Programa de Pós Graduação em Microbiologia Agrícola ESALQ/USP

APLICAÇÕES E PERSPECTIVAS

PARA A AGRICULTURA DO FUTURO

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PIRACICABA/SP | 2023

Aplicações e perspectivas para a agricultura do futuro: anais do V Simpósio de Microbiologia Agrícola

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Realização do Evento

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Dr. Thiago Rodrigues de Castro (Koppert, Brasil)

Dra. Valéria Maia Merzel (UNICAMP, Paulínia-SP)

Dr. Welington Luiz de Araújo (ICB-USP, São Paulo - SP)

Carta aos Participantes

O evento V SMAGRO foi organizado pelo Programa de Pós-Graduação em Microbiologia Agrícola da Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, de 11 a 14 de abril de 2023. A microbiologia agrícola é um campo amplo que engloba os setores de agronegócio e indústria que adotam microrganismos como parte fundamental. Envolve estudos nos campos da ecologia, bioprospecção, biorremediação, ciências genômicas, bioinformática, dentre outras. O principal objetivo deste simpósio foi promover discussões aprofundadas e a troca de experiências sobre tópicos cruciais no campo da Microbiologia Agrícola. Nosso objetivo foi promover a integração entre profissionais atuando na academia e no setor corporativo, abordando vários aspectos proporcionados pela microbiologia agrícola por meio de um programa com palestras, mesas-redondas e minicursos, todos conduzidos por profissionais especializados.

O tema escolhido deste ano foi "Aplicações e Perspectivas para a Agricultura do Futuro". O evento contou com palestras e debates conduzidos por palestrantes convidados especializados no campo. Além disso, foram realizadas apresentações de resumos com o objetivo de deliberar sobre tendências atuais e demandas no campo relacionadas ao agronegócio brasileiro. Este evento também serviu para lançar luz sobre as perspectivas futuras para enfrentar os desafios da agricultura do futuro, com ênfase nas preocupações ambientais e na segurança alimentar.

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

Cordialmente,

Profª Dra. Simone Possedente de Lira
Coordenadora do Programa de Pós Graduação em Microbiologia Agrícola ESALQ-USP

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

Minicursos

Estudos de comunidades microbianas com a utilização de plataforma KBase
Dr. Thierry Alexandre Pellegrinetti (CENA/USP)

Teoria à prática de extração, colonização, montagem e exame de esporos de Fungos Micorrízicos Arbusculares (FMAs).
Téc. Denise de Lourdes Colombo Mescolotti
Dr. Antonio Marcos Miranda Silva

Estatística Multivariada aplicada a dados biológicos
Me. Jhessica Letícia Kirch

Principais produtos microbiológicos utilizados na agricultura atual
Me. Isabella Tavares de Oliveira Silva

Abordagens e estratégias para o isolamento e purificação de compostos naturais bioativos
Prof. Dr. Roberto Gomes de Souza Berlinck – IQSC-USP

Palestras

Microbiologia Agrícola "Aplicações e perspectivas para a agricultura do futuro"
Dr. Nelson Lima (Universidade do Minho - Portugal)
Dr. Carlos Alexandre Costa Crusciol (UNESP)

Divulgação científica e popularização da ciência
Dra. Camila Manoel Crnkovic (FCF-USP)

SynCom: Modulação do microbioma para promoção de crescimento e proteção de plantas
Dr. Fábio Lopes Olivares (UENF)

Biológicos: onde estamos e para onde vamos?
Me. Sergio Zanon da Silva (Biotrop)

Microalgas para produção de bionanofertilizantes aplicados à agricultura sustentável
Dra. Flávia Vischi Winck (CENA-USP)

Energia Sustentável: benefícios e aplicações do biogás na agroindústria brasileira
Dra. Bruna de Souza Morais (UNICAMP)

Mesa redonda: Biogás: Panorama atual e perspectivas de geração de energia renovável
Dra. Leidiane Ferronato Mariani (Amplum Biogás)
Dra. Rose Maria Duda (UNESP)
Dr. Ricardo Luís Radis Steinmetz (EMBRAPA Suínos e Aves)

Uso de Controle Biológico na Agricultura Tropical
Dr. Thiago Rodrigues de Castro (Koppert)

Mesa redonda: Bioprospecção e Biotecnologia de microrganismos aplicada a agricultura
Dra. Suikinai Nobre Santos (Assessoria em Biotecnologia Microbiana)
Dra. Maria Carolina Quecine Verdi (ESALQ-USP)
Dr. Welington Luiz de Araújo (ICB-USP)

Overview sobre Regulamentação de Biopesticidas no Brasil
Ana Claudia Candido (Staphyt)

Freshwater Bioprospecting: An untapped 'El Dorado' for new biomolecules from eukaryotic microbes.
Dr. Paul Long (King's College - Inglaterra)

Interações microbianas e os mecanismos genéticos responsáveis pela sobrevivência dos micro-organismos em ambientes extremos
Dra. Valeria Maia Merzel (UNICAMP)

Ferramentas de biologia molecular para estudos ômicos no agro
Dr. Helder Teixeira de Freitas (Thermo Fisher Scientific)

Round Table: Abordagens ômicas para estudos de ecologia microbiana
Dra. Fabiana de Souza Cannavan Moraes (Metaquantion)
Dr. Jackson Antônio Marcondes de Souza (UNESP)

RESUMOS

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

SUMÁRIO

Study of the contribution of *cohLAB* genes to copper resistance in *Xanthomonas citri* subsp. *citri*

Gabriel Felicio Silva; Natália Alleoni; Mario Nicolas Caccalano; Henrique Ferreira.

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Abstract

Citrus canker is a disease caused by the bacterium *Xanthomonas citri* subsp. *citri*, which affects all the commercial citrus cultivars, leading to a decline in production and fruit quality, and representing a serious threat for Brazilian citriculture. Citrus canker has no cure, and disease control is mainly done by the pulverization of copper based bactericidal formulations in the orchards. However, this metal is bio-cumulative, and copper resistance has already emerged in *X. citri*, which imposes challenges for the control of citrus canker. Copper resistance is linked to the presence of *copLAB* or *copABCD* genes, while tolerance to the metal may be expressed by the increased expression of the homeostasis genes *cohLAB*. The latter are homologous to the *cop* genes, and their participation in copper tolerance was described very recently. Therefore, not much is understood about them, and, for many years, they were mistaken as resistance genes. In this study, we are characterizing the ability of *X. citri* mutants to tolerate different copper concentrations, through the deletion of *cohLAB* genes in resistant (A44) and sensitive (306) strains. The technique known as REMA was used to determine the minimal inhibitory concentration of copper strains can support. Through this method, it was possible to determine that the deletion of *cohLAB* genes enhanced the sensibility to copper in the strain 306. In the strain A44, the deletion had no effect, and the bacteria continued to endure high concentrations of copper. Those results indicated that these genes are in fact involved in copper bacterial metabolism.

Keywords: Alternative; Citric canker; Copper; Control.

Citrus canker management: *Xanthomonas citri* subsp. *citri* novel lytic bacteriophage and host range

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Abstract

Citriculture is amongst the most important agricultural activities in Brazil and its constantly threatened by diseases. Among them is Citrus Canker, caused by the Gram-negative bacterium *Xanthomonas citri* subsp. *citri* (*X. citri*) and causes significant economic losses. The disease management is mostly done using copper-based bactericides that contributes to long-term damage to the environment and the selection of bacterial resistance. Because of that new alternatives must be sought. In this context, control using lytic phage is an attractive, sustainable alternative. However very little is known regarding the diversity of phage infecting *X. citri*, their host range or basis of host interactions. Hence, we isolated and sequenced 84 *X. citri* bacteriophage obtained from canker infected citrus leaves collected from different orchards in Sao Paulo, to build a panel of novel lytic phage. These phage isolates were characterized for its host range against 102 *X. citri* historical isolates from different citrus producing areas around the globe, obtained from 1979 until 2002 – including two recent isolated copper resistant (CuR) *X. citri*. Phage genetic identification showed their distribution among three different families: Siphoviridae (3); Pokkenvirus (1) and Podoviridae (80). Furthermore, phage isolates showed a broad host range with 77,70% of bacterial isolates being susceptible to all phage tested and only 22,22% resistant, which can be attributed to distinct receptor specificities. The majority of phage resistant bacteria were not isolated in Brazil and/or are antique isolates, that can indicate a diversity change related to the recent *X. citri* population in the field.

Keywords: citrus canker; citriculture; bacteriophage; phage; copper

Financial support: CAPES; FAPESP 2017/50454-9

The obtaining of an extract containing guanitoxin for application on insect control.

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Abstract

Guanitoxin (GNT) is a molecule produced by freshwater cyanobacteria that has an inhibitory capacity for the enzyme acetylcholinesterase greater than or equal to certain synthetic organophosphates known on the market, showing potential applicability in controlling pests. Thus, the objective of this work was to evaluate the effect of the aqueous extract of *Sphaerospermopsis torques-reginae* (strain ITEP-024) on micro-tomato (*Lycopersicon esculentum CV MICRO-TOM*) with the species *Tuta absoluta*. First, the presence of GNT was confirmed by liquid chromatography coupled to mass spectrometry. The micro-tomato bioassay with the species *T. absoluta* was carried out in five different treatments (ultrapure water as a negative control, malathion insecticide as a positive control and three concentrations of the aqueous extract containing the lyophilized strain (62.5 mg/L; 125 mg /L and 250 mg/L)). The results obtained indicated that GNT was present in the samples and the acute exposure to the extract in *T. absoluta* showed significant results for the two extracts with the highest concentrations. It is the first time that a bioassay has been carried out to test the insecticidal potential of GNT. We conclude that the extract containing GNT in the highest concentrations was able to cause vital alterations in the insects, however it cannot be affirmed that the toxic effects caused in *T. absoluta* were attributed only to the toxin. As there is no analytical standard for the identification of GNT, its purification and isolation are not yet possible. In this sense, it is necessary to advance techniques for isolating this important neurotoxin.

Keywords: Cyanobacteria; Sustainable Agriculture; Guanitoxin; Anatoxin-a(s); Insecticides.

Financial support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Optimization of lignocellulolytic enzymes production under solid-state fermentation by *Bacillus subtilis* CCMA 0085

Clara Resende de Souza Castro[1]; Nayara Aparecida dos Santos Ribeiro[1]; Tainá Cearense Torres[1]; Victor Hugo Teixeira Buttrós[1]; Cristina Ferreira Silva[1]; Luciana Silva Ribeiro[1]

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Abstract

Bacillus subtilis is a Gram-positive and sporulating bacterium, considered a model system for biochemical, genetic, and physiological studies. Because it is a widely studied species, its biotechnological potential was and is explored in order to produce several metabolites of industrial interest such as, for example, biosurfactants and enzymes. The aim of this work was to optimize the production of lignocellulolytic enzymes (carboxymethylcellulase; manganese peroxidase and xylanase) from *Bacillus subtilis* CCMA 0085 through solid-state fermentation using sugarcane bagasse as substrate. A rotational central composite design (DCCR) was carried out using pH and temperature as variables, totaling 11 experimental trials. The inoculum was standardized at 10^7 CFU/mL and the SSF was carried out in sterile plastic bags for 120 hours. Samples were taken after 0h, 24h, 96h, and 120h and the enzymes were quantified. The best conditions for specific enzyme production were, respectively: CMCase (at pH 6, 42°C: 9.99 UI/mL); MnPase (at pH 3.9, 35°C: 165.31 UI/mL); Xylanase (at pH 8.1, 35 °C: 0.79 UI/mL). That said, although the results show that *Bacillus subtilis* CCMA 0085 was the best producer of MnPase, followed by CMCase and the least xylanase, further studies need to be done in order to optimize and enhance the production of each enzyme separately.

Keywords: Manganese peroxidase; Carboxymethylcellulase; Xylanase; bacteria; SSF; sugarcane bagasse.

Financial support: Minas Gerais Research Foundation (FAPEMIG), National Council for Scientific and Technological Development (CNPq) and Coordination for the Improvement of Higher Education Personnel (CAPES) (Financial Code 001).

Extract from the fungi *Aspergillus unguis,* inhibit the bacterium *Xanthomonas citri* subsp. citri, known causer of the citrus canker.

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Abstract

Citrus canker is a disease caused by the bacterium *Xanthomonas citri* subsp. *citri* (X. citri) that affect several species of citrus of commercial importance and causes significant economic losses in orchards in Brazil. Currently, citrus canker control measures include spraying of copper-based chemicals that, while effective, have bioaccumulative and toxic effects described, also, members of the genus Xanthomonas have already been described as resistant to copper chemicals. It becomes necessary to develop new control methods that are effective and less harmful to the environment and human health.In this light, the present research evaluated the inhibitory potential of the extract produced by *Aspergillus unguis*, a endophytic fungi isolated from *Passiflroa incarnata* leaves, against X. citri. The activity of the extract was evaluated using a Resazurin Microtiter Assay Plate (REMA) assay, which consists of microdilutions in 96-well microplates, using resazurin dye as an indicator of bacterial growth. The Minimum Bacterial Concentration (MBC) was determined from the incubation of an aliquot of the 96-well microplate in solid NYG plates, during the period of 48 hours. The values obtained in the REMA were used to construct a dose-response graph, identified IC90 and IC50 values, being 408.29 ug.ml-1 and 211.51 ug.ml-1, respectively. The MBC value corresponded to 525 ug.ml-1.

Keywords: Xanthomonas; citrus canker; endophytic fungus; antibacterial.

Financial support: 2022/10238-3, Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

Exploring the antimicrobial activity potential of cyanobacterial strains from soda lakes

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Abstract

Cyanobacteria are Gram-negative, photoautotrophic bacteria with a high capacity to produce specialized metabolites, including compounds with antimicrobial properties against human pathogens. This study aimed to evaluate the antimicrobial activity potential of cyanobacteria strains isolated from soda lakes of the Pantanal. The genomes of three strains (*Pantanalinema rosaneae* CENA516, *Geminocystis* sp. CENA526, and *Alkalinema pantanalense* CENA528) from the Pantanal were analyzed for the presence of genes related to biological activities against microorganisms using the software AntiSMASH. The production of bioactive compounds was investigated through disk diffusion assays using aqueous and ethanolic (50%) extracts of freeze-dried biomass from the strains. The tests were performed on *Escherichia coli* growing in LB culture medium. Ten microliters of resuspended dry extracts (10 mg/mL) were added to paper disks (6 mm), while sterilized deionized water was used as a negative control, and ampicillin (100 mg/mL) was used as a positive control. The genomes of the three strains contained genes for the biosynthesis of non-ribosomal peptide synthetase and polyketide synthetase, which are known to act in the synthesis of several metabolites with potential inhibitory action against microorganisms. Only the CENA 526 EtOH 50% and CENA 528 EtOH 50% extracts showed some inhibitory activity, with 9.05 mm and 7.94 mm inhibition zones, respectively. The result of this study suggests that the cyanobacteria strains isolated from soda lakes of the Pantanal have the potential to produce specialized metabolites with antimicrobial properties.

Keywords: Antimicrobial activity; *E. coli*; Specialized metabolites, Genome.

Enzyme profile of solid-state cultivation of *Pleurotus djamor*

Danilo Jose Machado Abreu [1]; Roberta Hilsdorf Piccoli1[2]. ; Tainá de S C Torres [2]., Clara Resende de Souza Castro[2], Anna Carolina Erlacher Medici[2], Victor Hugo Teixera Buttrós[2]

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Abstract

Fungi have demonstrated a significant capacity to degrade an extraordinary variety of organic compounds by means of lignocellulolytic and hydrolytic enzymes. The aim of this work is to quantify the enzymatic activity of *Pleurotus djamor* solid fermentation. Commercial cultures of *P. djamor* were obtained and incubated for 12 days at a temperature of 28°C. Samples were taken every three days for the preparation of the enzyme extract. For the extract, twenty grams of lignocellulosic substrate degraded by the fungus were used added to 150 mL of sodium acetate buffer solution (50 mM; pH 5.0), kept on an orbital shaker at 180 rpm for two hours at 25 °C. The contents were filtered, and the supernatant recovered by centrifugation at 8000 rpm for 20 min at 4°C. The enzymatic activities of lacase, xylanase, endoglucanase and total cellulase present in the extracts were quantified, which were expressed in U/mL. The experiments were performed in triplicate. It was verified that the maximum peak of the evaluated enzymes production was in the 9 day of solid state fermentation being: 1.92 U/mL (Xylanase), 0.11 U/mL (Endoglucanase), 0.05 U/mL (Total cellulase), 0.003 U/mL (β-glicosidase), 21.44 U/mL (Lacase). Evaluating the maximization of protein content and enzyme activity is a promising observation of lignocellulolytic and hydrolytic enzyme production by *P. djamor* species besides a preliminary study of the use of the crude extract.

