemble any other land use. Our results show the importance of understanding the relationship between soil C and microbial community composition in IAPS.

KEYWORDS: carbon, land use change, POM, MAOM

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Multi-omics for the identification of mycosporin-like amino acids (MAAs) in the cyanobacterium *Capilliphycus salinus* ALCB114379

Gabriel S. Passos[1], Rafael B. Dextro[1], Núbia P. da Silva[1], Marli F. Fiore[1], Ernani P. Junior[1]

1Center for Nuclear Energy in Agriculture, University of São Paulo, Piracicaba, Brazil.
E-mail: gabriel-passos@usp.br

Cyanobacteria have played a pivotal role in Earth's history, pioneering oxygenic photosynthesis and contributing to the formation of the ozone layer approximately 3.6 ± 0.2 billion years ago. Their extensive evolutionary journey has endowed them with remarkable adaptability to various stressors, thriving in environments with diverse luminosity, salinity, and air exposure. A prominent adaptive mechanism is the production of secondary metabolites. Collected from a supralittoral region profoundly influenced by environmental fluctuations, the cyanobacterium *Capilliphycus salinus* ALCB114379 may harbor a genetic reservoir potentially essential for stress mitigation and of biotechnological interest. This study aims to integrate genomics and metabolomics to identify biosynthetic gene clusters (BGC) for secondary metabolites and validate their production. After PacBio HiFi sequencing and genome assembly using HiFiASM, potential genes were annotated using Prokka and categorized into biological systems via BlastKOALA. Notably, within the "secondary metabolism" category, canonical genes for the biosynthesis of mycosporine-like amino acids (MAAs) - molecules that absorb UV light - were identified. In parallel, metabolomic analysis involved culturing *C. salinus* ALCB114379 for 45 days in a BOD chamber, followed by specific MAAs extraction. The extracts in triplicate were analyzed by LC/MS coupled to 6460 Triple-Quad MS with an electrospray interface. The mass spectrometer was operated in multiple reaction monitoring mode (MRM). Their MRM chromatograms showed the presence of Shinorine and Palythine. Future efforts include subjecting the cyanobacterium *C. salinus* ALCB114379 to inducible MAAs production conditions for comparative studies and efficiency evaluation, shedding light on its biotechnological potential.

KEYWORDS: Cyanobacterial adaptation, Genomic annotation, Metabolomic analysis, Secondary metabolite biosynthesis, UV radiation protection.

Cover crops and P sources modulate exoenzymes of C, N and P acquisition and soil multifunctionality in semi-arid conditions

L. N. S. Barbosa[1]*, J. H. S. Luz[1], P. S. Pavinato[1], V. R. Santos[2]

1Universidade de São Paulo, Escola Superior de Agricultura "Luiz de Queiroz"
2Universidade Federal de Alagoas (Campus Arapiraca).
*Email : luanabarbosa@usp.br

The introduction of cover crops in agricultural systems has been increasingly recommended as a management strategy beneficial to soil biology and functioning. The objective of this study was to investigate the effects of cover crops and P sources on the performance of C, N and P acquisition enzyme activity and on soil multifunctionality. The study was conducted in Alagoas, Northeast Brazil. The experimental design was in randomized blocks, with three replications, in a 3x7 factorial scheme. The first factor was the phosphate sources: soluble (SPS), reactive (FR) and phosphorus-free (Sem-P). The second factor was represented by the cover crops: *Crotalaria juncea*, *Crotalaria spectabilis*, *Cajanus cajan*, *Dolichos lablab*, *Canavalia ensiformis*, *Pennisetum glaucum* and a treatment without cover (fallow). The activity of the enzyme's acid phosphatase, β-glucosidase and urease, the carbon of the microbial biomass, the chemical attributes of the soil were determined, and the multifunctionality of the soil was calculated. P acquisition was superior under FR, especially with the *Dolichos lablab*. Similar results with *Dolichos lablab* were observed in C and N acquisition, with greater responses in the 0-5 cm layer, although N acquisition was higher in Sem-P, but did not differ from fallow. The addition of P favored microbial biomass, regardless of the layer. Fallow decreased soil multifunctionality, was negative regardless of P sources, and the best responses occurred with *Canavalia ensiformis* and *C. spectabilis*. The cover crops modulated the enzymatic activity of the soil, improving the chemical attributes, as well as activating the multifunctionality of the soil.

KEYWORDS: Phosphatase; Soil health, plant diversity, agricultural sustainability.

FUNDING: FAPESP, FAPEAL, CAPES

Bioprospection of endophytic bacteria able to control soil pathogen Fusarium oxysporum isolated from soybean and maize

L. L. M. Beraldo[12]*, H. S. Milano[2], S. H. Matis[2], M.C. Quecine-Verdi[1], M. N. Dourado- Ribeiro[2]

1Esalq. – Av. Pádua Dias, 11 – Cep 13418-900 – Piracicaba, SP
2 MicroGene – Av. Limeira, 1131 - sala 10 – Cep 13414-018 – Piracicaba, SP
*Contact : lucasberaldo@usp.br

The use of Bioinputs, such as endophytic bacteria, are a promising solution in sustainable agriculture. These microorganisms establish a mutualistic relationship with plants, providing benefits such as tolerance to environmental stress and growth promotion. Additionally, endophytic microorganisms can be used in biological disease control, reducing reliance on chemical pesticides. Their presence in plants interferes with pathogen development through competition for nutrients, production of hydrolytic enzymes, and induction of secondary metabolite production of the plant host. The current project aimed to search through a guided bank of 1600 isolates from Microgene Ltda startup, which were isolated from leaves, roots, and rhizosphere of soybean and maize plants. Subsequently, enzymatic, and physiological trials were conducted in triplicate using large petri dishes, including assays for cellulolytic, lipolytic, esterasic, and pectinolytic activities as well as Fusarium oxysporum control assay, a soil pathogen. Based on the trial results, 35% isolates were able to produce cellulase, 35% lipase, 44% esterase, and 35% pectinase. The top 73 bacteria multi traits were selected and submitted to identification through partial sequencing of the 16S rDNA gene. Among the bacteria, 14 distinct genera were identified, including *Bacillus, Priestia, Arthrobacter, Methylobacterium* and *Pantoe*a. Among those, 3 isolates (identified as *Priestia* and *Bacillus*) were able to control F. oxysporum which was selected to further in vivo tests, to develop a potential bioproduct to agriculture.

KEYWORDS: endophytes, biological control, hydrolytic enzymes, sustainable agriculture.

FUNDING: PIPE FAPESP.

Investigation of the storage of *Trichoderma harzianum* at different temperatures in containers suitable for small-scale producers

E. L. Correia[1]*, M. P. Bagagli[1]

1Instituto Federal de Educação, Ciência e Tecnologia de São Paulo, Campus Avaré, Av. Prof. Célso Ferreira da Silva, 1333 - Jardim Europa II, Avaré - SP, 18707-150
*eduarda.correia@aluno.ifsp.edu.br

On-farm production of microbial-based bioinputs has been growing in Brazil, providing a cost-effective alternative for producers. In this context, refining production techniques to prevent the proliferation of potentially pathogenic contaminants and to maintain the efficiency of the products is crucial. Thus, this study assessed the storage of Trichoderma harzianum propagule obtained through solid-state fermentation, in an aqueous suspension containing 0.05% polysorbate 80 and 0.85% sodium chloride, stored in sterile 20 mL syringes at three different temperatures (room temperature, 5°C, and -18°C) for use as inoculants in on-farm processes. The experiments were conducted in triplicate, with the quantification of the target microorganism on PDA agar. The initial indirect quantification of T. harzianum propagules was 4x107 CFU/mL. Storage for a period of 15 days at all three temperatures resulted in a significant reduction in the count (p-value < 0.05), with an average of 7x106 CFU/mL. The refrigeration temperature led to the greatest decline in propagule concentration. After 30 days of storage, no significant changes were observed compared to the 15-day assessment. Therefore, for the specific microorganism studied, stored in an aqueous solution in sterile syringes, freezing and room temperatures exhibited the lowest values of propagule losses. However, a significant reduction in propagules was observed at all evaluated temperatures.

KEYWORDS: Bioinputs, on-farm, Trichoderma harzianum, inoculum.

Characterization of different isolates of *Nostoc* spp. for biotechnological applications.

S. Alencar[1]*, T. Santos[1], L. Pimenta[2], G. Souza[2], F. Coelho[2], W. Duarte[1].

1 Department of Agricultural Microbiology, Institute of Natural Sciences, Federal University of Lavras (Universidade Federal de Lavras), MG, Brazil.
2 Department of Biology, Institute of Natural Sciences, Federal University of Lavras (Universidade Federal de Lavras), MG, Brazil.
* E-mail (corresponding author): samantha.alencar1@estudante.ufla.br

Cyanobacteria are prokaryotic microorganisms found in both aquatic and terrestrial environments due to diverse morphologies that provide adaptation capacity in various ecosystems. *Nostoc* spp. is a genus of cyanobacteria that has a gelatinous appearance due to the layer of exopolysaccharide and has the presence of heterocytes, cells with the capacity to assimilate atmospheric N2 in the absence of oxygen, which is one of the main reasons for interest in its use as a biofertilizer for plants. With the aim of investigating different characteristics of *Nostoc* spp., two strains of *Nostoc* like. (UFLA 37 and UFLA 19) and one strain of *Desmonostoc* (UFLA 11) were cultivated in 100 mL of $BG11_0$ medium (without nitrogen) in BOD, with a photoperiod of 12h at 23 ºC for 14 days, and the biomass growth analysis was carried out using absorbance, dry weight, total solids, chlorophyll a and exopolysaccharides (EPS) production. At the end of the experiment, a higher value of chlorophyll a (3.88 µg.mL-1) of UFLA 37 was confirmed, which was reinforced with the total solids (7X10-3 g.mL-1), which quantifies the biomass present in the medium, confirming that this strain showed greater growth. However, EPS production showed no difference between strains. Based on the results, it was observed that UFLA 37 was the strain that showed the best growth, so this study contributed to choosing the strain that will be used for future biotechnological applications.

KEYWORDS: Nitrogenase activity, *Nostoc* spp., Biofertilizer, Biomass

FUNDING: CAPES, CNPQ AND FAPEMIG.

The effect of *Bacillus* thuringiensis isolates by corn seed treatment and plant spraying on *Spodoptera frugiperda*

Reque. T.C[1]*, Iwanicki. N.S.A[1], Lopes. E.C.M[1], Gomes. L.M[1], Veloso. L.F[1], Pires. I.C.R[1], Delalibera Jr. I.[1]

1 University of São Paulo, "Luiz de Queiroz" College of Agriculture (ESALQ) Av. Pádua Dias, 11 – P.O. Box 9 – CEP, 13418-900 Piracicaba, S˜ao Paulo, Brazil
*tais.cabrera@alumni.usp.br

Bacillus thuringiensis (Bt) is a bacterium traditionally used in agriculture as a biological control tool for pests. This bacterium is found in the soil and is capable of endophytically colonizing plants. In this study, we evaluated the effect of corn plants, whose seeds were treated with spores of four Bt isolates, and plants whose seeds were not treated but whose leaves were sprayed with spore suspensions of the same isolates, on the weight of Spodoptera frugiperda that fed on these leaves. Spores of the HD-1, 42, 49, and 368 isolates of Bt were obtained in LB medium supplemented with salts. Conventional corn seeds were treated with suspensions of 107 spores/mL. When reaching the V2-V3 stage, plants whose seeds were not treated were sprayed with the Bt isolates at a concentration of 107 spores/mL. After spraying, neonatal S. frugiperda larvae were transferred to the plants, and the experiment was conducted in a greenhouse following a randomized block design. Seven days after spraying, the larvae were removed from the plants and weighed. Larvae that fed on seeds treated with isolate 49 and sprayed with the same isolate showed significantly lower weight compared to larvae that fed on untreated seeds and plants. For isolate 49, seed treatment had a more impactful effect on weight reduction compared to foliar spraying. For isolate HD-1, only the foliar spraying had an impact on larval weight. The sublethal effects of Bt on larvae depend on the isolate and method of application.