Keywords: Mushroom; Basideomycete; Enzyme extracts; Solid state fermentation; Lignocellulolytic and hydrolytic enzymes.

Financial support: Minas Gerais Research Foundation (FAPEMIG), National Council for Scientific and Technological Development (CNPq) and Coordination for the Improvement of Higher Education Personnel (CAPES) (Financial Code 001).

Identification of mercury resistant filamentous fungi

Tainá de S C Torres [1]; Judith Liliana Solózano Lemos[2]; Danilo Jose Machado Abreu [2]., Clara Resende de Souza Castro[2], Anna Carolina Erlacher Medici[2],Victor Hugo Teixera Buttrós[2]

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Abstract

Large cities have seen the majority of Brazil's industrial growth in recent decades, which is indicative of the dramatic rise in pollution levels mostly brought on by industrial activity. One of the biggest pollutants is mercury, which is more hazardous than inorganic ions and can bioaccumulate after being consumed in tainted food, leading to serious issues. In order to exploit Aspergillus and Penicillium as bioremediation agents for this metal, the current investigation evaluated their potential as fungi for removing Hg2+. 14 fungal strains were therefore tested at 10 mg Hg2+/L for resistance, and the strains that performed best were then tested at higher doses, i.e. 15, 20, and 25 mg Hg2+/L. Aspergillus flavus IOC 4123, Aspergillus flavus IOC 4133, Aspergillus niger IOC 4003, Aspergillus niger IOC 4616, Aspergillus versicolor IOC 4266, and Penicillium corylophilum IOC 4209 all produced satisfactory results after the first phase. A. niger IOC 4003 showed promise for resistance to Hg2+ in the Minimum Inhibitory Concentration (MIC) study, growing at a concentration of 75mg Hg2+/L. However, at a concentration of 150 mg Hg2+/L, it was entirely blocked. The results for the Q value were analyzed using linear regression derived from an experimental design matrix: decreases in ionic strength and inoculum size, as well as increases in the initial concentration of Hg2+ and contact time, would encourage an increase in the mass of mercury retained per mass.

Keywords: Mercury; Filamentous fungi; Wastewater treatment; Bioremediation; Biosorption

Financial support: Rio de Janeiro Research Foundation (FAPERJ)

Dynamics of encapsulated microbial inoculant in soil under different salinity conditions

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Abstract

Phosphate fertilizers are widely used in order to achieve high crop productivity, but their production processes are expensive and can cause environmental damage. An alternative for fertilization is the use of P-solubilizing microorganisms (PSMs). However, a challenge during the inoculation with microorganisms is the high complexity of the soil in relation to biotic and abiotic factors. To overcome this limitation, a strategy to improve the efficiency of PSMs is the use of encapsulated microorganisms as an inoculant. This strategy can protect the microorganisms from adverse environmental effects, physical and chemical factors of the soil, and increase the shelf-life time. The present study evaluated the effectiveness of free and encapsulated *Bacillus subtilis* in the colonization of clay soil under different salinity conditions at 30 ºC during 7 days. A polymeric gelatinized starch matrix was used for microbial encapsulation. The results showed that the encapsulated *B. subtilis* had a higher abundance of colonization in comparison with the free microorganism, indicating that encapsulation can improve the efficiency of PSMs in salinity stress. However, the abundance of colonization decreased with high salinity condition (10 mM and 20 mM), suggesting that salinity stress affects the microorganism's development. Overall, the study confirmed the potential of encapsulated *B. subtilis* as a good strategy for fertilization, particularly in providing protection of the microorganisms in adverse abiotic conditions. This approach could be a more sustainable alternative to traditional phosphate fertilizers. Further research is needed to evaluate the long-term effects of encapsulated microorganisms on crop productivity and soil health.

Keywords: *Bacillus subtilis*; encapsulation; inoculation; soil; abiotic conditions.

Financial support: Embrapa, CNPq, FAPESP and CAPES.

Determination of the Host-Range of 24 *Xanthomonas citri* bacteriophages.

João Vitor Alves Balieiro[1]; Mario Nicolas Caccalano[1]; Natalia Alleoni[1]; Caio Felipe Zamuner[1]; Henrique Ferreira[1].

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Abstract

Citrus canker is a disease caused by *Xanthomonas citri* (*X. citri*) that decreases citrus production. Over the last seventy years, this disease has widely spread amongst citrus orchards in Brazil, which led canker to be a major problem for Brazilian citriculture. The main strategy regarding the disease prevention is the use of copper-based sprays in citrus crops. The use of copper-based formulations in citrus-growing areas has been associated with the contamination of water resources and organisms that live within these areas, given the fact that copper can be cytotoxic in high concentrations and is bioaccumulative. Furthermore, copper-resistant strains of *X. citri* have been reported. Based on this perspective, it is essential that less toxic management techniques for canker are developed. Bacteriophages are viruses capable of infecting bacteria and are being explored as an alternative method for the control of different diseases. Moreover, host-bacteriophages interactions are highly specific, making bacteriophages a potential alternative management technique for citrus canker. The present work verified the host-range of 24 bacteriophages against a panel of 24 strains of *X. citri* isolated from different regions of São Paulo state. The results have provided a range of bacteriophages, such as P180 and P186, that were highly effective in infecting different *X. citri* isolates, whilst others, such as P74, were unable to do so. Therefore, these two isolates could be used in further studies to develop new formulations for combating citrus canker from different regions.

Keywords: Citrus canker; Copper-resistance; Bacteriophages.

Metagenomics unveils shifts on microbial assemblages on sugarcane rhizospheres under contrasting farming systems

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Abstract

Synthetic agrochemicals widely used in sugarcane cultivation may cause impacts on soil microbial diversity, moreover, it has been associated with high production costs. Therefore, organic farming is presented as a sustainable alternative, in which productivity is increased while environmental impacts are reduced. In this context, the rhizosphere microbiota is extremely important for plant development and could be benefited by organic management. Through culture-independent methods, such as metagenomics, it is possible to study the diversity and genome functionality of microbiota, which allows for the discovery of new species, biomolecules, and biotechnological solutions. The present study aimed to compare the impact of organic and conventional cultivation on the microbial community of sugarcane rhizosphere. Soil samples were collected, and DNA extraction and sequencing were performed using high-throughput technology, followed by bioinformatic analyses. By comparing the metagenomic data of the two farming systems, it was possible to evaluate the shifts caused in their microbial assemblages, including viruses, bacteria, archaea, fungi, and other eukaryotes. The quantitative analysis revealed subtle but persistent differences in microbial diversity, suggesting that management is an important driver of plant-associated microbiome. These findings advance knowledge about the microenvironment and can be used to conceive field technologies, such as bioinoculants or soil transplantation, to improve bioremediation and promote more sustainable sugarcane growth. In addition, microbiota studies can lead to more efficient soil treatments that could lead to both a productive and sustainable agriculture, by reducing the application of synthetic compounds harmful to the environment and public health.

Keywords: sugarcane cultivation; organic farming; microbial community; rhizospheric microbiota; metagenomes.
Financial support: This study was financed in part by the Coordenação de perfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

Microbial diversity of soybean rhizosphere and root nodules associated with exogenous application of *Bradyrhizobium* sp.

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Abstract

Seed treatment with exogenous nitrogen-fixing bacteria (NFB) is an essential and efficient practice to improve nitrogen availability and reduce the use of synthetic nitrogen-based fertilizer in the soybean production chain. However, the interaction between exogenous *Bradyrhizobium* and the soybean rhizosphere microbiome is unknown. In this regard, the objective of this study was evaluate the effect of *Bradyrhizobium* application in the soybean-associated microbiome. For this, soybean seeds were sowed in nine soils collected from different soybean production areas under greenhouse conditions. During the evaluations, were observed seedlings with high vigor in three different soils, being these soils selected for microbial community investigation. Thus, 16S rRNA gene metabarcoding analysis was performed in the **i)** soil before and after test, **ii)** rhizosphere around the surface of the nodules and **iii)** inside the nodules. The results obtained showed a similarity of the microbial community both in the soil samples before and after the test, as well as in the surface area of the nodules. Meanwhile, the microbiome inside the nodules was composed exclusively of *Rhodopseudomonas* with greater abundance, followed by *Bradyrhizobium* with lower abundance, regardless of the tested soil. This result can guide further studies involving the co-inoculation of NFB and other beneficial bacteria in soybean crops, improving yield and reducing the environmental impact of artificial nitrogen fertilizers application.

Keywords: Soybean; nodules; 16S rRNA gene metabarcoding; microbial association; *Bradyrhizobium*.

Plant development potential of endophytic fungi from Brazilian Cerrado

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Abstract

Endophytic fungi are microorganisms that inhabit plant tissues and have attracted significant attention in agriculture due to their ability to enhance plant growth and development. In this study, we aimed to prospect endophytic fungi from Cerrado forage plants and evaluate their potential to promote plant growth. Forage plant roots (for example, *Panicum maximum*, different species of *Brachiaria*, as well as spontaneous grasses from collection areas) were collected from two organic farms located in the cities of Entre Rios de Minas and São Vicente de Minas in Minas Gerais, Brazil. Four lots were selected from each farm, and roots were collected from four points in each lot. The roots were surface disinfected, cut into small fragments, and placed onto Petri dishes containing PDA medium. Fungal strains were isolated, and screened for their potential to solubilize phosphate, mineralize phytate and produce indole compounds. We obtained 254 fungal isolates (193 filamentous fungi and 61 yeasts), 90 showed positive effects in either phytate mineralization (21 isolates) or phosphate solubilization (69 isolates), and 12 isolates showed positive effects in both tests. All 90 isolates with positive results for either test also exhibited indole compounds production, with three isolates (245, 11, and 20) exhibiting particularly high levels of indole production (196,85 ug/ml, 148,87 ug/mL, and 145,3 ug/mL, respectively). These findings suggest that the endophytic fungi extracted from Cerrado forage plants show great promise in promoting plant growth and development, but further research is required to fully elucidate their potential.

Keywords: forage; endophytic microbiota; plant-associated fungi; plant growth; bioinoculant

Financial support: Rural Sutentável project, from Brazilian Institute of Development and Sustainability (IABS) and CAPES.

Technological development for the cultivation of the mushroom *Agrocybe aegerita* in Brazil

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Abstract

This project aimed to analyze the cultivation of *Agrocybe aegerita* using different raw materials as substrate, with or without protein supplementation. The raw material used consisted of bulk materials (rice and wheat straw, sawdust and sugarcane bagasse), with percentages between 80-100%, and concentrated materials (homogenized wheat and rice brans), with doses between 0-20 % of supplement. The substrate was moistened (65%), distributed in polyethylene plastic bags, with a mass of 0.8 kg of moist substrate. After that, they were autoclaved at 121°C for 4 hours and inoculated in a laminar flow chamber with 2% inoculum. During production, two transverse cuts (10 cm) or four horizontal cuts (10 cm) were made on the surface of the plastic bag, according to the vigor of the colonized substrate. The cultivation lasted 117 days, with manual harvesting and 3 harvesting flows. During this period, the air temperature remained at 23 ± 2°C and the relative humidity at 80 ± 5%, in a controlled environment. The following agronomic parameters were evaluated: productivity (%), precocity, average mass of mushrooms (g), number of mushrooms (u), and block contamination index. The results obtained were evaluated by analysis of variance (ANOVA) and the means compared by the Tukey test at 5% probability. Rice straw substrates obtained the best results compared to other raw materials. No significant difference was observed regarding the average mass of the mushrooms. Sawdust was the worst raw material in all analyzed segments.

Keywords: Straw, bran, substrate, supplement, productivity.

Isolation of microorganisms from Brazilian green propolis

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Abstract

Brazilian green propolis is a mixture of resinous substances, composed mainly of resins of unexpanded cauline apexes of *Baccharis dracunculifolia*. The biological activity of this propolis has already been well reported, such as antimicrobial activity, but there are no studies on the associated microbiota. Therefore, the objective of this work was to isolate microorganisms from Brazilian green propolis and resin of botanical origin. The collection of propolis and stem tips of *B. dracunculifolia* was carried out in Minas Gerais. The propolis was stored in a falcon tube and the shoot apexes in an eppendorf tube in saline solution. Then, the samples were homogenized and, with an aliquot of 1 mL, serial dilutions were made up to 10^{-6} for bacteria and 10^{-4} for fungi and actinobacteria, followed by plating in culture medium. The plates were incubated at 28 °C and inspected daily, as the microorganisms were visible, and transferred to new plates until total purification of isolates. After 24 hours of incubation, it was possible to observe the growth of bacteria, while fungi and actinobacteria began to grow one week after plating. A total of 44 microorganisms from propolis and 24 from *B. dracunculifolia* buds were isolated. Of these, only one and two filamentous fungi were isolated from propolis and buttons, respectively. The identified morphotypes are brown, yellow, white and gray. With this, we realize that there are microorganisms associated with propolis that require further investigation to understand this complex system.

Keywords: *Baccharis dracunculifolia*; Resins; Morphotype; Biological Properties

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Biogas production from sweet sorghum broth in UASB reactors

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ABSTRACT

Sweet sorghum has often been used to optimize ethanol production, especially in the sugarcane off-season, to maintain high levels of productivity in the sugar-energy sector. However, there is the possibility of producing biogas from sweet sorghum juice, which would avoid the generation of vinasse, derived from ethanol production. Vinasse has a high polluting potential, due to its high organic load, therefore offering risks of environmental impact. The objective of this work was to evaluate the potential for biogas production from the anaerobic digestion of sweet sorghum juice in two upflow anaerobic sludge blanket reactor (UASB - R1 and R2), in series, with volumes of 3.5 and 6.0 L, respectively, applying a hydraulic detention time of 28.3 h to the system (R1 + R2). The organic load rate applied to R1 and R2 ranged from 0.25 to 14.77 g total COD $(L\ d)^{-1}$ and from 0.36 to 8.7 g total COD $(L\ d)^{-1}$, respectively. The mean ratio between intermediate alkalinity and partial alkalinity (IA/PA) observed was 3.2 for R1 and 3.32 for R2, and the anaerobic system remained stabilized. Furthermore, an average volumetric methane production of 0.58 and 0.39 L CH_4 $(L\ d)^{-1}$ was observed for R1 and R2, respectively, from the 90^{th} to the 215^{th} day of operation. Denoting, therefore, that the anaerobic digestion of sorghum juice can represent an important and effective alternative for energy generation (biogas), and this can exclude the risks of environmental impact that are offered in the generation of ethanol, from this same raw material.

Keywords: Sweet sorghum; anaerobic digestion; biogas.

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Startup of a UASB reactor for anaerobic digestion of sorghum vinasse

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Abstract

Population and industrial growth have increased the need to use natural resources, however, the impact of intense industrialization has made ecosystems unstable, so there is a growing interest in developing alternatives and adopting sustainable development practices. Sweet sorghum [Sorghum bicolor (L.) Moench] has proved to be a highly relevant alternative in the generation of biofuels, due to its adaptability and similarity to sugarcane, which can reduce downtime, of sugar-energy plants in the off-season. In this work, vinasse obtained from the distillation of sorghum ethanol was used. Anaerobic digestion can considerably reduce the organic load of sorghum vinasse. A conventional 15.2 L UASB reactor was used, operated in the mesophilic controlled temperature range, for 65 days, with a hydraulic detention time of 12 h and organic load rate of 0.33 to 1.23g COD $(L\ d)^{-1}$, evaluating system behavior during startup. The maximum chemical oxygen removal efficiency was 85%, being observed during the sludge adaptation process. The pH of the influent was corrected with $NH_4\ HCO_3$, so that the alkalinity of the system increased, reaching an intermediary alkalinity and partial alkalinity IA/PA) ratio of 0.3 and mean values of volatile acids from 37 to 150 mg L^{-1}. With the help of an alkalizing agent, the microbiota was able to adapt and remove the organic material from the sorghum vinasse.

Keywords: Anaerobic digestion; UASB reactor; Vinasse Sorghum; Biofuel.