KEYWORDS: entomopathogens, biological control, microorganisms, maize

Action of Metarhizium anisopliae metabolites against the brown stink bug *Euschistus heros*

L. A. Miyamoto[1], A. N. Silva[1], J. B. Seibert[1], I. D. Junior[1]

1Department of Entomology and Acarology, Escola Superior de Agricultura 'Luiz de Queiroz', University of São Paulo (ESALQ-USP), Av. Pádua Dias, 11, Piracicaba, São Paulo,13418-220, Brazil. lilianmiyamoto@usp.br

Euschistus heros (Hemiptera : Pentatomidae) is an economic pest in soybean fields that causes damage in grain production. *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae) is an entomopathogenic fungus used throughout the world as agent control of pests, however, its use is restricted due to the short shelf life. Aiming to control brown stink bug in a disruptive approach, the objective of this work was to evaluate the insecticidal action of *M. anisopliae* (E9) metabolites produced in liquid fermentation compared to conventional control, through conidia. The production of the microorganism was evaluated using three different culture media focusing on biomass, and the modified Adamek medium was selected. Bioassays were conducted with intracellular and extracellular metabolites at two concentrations sprayed on adult insects. A suspension at 1x107 con/mL was used for aerial conidia. The highest mortality rate (40%) was found for the extracellular metabolite sample at concentration of 10%, which was lower than the treatment of aerial conidia (61%). Furthermore, the insecticidal potential of the metabolites was also evaluated using an ingestion methodology. However, lower mortality values were found when compared to the contact methodology and this fact may be related to its feeding habits. These results suggest that metabolites produced by *M. anisopliae* are effective as conidia, with the advantage of not requiring special storage conditions.

KEYWORDS: entomopathogenic fungi, biological control, metabolites, microorganisms, insecticidal activity.

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Effect of glucose on expression of lactose genes in the yeast *Papiliotrema laurentii*

J. V. M. G. ASSIS[1]*, E. L. M. Almeida[1], A. C. N. Souza[1], J. S. C. Fonseca[1], F. P. M. Freitas[1],W. B. Silveira[1]

1 Department of Microbiology, Universidade Federal de Viçosa, Minas Gerais, Brazil
*joao.v.goncalves@ufv.br

Papiliotrema laurentii is a Basidiomycota yeast that can produce up to 9.3 g/L of lipids when cultivated in ricotta whey, which contains lactose as the main sugar. Previously, we identified the genes encoding β-galactosidase, lactose permease and a transcription factor, which were clustered in the genome of *P. laurentii*. Here, to evaluate the effects of glucose on the growth and expression of these genes, we cultived in minimal medium containing glucose plus lactose and glucose plus galactose as carbon sources. We collected points every hour for 20 h and then at 26 h to evaluate growth and sugar consumption. At the same time, we collected points at 3, 7, 13, 14, 18, 20, and 26 h for RNA extraction followed by RT-qPCR analysis. We found that *P. laurentii* presented diauxic growth under these conditions and the second lag phase was longer for lactose than for galactose. Additionally, the metabolic genes were repressed by glucose The highest expression of the three genes was observed at 20h in mininal medium containing only lactose, after glucose consumption. Galactose induced the expression of the transporter and, in contrast to previous findings, also induced the expression of β-galactosidase. The expression of the transcription factor did not follow the same pattern of the metabolic genes. In summary, we described the glucose-induced repression of the lactose cluster genes in the Basidiomycota yeast *P. laurentii*.

KEYWORDS: lactose, glucose, gene expression, repression, oleaginous yeasts

FUNDING: FAPEMIG

Effect of *Bacillus* spp. isolates on the in vitro growth of the fungus *Corynespora cassicola*

Katherine Anders Lins de Carvalho[1]*; Maria Eduarda Carvalho Rezende[1]; Jéssica Maria Israel de Jesus[1]; Pedro Renato Bodo de Paiva[1]; Luis Eduardo Aranha Camargo[1]

[1]Universidade de São Paulo, Escola Superior de Agricultura "Luiz de Queiroz", Piracicaba, São Paulo, 13400-970, Brazil
E-mail: katherine.anders@usp.br

Soybean (*Glycine max*) is one of the most important crops globally, with Brazil being among the world's largest producers of this agricultural commodity. However, its production may face challenges due to attacks by phytopathogens, such as the fungus *Corynespora cassiicola*, causing target leaf spot and reducing soybean productivity by up to 45%. Therefore, it is essential to search for more sustainable practices of disease control in plants, such as the use of biological control. The aim of this study is to analyze the effect of two bacteria from the genus *Bacillus* on the mycelial growth of the *C. cassiicola* fungus in vitro. To this end, the double pairing technique was used. The experiment was conducted in a Completely Randomized Design (CID), consisting of three treatments and ten repetitions: T0 - control (containing only the fungus); T1 – fungus + LGM1 (*Bacillus* sp.); T2 – fungus + LGM3 (*Bacillus* sp.). Each repetition involved a Petri dish containing PDA (Potato Dextrose Agar). The plates were incubated in a B.O.D. growth chamber at 28 ºC with a 12h/12h photoperiod for 15 days. The mycelial growth of the fungus was measured daily by measuring the diameter using a ruler. It was observed that both *Bacillus* isolates inhibited the mycelial growth of the fungus *Corynespora cassiicola*. These results suggest the potential use of these isolates for the biological control of this fungus. Further studies are needed to evaluate the potential of these bacteria in vivo control of target leaf spot.

KEYWORDS: soybean, plant pathogen, mycelial growth, biological control.

Selection of resident microorganisms isolated from soybean plants for biocontrol of asian soybean rust in vitro

D.E.H. Souza[1], L. J. Silva[2], I. S. Melo[2]

1Escola Superior de Agricultura "Luiz de Queiroz", Piracicaba- São Paulo.
2Embrapa Meio Ambiente, Jaguariúna- São Paulo.