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Inoculation of phosphate-solubilizing bacterial strains in maize grown under different types of phosphate fertilization management

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Abstract

The use of microbial inoculants is an ecologically sustainable alternative for crop production. BiomaPhos® is a commercial inoculant composed of two phosphate solubilizing strains (B119 and B2084), which promotes yield increase for different crops. This work aimed to evaluate the effect of BiomaPhos® on the genetic diversity of the rhizosphere microbiota of maize grown under different types of phosphate fertilization management. It was evaluated two areas, one of consolidated no-tillage systems (NTS) and the other with newly adopted NTS in MS state, Brazil. The experimental design was a randomized complete block with three replications with a factorial of 2x5, with and without BiomaPhos® and five doses of P_2O_5 (0, 25, 50, 100 and 125%). Rhizosphere and non-rhizosphere soil were collected from maize plants from each plot and analyzed by terminal restriction fragment length polymorphism. Total DNA was extracted and *16S* (for bacteria) and *28S rRNA* (for fungi) genes were amplified and digested with restriction enzymes. PCR digested products were genotyped on Genetic Analyzer 3500XL with GeneMapper 5.0 software and analyzed with T-REX program. A significant difference was observed between the bacterial and arbuscular mycorrhizal fungi (AMF) communities of the rhizosphere and non-rhizosphere soils, and between the two areas. It was observed a genetic diversity difference between inoculated and non-inoculated within each dose of P_2O_5. However, for AMF it was observed a difference only on consolidated NTS regarding P_2O_5 dose. Our results expand knowledge on the microbial community structures from maize inoculated and grown in soil under different phosphate fertilization conditions.

Keywords: T-RFLP, BiomaPhos®, plant growth-promoting bacteria.

Financial support: Embrapa, Capes, CNPq, Fapemig and Finep.

Evaluation of cyanobacterial strains producing mycosporine-like amino acids (MAAs) for possible application in sunscreen

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Abstract

Cyanobacteria are photossyntetic prokaryote, which produce a wide variety of bioactive compounds with biotechnological potential. Mycosporine-like amino acids (MAAs) are compounds, produced by cyanobacteria when exposed to ultraviolet radiation (UVR), which have photoprotective and antioxidant functions. These compounds have been widely researched for possible application in sunscreens and anti-aging products. The aim of this work was to evaluate the production of MAAs by different cyanobacterial strains, for sunscreen application. Cyanobacterial cultures was cultivated in ASM-1 medium or BG-11 medium at 24 °C ± 2 °C under light:dark cycle (12h:12h) with white fluorescent illumination (80 μmol photons.μm^{-2} s^{-1}). Cyanobacterial suspensions were concentrated by centrifugation and lyophilized. Then, cells were extracted with formic acid solution + NH_4OH (pH 3.15). Cells were centrifuged and supernatants were collected to obtain the partially purified MAAs. Aqueous partially purified MAAs extracts were analysed by LC/MS coupled to 6460 Triple-Quad MS with electrospray interface. Separation was achieved in a column Synergi 4μ Hydro-RP 80 A (150 x 2,0 mm) at 30 °C. Buffer A was 0.2% formic acid solution + NH_4OH (pH 3.15), buffer B was ACN/H_2O (9:1) + 0.1% formic acid and the flow rate was 0.4 mL min^{-1}. The mass spectrometer was operated in a multiple reaction monitoring mode (MRM). The source was operated in the positive ion mode. MAAs were identified by comparison with standards. We report MAAs production in thirteen cyanobacteria. Their MRM chromatogram are comparable to those of shinorine, palytine and porphyra-334.

Keywords: Cyanobacteria; mycosporine-like amino acids; LC-MS/MS; sunscreen application.

Financial support: CAPES, CNPQ, FUSP e FAPESP.

Soil Bacteria Adaptability for Microplastic Biodegradation in a Simulated Marine Environment: A Respirometry and Colorimetry Study

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Abstract

Soil microbial diversity corresponds to hundreds or thousands of species, and their survival mechanisms are diverse as they can adapt to extreme environments, for this reason they are degrading agents with great enzymatic potential for plastic biodegradation, so the present experiment seeks to test the adaptability of soil bacteria for the biodegradation of microplastics in a simulated marine environment as an alternative to optimize for marine depollution. As an initial test, the halophilic induction of soil microorganisms was proposed with the intention of adapting them to the conditions found in the marine environment and subsequently evaluating the biodegradation through respirometry technique to determine the best size and proportion of microplastic. Additionally, we tested the bioavailability of potential microorganisms selected from the soil inoculum and grown on marine agar through colorimetry using the 2,6-Dichlorophenolindophenol as a redox color indicator for microbial metabolism. In the respirometric assays, we used polypropylene microplastics as a pollutant in amounts of 1.1 g, 2.2 g and 4.4 g in two different solutions: simulated marine solution and seawater (100 mL) with soil inoculum. After 77 days of weekly titrations, the degradation efficiency of the microorganisms present in each respirometer sample, not showing significant degradation in any of the tests. The size may be a limiting factor for biotic degradation, requiring sizes smaller than 8mm. However, the colorimetry assay with the four strains AS1, AS3, AS4 and AS5 selected from the soil inoculum, found a metabolic interaction, not necessarily biodegradable, of microorganisms with the polypropylene microplastic, mainly the AS5 sample with 45 minutes less than others, this makes for a more robust enzymatic alternatives to improve the efficiency of the biodegradation process, which will be part of subsequent follow-up experiments.

Keywords: microplastics; soil microorganisms; biodegradation; marine; adaptation.

Study of the biological control of phytopathogenic fungi using eugenol from essential oils

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Abstract

The use of essential oils on phytopathogenic fungi has been shown to be effective in reducing the incidence and severity of these pathogens, making it an alternative method for biological control. In addition, essential oils have low toxicity for humans and the environment, and are biodegradable, which makes them an excellent alternative to the use of chemical pesticides. Some compounds present in essential oils can act as membrane disruptors, interrupting the structural integrity of the membrane and allowing unwanted substances to enter. These interactions can lead to cell death as they can destabilize the membrane or inhibit or alter cell functions. In this study, 9 phytopathogenic fungi (*Rizoctonia solani AG4, Colletotrichum lindemuthianum, Phomopsis sojae, Fusarium brasiliense, Sclerotinia sclerotiorum, Macrophomina phaseolina, Colletotrichum truncatum, Cercospora kikuchii, Aspergillus flavus*) were used, each fungus was added to a Petri plate containing 10 ml of BDA and 10 µl of commercial Eugenol incorporated into the medium. They were stored in the BOD at 28°C for 7 days and it was possible to observe that Eugenol led to cell death totally inhibiting the growth of the 9 fungi, demonstrating that it has excellent efficacy as a biological control.

Keywords: Essential oil; Microorganisms; Eugenol.

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Mycelial growth and sporulation of *Colletotrichum falcatum* submitted to different culture media and photoperiods

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Abstract

Sugarcane (*Saccharum* spp.) is a widely cultivated crop in Brazil and in different parts of the world. However, phytosanitary factors such as the red rot disease, caused by the fungus *Colletotrichum falcatum*, can cause damage. For the cultivation of fungi *in vitro*, knowledge of the physiology of the phytopathogen is important, as nutritional and environmental factors can induce or inhibit mycelial growth and sporulation. Therefore, the objective was to evaluate the effects of different culture media and photoperiods on mycelial growth and sporulation of *C. falcatum*. Ten culture media were evaluated: Oat (O), Potato-dextrose-agar (PDA), Potato-sucrose-agar (PSA), Carrot (C), Sugarcane leaf extract (LE), Sugarcane stalk extract (SE), Tomato Extract (TE), Corn (M), Nutri (N) and YBA. Incubation in a growth chamber (BOD) at a temperature of 25±1°C was carried out under the following conditions: continuous light (24h), alternating periods of light (12h) and continuous dark (0h). The experiment was conducted in a completely randomized design (CRD) following a 10x3 factorial scheme with four replications (plates). The means of mycelial growth and conidial production were submitted to ANOVA and, when significant, compared by Tukey's test ($p \leq 0.05$). Significant differences were observed in mycelial growth and conidial production between different treatments. Six media showed the best mycelial growth rates (O, PSA, C, SE, M and N). As for the production of *C. falcatum* conidia, the most favorable conditions were PSA medium in 12h or 24h and Oat medium in 0h.

Keywords: Red rot of sugarcane; Conidia; Light; *in vitro* growth.

Financial support: FAPEG/CAPES.

Microbial volatile organic compounds with inhibition capability of Rhizoctonia solani

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Abstract

Volatile organic compounds (VOCs) are small molecules, with high diffusibility, mediators of interactions between organisms at short and long distances. Microbial VOCs are applied in agriculture, the antagonism of VOCs against phytopathogenic fungi shows promise. The potential of VOCs from rhizospheric bacteria in the biocontrol of the fungus *Rhizoctonia solani* was evaluated. Samples of beans, wheat and sugar cane were collected in agricultural areas, separating the soil adhered to the root system, serial dilution 1:10 and growth on plates with tryptone soy broth. Distinct colonies were purified and the collection of isolates was submitted to fungus inhibition test. The isolates were inoculated in Petri dishes with potato dextrose agar, each plate with four tested simultaneously on the sides and a mycelium disc of the pathogen was added to the center. The evaluation was made when the control reached the edge of the plate. Inhibitor isolates were submitted to the VOC production inhibition test. In bipartite Petri dishes (ensuring that the microorganisms grew in the same atmosphere, but without direct contact between them), the isolates were peaked on one side, the other side received a mycelium disc. The growth of the pathogen compared to the control was measured. From the collection of 180 isolates, 60 inhibited the fungus and proceeded to VOC evaluation, ten of which demonstrated the desired effect. Three completely prevented growth, three showed inhibition greater than 75% and four in the 50-75% range. The emission of VOCs proved to be a relevant mechanism in the inhibition.

Keywords: rhizospheric bacteria; biocontrol; volatile organic compounds.

Organic amendments enhance soil enzyme activity and chemical attributes in intensively degraded Cerrado soil

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Abstract

In order to establish a thriving ecosystem in a degraded Cerrado biome, we are restoring a degraded site located in Southeast of Mato Grosso do Sul. The site had suffered severe soil degradation during the construction of a Hydroelectric Power Plant (HPP) in the 1960s, which resulted in the removal of the soil's surface layer and exposure of the subsurface layer. This has led to the complete absence of vegetation and the accumulation of soil erosion, rendering the area unstable and unsuitable for supporting any ecosystem. Our research is focused on identifying a combination of soil amendments that can effectively improve the composition of Cerrado's soil (Oxisol) for restoration purposes. To achieve this goal, the area received different levels of biomass introduction and 12 treatments were implemented, ranging from no manipulation to the addition of various organic compounds, sheep residue, twigs, and native seed species. Soil samples were collected from a depth of 0.0 to 0.1 m, 24 months after the treatments were introduced. To assess microbial activity, we measured soil enzyme activity, including beta-glucosidase, phosphatase, and total enzyme activity (FDA), as well as other soil chemical aspects like phosphorus, carbon, nitrogen, and pH, which can be improved by the amendments used. By analyzing these methods, we aim to determine which treatment will respond most favorably to the different recovery techniques and residues added. Our ultimate goal is to apply these findings to other degraded areas and restore their once-thriving ecosystems by replicating these successful methods.

Keywords: Soil enzyme activity; Nutrients; Degraded soil; Cerrado Biome; Amendments.

Financial support: CAPES.

Efficiency of the Reconstructed Human Epidermis (RHE) in the classification of biological product by the *in vitro* irritation and corrosion tests (OECD 439 and 431).

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Abstract

Following the use of alternative methods and especially Art 6 of RDC 294 (July 29, 2019) from Brazilian Regulation, we evaluated the in vitro skin irritation (OECD 439) and corrosion (OECD 431) methods to classify biological products, using reconstructed human epidermis (RHE, SkinEthic^{TM}) provided by Episkin Brasil. This model represents the in vitro target organ. A biological product has been tested according to OECD 439 and 431. Cell viability in RHE model was measured by the vital dye MTT, the formazan salt was quantitatively measured after extraction from tissues after biological product exposure. The viability results obtained in vitro (mean ± SD) were NC: 100 ± 3.27; PC: 1.92 ± 0.07; Biological Product: 104.05 ± 7.92 for the irritation test. For corrosion were NC: 100 ± 7.90; Biological Product: 99.62 ± 1.19 for 3 minutes of exposure and NC: 100 ± 1.65; PC: 2.75 ± 0.13; Biological Product: 94.63 ± 0.46 for 60 minutes of exposure. Our results were within the acceptance criteria. As expected, the positive control demonstrated a significant relative cell viability reduction when compared to the negative in both tests. In addition, according to the results obtained, the Biological Product was classified according to UN GHS Category as "No Category" and "Non-Corrosive", corroborating with *in vivo* classification tests. Therefore, the RHE could be used for classification of biological products, but some care must be taken when handling the biological product in order to avoid cross contamination or loss of effectiveness of the product.

Keywords: Alternative methods; Biological products; Skin irritation; Skin corrosion.

The effects of *Bacillus spp.* inoculation to the mitotic index and parenchyma cell size in tomato

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Abstract

Rhizobacteria are groups of bacteria that have the ability to, directly or indirectly, associated with plants' rhizosphere region, promoting a stimulus in the development and growth of their host. One of these actions is indole acetic acid production, which promotes greater root growth when interacting with root cells. However, few studies have related how the auxin influenced the promotion of root growth. To better understand this question, we analyzed whether auxin influences the root cell size or the cell division speed. Mitotic index analyses and cortex parenchyma cell size measurements were needed to address this question. A collection of 21 strains of *Bacillus* spp. obtained by the Genetics of Microorganisms Laboratory "Prof João Lúcio de Azevedo" were used. We inoculated the strains in seeds of tomato cv. MicroTom and roots were collected on two different days – 3 and 6 days after inoculation. The meristematic tips of the roots were stained by Feulgen's method to determine the mitotic index. The region of the root cortex was stained with Fast-Green and photographed in the microscope to measure the size of the parenchyma cells. Strains RZ3MS27 obtained the highest mitotic index after three days, while FS3-7 obtained the highest mitotic index after 6 days of inoculation. We also emphasize that on the sixth day, the mitotic index decreased considerably compared to the third day in all strains. However, the parenchyma cell size was not different among RZ3MS27, FS3-7, and the controls. The results suggest a direct connection between the mitotic index and bacteria than during subsequent cell differentiation.

Keywords: *Bacillus*; rhizobacteria; cell division; root.

Financial support: GFS by CAPES, TC by PET-MEC, and MM is a PET-MEC fellow.

In vitro selection of multifunctional microorganisms aiming the control of *Colletotrichum lindemuthianum*

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Abstract

Developing technologies based on beneficial microorganisms for productive agricultural systems with low negative impact on the environment is one of the goals of sustainable agriculture. Beans are a component crop of production systems in the Cerrado Biome and among the diseases that affect bean production, anthracnose (*Colletotrichum lindemuthianum*) stands out. The objective was to evaluate the in vitro potential of bacterial isolates antagonistic to *C. lindemuthianum*. Twelve isolates were used: *Pseudomonas* sp., *Bacillus cereus, B. thuringiensis, Stenotrophomonas maltophilia, S. nitritireducens, Enterobacter hormaechei* and *Priestia megaterium*. 5 mm discs containing mycelium of *C. lindemuthianum* were deposited in the center of Petri dishes containing PDA (Potato-Dextrose-Agar) culture medium. After 19 days, cell suspensions of four bacterial isolates were transferred and arranged in equidistant lines and incubated for 20 days at 28°C under constant light. For the control, only pathogen mycelium disks were deposited. The design was completely randomized, with five replications. The evaluation was carried out with the aid of a digital caliper, to determine the radius of the pathogen colonies. Data were subjected to analysis of variance and means compared using the Scott-Knott test ($p \leq 0.05$). Among the treatments, the isolates *Pseudomonas* sp., *B. cereus, S. nitritireducens* and *maltophilia, E. hormaechei* and *P. megaterium* were statistically similar in terms of inhibition of mycelial growth of *C. lindemuthianum*. Among them, the isolates *E. hormaechei* and *B. cereus* stood out, both with 44.9% reduction in the mycelial growth of the pathogen, showing potential for studies of suppression of bean anthracnose.

Keywords: Bean; Disease; Phytopathogenic fungus; Biological control; Antagonism.