Soybean farming is an important agricultural activity in Brazil, occupying nearly 41 million hectares in the 2021/22 harvest. Directly impacting soybean productivity in the country, the phytopathogen *Phakopsora pachyrhizi*, the causative agent of asian soybean rust (SBR), can cause losses of up to 70% in productivity. Furthermore, due to the emergence of different races, traditional methods based on the application of fungicides and the use of resistant cultivars have lost their efficiency over time. Microbial inoculants are a possible alternative for controlling the disease, considering their lower environmental impact when compared to traditional fungicides and the evident success of other agents already used in soybean cultivation as growth promoters. Therefore, this project proposes the bioprospecting of microorganisms for the selection of biological agents capable of helping to control SBR in Brazil. Thus, bacteria and fungi isolated from the soybean phyllosphere deposited in the collection of Cultures of Microorganisms of Agricultural and Environmental Importance (CCMA) at Embrapa Meio Ambiente were reactivated, identified and had their antibiosis evaluated through in vitro tests to inhibit the germination of urediniospores of *P. pachyrhizi* in concave microscope slides and detached leaves. Out of the 63 isolates tested in the concave slides, 24 had a germination inhibition above 90% and were selected for the detached leaves essay in which 9 isolates promoted a reduction in the severity of Asian rust. Finally, the isolates selected in the previous steps will be used in subsequent tests in a greenhouse under partially controlled conditions.

KEYWORDS: Soybean rust, Bioprospecting, Biological Control, Microbial inoculant.

FUNDING: COORDINATION OF SUPERIOR LEVEL STAFF IMPROVEMENT (CAPES)

Physiological and Molecular Characterization of Phosphorus Solubilization and Mineralization by the Bacterium *Pantoea agglomerans* 33.1

Renan Fantine[1]*, Gladys A Apaza-Castillo[1], Carolina A A Hayashibara[1], Maria Carolina Quecine[1]

1 Genetics of Microorganisms Laboratory "Prof. João Lúcio de Azevedo", Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba-Brazil
*renanfantine9@usp.br

Phosphorus (P) is a vital element for plant development. However, a few portion of all phosphorus present in the soil is available by plants, as it is often linked to other elements. Phosphate-solubilizing bacteria, known as PSB, play a crucial role in making phosphorus soluble, facilitating plant absorption and promoting the plant growth. For this reason, PSB have gained prominence in research involving sustainable agriculture. Among the various PSBs, *Pantoea agglomerans* 33.1 stands out for its proven ability to solubilize nutrients and stimulate plant growth. Given this context, the present study aimed to characterize, physiologically and molecularly, the process of phosphate solubilization and mineralization by this bacterium. This involved an analysis of genomic, phenotypic and genetic responses of *Pantoea agglomerans* 33.1 when grown in different concentrations of soluble phosphorus. For this, 33.1 was cultivated in a simple phosphate medium for solubilization and a medium with phytic acid for mineralization, both supplemented with different soluble phosphorus concentrations. Then, genes were selected to evaluate their expression using the RT-qPCR technique. The results reveal that soluble phosphorus can modulate the expression of genes associated with phosphate transport, metabolism, and mobilization. The phosphate regulator (pho), a global regulatory mechanism related to phosphate management in bacteria, is particularly noteworthy, controlled by the PhoB/PhoR Two-Component System. As a result, this study provides starting points for further investigations, deepening the genetic understanding of phosphate solubilization in different treatments to optimize this process for the benefit of sustainable agriculture.

KEYWORDS: Phosphorus, Solubilization, *Pantoea agglomerans*, Gene expression

FUNDING: São Paulo Research Foundation (FAPESP), process no. 2021/12378-4 and 2023/07799-6 ; National Council for Scientific and Technological Development (CNPq)

Soybean endophytic bacteria RS11 as a promoter of plant growth and in the biocontrol of *Macrophomina phaseolina*

S. Gomes[1]* , E. Morais[1], B. Sousa[1], J. Beserra Junior[2], F. Araújo[1]

[1]Campus Professor Cinobelina Elvas, Federal University of Piauí, Bom Jesus, PI, 64900-000, Brazil
[2]Campus Petrônio Cortela , Federal University of Piauí, Teresina, PI, 64900-001, Brazil
*saralago487@gmail.com

Endophytic bacteria have potential for biocontrol and promotion of plant growth by offering ecological benefits when interacting with host tissue. Therefore, this work aimed to evaluate the potential of the bacterial RS11, isolated from soybean plants cultivated in Southwest Piauí, in promoting plant growth and biocontrol of *Macrophomina phaseolina* (Tassi) Goid., causing charcoal rot in soybean plants. Bioassays were carried out in vitro with soybean seeds and greenhouse experiments, with treatments: untreated seeds (T1); seeds + RS11 (T2); seeds + RS11 + *M. phaseolina* COUFPI11 (T3); seeds + biological fungicide + *M. phaseolina* COUFPI11 (T4); seeds + chemical fungicide + *M. phaseolina* COUFPI11 (T5); seeds + *M. phaseolina* COUFPI11 (T6). The severity in soybean seeds in in vitro bioassays was significantly reduced in the treatment with RS11 (T3), which also stimulated root growth and the percentage of seed germination. Plants grown in a greenhouse infected by the phytopathogen (T6) showed maximum incidence of the disease, while treatment with the bacterial strain (T3) significantly reduced the severity. Physiological parameters such as plant height (29 cm), stem diameter (4.86um), dry mass (1.12g; 1.11g; 1.43g) and fresh mass (12.6g; 6.4g; 6.30g) of the aerial part, roots and leaves respectively and the number of leaves improved with the RS11 strain, indicating its role in promoting plant growth. The results reveal the potential of the RS11 strain in the biocontrol of *M. phaseolina* COUFPI11 and in promoting the growth of Soybean.

KEYWORDS: Biological control, Charcoal rot, *Glycine max*, Growth promotion.

Assessment of the Potential of the *Bacillus* Genus in Suppressing Fungal Diseases in Orchards

G. T. Santos[1],3*, H. S. Lima[1], G. B. Peruchi[1],[2], N. Mancine[1],[2], L. M. N. Massone[1],[2], H. D. Coletta-Filho[1]

[1] Centro de Citricultura Sylvio Moreira, IAC - Cordeirópolis, SP, Brasil
[2] Universidade Federal de São Carlos, Araras, SP, Brasil
3 Fundação Hermínio Ometto-FHO
*gabriellytramontelli@gmail.com

The genus *Bacillus* spp, renowned for its substantial biocontrol efficacy across diverse agricultural and environmental settings, particularly stands out for species like *Bacillus subtilis*, which demonstrate antagonistic properties against various phytopathogens. These bacteria aid in disease management in plants through the synthesis of antibiotics, enzymes, and other bioactive compounds that impede the proliferation of pathogenic agents, presenting an environmentally sound and effective alternative to chemical pesticides. This study aims to evaluate the potential of *Bacillus* spp. for biological control against four phytopathogenic fungi: *Alternaria* sp., *Phyllosticta citricarpa*, *Phytophthora infestans*, and *Colletotrichum sp*.Rhizosphere soil samples were collected from 40 healthy citrus trees in four distinct geographical regions of São Paulo State, Brazil, under varying environmental conditions during dry and rainy seasons. Microorganisms were isolated from plant roots (2-5mm) using four culture media and cryopreserved. Subsequently, total DNA was extracted from each isolate and subjected to 16S rRNA sequencing. Of the 2880 isolates obtained, 65 were identified as *Bacillus* genus members. Notably, 56,92% of isolates originated from the dry season, with 43,08% from the rainy season. These isolates are currently undergoing laboratory evaluation for their biocontrol potential against the aforementioned pathogens. Biological control presents a promising avenue for managing fungal diseases in plants, fostering sustainable and eco-friendly agricultural practices. By minimizing reliance on synthetic pesticides, it contributes to biodiversity preservation and soil health enhancement. This ongoing investigation aims to advance more efficient and sustainable biological control strategies, with the ultimate goal of mitigating losses caused by fungal diseases and promoting robust agricultural production systems.

KEYWORDS: Biological control, Bacillus,Microorganisms,Phytopathogens.

FUNDING: Fapesp (2020/14584-8)

Molecular phylogenetic analysis reveals two new fungal species belonging to the order Hypocreales from caves in the Serra da Ferrugem Natural Monument, Minas Gerais

Ana Flávia Leão[1]*, Thiago Oliveira Condé[1], Fábio Alex Custodio[2], Olinto Liparini Pereira[2]*

1Departamento de Microbiologia, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.
2Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.
* Contact of the correspondent author: ana.f.leao@ufv.br, oliparini@ufv.br

The Serra da Ferrugem Natural Monument is a preservation area located in the municipality of Conceição do Mato Dentro that is formed by iron rocks and possesses several cavities. The objective of this study was to identify and describe three fungal isolates found in three ferruginous cavities. The isolate COAD 3669 was obtained from leaf litter of cave CSF0386, whereas isolates COAD 3667 and 3668 were obtained from soil samples of caves CMN16 and CSF 0804, respectively. Phylogenetic analysis using five loci (ITS, LSU, RPB2, TEF1-α, and TUB) were performed using Bayesian inference in MrBayes v.3.2.7 and maximum likelihood in IQTREE v.2. Macroscopic and microscopic characterizations of the fungal isolates were also performed. Isolate COAD 3669 clustered in the genus *Cylindromonium* and did not group with any previously described species, forming a sister clade to *C. eugeniicola*. The isolates COAD 3667 and 3668 belonged to the genus Sesquicillium and were grouped into a distinct and well-supported clade from other known species, being closest to S. lasiacidis and S. candelabrum. Morphologically, isolate COAD 3669 is different from other *Cylindromonium* species by producing monophialidic conidiophores, which are sometimes reduced to conidiogenous cells and 1-septate conidia. The isolates COAD 3667 and 3668 are different from other Sesquicillium species due to the development of conidiophores with only solitary terminal phialides and subglobose, shorter, and wider conidia. These isolates will be proposed as new species following the International Code of Nomenclature for Algae, Fungi, and Plants. This study provides insights into taxonomical novelties found in Brazilian cave environments.

KEYWORDS: Bionectriaceae, Niessliaceae, biospeleology, mycospeleology.

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Diversity of citrus rhizosphere bacterial community and the water stress

G. B. Peruchi[12]*, N. Mancine[12], H. S. Lima[1], G. T. Santos[13] , L. M. N. Massone[12] , H. D. Coletta[1]

1 Centro de Citricultura Sylvio Moreira, IAC - Cordeirópolis, SP, Brasil
[2] Universidade Federal de São Carlos, Araras, SP, Brasil
[3] Fundação Hermínio Ometto – FHO, Araras, SP, Brasil
*gibetinperuchi@gmail.com

The rhizosphere microbiome is crucial to plant resistance. Diverse microbial communities contribute to drought tolerance by modulating phytohormone levels in the rhizosphere and producing water-sequestering biofilms. Recognizing microbiomes can help plants resist drought and the serious impacts of water stress in orange production, the main objective of this work is to carry out a comparative analysis of rhizobacteria populations in sweet orange plants that suffer different levels of water stress in different seasons. Rhizosphere soil samples from the Valencia variety grafted onto Citrumelo Swingle rootstock, exhibiting contrasting water deficiency symptoms, were collected in the Northwestern region of the State of São Paulo in winter and summer. Total DNA extraction was followed by Illumina NovaSeq 250x250bp sequencing of the 16S rRNA V4-V5 region using. Raw data processing and statistical analyzes were performed using R software. The results showed a significant difference in the α-diversity of the rhizosphere bacterial community of plants with different water deficiency symptoms (Shannon index, $p < 0.01$). Comparing the different seasons, α-diversity did not show a significant difference. β-diversity (ANOSIM) showed significant differences in the rhizosphere bacterial community between different levels of water stress and seasons (R^2=0.14748, P= 0.001; R^2= 0.27665, P= 0.001). The differences in rhizosphere bacterial communities under different water stress levels and seasons indicate their pivotal role in influencing water deficiency symptoms. These findings contribute to understanding how rhizosphere bacterial communities contribute to plant resilience, shedding light on effective strategies for mitigating water stress impacts in orange production.

KEYWORDS: Microbiome, Orange, Drought, Roots.