Characterization of endolysins from *X. citri* bacteriophages

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Abstract

Brazil is the major producer of sweet oranges, one of the most important commodities for the Brazilian economy. However, this economic activity is threatened by several infectious diseases such as citrus canker, which is caused by the Gram-negative bacterium *Xanthomonas citri* subsp. *citri* (*X. citri*). The main form of control of citrus canker is done by recurrent sprays of copper-based formulations. Copper is a heavy metal toxic to several organisms, and, in addition, resistant strains of *X. citri* have already emerged. Therefore, alternative control measures that minimize or substitute copper are urgently needed. Bacteriophages are viruses capable of infecting bacteria, and, in their lytic cycle, they produce endolysins responsible for the degradation of the bacterial peptidoglycan to free the virus progeny. Here, we produced and purified an endolysin encoded by a bacteriophage able to infect *X. citri*. Protein was expressed in an *E. coli* based system and carries a His6-TAG on its N-terminal domain. Subsequently, the fusion was purified to nearly homogeneity by IMAC using a Ni2+ charged resin. We evaluated the secondary structure of the enzyme using circular dichroism, and observed that it contains ~40.8% of alpha-helix content. Thermal stability assays ranging from 20ºC to 90ºC showed that the endolysin lost its structure at 50ºC, but apparently re-folded when the temperature was decreased regaining its structure. This study provides the basis for future research in which we intend to evaluate the potential of endolysins as an alternative to copper for the control of citrus canker.

Keywords: Citrus canker; Bacteriophages; Endolysin

Financial support: FAPESP. Process: 2022/01814-0

Agroforestry as a sustainable alternative to monoculture for soil health in the Amazon

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Abstract

Forest conversion for agriculture and livestock is the primary cause of deforestation in the Amazon. Agroforests can mitigate the negative impacts of deforestation on soil and biodiversity loss, making them a crucial choice for degraded areas. This study aimed to investigate the effects of converting forest to agroforestry and monoculture on soil attributes over 30 years. For this, soil samples were collected at three depths using a five points grid with five subpoints each, in three parallel areas of Manacapuru - AM. Results indicated that land-use change caused alterations in soil nutrient cycling dynamics, as assessed by soil enzymes. Agroforest soils showed higher beta-glucosidase activity than other areas, and similar acid phosphatase activity to forest soil. In contrast, the arylsulfatase enzyme was lower in forest soils but higher in agroforestry. All enzymes had higher activity in topsoil layer, suggesting strong C and P cycling in forest and agroforestry soils, while monoculture soil showed lower activity for all enzymes, especially in lower layers. The physicochemical attributes of the soil were significantly altered over 30 years, with monoculture soil differing from forest and agroforestry soil, which remained similar. The parameters Ca, Mg, pH, and Al^{3+} primarily contributed to changes in the soil, while Fe, K, Bo, and organic matter were secondary factors. The study concludes that replacing monoculture with agroforestry is essential for supporting high soil functionality. Forest soils are in equilibrium and may show less enzymatic activity, while monoculture soils, may become depleted over time, and show even less nutrient cycling.

Keywords: Land-use change; Tropical rainforest; Climate change; Belowground interactions; Sustainable agriculture.

Financial support: This study was supported by a grant from FAPESP-FAPEAM (2020/08927-0) and scholarship from CAPES.

Genomic analysis and annotation of biosynthetic gene clusters of Brazilian cyanobacteria of the order Nostocales

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Abstract

Cyanobacteria that belong to the order Nostocales are phototrophic bacteria, capable of fixing atmospheric nitrogen, which plays a crucial role in both aquatic and terrestrial biogeochemical cycles. Members of this order are known for their ability to synthesize a diverse range of specialized metabolites that are of biotechnological interest, with several genera forming blooms in aquatic environments. This study aimed to conduct genomic analyses of six cyanobacterial strains that belong to the order Nostocales and were isolated from various environments in Brazil. To uncover the potential of these nostocacean strains for the production of specialized metabolites, their genomes were sequenced and assembled. Genomic libraries were constructed and the sequencing were performed using MiSeq(Illumina), HiSeq (Illumina), PacBioHiFi, and Nanopore Mini Ion platforms. The genomes were assembled independently and by reference and biosynthetic gene clusters (BGCs) were predicted and annotated using available computational tools (antiSMASH and BiG-SCAPE). The genomes contained a large number of contigs, which was expected due to the homopolymeric regions of cyanobacterial genomes. Despite an average of 166 contigs, the other quality parameters were excellent, with good genome completeness (above 80%) and a low contamination rate (below 10%). A total of 128 BGCs were obtained from the annotation and predicted data. The data generated by this study will help to understand the evolution, genetic and ecological factors related to the production of bioactive substances by cyanobacteria and their adaptation to different environments.

Keywords:genomics; metabolites; Nostocales; prediction.

Financial support: CAPES

Evaluation of Tomato growth promotion by unconventional inoculants.

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Abstract

The world's population is increasing; as well as the demand for food, the production of these food must increase productive and in a sustainable way, aiming to reduce the use of pesticides that leave residues in the environment, a big challenge for modern agriculture. In this scenario, the use of bacteria that promote plant growth is a promising alternative to increase the efficiency of these biological products, resulting in increased productivity, that is, increasing production in the same area. Plants interact with a wide range of microorganisms, but few are used commercially as inoculants. Thus, in previous works, pasture endophytic bacteria were isolated and auxin production, siderophore production and phosphate solubilization were evaluated, allowing the selection of some isolates and consequently their identification by 16S rDNA sequencing. Thus, the objective of the present work was to test the selected microorganisms in tomato culture. As an inoculation method, 15 different microorganisms were applied directly on the seed, through submersion in a liquid medium. As a result, the bacteria: *Methylobacterium* sp. and *Rhizobium* sp, promoted an increase of 37% and 41% sequentially, in tomato dry mass after 60 days of germination, compared to the control. Showing great potential for promoting plant growth in parallel with the phenometric results of the other tested strains, the use of these new biological products will increase plant productivity, reducing the use of inputs that leave residues in the environment, contributing to a more sustainable production and with lower cost.

Keywords: *Methylobacterium* sp.; *Rhyzobium* sp.; Plant growth promoting bacteria; bioproduct.

How Can Microbial Resources Contribute to a Sustainable Agriculture and Food Security?

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Abstract

In the present work, it is discussed how microbial resources can contribute to sustainable agriculture and food security. For this purpose, contextualization of the importance of agriculture for human consumption and the need to have food for all will avoid, at the same time, the deterioration and greater segmentation of natural ecosystems. To achieve this ambition, it is discussed what roles microbial resources, preserved *ex-situ* in culture collections or *in-situ*, such as microbes-plants interactions (holobiontes) and soils microbiomes, play as well as in the participation in resolving, in part, the great societal challenges facing by the societies and the Earth. Alignments with the goals of sustainable development of the United Nations, as well as regional policies such as the "European Green Deal" serve as terms of reference to present possible solutions and paradigm shifts towards a more circular economy based on microbiological processes. The Microbial Resource Research Infrastructure (MIRRI) Strategic Research and Innovation Agenda for 2021-2030, with the *motto* of "microbial resources for a green, healthy and sustainable future", intends to anticipate major trends and opportunities for the valorisation of microbial resources and current examples will be given. It is concluded that the role of microorganisms and microbiomes is a treasure yet to be explored and that it is urgent to integrate them into innovative solutions for a brighter and sustainable future, including more intelligent and resilient agriculture activities, and food security.

Keywords: Food security; Intelligent and resilient agriculture; Microbial resources; Microbiomes; SDGs.

Financial support: This research had the partial financial support of Portuguese funds through the FCT (Foundation for Science and Technology) within the framework of the CIEC project UIDB/00317/2020 and CEB project UIDB/04469/2020. It also had the partial support of the European Union's Horizon 2020 research and innovation programme under grant agreement No 871129 - IS_MIRRI21 Project.

Microbial Diversity of Amazonian Dark Earths in Different Land Uses

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Abstract

The soil microbial diversity from natural environments is the starting point for biotechnological solutions that improve plant development. For restoration ecology, it is important to understand how microorganisms from the natural environment to be restored should act in an active restoration process. For that reason, we conducted a study to analyze the soil microbial diversity in Amazonian Dark Earths (ADE) and its potential for improving plant development and restoration ecology. We collected 18 soil samples from three environments (secondary forest, cassava crop, and degraded pasture) and analyzed their chemical composition and microbiological patterns, by the 16S rDNA. Our findings showed that the forest ADE had the lowest microbial diversity, likely due to its stability, while the ADE cassava crop and the degraded pasture had higher levels of microorganisms adapted to stress. The forest ADE also had the most nutrient-rich soil. The main microorganisms found in ADE were nitrogen-fixing Bacteria and Archaea such as *Rhodoplanes*, *Pseudorhodoplanes*, *Rhodopseudomonas*, *Nitrososphaera*, *Bosea*, *Nitrospira*, *Rhizobium*, and *Pseudolabrys*. The ADE cassava crop had higher amounts of *Paenibacillus*, and the pasture had higher levels of low-nutrient content adapted to microorganisms like *Mycobacterium*, *Shpingomonas*, and *Terriglobus*. The high microbial diversity found in ADE suggests that these soils may have the potential to provide microorganisms to help the ecological restoration of degraded pastures in the Amazon. The study of soil microbial diversity in ADE can lead to biotechnological solutions that enhance plant development and soil health, making a significant contribution to restoration ecology.

Keywords: Environmental DNA; Microbiome; NGS; Soil Quality.

Financial support: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Finance Code 001. It was also financed by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) – Process 2021/10626-0.

Deletion of *zapA* and *zapB* in *Xanthomonas citri* produces a cell division phenotype

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Abstract

Xanthomonas citri subsp. c*itri* (*X. citri*) is the causal agent of citrus canker, one of the main infectious diseases affecting citrus plantations in Brazil. Currently, control of citrus canker is done by management practices that include the use of copper as a bactericide. Our group showed recently that chemical compounds able to inhibit cell division in *X. citri* can preclude the ability of this plant pathogen to infect citrus. Therefore, understanding better cell division can potentially help the development of alternatives to control citrus canker. This study investigated the function of two proteins that are part of the division apparatus of *X. citri*, ZapA and ZapB. The knockout of both genes encoding for these proteins led to significant alterations ($p<0.0001$) in cell length, where the average cell length of the mutant being 3.297 µm (SD 3.184), while the wild-type cells had 1.533 µm (SD 0.4222). In addition, the presence of filaments and chains in mutant cells was observed, indicating typical phenotypes of cell division errors. We could not detect any alteration in the organization of the bacterial nucleoid by using DAPI staining. Although ZapA and ZapB have a function in cell division of *X. citri*, these proteins are not essential for cell viability.

Keywords: citriculture; citrus canker.

Financial support: CNPq (165770/2021-0) and FAPESP (2022/01768-9)

Behavior of bacteria of the genus Lactobacillus against hydroalcoholic solutions

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Abstract

In the sugar-energy sector, the occurrence of contamination in high concentrations directly influences the productivity of fuel ethanol plants. The aim of the study was to investigate the antimicrobial action of hydroalcoholic solutions on contaminants of the genus Lactobacillus, which are the main contaminants found in distilleries in Brazil. It was used a miniaturized assay in transparent, flat-bottomed, 96-well microplates, in a multifunctional microplate reader (TECAN), with 200 □L of each treatment added in triplicate, lasting 24 hours and readings taken every 30 minutes, at 30°C, agitation for 2 seconds before each reading and a wavelength of 540 nm was used.The treatments used were hydroalcoholic solutions (10 (a), 15 (b), 20% (c) and 25% (d)), MRS culture medium, and the microorganisms used were *Limosilactobacillus Fermentum* and *Lactiplantibacillus Plantarum*. Treatments T1 to T5 used hydroalcoholic solutions together with MRS, while T6 was the control in the test, containing only the culture medium (MRS), both bacteria being inoculated in all treatments. Based on the results obtained, it was concluded that T5, a treatment containing 25% ethanol, was more efficient in causing a bactericidal effect on contaminants, resulting in the death of bacteria. This study suggests that the use of hydroalcoholic solutions could be an effective alternative to the acid treatment currently used during the cellular recycling of yeasts in fuel ethanol production, which could have positive economic and socio-environmental impacts.

Keywords: Lactobacillus, acid treatment, alternative treatments, bactericidal, ethanol.

Spent mushroom substrate of *Pleurotus ostreatus* increases soil enzyme activities, glomalin content and maize biomass

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Abstract

Spent mushroom substrate (SMS) is one of the emerging biofertilizer to enhance soil health and plant productivity. This study aimed to determine the effects of SMS of *Pleurotus ostreatus* on soil enzyme activity, glomalin content and plant growth parameters. An undisturbed soil-core cultivation (a depth of 20cm; a diameter of 10 cm) was set up considering the following treatments: i) cores with maize plant (R), ii) cores with SMS (SMS), iii) cores with maize and SMS (R+SMS), and iv) control cores without maize and SMS (C). SMS was added on the top soil (0-5 cm) in SMS treatments. Maize was planted three times in the same core to take into account the decomposition period of SMS. Soil was sampled from 0-5 cm (top), 5-10 cm (middle), and 10-20 cm (bottom) in each core. Soil enzyme activities (urease, β-glucosidase, and phosphatase), glomalin content, and plant biomass and length were measured 26 days after planting. Maize biomass and length were significantly higher in the presence of SMS when compared to those without SMS. In the top soil, all of the soil enzyme activities were higher in the SMS and R+SMS treatments than in the C and R treatments. Additionally, urease activity in R+SMS was 2-fold higher than in SMS. There was no difference in soil enzyme activities of the bottom soil. Glomalin contents showed a similar tendency of β-glucosidase. Therefore, we concluded that the addition of SMS improved soil enzyme activity and glomalin content in the soil surface, promoting better plant growth.

Keywords: Urease; β-glucosidase; phosphatase; glomalin; spent mushroom substrate.

Financial support: the Joint International Research Program with Tokyo University of Agriculture for FY2021-FY2023.

Germination and sporulation of *Metarhizium rileyi* microsclerotia (Hypocreales: Clavicipitaceae) on plants surfaces

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Abstract

Microsclerotia are described as melanized hyphae aggregates with endogenous nutritional reserves. This structure can be produced by *M. rileyi*, a fungus that is very virulent to Noctuidae species, and could be used in the inoculative biological control of these pests. This study aimed to evaluate the sporulation of microsclerotia on different surfaces: water-agar, maize, and soybean leaves, which are crops that this fungus could be used to control caterpillars. Microsclerotia produced by liquid fermentation were prepared in a dry mixture with diatomaceous earth, containing 1 x 10^5 microsclerotia/g. Sterile fragments of maize and soybean leaves were placed under water-agar plates. An aliquot of 0,02 g of the formulation was spread directly onto water-agar plates and on the plates containing leaf fragments. Plates were incubated at 26 ºC and 100% UR for six days. Microsclerotia germination was evaluated on the first three days and conidial production on days 4, 5, and 6. The propagules germinated faster on plant surfaces (maize 94,9% ± 0,45; soybean 92,8 % ± 0,94; water-agar 29,3 ± 2,45 within 24 hours of incubation) and also produced conidia faster (maize 5,1 x 10^8 ± 6 x 10^7; soybean 4,4 x 10^8 ± 8 x 10^7; water-agar 4 x 10^7 ± 4,6 x 10^6 within 96 hours of incubation). The greater development of microsclerotia on leaf surfaces is new and can indicate its potential to sporulate and infect caterpillars in field conditions.

Keywords: Liquid fermentation; Maize; Soybean; Entomopathogenic Fungi;

Financial support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Use of *Metarhizium rileyi* microsclerotia to control the fall armyworm *Spodoptera frugiperda* (Smith, 1797) in laboratory

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Abstract

Metarhizium rileyi is an entomopathogenic fungus mostly known for its high virulence to Lepidoptera (Noctuidae) and it could be promising to control the fall armyworm, *Spodoptera frugiperda*. Microsclerotia are melanized hyphae aggregates with endogenous nutritional reserves and in a favorable environment, they germinate and sporulate, producing infective conidia. This study aimed to evaluate the inoculative use of microsclerotia to control *S. frugiperda*. Microesclerotia produced by liquid fermentation were prepared in a dry mixture with diatomaceous earth, containing 1 x 10^5 microsclerotia/g. The controls used were distilled water (T1), water + silweet 0,02% (T2) and diatomaceous earth for the negative control (T3). The treatments tested were: conidial suspension (1 x 10^7) applied onto maize leaves and then positioning the caterpillar under the leave (T4), the conidial suspension applied directly on *S. frugiperda* (T5) and microsclerotia formulation applied on sterile maize leaf fragments (T6) (0,02 g, producing 1 x 10^7 conidia after four days). It was observed that sporulated microsclerotia caused high mortality within four days (T4 = 12,5% ± 0,44; T5 = 10% ± 0,32; T6 = 90,8% ± 0,34). This is probably associated to the high concentration of conidia produced and the diatomaceous earth presence (an abrasive component), acting faster than the conidial suspension. This study proves in laboratory conditions that microsclerotia could be explored to control *S. frugiperda*, and the next step is to develop bioassays in greenhouse and field environments.

Keywords: Liquid fermentation; Maize; Entomopathogenic fungi; *In vitro*.

Financial support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Effect of the inoculation of different doses of phosphate-solubilizing bacterial strains on sorghum microbiota

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Abstract

The use of microbial inoculants is a technology that meets the precepts of ecological intensification of agriculture. BiomaPhos® is a commercial inoculant composed of two phosphate solubilizing bacterial strains (B119 and B2084), which promotes yield increase for different crops. This work aimed to evaluate the effect of different doses of BiomaPhos® on the genetic diversity of the rhizosphere microbiota of sorghum. It were evaluated two areas in Sete Lagoas - MG, Brazil (Embrapa Maize and Sorghum and Federal University of São João del-Rei - UFSJ). The experimental design was a randomized complete block with three replications and seven treatments, T1: 0% P_2O_5, T2: 50% P_2O_5, T3: 100% P_2O_5, T4-T7: 50% P_2O_5 with 80, 100, 120 and 200 mL of BiomaPhos® for 200,000 seeds.ha^{-1}, respectively. During the flowering stage, rhizosphere and non-rhizosphere soil were collected from three sorghum plants from each plot and analyzed by terminal restriction fragment length polymorphism (T-RFLP). Total DNA was extracted and *16S* (for bacteria) and *28S rRNA* (for fungi) genes were amplified and digested with the restriction enzymes *Alu*I, *Hha*I and *Hae*III. PCR digested products were genotyped on Genetic Analyzer 3500XL with GeneMapper 5.0 software and analyzed with T-REX program. The relative abundances of the microbial species were determined by the average T-RF size values of digestions with the restriction enzymes. A significant difference on genetic diversity was observed in the bacterial communities in different P_2O_5 doses at Embrapa and UFSJ. However, for bacteria and arbuscular mycorrhiza fungi (AMF) there were significant differences only between inoculated and non-inoculated at Embrapa. Moreover, root AMF colonization was significantly higher for all treatments compared to T1 at Embrapa and between non-inoculated (T1-T3) compared to inoculated treatments (T4-T7) at UFSJ. Our results expand knowledge on the sorghum microbiota under different doses of BiomaPhos® and P fertilization conditions.

Keywords: *Sorghum bicolor*, T-RFLP, BiomaPhos®, plant growth-promoting bacteria.

Financial support: Embrapa, Capes, CNPq, Fapemig and Finep.

Sorgoleone concentration influences mycorrhizal colonization in sorghum

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Abstract

The low availability of phosphorus (P) in the soil is one of the main constraints for crop production. Plants developed different strategies to increase P use efficiency, such as root morphology modifications, exudation of different compounds and association with microorganisms, such as arbuscular mycorrhizal fungi (AMF). The objective of this work was to investigate the effect of sorgoleone on AMF colonization and consequently on P uptake and sorghum growth. The experiment was carried out in a greenhouse, using sorghum genotype P9401 (low sorgoleone production), in a completely randomized experimental design with four replicates, with inoculum of the fungus *Rhizophagus clarus* (500 spores/plant) and without inoculum (negative control). In addition, three doses of purified sorgoleone (20 μM, 40 μM and 80 μM) were added to the low-P soil (P = 2.8 mg dm^{-3} Mehlich 1). The plants were collected 45 days after treatments started. There was no significant increase in mycorrhization with the addition of 40 and 80 μM of sorgoleone. The results showed a significant increase in total biomass in the treatment with inoculation of 20 μM of sorgoleone and *R. clarus*. These results indicated that 20 μM sorgoleone was able to influence mycorrhization in sorghum under low-P, suggesting that sorghum genotypes with higher sorgoleone exudation may develop a denser mycorrhizal network, promote greater nutrient uptake and higher grain yield.

Keywords: Phosphorus, arbuscular mycorrhizal fungi, *Sorghum bicolor*.

Financial support: Embrapa, Capes, CNPq and Fapemig.

Antibiotic resistance genes in soils with long-term manures application

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Abstract

Manure application can promote spread of antibiotic resistance genes (ARGs) in the soil, facilitating transmission of antibiotic resistance between environmental and pathogenic bacteria. Thus, the objective of this work was to evaluate the effect of long-term application of composted manure on ARGs in the soil. The identification of ARGs was performed through metagenome sequencing. Two soils were considered, one with composted poultry manure and the other with stockpiled pig manure applications annually, both contrasted with parallel areas without manure applications. ARGs' identification was done using the DeepArg software. The 25 most common ARGs and 25 ARGs of clinical interest were evaluated through Heatmaps and Walsh T tests. There were differences in abundances of the most common genes, *i.e.*, abundances of 10 and 16 ARGs changed in soils amended with stockpiled pig and composted poultry manures, respectively. Among ARGs of clinical interest, only one gene (sul1) differed within the area treated with composted poultry manure. Abundance differences for the most common ARGs could be explained by physicochemical alterations observed in fields with manure application. However, application of composted manure to soils promoted only transient increases in the abundance of ARGs, which may explain the absence of a greater abundance of ARGs of clinical interest in soils with application of manure. Therefore, the use of composted animal manures pose less environmental risks to human health, and thus being a better strategy to dispose these residues in agriculture.

Keywords: ARGs; soil; manure.

Financial support: CAPES and FAPESP.

Inoculation of sorghum genotypes with phosphate solubilizing bacteria in clayey and sandy soils

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Abstract

The cultivation of sorghum in Brazil has been expanding to sandy and medium textured soils, however phosphorus (P) availability is low in these soils. Thus, the use of plant growth promoting bacteria could be an alternative to increase crop yield. This work aimed to evaluate the effect of different doses of P-solubilizing bacteria (BiomaPhos®) inoculated in two sorghum genotypes grown in sandy and clayey soils. Seeds of sorghum genotypes (BRS373 and BRS332) were inoculated with five doses of BiomaPhos® (0, 50, 100, 200 and 500 $mL.ha^{-1}$ per 200,000 seeds) and reinoculated after 15 days in two types of soils (sandy soil: Trijunção Farm - Jaborandi/BA, clayey soil: Embrapa Maize and Sorghum - Sete Lagoas/MG) in greenhouse condition. After 40 days, roots were washed, scanned with WinRhizo and dried to constant weight. For the BRS373 genotype, root parameters and dry weight were lower in treatments with inoculation compared to the non-inoculated control, in both soil types. For the BRS332 genotype, bacterial inoculation promoted a significant increase in root diameter in clayey soil. The dose of 50 $mL.ha^{-1}$ of BiomaPhos® per 200,000 seeds promoted a significant increase in root, shoot and total dry weight, length and surface area of roots with diameter between 1-2 mm, both in clayey and sandy soil. Therefore, it is essential to consider both the sorghum genotype and the inoculant dose in different soil textures, in addition to carrying out validations under field conditions, aiming at optimizing the inoculant in different regions of Brazil, especially in agricultural frontiers.

Keywords: phosphorus, *Sorghum bicolor*, bacterial inoculant, soil texture

Financial support: EMBRAPA, CNPq, FAPEMIG and CAPES.

Inoculation of sorghum genotypes with phosphate solubilizing bacteria in sandy soil

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Abstract

The cultivation of sorghum in Brazil has been expanding to sandy and medium textured soils, however phosphorus (P) availability is low in these soils. Thus, the use of P-solubilizing bacteria could be an alternative to increase crop yield. This work aimed to evaluate the effect of different doses of the inoculant BiomaPhos® on the genetic diversity of the rhizosphere microbiota of sorghum. The experiment was carried out at a sandy soil on Trijunção Farm - Jaborandi/BA. The experimental design was a randomized complete block with four replicates with a factorial of 2x5, two sorghum genotypes (BRS373 and BRS310) inoculated with different doses of BiomaPhos® (0, 100, 200, 300 and 500 mL.ha^{-1} per 200,000 seeds). During the flowering stage, rhizosphere and non-rhizosphere soil were collected from three sorghum plants from each plot and analyzed by terminal restriction fragment length polymorphism (T-RFLP). Total DNA was extracted and *16S* (for bacteria) and *28S rRNA* (for fungi) genes were amplified and digested with the restriction enzymes *Alu*I, *Hha*I and *Hae*III. PCR digested products were genotyped on Genetic Analyzer 3500XL with GeneMapper 5.0 software and analyzed with T-REX program. The relative abundances of the microbial species were determined by the average T-RF size values of digestions with the restriction enzymes. A significant difference on bacterial genetic diversity was observed for the two sorghum genotypes. Moreover, it was observed differences within BiomaPhos® doses. For BRS310 and BRS373, the highest and lowest doses, respectively groups together and separated from others. However, for arbuscular mycorrhiza fungi (AMF) there were not significant differences. Root AMF colonization was significantly higher in the highest dose of BiomaPhos® for both genotypes. Our results expand knowledge on the sorghum microbiota and highlight the importance to consider both the sorghum genotype and the inoculant dose to optimize the inoculant use in agricultural frontiers.

Keywords: phosphorus, *Sorghum bicolor*, bacterial inoculant, soil texture

Financial support: Embrapa, Capes, CNPq, Fapemig and Finep.

New technological approaches to identify microalgae-nanoparticles interaction and agricultural traits

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Abstract

The employment of titanium nanoparticles in industrial products has increased based on its optical, catalytic, and scaffold-behavior properties. On the other hand, the effect of these nanoparticles, mainly the physical interaction of this material with plant cells and microorganisms, is poorly understood. This study aimed to investigate internalization, cell-nanoparticle interaction, and its impacts on the microalgae metabolism, including growth and photosynthetic-related responses. Titanium dioxide nanoparticles were obtained from the titanium isopropoxide hydrolysis and characterized by TEM, DLS, UV-VIS and XRD techniques. Axenic microalgae *Chlamydomonas reinhardtii* CC503 cw92 mt+ strain was cultured in mixotrophy with Tris-Acetate-Phosphate (TAP) medium, and TAP supplemented with 0.05 and 1.5 g L^{-1} of nanoparticle dispersion. The algae growth was monitored daily by cell counting for 8 days. The cells suspension was analyzed by multispectral imaging, CytoViva dark-field confocal microscopy and X-ray Fluorescence quantification of Titanium. The concentration of titanium detected intracellularly by X-ray fluorescence spectrometry is lower than the amount surrounding the cells, illustrated by CytoViva dark-field imaging. Significant changes were observed in microalgae multispectral reflectance patterns under nanoparticle treatments. Our results, therefore, indicate that titanium dioxide nanoparticles can modulate microalgae growth and photosynthetic behavior by yet unknown mechanisms, likely taking place in the cells surrounding area affecting light capture and oxidative state. These aspects need to be further investigated.

Keywords: Nanotechnology; Microalgae; Multispectral Imaging.

Financial support: PRP-USP, FAPESP (Grant#2016/06601-4; #2018/03793-5; #2022/15431-6)

Evaluating the protective effect of different matrices in encapsulating *Bacillus*

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Abstract

Microbial inoculants are a sustainable and effective alternative to chemical fertilizers and pesticides in promoting plant growth, solubilizing insoluble phosphorus, and controlling plant diseases. Bead encapsulation is a widely used technology for encapsulating microorganisms due to its numerous advantages over other methods. Recently, *Bacillus* bacteria have been the focus of research due to their positive effects on plant growth and biocontrol of plant diseases. This study aimed to produce water-oil emulsion spheres using Polyvinyl Alcohol and Cationic Starch as the base matrix for encapsulating *Bacillus megaterium*. We also evaluated the effects of citric acid (F2) and Sodium Trimetaphosphate (F3) as cross-linking, as well as the matrix without cross-linking (F1). The encapsulated bacteria was exposed to heat (65°C/24h), salinity (50% w/v, 25°C/2h), fungicide (commercial fungicide Cruiser, 25°C/24h), and ultraviolet light (20 min). Matrix F3 exhibited the highest level of protection, with 100% survival after exposure to heat and salinity. Matrix F1 provided about 81% protection against the fungicide. Matrix F2 performed satisfactorily in protecting the encapsulated bacteria against salinity, with approximately 82% survival. However, none of the matrices showed satisfactory performance against exposure to ultraviolet light, with values ranging between 14% and 37%. The findings demonstrate the specificity of each matrix in protecting microorganisms and their potential use as microbial inoculants. The development of effective encapsulation methods will contribute to promote the use of microbial inoculants for more sustainable agricultural practices.

Keywords: water-oil emulsion spheres; SMTP; sustainable agriculture; Microbial inoculants; PVA.

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

Sulfate and sulfide removal in the UASB reactors used for the production of biogas from vinasse, molasses and filter cake

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Abstract

The perspective of environmental control and energy sustainability, new proposals for biotechnological solutions for the treatment of vinasse and other by-products of ethanol production have been intensified, aiming at the production of biogas. In this work, two upflow anaerobic sludge blanket reactors (UASB) will be studied in series. Three treatment systems were tested in parallel: System I – anaerobic co-digestion of vinasse and sugarcane molasses and systems II and III – anaerobic digestion of molasses. For nitrogen (N) and phosphorus (P) supplementation, the use of filter cake, a by-product of sugar production, was studied in the affluent of systems I, II and III. The effluent from system III, before recirculation, was submitted to the advanced Fenton oxidation process, to improve biodegradability and decrease toxicity. Sulfide toxicity is a potential problem for anaerobic treatment. Therefore, the objective of this work was to determine the presence of sulfide and sulfate ions in the influent and effluent of the UASB reactors of systems I, II and II. Sulfate ion removals were observed in the three sets of UASB reactors in series (Set I, II and II) and values of sulfide ions below the toxicity limits were observed. The strategies for operating the UASB reactors and the determination of sulfate and sulfide will allow the stable and continuous obtainment of methane in the UASB reactors, providing solutions for the reuse of by-products from the production of ethanol, an important Brazilian sector.

Keywords: Anaerobic digestion; Biogas; Nutrients; Vinasse

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Potential of bacterial strains isolated from solar panels in biosurfactant production and oil degradation

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Abstract

With representatives in all three domains of life, extremophiles are microorganisms that, through adjustments in their cellular machinery, are adapted to survive in environments in which the physicochemical properties are hostile to most living organisms. Thermophiles and hyperthermophiles inhabit natural environments characterized by high temperatures, such as hot springs, volcanic sites, tectonically active faults, and deep-sea hydrothermal vents. The presence of thermophiles, however, is not restricted to natural environments , as they can also colonize artificial environments such as photovoltaic panels. Due to their pigments and resistance to radiation and desiccation, these microorganisms usually synthesize metabolites of biotechnological interest; therefore, the ability to produce biosurfactants was evaluated. The production of these metabolites was analyzed from a design of experiments (DOE) that considered deformations, time, and medium as factors and emulsion index (%E_{24}) and ability to reduce surface tension (ST) as responses to optimize strain with the molecule with the highest emulsion and lowest ST reduction. Analysis of variance (ANOVA) with Tukey's adjustment was applied to the pairwise comparisons to determine the significant difference ($p < 0.05$) between the average strain growths. The optimal condition was selected for the biosurfactant production process. *Psychrobacter* sp. was selected and maintained in Bushnell-Hass medium for 168h, achieving 90.41% of the emulsion and reducing the ST from 72 to 38.44 mN/m. These results show significant biotechnological potential, as biosurfactants can be applied to remediate sites contaminated by various compounds, in addition to favoring biodegradation processes through emulsification, mobilization or solubilization.

Keywords: Biosurfactants; Extremophilic; Extreme environments; Emulsifier; Bacteria

Financial support: This study was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior — Brasil (CAPES).

Content of Lipids, Fatty Acids, Carbohydrates and Proteins in Cyanobacteria

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Abstract

Due to anthropic eutrophication, the increase in cyanobacterial biomass is a phenomenon known as bloom. This phenomenon presents an environmental problem, causing an imbalance in aquatic ecosystems. However, the biomass produced by cyanobacteria has excellent biotechnological potential due to the nutritional potential of primary metabolites (fatty acids, lipids, proteins, and carbohydrates) produced by cyanobacteria. Among the possibilities of applications, its use in the agro-industrial and bioenergy system stands out. Thus, there is a need to compile data regarding the composition of fatty acids, lipids, proteins, and carbohydrates in freshwater cyanobacteria, to evaluate the biotechnological possibilities of different strains of cyanobacteria. For this, they were obtained from a literature review, between 2000-2023, in the main databases (e.g., ScienceDirect, PubMed, Web of Science), indicating the strains that have a greater source of these macronutrients and, consequently, greater potential biotech. The data obtained can be used by several researchers interested in generating value-added products from cyanobacterial biomass.