FUNDING: FAPESP (2020/14584-8)

The use of *Azospirillum* as a fertilizer alternative in cotton cultivation

Luana Beatriz Gonçalves[1]*, Carlos Henrique Barbosa[1], Edvan Teciano Frezarin[1], José Alyson Rocha Pismel[1], Dalilla Berlanda de Lima Gonilha[1], Everlon Cid Rigobelo[2]

1Postgraduate Program in Agricultural Microbiology, Faculty of Agricultural and Veterinary Sciences, UNESP/FCAV, Jaboticabal, SP, Brazil;
2Agricultural Microbiology Laboratory, Department of Plant Production, Faculty of Agricultural and Veterinary Sciences, UNESP/FCAV, Jaboticabal, SP, Brazil.
*luana.b.goncalves@unesp.br

The cotton industry in Brazil is essential because it leads both globally in production and domestically in the market. However, to increase productivity, the use of chemical fertilizers has become prevalent, resulting in environmental concerns. An alternative is the use of plant growth-promoting bacteria (BPCP), which can enhance soil and plant quality while reducing the need for chemical fertilizers. This study aimed to evaluate the impact of *Azospirillum* spp. on cotton development. The research was conducted in a field environment at the UNESP-FCAV experimental farm in Jaboticabal-SP, Brazil. The soil was fertilized and the area was divided into five treatments: a control, one with nitrogen, and three with varying concentrations of Azospirillum. Plants were harvested during flowering and their nitrogen concentration was assessed. The results demonstrated that, irrespective of the concentration of Azospirillum, there were no significant discrepancies in the concentration of nitrogen in the shoots and grains of cotton plants. These findings align with those of prior studies that discovered no advantages of *Azospirillum* in other crops, such as corn. Nonetheless, this study emphasizes the necessity of investigating new methods to decrease the use of chemical fertilizers and promote sustainability in the cotton industry. Further research is required to better understand the potential of plant growth-promoting bacteria in this context.

KEY WORDS: Chemical fertilizers, Sustainability, Plant development, Zero results.

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Assessment of *Beauveria bassiana* growth in culture medium with chlorine concentration

A. B. Q. Aguiar [1]*, V. A. R. Moreira[2], H. O. Ribeiro [1], M. P. Bagagli[1].

1 Instituto Federal de Educação Ciência e Tecnologia de São Paulo- Campus Avaré. Av. Prof. Célso Ferreira da Silva, 1333 - Jardim Europa II, Avaré - SP, 18707-150.
2Escola Superior de Agricultura Luiz de Queiroz da Universidade de São Paulo – USP. Av. Pádua Dias, 11 - Agronomia, Piracicaba - SP, 13418-900.
*annabeatrizqueirozaguiar@gmail.com

The quest for more economical and sustainable options for pest and disease control in agriculture has led to an increased use of bioinputs. This study aimed to use sodium hypochlorite to reduce microbial contamination of water used in the preparation of on-farm fermentation culture media, investigating whether the residual compound interferes with the growth of the target microorganism, in this case, Beauveria bassiana. The culture medium was based on sugarcane molasses and yeast extract, prepared in one-seventh of the planned water volume, and autoclaved at 121 °C for 15 minutes. The remaining aqueous phase was a 200-ppm sodium hypochlorite solution, aerated for 24 hours with a submersible pump in a hermetically sealed plastic container, and mixed into the culture medium under laminar flow. The water's chlorine content was measured by iodometry (209.49 ± 9.65 ppm). The medium (50 mL) was inoculated with 3 PDA agar cylinders (0.6 cm in diameter), with the fungus cultivated for 72 hours at 28°C. The assays were conducted in triplicate, incubated at 28°C, 200 rpm for 72 hours. The colony-forming unit count of molds on PDA agar indicated an increase from 104 CFU/mL to 107 CFU/mL throughout the fermentation. Optical microscopy revealed the presence of hyphae, spores, and mycelia characteristic of Beauveria bassiana. However, atypical colonies were observed on PDA plates, indicating contamination of the medium for the evaluated system. In this manner, it is imperative to assess alternative concentrations of the sanitizing agent.

KEYWORDS: Beauveria bassiana, on-farm, sodium hypochlorite, sanitization

Biosurfactant production by *Rhodotorula mucilaginosa* and *Psychrobacter* sp. and its applications in the agricultural area

J. Baccarat[1]

1Federal University of São Carlos, Rod. João Leme dos Santos, Km 110, Sorocaba - SP, 18052-780

Biosurfactants are secondary metabolites, produced from microorganisms, whose main characteristics are their amphiphilic nature, the reduction of surface tension and the formation of emulsions. In the agricultural sector, the use of synthetic chemical surfactants has been very common in the composition of pesticides. But because they have a synthetic origin and low biodegradability, they end up affecting groundwater, the soil and even causing harm to humans. Therefore, a possible ecologically correct method to make this replacement would be the use of biosurfactants. The project aims to investigate the production of biosurfactants by *Rhodotorula mucilaginosa* and *Psychrobacter* sp. and its applications in agricultural phytopathogens. Thus, addressing the possibility and effectiveness of using biosurfactants as biocontrol agents, in reducing the incidence of fungal phytopathogens from Fusarium sp., a soil fungus that is associated with several plant diseases, and *Alternaria alternata*, a foliar pathogen fungus. Therefore, to test the efficiency of these biosurfactants and explore these applications, tests will be carried out using the disk diffusion technique against selected fungal microorganisms. Furthermore, the biosurfactants produced will be partially characterized by Fourier Transform Infrared Spectroscopy (FTIR), partially elucidating their chemical structure. Based on the tests and analyses, the expected results of this project are that the growth of agricultural phytopathogens will be suppressed when they come into contact with the biosurfactants produced. This makes them more environmentally friendly options to replace the synthetic surfactants currently used in the agricultural sector.