Keywords: biofuel; biomass; cyanobacteria; environmental waste; metabolites; review

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Assessing *Fischerella* potential as candidate for bioprospection of photoprotective metabolites

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Abstract

Several genera of cyanobacteria are underrepresented in biotechnological studies. The search for diverse biological models challenges the more commonly explored genera by comparison of efficiency and production yield. *Fischerella* is a cosmopolitan genus described in 1895. Strains of this branched filamentous and nitrogen-fixing genus grow well in laboratory and can be candidate for bioprospection. Apart from a few studies exploring antibacterial and antitumoral compounds, *Fischerella*'s metabolic potential remains belittled. To evaluate its aptitude as producer of photo protective compounds (namely total carotenoids and mycosporine-like amino acids, MAAs), all genomic assemblies belonging to *Fischerella* deposited on the National Center for Biotechnology Information (NCBI) were assessed. Gene annotation showed that only 20% of the available genomes have the MAA-related targeted genes organized in a colinear cluster, which occurred in three distinct arrangements. Literature data reporting production of carotenoids and mycosporine-like amino acids by *Fischerella* strains were compiled and compared to the biosynthesis measured by spectrophotometer and High-Performance Liquid Chromatography (HPLC), respectively, in *Fischerella* sp. CENA161 isolated in Piracicaba (SP, Brazil). The modest total carotenoid concentration measured (0,47 ± 0,05 µg mL^{-1}) might indicate that these compounds are not a key photoprotective strategy of CENA161. Conversely, the constitutive production of shinorine (3,62 ± 0,46 μg_{Shi} $mg_{Biomass}^{-1}$) detected in this strain is comparable to values reported on other genera of cyanobacteria, highlighting its potential as specialized metabolite producer. Still, the gene cluster's low frequency indicates that only a few of the currently known *Fischerella* strains are prospective candidates for MAA biosynthesis.

Keywords: Biosynthetic Gene Cluster; Cyanobacteria; HPLC; Shinorine; UV-absorbing.

Financial support: CNPq (#140892/2020-6)

Genomic analysis of plant growth-promoting bacteria and development of strain-specific molecular markers

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Abstract

The use of plant growth-promoting bacteria (PGPB) is beneficial for a more sustainable agriculture. The objective of this work was to characterize the genome of three PGPB strains and to develop and test specific molecular markers for their monitoring in the maize seedlings after seed inoculation. The genome sequencing of the B116, B119 and B2084 strains from Collection of Multifunctional Microorganisms of Embrapa Maize and Sorghum was performed using the Illumina HiSeq 4000 platform and 150-paired end strategy. The softwares RAST and PROKKA were used for the genome functional annotation. Concatenated sequences were aligned using ClustalW program and phylogenetic trees were constructed using MEGA X software with a distance-based ML method calculated by GTR model with discrete GD and 1000 bootstraps, as directed by the JModelTest software. The Kmer methodology was used to identify unique genomic regions and Primer3plus was used to develop specific primers for real-time PCR analysis. The primers were validated and tested in maize plants grown in hydroponics conditions until 22 days after inoculation. All strains presented different PGPB categories, including phosphorus metabolism, amino acids and derivatives, nitrogen metabolism, secondary metabolites, iron metabolism and acquisition and motility and chemotaxis. The strain B116, B2084 and B119 were classified as *Bacillus thuringiensis*, *B. subtilis* and *Prestia megaterium*, respectively. The strain B119 was observed on seeds after one day, while B116 and B2084 strains after three days pos-inoculation. The B2084 was more abundant in the shoot after 10 days, while the other strains were more abundant in the seed than in the root and shoot. The results indicated that these strains have different genes associated with plant-growth mechanisms and colonization pattern.

Keywords: genome; qPCR; monitoring; nutrient solution

Financial support: Embrapa, Capes, CNPq,Fapemig and Finep.

Residual effect of phosphate fertilization on Black Oat productivity, with or without bacterial inoculant

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Abstract

Plant Growth Promoting Bacteria (PGPB) can enhance plant productivity while reducing fertilizer use through biological nitrogen fixation (BNF) that when combined with Integrated Crop-Livestock-Forest Systems (ICLFS) and no-till farming. This is because PGPB help maintain soil fertility, especially in the presence of straw, which promotes nutrient cycling in the environment. The objective of this study was to evaluate the dry matter (DM) productivity of Black Oats (*Avena strigosa*) cv. EMBRAPA 29 with or without inoculation with *Azospirillum brasilense*, under the residual effect of phosphorus fertilization. The experiment was conducted under field conditions from June to October 2020 in Selvíria/MS, in a typical clayey Red Dystrophic Latosol with a history of crop cultivation using no-till farming for 16 years, in an area irrigated by sprinkler (central pivot). Black Oats were sown following a randomized block design in a 5 x 2 factorial scheme, with four doses of monoammonium phosphate (MAP) (30; 60; 120 and 240 kg ha^{-1} of P_2O_5) and a control (without fertilization) with or without *A. brasilense*, with four replicates. The data were analyzed using SISVAR® Software and subjected to F test ($p<0.05$), T test ($p<0.05$), and regression. The results indicated that *A. brasilense* increased DM productivity and promoted greater phosphorus accumulation in the plants.

Keywords: *Avena strigosa*; *Azospirillum brasilense*; integrated systems of agricultural production; no tillage system; Phosphorus dynamics.

Combination of inoculation or not of growth-promoting bacteria on the productivity of Black Oat and residual effect of phosphorus fertilization

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Abstract

The increasing need for sustainable production systems makes the agribusiness sector search for alternatives to solve the high demand for raw materials that add benefits to the environment, economic and social system. The bacteria *Azospirillum brasilense* is standing out in the national and international scenario because it provides increases in productivity dry matter and/or grain crop when inoculated, in addition to reducing the use of fertilizers. Based on this premise, the objective of this study was to evaluate the dry matter productivity of Black Oat (*Avena strigosa*) cv. EMBRAPA 29 in the absence or presence of inoculation with *Azospirillum brasilense* under the residual effect of phosphorus fertilization. The experiment was conducted at FE/UNESP under field conditions from June to October 2021, in a sprinkler-irrigated area (central pivot) in Selvíria/MS, in a typical clayey dystrophic Red Latosol with a 17-year experimental history of annual crop cultivation under No-Till System. Black Oat was sown under No-Till System following a randomized block experimental design in a 5 x 2 factorial scheme, consisting of 4 MAP doses (30; 60; 120 and 240 kg ha^{-1} of P_2O_5) and control (without fertilization) in the absence or presence of *A. brasilense*, with four replicates. The data were analyzed using SISVAR® software and subjected to F-test ($p<0.05$), t-test ($p<0.05$), and regression. According to the results obtained, the inoculation of Black Oat with *Azospirillum brasilense* increased the productivity of dry matter, and the inoculation helped with the assimilation of phosphorus by the plants.

Keywords: *Avena strigosa*; *Azospirillum brasilense*; chemical quality; straw; phosphorus fertilization.

Avaliação do potencial bioestimulante e antimicrobiano de microrganismos isolados de painéis fotovoltaicos e seus subprodutos na germinação de sementes de tomate (*Solanum lycopersicum*)

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Abstract

Microorganisms isolated from extreme environments can be interesting in biotechnological processes. Among the extreme environments, photovoltaic panels stand out in this study due to the incidence of radiation, temperature fluctuation and desiccation. Microorganisms can grow adhered to these surfaces forming biofilms, but also show tolerance to desiccation and ultraviolet radiation. Another characteristic of the microbiome of photovoltaic panels is the presence of microorganisms rich in pigments, which represents a product of biotechnological interest. The application of biopigments in the cosmetic and food industry has been investigated recently, however, there is still a gap in the use of these pigmented microorganisms and their by-products in the agro-industrial sector. Thus, the present study aims to investigate pigmented microorganisms isolated from photovoltaic panels, regarding their ability to stimulate seed germination of tomato (Solanum lycopersicum) and their antimicrobial effect against those that cause diseases that affect the cultivation and development of the fruit. Pigments extracted from these microorganisms will also be evaluated for their influence on germination. It is also intended to synthesize bionanoparticles of Zinc oxide (ZnO) from these microorganisms and verify whether they have potential as a biostimulant in seed germination. Thus, it is intended to advance in the research of pigmented extremophile microorganisms isolated from photovoltaic panels in tropical regions in order to direct them to the agro-industrial sphere, extract their potential and contribute to studies of biostimulant.

Keywords: pigmented microorganisms; agroindustrial; bioestimulant; germination; nanoparticles.

Financial support: CAPES

Anaerobic co-digestion of sugarcane by-products for biogas production

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Abstract

More and more sources of renewable energy are being sought, with Brazil being the second largest producer of sugarcane ethanol in the world. However, in the production of ethanol, large volumes of vinasse are generated, in addition to other by-products of sugarcane, such as molasses and filter cake. And that means risk of environmental contamination and high costs with the management of these liabilities, which makes the anaerobic digestion of sugarcane by-products for the production of biogas in UASB reactors a strong option for this problem. Therefore, the objective of this work is to evaluate the performance of two UASB reactors (R1 12L and R2 5.6L), in series, using vinasse and molasses (50%V and 50%M in terms of chemical oxygen demand) supplemented with filter cake for biogas production. The organic load rate applied in R1 ranged from 1.1 and 24.0g total COD $(L\ d)^{-1}$, respectively. The analysis of the parameters of volatile acids and total alkalinity, shows that the system presents stability. When alkalinity is analyzed in its intermediate (AI) and partial (AP) components, it is observed that the average AI/AP ratio is 0.3, and 0.13, for R1 and R2 respectively. The total and dissolved oxygen chemical demand removal efficiencies for the system (R1+R2) were 62 and 30%, respectively. Volumetric methane productions of up to 5.794 and 1.288 L CH_4 $(L\ d)^{-1}$, respectively, were observed in R1 and R2, respectively. This indicates that it is possible to co-digest vinasse and molasses supplemented with filter cake to obtain biogas in UASB reactors, in series.

Keywords: Biomethane; Vinasse; Molasse; UASB reactors; COD.

Financial support: This study was financed by Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (Processo n. 2019/19443-6); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001".

Soil health under different managements in Zea Mays cultivation in relation to microbiological and environmental parameters.

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Abstract

Due to the growing demand for food and the call for more sustainable soil management, there is diversification of management, introducing organic and natural management in variation to conventional management. Such managements may differ from each other in terms of soil quality responses that integrate chemical, physical and biological indicators of the soil which interfere with productivity, greenhouse gas emissions and microbiological parameters showing sensitivity to changes in soil function and its ecosystem services. In this context, this work aimed to evaluate the soil quality under organic, natural and conventional management in corn cultivation and to correlate the soil quality indices as well as the ecosystem functions integrated in the indices for each management with the emission of greenhouse gases. and activity of soil functional genes through multivariate analysis by a PCA. The results showed that the functions of the soil related to soil structure, chemistry and soil biology had a greater correlation with the activity of functional genes *amoA*, *nifH* and *phoD*, being more correlated with natural management. Organic management showed a positive relationship with the function related to carbon cycling and CO_2 emission and a negative relationship with CH_4 emission, with conventional management less grouped with the variables when compared to the other soil managements. Natural and organic management can present greater relationships with indicators of sustainability and soil quality.

Keywords: Functional-genes;Greenhouse-effect;PCA;Correlation;soil-quality.

Financial support: CAPES; CNPQ ;FAPESP.

Entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* play roles in maize (Zea mays) growth promotion

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Abstract

Recently, there has been an increasing interest in using beneficial microorganisms to improve plant health and productivity. Some species of entomopathogenic fungi (EPF), such as *Beauveria* spp. and *Metarhizium* spp., are commonly employed as biological control agents against various pests due to their wide host range, simple production, and virulence. Recently, they have been identified as plant root colonizers and provide multiple benefits (growth promotion, drought resistance, nitrogen acquisition). This study aimed to evaluate the effect of potential EPF in promoting maize (*Zea mays*) growth. Maize seeds were treated separately with ten *Metarhizium anisopliae* and *Beauveria bassiana*, prepared at three concentrations of aerial conidia (10^7, $10^{8,}$ and 10^{10} conidia.mL^{-1}) and uninoculated control. Afterward, in a greenhouse, each treatment group was placed in separate plastic pots containing a mix of clay and sandy soil. The ability of the fungi to promote maize growth was evaluated at 21 days. The plant growth indexes include root length, root, and aerial part dry weight. Two *M. anisopliae* isolates at 1 x 10^{10} conidia.mL^{-1} and four B. *bassiana* isolates at 1 x $10^{7,}$ and one isolate at 1 x 10^{10} conidia.mL^{-1}were more efficient in increasing dry weight of the aerial part (28% and 20%) than the control. Inoculation of one *M. anisopliae* at 1 x 10^{10} conidia.mL^{-1} and one *B. bassiana* at 1 x 10^7 conidia.mL^{-1} increased stem diameters (41% and 56%) than the control. These findings prove and bring one more perspective on the benefits promoted by EPF when employed as bioinoculants.

Keywords: microorganisms; growth promotion; entomopathogenic fungi

Acid phosphatase and microbial biomass after inoculation of P-solubilizing microorganisms in soil tillage and sources P

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Abstract

The high P input due to the low efficiency of phosphate fertilization in Brazilian tropical soils demands the search for sustainable strategies to increase the P-use efficiency. The objective was to evaluate the inoculation of P-solubilizing microorganisms (PSM) that can be influenced by P sources and soil tillage on the activity of acid phosphatase and microbial biomass in the long-term. The study was in a long-term trial established in 1999 at EMBRAPA Cerrado in an Oxisol with 57% clay. The experimental design was randomized blocks in a factorial scheme (2x3x3), with three replications. The first factor was inoculation or not of *Bacillus subtilis* and *B. megaterium* in corn seed. The second factor of sources P (50 kg ha^{-1} of P with rock phosphate (RP), triple super phosphate (TSP) and without P (Nil-P)). The third factor by soil tillage system (conventional tillage (NT), No-tillage with millet (NT-GRASS) or with black velvet (NT-LEGU). After nine years of phosphate suppression (2020/21 crop), it was determined acid phosphatase activity and soil microbial biomass in the 0-10 cm depth. There was a triple interaction of factors in acid phosphatase (p=0. 0001), where the highest activity was inoculation in NT-GRASS under Nil-P (2713 µmol g^{-1} h^{-1}) and TSP (2889 µ mol g^{-1} h^{-1}), and NT-LEG under RP (2724 µmol g^{-1} h^{-1}). In microbial biomass, there was only interaction between sources P x soil tillage, and the curiously simple effect of inoculation, with 60% reduction with inoculation (118 vs. 72 µg g^{-1}).

Keywords: Bioinputs; Enzymatic activity; Phosphate mobilization.

Study of the tolerance of *Saccharomyces cerevisiae* in high zinc concentrations

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Abstract

Yeasts are microorganisms that present promising properties, such as the ability to incorporate the minerals necessary for animal nutrition. Among these, zinc stands out for regulating several biochemical and physiological processes in living beings. This work aims to define the maximum zinc concentration that the yeast *Saccharomyces cerevisiae*-Thermossac tolerates in the culture medium. The yeast was inoculated on Petri dishes with YEPD medium containing the following concentrations of zinc sulfate ($ZnSO_4$): (T0) 0 mg L^{-1}; (T1) 250 mg L^{-1}; (T2) 500 mg L^{-1}; (T3) 750 mg L^{-1}; (T4) 1000 mg L^{-1}; (T5) 1250 mg L^{-1} and (T6) 1500 mg L^{-1}. The assay was performed in quadruplicate and the plates were incubated at 30°C for 48 h. The parameters analyzed were the total count of microorganisms (CFU mL^{-1}) and the macromorphological characteristics of the colonies. As a result, regarding the total count, treatments T0 to T5 presented colony growth, while T6 did not showed growth. It was noteworthy that the counts decreased with the increase of zinc concentration in the medium, because the cells suffered stress caused by the conditions of the culture medium. As for the macromorphological characteristics, treatments T3, T4, and T5 presented smaller colonies when compared to the others, showing that the inoculated yeasts went through fewer reproductive cycles due to the inhibition of high concentration of zinc. In conclusion, the studied yeasts were tolerant until 1250 mg L^{-1} of $ZnSO_4$. However showed alterations in the sizes of the colonies in the medium with high zinc concentrations.