Key Words: Biosurfactant; biological control; *Psychrobacter* sp.; applications in the agricultural area; *Rhodotorula mucilaginosa*

Characterization of Streptomyces isolates as auxins producers

Mariana V. Françoes[1]*, Leandro V. Astarita[1]

[1]Pontifical Catholic University of Rio Grande do Sul, PUCRS, School of Health and Life Sciences, Plant Biotechnology Laboratory, Porto Alegre, RS, Brazil
*E-mail (corresponding author): mariana.francoes@edu.pucrs.br

Actinobacteria *Streptomyces* is one of the most studied microrganisms for biotecnological purpose. Auxins are phytohormons envolved in plant cells division and differentiation. Streptomyces are shown to synthesize high amounts of indolics, like Indole-3-acetic acid (3-IAA), which works as plant biostimulant and represents an alternative for crop management. The aim of this study is to characterize Streptomyces isolates as auxin producers. Isolates CLV45, CLV91, CLV104 and CLV194 were selected based on previous results of growth promotion in soybean plants. Isolates were grown in liquid medium (30º C, 200 rpm) and the parameters: i) auxins synthesis in 96h; ii) auxin synthesis and cell viability throughout 7 days; iii) auxin synthesis in four culture media, with different combinations of carbon and nitrogen sources, were analyzed. All isolates have been confirmed through phylogenetic analysis (16S rDNA) to be closely related to other Streptomyces species. All isolates were capable of auxin synthesis. CLV91 and CLV194 were the most promising ones, with 550 µg/mL and 497 µg/mL. The highest auxin level was at 7 days for isolates CLV45 and CLV104. Isolates CLV91 and CLV194 synthetized higher amounts of auxins from 3-5 days. The highest cell viability was observed at 4 days for CLV45 and CLV91, 6 days for CLV104, and 7 days for CLV194. All isolates were able to synthetize auxins in ISP2 medium. Only CLV91 and CLV104 synthetized auxins in Gause nº1 medium and only CLV91 was capable of auxins synthesis in ISP3. Experiments are being carried out to optimize auxins synthesis of Streptomyces isolates.

KEYWORDS: Actinobacteria, plant growth, indolic compounds.

FUNDING: CAPES

Bacillus velezensis BIB 0110 colonizes endophytically sugarcane and improves its growth

I. Carvalho[1]*, I.C.P. Paz[1], A.M.Guimarães[2], M.L. Bonatelli[3] , M. Mondi[1], M.C. Quecine[1]

1Department of Genetics, Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba, Brazil
2Biota Innovations, Uberaba, Brazil
3Department of Environmental Microbiology, Helmholtz Centre for Environmental Research—UFZ, Leipzig, Germany
*E-mail (correspondent author): isabelacarvalho@usp.br

Abstract

Plant growth-promoting bacteria (PGPB) in their interaction with plants have potential for biofertilization and biocontrol, a fact that makes them promising alternatives for reducing fertilizers and pesticides in agriculture, maintaining productivity and crop safety. Among these bacteria, *Bacillus velezensis* strain BIB 0110, an endophytic bacterium isolated from Eucalyptus, plays a notable role in phosphate solubilization, atmospheric nitrogen fixation and production of indole acetic acid in vitro, in addition to its ability to promote plant growth and control phytopathogens. This work aimed to evaluate the effects of BIB 0110 inoculation on sugarcane growth promotion and monitoring its endophytic colonization using BIB 0110 GFP-tagged strain. BIB 0110 was transformed with the plasmid *pNKGFP* by electroporation. Sugarcane was inoculated by fertigation with BIB 0110 and, after 60 days in a greenhouse, the plants were evaluated. The shoot and root dry mass were evaluted. The sugarcane colonization by BIB0110-GFP were analyzed under optical fluorescence microscopy (MOF). The inoculation was able to improve the dry weight of the sugarcane shoot by 48% and shoot height by 30%. Using MOF, it was possible to identify the bacteria in diverse plant tissues. These results suggest the promising action of BIB 0110 for sugarcane growth promotion and proved the endophytic behaivor of BIB 0110 during sugarcane interaction, in addition to allowing a better understanding of the biology of this bacterium as a broad-spectrum colonizing host and expanding the range of its application.

KEYWORDS: Plant growth promoting bacteria, Endophytic; pNKGFP, MOF.

Exploring Riboflavin Production by *Meyerozyma caribbica* UFV-1: Effects of Cultivation Conditions

F. P. M. Freitas[1]*, J. V. M. G. Assis[1], E. L. M. Almeida[1], W. B. Silveira[1]

1Departmant of Microbiology, Universidade Federal de Viçosa, Minas Gerais, Brazil
*fernanda.pinheiro@ufv.br

Food processing can increase the loss of nutrients, mainly water-soluble vitamins, such as riboflavin (vitamin B2). Riboflavin is produced by different Fungi, including non-Saccharomyces yeasts like *Meyerozyma* sp. Herein, we evaluated the capacity of *Meyerozyma caribbica* UFV-1 to produce riboflavin and the effects of the culture medium, carbon source and temperature. We cultivated this yeast at 30 and 40 ºC in YNB medium [6.7 g/L (w/v)] with glucose or xylose (30 g/L) (YG30, YG40, YX30 and YX40, respectively) and SS2 [Yeast extract 1.0, (NH4)2SO4 0.9, NaCl 0.1, CaCl2 0.1, MgSO4 0.5 g/L] (SG30, SG40, SX30 and SX40, respectively) and incubated at 200 rpm/72 h. We monitored the yeast growth using the optical density at 600 nm and determined the presence of riboflavin by spectrophotometry based on the excitation peaks at 358 and 439 nm. The yeast presented similar specific growth rates (h-1) in the media containing xylose at both temperatures tested. However, for glucose, there was a reduction in growth rate at 40 °C. Regarding riboflavin production, regardless of the carbon source, we observed higher values at 40 ºC for both wavelengths. This result indicates that higher temperatures favor the riboflavin synthesis by *M. caribbica* UFV-1. Higher peaks were observed in YNB (minimal medium) with xylose at both temperatures compared to SS2 medium (C:N ratio = 100). For glucose, this behavior was only observed for cultures in YNB at 30 ºC. The results obtained in this study will be useful to further studies of riboflavin production by *M. caribbica* UFV-1.

KEYWORDS: riboflavin, high-temperature, non-Saccharomyces

FUNDING: CNPQ, CAPES, FAPEMIG

Soil microbial activity in degraded areas: assessing predictions functions related to nitrogen cycling during rehabilitation.