Keywords: colonies; macromorphological; micronutrient; yeasts; zinc sulfate.

Financial support: Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Financing Code 001.

Impact of pH and ethanol on the kinetic behavior of yeasts in mixed must

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Abstract

Cooperation between industries is essential to improve various production processes. The production of ethanol from starchy sources plays a crucial role in providing energy both in Brazil and around the world. Therefore, this activity is fundamental to ensure global and local energy sustainability. Thus arising the objective of this work, which was to investigate the influence of pH and ethanol on Thermosacc yeasts at the concentration of 100 g L-1 of A.R.T. in hydrolyzate with syrup. The assay was miniaturized using transparent, flat-bottomed, 96-well microplates, in a multifunctional microplate reader (Tecan), with 200 □L of each treatment added in triplicate, lasting 24 hours and readings taken every 30 minutes, at 30°C at 570 nm. The pHs were adjusted with H2SO4 to pH = 2.1 (a); 4.3 (b) and 5.5 (c) and ethanol concentrations at 5, 10. As a result, at pH 2.1 all ethanol concentrations negatively influenced growth kinetics when compared to control. At pHs 4.3 and 5.5, it was possible to observe that the effect of ethanol stands out in relation to the pHs, acting negatively on the growth kinetics in relation to the control. Understanding the kinetic behavior of yeasts in relation to ethanol and pH variations is essential to optimize fermentations. This is because acidic environments and high levels of ethanol can lead to poor fermentation yields. Therefore, it is crucial to study these factors to maximize the efficiency of the fermentation process.

Keywords: Energy sustainability; starchy source; ethanol; hydrogen potential; performance.

Financial support: CAPES.

Antimicrobial activity of *Streptomyces* against fungal pathogens causing diseases in soybean culture

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Abstract

Agricultural crop management is crucial to meet the increasing demand for food due to the growing global population and industrialization. Soybean is a vital crop for food and animal feed production, but microorganism-induced diseases adversely affect production. Chemical control has negative effects on the environment and human health, and biological control offers a safer alternative for managing plant diseases. Actinomycetes, Gram-positive bacteria found in soil, produce bioactive compounds and inhibit microorganisms, making them a promising source of antimicrobial agents. Streptomyces, a primary group of actinomycetes, produces 50% of all known antibiotics and can be used as biological control. This study evaluated the inhibitory activity of Streptomyces spp. isolates on phytopathogenic fungi using a dual culture plate assay. The results of the assay conducted with Rhizoctonia solani and Streptomyces isolates 92 and 116 showed observed inhibition percentages of 60% and 50%, respectively. These findings indicate the potential of Streptomyces spp. isolates as a biological control agent for soybean diseases caused by fungal pathogens. This study provides valuable information on the inhibitory activity of Streptomyces spp. isolates on necrotrophic fungi, contributing to the development of alternative strategies for plant disease control in soybean production. The results highlight the opportunities in the expanding market for microbiological products with agronomic efficacy for managing soybean diseases, providing a more sustainable and safer approach to crop management. It is important to note that this is a preliminary result from a larger study with more phytopathogenic fungi and other methods of evaluation of the biocontrol potential.

Keywords: biological control; phytopathogenic fungi; *Streptomyces*.

Financial support: CAPES.

Bioprospecting of lignocellulosic fungi in a medium enriched with sugarcane bagasse

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Abstract

Bioprospecting lignocellulosic fungi has emerged as a promising strategy for developing more efficient and sustainable industrial processes, particularly for biofuel production. Sugarcane bagasse is an abundant and renewable source of carbon, making it a prime substrate for cultivating these fungi. This study aimed to develop selective culture media for lignocellulosic fungi enriched with sugarcane bagasse, with the goal of identifying and isolating strains capable of producing lignocellulose-degrading enzymes. Sugarcane bagasse samples were collected from UFSCar, Araras - São Paulo, and subjected to drying at 80°C for four days, and grinding to standardize the grain size, thereby removing impurities and obtaining the purest fraction of biomass. Culture media containing 10% sugarcane bagasse were then prepared, some of which were subjected to pre-treatment strategies through autoclaving. After inoculation with the collected samples, the fungi were cultivated at a temperature of 30°C and evaluated for their colony growth capacity in selective media. The results indicate that using sugarcane bagasse as a substrate for selecting lignocellulosic fungi is a promising strategy for bioprospecting strains with industrial potential, with a 55.8% increase in the growth of strains with potential for enzyme production. Moreover, the preparation of selective culture media enriched with specific nutrients can increase the efficiency of biofuel production processes from lignocellulosic biomass. These findings suggest the potential for the development of more efficient and sustainable industrial processes for the production of second-generation ethanol.

Keywords: bioprospecting; fungi; lignocellulosic.

Influence of supplementation with trace elements on the removal of organic matter in the process of anaerobic digestion of vinasse

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Abstract

The sugarcane industry faces challenges in the sustainable disposal of vinasse due to its large volume and complex physicochemical composition. A promising solution is the use of anaerobic digestion (AD), a biological process that converts organic matter into biogas through the action of microorganisms. However, nutritional deficiencies in the substrate can affect process productivity and yield. To improve AD performance, favorable conditions for the microbial population must be ensured, which can be achieved through supplementation techniques. This study evaluated the removal of organic matter (ROM) from vinasse using the AD process with the addition of trace elements such as Nickel (Ni), Selenium (Se), Molybdenum (Mo), and Cobalt (Co). Reactors (500ml) were used, inoculated with mesophilic granular sludge and vinasse, passivated with N2 gas, and maintained at 38°C under agitation for 42 days. ROM was evaluated by the Biochemical Methane Potential assay using a rotational central composite design (DCCR) using 4 factors with central point supplementation of 9, 16, 16, and 40 µg/g COD and increments of 3, 4, 4, and 10, respectively, resulting in 27 treatments. The lowest ROM efficiency (82.59%) was expressed in the assay containing 9, 16, 24, and 40 µg/g COD of Ni, Se, Mo, and Co, respectively, while the highest ROM (92.84%) was in the assay with 12, 12, 20, 50 µg/g COD of Ni, Se, Mo, and Co, respectively. It was concluded that supplementing vinasse with 9, 16, 24, and 40 µg/g COD of Ni, Se, Mo, and Co, respectively, increased ROM efficiency

Keywords: Sugar-energy; micronutrients; microorganisms; organic matter.

Financial support: National Council for Scientific and Technological Development - CNPq (Project 142268/2020-8)

Yeast production through aerobic metabolism in medium supplemented with sodium selenite

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Abstract

The yeasts have various biotechnological applications, mainly due to their nutritional composition, metabolic versatility and for being capable of absorbing micronutrient molecules, as in the case of selenium, generating antioxidant enzymes. The objective of the work was to evaluate cell multiplication through aerobiosis, under different concentrations of sodium selenite (Na_2SeO_3). The assay was performed in fed-batch, using the yeast *Saccharomyces cerevisiae* - Thermossac, in a prototype developed for 16 h, temperature of 28˚C and oxygen flow rate 1 v v^{-1} min^{-1}. The wort was corn hydrolysate with 0.1g L^{-1} sugar, and the feed wort 130 g L^{-1} sugar, both varying the concentration of Na_2SeO_3: T1 (0 mg L^{-1}), T2 (200 g L^{-1}) and T3 (400 g L^{-1}), being performed in quadruplicate. The growth started with 3% yeast and 300 mL of wort, with feed flow 0.18 mL min^{-1}, increasing according to the equation: $F= (\mu (t)*[X(t)*V(t)]) / (Yx/s(t) (Sf -Sm)$. As parameters, the produced biomass and cell yield were analyzed. In the results we obtained that T1 produced more biomass, of 33.7± 0.1 g and obtained the best yield of 0.64±0.02 g of yeast g^{-1} of sugar consumed ($p<0.05$), compared to the other treatments. Thus, it can be inferred that the osmotic stress caused by the high concentration of Na_2SeO_3 alters the reproduction cycle of the microorganism, taking more time for reproducibility. It can be concluded that T1 was able to produce more biomass and had better yield because it did not contain the limiting factor Na_2SeO_3.

Keywords: Selenium; Micronutrient; Yeast; Aerobic metabolism.

Financial support: Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Funding Code 001.

Can microbial enzymes for the acquisition of C-N-P from soil be modulated by cover crops and P sources in the Alagoas semiarid?

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Abstract

Soil microbial enzymes play a key role in nutrient cycling and have the potential to maintain soil fertility by favoring the availability of nutrients for plants. The objective was to evaluate the influence of cover crops and phosphate sources on microbial exoenzymes of soil C, N, and P acquisition in Alagoas semiarid. The study was conducted in the trial at Federal University of Alagoas, Arapiraca Campus on sandy soil in short-time. The design was randomized blocks, with three replications, a factorial scheme 3x6. The first factor was soluble phosphate (SSP), rock phosphate (RP), and without P (Nil-P), and the second factor by cover crops (Crotalaria juncea, C. spectabilis, Cajanus cajan, Dolichos lablab, Canavalia ensiformis, Pennisetum glaucum) and fallow. Determine the activity of urease, acid phosphatase, and β-glucosidase in 0-5 and 5-10 cm depth after the second crop. In general, in urease there was a higher response in the 0-5 depth under Nil-P (83 μmol g-1 h-1) in all plants, except for P. glaucum, C. cajan, and fallow. In acid phosphatase, the highest activity was observed in FP under Nil-P (2202 μmol g-1 h-1) in the first layer, followed by D. lablab associated with PR. As well as n to β-glucosidase, D. lablab associated with RP at 0-5 cm (690 μmol g-1 h-1) was higher in cover crops and an increase of 80% compared to fallow. The enzymatic activities of acquisition C, N, and P of microbial are modulated by cover crops and P sources in short-time in the Alagoas semiarid.

Keywords: Soil health; Exoenzymes; Agricultural sustainability

Financial support: FAPEAL; FAPESP; CAPES.

Potential degradation of glyphosate by a herbicide tolerant *Streptomyces* strain

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Abstract

Glyphosate, N-(phosphonomethyl)glycine, may cause inhibitory effects in microorganisms, therefore affecting the soil microbiome. Rhizobacteria from the genus *Streptomyces* are well-known as microorganisms able to promote plant growth; moreover, they have shown potential use in the biodegradation of pesticides. This study aimed to characterize an isolate of *Streptomyces* on the production of metabolites and its capacity to degrade glyphosate. *Streptomyces* CLV322, obtained from rhizosphere of soybean [*Glycine max* (L.) Merrill] from a field treated with pesticides was evaluated for its cell viability and synthesis of metabolites under pesticide stress in the concentrations of 0.002 to 7.2 mg mL^{-1}. Medium without glyphosate was used as a control. Synthesis of siderophore, índole-3-acetic acid (IAA), NH_3 and phenazines, and phosphate solubilization were the parameters analyzed. Exposure to the pesticide reduced bacterial biomass and viability significantly from the concentration of 1.8 to 7.2 mg mL^{-1}. The strain showed the highest siderophore synthesis under pesticide stress at 0.002 and 0.45 mg mL^{-1}. IAA synthesis and phosphate solubilization decreased in bacteria treated with 3.6 and 0.9 to 7.2 mg mL^{-1} of glyphosate, respectively. The quantification of phenazines, performed by HPLC, demonstrated that pyocyanin production occurs up to a concentration of 7.2 mg mL^{-1}. Experiments are being carried out to assess the decomposition of glyphosate by the *Streptomyces* strain.

Keywords: Actinomycetes; PGPR; secondary metabolites; pesticides.

Financial support: CNPq

Influence of specific growth rate on yeast production in a fed-batch system

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Abstract

Yeasts can be used as a nutritional supplement in animal feed because they have high levels of proteins. Yeasts can be produced by anaerobic and aerobic processes. However, this depends on how glucose is added incrementally during the course of yeast growth to maintain a low sugar concentration (0.1 g L^{-1}). The study aimed to verify the influence of the specific growth rate used to calculate the wort feed rate to maintain the ideal glucose rate on aerobic cell growth in fed-batch. The cell growth was carried out, using *Saccharomyces cerevisiae*, strain Thermossac, in a bioreactor for 16 hours at a temperature of 30˚C, pH 5 and oxygen flow 1 v v $^{-1}$ min^{-1}. The feed wort was corn hydrolyzate (130 g L^{-1}) and the specific growth rate tests (quadruplicates) were the following treatments: T1 (μ = 0.2 h^{-1}), T2 (μ = 0.15 h^{-1}) and T3 (μ = 0.12 h^{-1}). At the end of cell growth, the T3 provided the highest cellular biomass yield (Yx/s = 0.44, ±0.01 g of yeast per g of sugar) ($p<0.05$). While, T1 and T2 obtained respectively Yx/s of 0.08±0.01 and 0.22±0.01 ($p <0.05$). The results show that T2 and T3 grew by aerobiosis because in the aerobic process, 0.2 to 0.5 grams of yeast are produced for each gram of sugar consumed. While T1 grew by anaerobiosis. Thus, it is possible to verify that increasing the specific growth rate to calculate the wort feed provides a change in the metabolic route to anaerobiosis.

Keywords: Aerobiosis; Biomass; Cell growth; Metabolism.

Potassium solubilization of granitic rock by *Bacillus spp*.

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Abstract

The slow release of nutrients is a limitation for the use of rock powders. An alternative is biosolubilization based on microbial action. Based on this, we aimed to evaluate the potassium (K) solubilization of granite from the activity of *Bacillus subtilis* and *Bacillus pumilus*. For this, falcons received 45 mL of modified Aleksandrov medium and supplemented with 3g L^{-1} of granite powder as the only source of K. The pH was adjusted to 7.0 and each treatment received a colony of the respective isolate. Thus, 3 treatments (2 isolates and 1 control only with the K source) and 5 replications were established. After 10 days under agitation at 120 rpm and a temperature of 28°C, the tubes were centrifuged at 7,000 g for 11 minutes to separate the supernatant. Afterwards, the supernatant was filtered through nº 42 paper, the pH was measured using a potentiometer and the K concentration was determined by flame spectrophotometry. The means were compared by Tukey's test at 5% probability in the Sisvar 5.6 program. *B. pumilus* showed a higher concentration of K (6.12 mg L^{-1}) and *B. subtilis* (5.84 mg L^{-1}) did not differ from the control (5.60 mg L^{-1}). The lowest pH value (5.68) was observed with *B. subtilis*, while *B. pumilus* did not differ from the control. There was no correlation between the pH reduction and the increase in K biosolubilization. These results suggest that *B. pumilus* has the potential as a K biosolubilizer.

Keywords: Biosolubilization; rock powder; microbial activity.

Financial support: CAPES

Does composted sewage sludge associated with cover crops increase soil easily extractable glomalin, porosity and P availability in the Cerrado region?

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Abstract

The use of organic fertilizers produced from the reuse of (agro)industrial and urban waste combined with crop rotation has become an important alternative source of management in the Cerrado region. However, the benefits of applying organic fertilizers go beyond the chemical improvement of the soil, as these materials also contribute to improve soil physical and biological attributes. The aim of this study was to evaluate how cover crops amended with mineral or organic fertilizers (composted sewage sludge–CSS) affect soil health through analysis of P availability, soil porosity, and easily extractable glomalin. The soil used was classified as Rhodic Haplustox (clayey Oxisol). The treatments were arranged in a factorial scheme, in randomized complete block, as follows: two crop rotation systems (CI–soybean, maize, bean, marandu grass, bean, marandu grass, soybean, oat, maize, marandu grass) and (CII–rice, bean, marandu grass, maize, marandu grass, a mix= forage turnip, crotalaria, and millet, maize, mix) and four fertilization practices (*i*= no CSS and mineral fertilizer application, *ii* = conventional mineral fertilization only, *iii* = application of 15 t ha^{-1} of CSS (wet basis) and iv = application of 30 t ha^{-1} of CSS (wet basis). Soil health indicators were evaluated in samples collected from the 0–0.1 layer. Soil samples and cover crop biomass were collected 60 days after plant emergence. Soil attributes responded positively to the combination of CSS with cover crops. Shoot dry biomass of cover crops increased with the application of both CSS and mineral fertilizer.

Keywords: Biosolid; Organic fertilizer; Crop rotation; Soil health; Arbuscular mycorrhizal fungi.

Financial support: Coordination for the Improvement of Higher Education Personnel (CAPES) and Agrarian Studies Foundation Luiz de Queiroz (FEALQ–Agrisus).