L. S. Ferreira[1]*, H. S. Lima[2], A. G. de Castro1, I. G. O. Prado[1], I. R. de Assis[1], C. C. da Silva[1]

1Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brasil.
2Centro de Citricultura Sylvio Moreira, IAC - Cordeirópolis, SP, Brasil.
*leticia.ferreira@ufv.br

Degraded areas typically exhibit diminished microbial activity due to various factors. Implementing rehabilitation practices is crucial for restoring soil health and microbial activity, especially for nutrient cycling, as Nitrogen, that is a fundamental element to plant growth and viability. This study aimed to assess soil microbial predicted activity related to the nitrogen cycle in areas undergoing rehabilitation. Samples were collected from two sites in the affected area: DA1, which underwent significant engineering interventions; DA2, adjacent to the forest; and a reference area (REF) during dry and rainy seasons. Total DNA from the samples was extracted, and 16SrRNA sequencing was conducted. Functional prediction analysis was used to evaluate functional changes using the FAPROTAX database, LEfSe, and Log2-Fold-Change analysis. Results showed that nitrogen metabolism pathways were significantly ($p<0.05$ and $Log2FC>|1|$) affected during the dry season, with 3 functions related to the nitrogen cycle being depleted in area DA1, while both DA1 and DA2 exhibited enrichment of function associated with aerobic ammonia oxidation in the rainy season. Biomarker analysis revealed a greater number of nitrogen cycle-related functions in REF during the dry season and DA2 in the rainy season ($p<0.05$ and $LDA>2$). It was possible to observe that DA2 increased functions related to the nitrogen cycle in the rainy season, suggesting an improvement in soil microbial functions over time. The study highlights the importance of rehabilitation in boosting soil microbial activity, especially in the nitrogen cycle, however, more analyses are needed to validate the real impact of microbial activity in degraded soils.

KEYWORDS: nutrient cycle, 16SrRNA, FAPROTAX, biomarker.

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Metabolomics guided discovery of specialized metabolites from *Streptomyces lunalinharesii* A54A, a promising biological control agent of crop phytopathogens

Dr. Jairo I. Quintana-Bulla[1], Gabriel Luiz Padoan Gonçalves[1], Roberto G. S. Berlinck[1], Simone Possedente de Lira[1]*

1Department of Math, Chemistry and Statistics. Luiz de Queiroz College of Agriculture. University of São Paulo – CEP 13418-900, Piracicaba, SP, Brazil
2São Carlos Institute of Chemistry. University of São Paulo – CEP 13560-970, São Carlos, SP, Brazil
* Lira splira@usp.br

The assault from fungal and bacterial pathogens on crops causes great losses on food production worldwide. Traditional methods have heavily relied on chemical pesticides for their control, with detrimental effects on environment and human health as well as creating pest resistance. In this regard, biological sources have emerged as an alternative for crop pest management, particularly actinobacteria because of their capacities for the biosynthesis of specialized metabolites with antimicrobial properties. This study investigates the use of the endophytic actinobacterium *Streptomyces lunalinharesii* A54A, obtained from the leaves of the plant *Anthurium urvilleanum* from São Paulo Coast, as a biological control strain against various phytopathogenic bacteria and fungi. In vitro bioassay-guided fractionation revealed the potent growing inhibiting fractions, demonstrating its broad antimicrobial spectrum against both bacterial and fungal phytopathogens. Furthermore, a high-resolution tandem mass spectrometry (HRMSMS) untargeted metabolomics approach was implemented in order to investigate chemical space produced by *S. lunalinharesii* A54A. Statistical multivariate analysis and molecular networking (GNPS) analysis showed diverse chemical compound classes such as nonribosomal peptides and polyketides in the bioactive fractions. This approach made possible to target the potentially new antimicrobial compounds for their isolation in further stages. Hence, these results indicate the potential of *S. lunalinharesii* A54A as a biological control agent of a wide range of microbial pythopathogens. Finally, the merged activity-metabolomics approach proves to be a powerful tool to accelerate the bioprospection of microorganisms for their subsequent application for crop protection.

KEYWORDS: Actinobacteria, untargeted metabolomics, specialized metabolites, biological control, phytopathogens, crop protection

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Production of Organophosphate by Cyanobacteria Through Different Cultivation Conditions Aimed at Applications in Agroindustry

M. G. T. S. Souza[1]*, R. D. Aragoni[1], E. Pinto[1], D. Oliveira[2], K. A. Fernandes[1]

1Centro de Energia Nuclear na Agricultura da Universidade de São Paulo (CENA/USP), Av. Centenário, 303 - São Dimas, Piracicaba - SP
2 Universidade Tecnológica Federal do Paraná, Av. Sete de Setembro, 3165 - Rebouças, Curitiba - PR

Cyanobacteria, photosynthetic organisms of great ecological and economic importance, are distinguished by their ability to produce valuable secondary metabolites. In this study, we employed the cyanobacterium Sphaerospermopsis torques-reginae (strain ITEP-24), notable for synthesizing value-added compounds, including Guanitoxin (GNT), a natural organophosphate with neurotoxic effects. We assessed biomass and GNT production in various culture media (BG-11; BG-11 + 5% N; ASM-1; ASM-1 + molybdenum and 5% N; ASM-1 + 5% N; and Z8) under aeration and a 12h:12h photoperiod over 52 days. Samples were collected at intervals of 4, 5, and 7 days (n=4) for cell count, chlorophyll-a concentration and GNT identification. The samples for cell counting were fixed in formaldehyde (4%) and quantified using a Neubauer chamber. Extraction of Chlorophyll-*a* and GNT was performed in 90% Acetone and 70% Methanol, respectively, followed by ultrasonication and centrifugation. Chlorophyll-a quantification was conducted through spectrophotometry (at 652 nm and 665 nm), and GNT identification by LC-MS/MS in MRM mode (253>58 and 253>159 [M+H]+). The results indicated that the BG-11 + 5% N medium maximized cell concentration, while for chlorophyll-a, the BG-11 + 5% N and Z8 media were outstanding. On the other hand, no statistical difference was found between the BG-11 and modified ASM-1 and Z8 media in LC-MS/MS analyses. However, we can conclude that increasing the concentration of N and other nutrients in the medium can significantly enhance biomass and GNT production, representing a promising advance for industrial applicability.

KEYWORDS: Cyanobacteria, organophosphate, agroindustry, biopesticide.

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