Inoculation of *Curvularia* spp. and P rates affecting nutrients absorption and fungal root colonization on initial development of maize

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Abstract

The maize production in Cerrado may be restricted by several factors such as low nutrient and water use efficiency. Soil microorganisms have been used to help plants in this environment. Dark Septate Endophytes (DSE) can grow in biotrophic and saprophytic forms. DSE fungi can function as substitutes or complements for arbuscular mycorrhizae fungi (AMF) in environments under different stresses. The ability of these fungi to dissolve soil phosphates is poorly understood, although there are examples referring to the effect of DSE fungi on the absorption of P and other nutrients by plants. DSE species adapted to the Cerrado soil and environmental conditions can improve maize production. The aim of this work was to evaluate the effect of inoculation by *Curvularia* spp. in maize seeds in function to the management of P rates, analyzing the P levels in the soil and roots and root colonization by DSE and indigenous AMF thirty days after plant emergence. The soil used was classified as Rhodic Haplustox (clayey Oxisol). The experimental design was completely randomized, with four replicates, arranged in a 2 x 4 factorial scheme. The treatments were consisted of two P rates (0%; 25%; 50% and 100% of recommendation of P) and two seed inoculation with *Curvularia* spp. (without or with). *Curvularia* ssp was isolated from rice roots using agar-malt culture medium with $Ca_3(PO_4)_2$. The P available in the soil and the P rates in roots were higher when DSE was inoculated. Root colonization by AMF was lower when DSE was inoculated.

Keywords: *Zea mays* L.; Dark septate endophytes; Arbuscular mycorrhizal fungi; Crop nutrition.

Production of a bioactive orange pigment by *Arthrobacter* sp. strain mono58

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Abstract

The objective of this work was to identify an endophytic isolate from an Antarctic seaweed and to evaluate the bioactivity of a pigment produced by it. The MONO58 bacteria is an endophyte from Monostroma hariotti seaweed and was provided by Brazilian Agricultural Research Agency. The identification was carried out by 16S gene sequencing. For pigment production, the isolate was cultured in marine broth media (28^{o}C, 150 rpm) for 72 hours. The biomass was collected and the cells disrupted by acid hydrolysis (3N HCl), extracting the pigment with acetone. The pigment pH was neutralized by dialysis using an anion exchange membrane. Antimicrobial assays were performed in 96-well microplates with 256 µg mL^{-1} of the pigment against six bacterial pathogens from human and plants. The isolate MONO58 was identified as *Arthrobacter* sp. (Micrococcaceae), having shown 99.7% similarity to *Arthrobacter psychrochitiniphilus* species. The chemical analysis presented a compound with optimal UV absorption in 450 nm in t_R 13,18 minutes. The compound MS spectra showed the ions at m/z 328 and 368. The extracted pigment showed bioactivity against the phytopathogens *Ralstonia* sp. BL1 (66.21%) and *Pseudomonas* sp. BSE238 (53.16%), lineages responsible for damage to commercial crops. The endophyte *Arthrobacter* sp. strain MONO58 can produce a bioactive intracellular pigment able to inhibit the growth of phytopathogens with importance to agriculture, being useful for products development. Now it is necessary to purify the pigment as well as carry out the structural elucidation.

Keywords: Microbial pigments; *Arthrobacter*; Antarctic bacteria.

Financial support: National Council for Scientific and Technological Development (CNPq) - Scholarship 141501/2020-0 to M.C.P.r. Coordination of Superior Level Staff Improvement (CAPES) – Finance Code 001 and scholarship to M.C.P.Jr. São Paulo State Funding Agency, Brazil (FAPESP) - Research grant 2019/17721-9.

Influence of *Bacillus* spp. in root growth of tomato cv. Micro-Tom (*Solanum lycopersicum* L. cv. Micro-Tom)

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Abstract

The genus *Bacillus* includes rod shape bacteria with high spore production and genotypic and phenotypic diversity. Some species are found in the soil and are capable of interacting with the plant's roots promoting its growth. These bacteria are called plant growth-promoting rhizobacteria (RPCPs). In that regard, there are already products based on these bacteria, standing out because of their sporulation capacity, which improves product shelf-life. The objective of this work was to evaluate tomato cv. Micro-Tom (*Solanum lycopersicum* L. cv. Micro-Tom) root growth speed and size to select promising RPCP strains. A group of 21 strains of *Bacillus* was inoculated (10^8 UFC/mL) in 5 seeds for each treatment for 30 minutes and germinated in Petri dishes with soaked filter paper for three days. After three days of germination, the seeds were sowed in Petri dishes with agar and maintained in the B.O.D at 28°C, following the radicular growth for the next 4 days. The samples were imaged by scanner every 24 hours and measured with ImageJ software. The strains FS3-7 and RZ3MS27 showed the best performance overcoming the controls composed by samples growing in water, culture media, and the strain RZ2MS9, already studied as tomato cv. Micro-Tom RPCPs. The FS3-7 and RZ3MS27 strains are promising candidates for further studies about your bioinoculant capability for tomatoes.

Keywords: *Bacillus*; plant growth promoting rhizobacteria; tomato; root

Financial support: TC by PET-MEC and FEALQ, GFS by CAPES and MM is a PET-MEC fellow.

From the cave to the field: prospecting species of *Penicillium* section *Citrina* against phytopathogenic fungi

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Abstract

Caves are an ecosystem that recently have scientific attention because the discovery of a big fungal diversity living in that places. *Penicillium*, a cosmopolitan genus with several uses, is among the most found fungi in these environments. This study aimed to investigate isolates of *Penicillium* section *Citrina* as biocontrols agents of phytopathogenic fungi. Isolates of *Penicillium* were obtained from sediment and air of the cave Lapa do Boqueirão, Vila Propício-GO. The phytopathogenic fungi were obtained from the collection of the Laboratório de Micologia e Etiologia de Doenças Fúngicas de Plantas, UFV, Viçosa-MG isolated from soy (*Macrophomina phaseolina*), tomato (*Fusarium oxysporum*), and *Cattleya nobilior* (*Lasiodiplodia* sp.), they were identified in previous studies using phylogenetic analysis and/or morphology. *Penicillium* isolates were identified in six species, *P. shearii*, *P.* cf. *tropicum*, *P. citrinum*, *P. sumatrense*, *P. terrigenum*, and *P. copticola*, based on morphological features and phylogenetic analysis of ITS rDNA sequences using the Maximum Likelihood. After 10 days of inoculation, the antagonism of *Penicillium* against the pathogens was evaluated. *Penicillium citrinum* was the only species with a clear antagonist action against the three pathogens, being a species known for its biocontrol potential. *Penicillium shearii* had a possible antibiosis effect against *M. phaseolina* and *F. oxysporum*, and *P. sumatrense* against *M. phaseolina* and *Lasiodiplodia* sp. The antimicrobial effect of metabolites from *P. shearii*, *P. sumatrense* and other *Penicillium* spp. is reported in the literature. This study showed that cave fungi have an important biotechnological potential to be investigated as antagonists of phytopathogens.

Keywords: Eurotiales, Biocontrol, Fusarium, Botryosphaeriaceae.

Financial support: CNPq, CAPES, FAPEMIG, FAPEG

Dark septate endophytes on the spotlight: Two new species of *Cladophialophora* associated with roots of *Cattleya locatellii* in Brazil

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Abstract

Cattleya locatellii is an endangered species that belongs to the Orchidaceae family. Endemic to Minas Gerais, this rupicolous species was first described in 2016 and little is known about the fungi that compose its endophyte microbiome. Dark septate endophytes (DSE) can be found on the roots of many plants, including orchids, and are known to being able to reduce the abiotic and biotic stresses. Therefore, this study aimed to isolate and to characterize the DSE associated with the roots of *C. locatelli*. Samples of healthy roots were collected in Araponga, MG, and brought to the *Laboratório de Micologia e Etiologia de Doenças Fúngicas de Plantas*, at Universidade Federal de Viçosa. Fungal endophytes were isolated using the dilution-to-extinction method and cultivated in PDA and MEA. Fungal DNA was extracted using the Wizard Genomic DNA Purification kit (Promega Corporation, WI). The rDNA-ITS region was amplified by PCR using the ITS5/ITS4 primers and sequenced by Macrogen. Sequences were compared with the GenBank database by megaBLAST. Four isolates were identified as belonging to the genus *Cladophialophora*. Maximum Likelihood and Bayesian inferences were performed for phylogenetic analyses. Two new species of *Cladophialophora* were identified on both analyses, with strong support. They form a sister clade related to the species *Cladophialophora inabaensis* and *C. lanosa*, two soil born species. The fungal diversity associated with Brazilian endemic flora is mostly unknown and represents a biotechnological potential to be discovered. In addition, this study contributes to the knowledge about the diversity of DSE associated with the *C. locatellii*.

Keywords: Orchidaceae; Herpotrichiellaceae; Phylogeny; Taxonomy.

Financial support: CNPq, CAPES and FAPEMG

Secret friends: Four new species of mycorrhizal *Serendipita* associated with the orchids *Bifrenaria* sp. and *Cattleya locatellii*

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Abstract

Serendipita species are known to form ectomycorrhiza and orchid mycorrhiza. In orchids, this association is essential, once those seeds are unable to germinate without the fungal association. Therefore, this study aimed to explore the diversity of mycorrhizal *Serendipita* associated with native orchids. Healthy roots of *Bifrenaria sp*. and *Cattleya locatellii* were collected in Araponga-MG, Serra do Brigadeiro. Four isolates were obtained from direct isolations from *pelotons*, two from *Bifrenaria* sp. and two from *C. locatellii*. Other three isolates from *C. locatellii* were obtained using the dilution-to-extinction isolation method. Fungal DNA was extracted using the Wizard Genomic DNA Purification kit (Promega Corporation, WI). The rDNA-ITS region and the large subunit of rDNA were amplified by PCR using the ITS5/LR6 primers and sequenced by Macrogen. Sequences were compared with the GenBank database by megaBLAST. Slides were made in lactoglycerol to observe fungal structures. The seven isolates of this study were identified as belonging to the mycorrhizal genus *Serendipita*. Phylogeny using Maximum Likelihood and Bayesian inferences indicates four new species, one from *Bifrenaria* sp. and three from *C. locatellii*. Three of the new species (two from *C. locatelli* and one from *Bifrenaria* sp.) form a sister clade related to *Serendipita restingae* and *S. petricolae*, while another one found in *C. locatellii* form an external clade to those mentioned above. Although they are essential for the survival of orchids, few species of *Serendipita* associated with them are known nowadays. This study contributes to the knowledge about the diversity of *Serendipita* associated with Brazilian orchids.

Keywords: Serendipitaceae; Orchidaceae; Phylogeny; Taxonomy.

Financial support: CNPq, CAPES and FAPEMG

Evaluation of germination of tomato seeds encapsulated with medium-viscosity sodium alginate.

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Abstract

Encapsulation is the technology of encapsulating solid, liquid, and gaseous materials in small capsules that release their contents under controlled conditions. The choice of encapsulating agent depends on several factors, among them its non-toxicity. Sodium alginate is a polysaccharide, originating from brown seaweed and commonly used to formulate edible coatings, and coatings for transport of numerous substances due to its biodegradability, biocompatibility and low toxicity characteristics. The objective of the present work is to evaluate the germination of tomato seed encapsulation, so that in future projects this method can be used as a carrier for biological products, along with the seed. The methodology of encapsulation of the seeds with sodium alginate of medium viscosity (Agmv) in different concentrations (1.5% and 0.75%) with and without sodium chloride, by ionotropic gelling, was used, and then they were sown in a sowing field and the germination obtained was evaluated. As a result, it was observed that the seeds encapsulated with 1.5%Agmv+NaCl obtained a 44% increase in germination and a 45% increase in initial growth, when compared to the control. Through, it is concluded that the encapsulation of the seed promoted the protection of the plant, aided plant growth and is an alternative in the treatment of seeds.

Keywords: Encapsulation; Ionotropic Gelling; Germination Test; Sodium alginate.

Evidence Of Multiple Plant Growth-Promoting Traits Of Isolates From Sugarcane Associated Bacterial Communities

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Abstract

The usage of agricultural inputs, both for plant fertilization and pest control, has intensified in recent years, characterizing intensive agriculture systems, which can cause several damages, both for the agricultural ecosystem and for the health of consumers. In contrast, biological farming inputs have shown themselves as a more sustainable alternative once they enable the reduction of synthetic agrochemicals usage and their adverse outcomes. In this sense, the prospection of bacteria with plant growth-promoting traits is a type of strategy that has gradually gained prominence to recognize beneficial microorganisms that could be used as bioinoculants to the development and productivity of crops. Thus, this study aimed to investigate 36 isolates obtained from six bacterial communities from epiphytic or endophytic environments in sugarcane. These bacterial now the isolates were characterized by considering direct and indirect mechanisms. From the analysis of the 16S rRNA gene sequences, it was possible to carry out the taxonomic assignment, at least at the genus level, with a suggestion of species for each isolate. In this sense, we highlight the species of the genus *Enterobacter (E. mori, E. asburiae and E. cancerogenu*s), in addition to the species Serratia surfactantfaciens, *Agromyces iriotenses* and *Stenotrophomonas maltophilia*. By in vitro assays, these isolates showed promising results, with highlights the high ammonia production, phosphate solubilization, ACC-deaminase, and siderophore production. In conclusion, we identified many and various functional characteristics associated with several bacterial isolates, with the potential to promote healthy plant development.

Fundin: This study received financial support from the "Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)" (Process number 2017/09008-5) and "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES)" - Finance Code 001.

Two new *Tulasnella* species isolated from roots of endangered Brazilian Atlantic Rainforest orchids

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Abstract

The Atlantic Rainforest is a biodiversity hotspot in Brazil, recognized for containing endemic and endangered orchids, such as *Cattleya locatelli* and *Zygopetalum pabstii*. In natural habitats the orchids associate with orchid mycorrhizal fungi to germinate and grow, and *Tulasnella* is know to be one of these mycorrhizal genus. This study aims to investigate the diversity of *Tulasnella* species associated with Atlantic Rainforest orchids. Healthy roots was collected from *C. locatelli* and *Zygopetalum* sp. native to Parque Estadual da Serra do Brigadeiro - MG. One isolate was obtained from direct isolations from *Zygopetalum* colonized root cells. Other two isolates were obtained using the dilution-to-extinction isolation method from *C. locatelli* roots. Fungal DNA was extracted using the Wizard Genomic DNA Purification kit (Promega Corporation, WI). The ITS and LSU rDNA were amplified by PCR using the ITS5/LR6 primers and after sequenced. The sequences were edited and analyzed by Baysian inference. Sequences were compared with the GenBank database by megaBLAST. Slides were made from cultures grown in Corn Meal Agar, mounted in lactoglycerol to observe fungal structures. Each of the three isolates were identified as *Tulasnella* and the phylogenetics analysis indicates two new species, one from *C. locatelli* (clade 2), and other from *Zygopetalum* sp. (clade 3) and one *Tulasnella* cf. *calospora* (clade 2). This study contributes to knowledge of the *Tulasnella* species associated with endangered Brazilian orchids and with a potential to be used for orchid commercial production, and for re-introduction programs.

Keywords: Mycorrhiza, Taxonomy, Phylogeny, Orchidaceae

Financial support: CNPq, FAPEMIG and CAPES

Inoculation of endophytic fungi to control the severity of Ramulose in plants of *Gossypium hirsutum*

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Abstract

Phytopathogenic microorganisms such as *Colletotrichum gossypii* can attack *Gossypium hirsutum* (cotton) plants, compromising their physiological potential, but it is known that endophytic microorganisms compete with phytopathogens for ecological niches. In view of this, the hypothesis was raised that fungi symbiotic with the *Butia purpurascens* palm tree could be used to protect cotton, reducing the effects of ramulose caused by *Colletotrichum gossypii*. For this, plants inoculated with four types of symbiotic fungi, previously selected by in vitro antibiosis tests, were evaluated. Seeds infested by *Colletotrichum gossypii* and associated with endophytic fungi were cultivated in a greenhouse system and analyzes were carried out in the vegetative phenological stage, evaluating biometric characters of growth, dry and fresh biomass, synthesis of photosynthetic pigments and fluorescence of chlorophyll *a*. The obtained data were submitted to analysis of variance and mean test to verify the effect of the presence of endophytic fungi in plants with ramulose. A decrease in the deleterious effects of ramulose on the physiological characteristics of the plant was observed. Overall, the endophytic *Hamigera insecticola* (BP33EF) showed better performance in controlling ramulose symptoms, inducing accumulation of dry and fresh biomass and photochemical performance. Plants inoculated with the fungus *Codinaeopsis* sp. (BP328EF), however, tended to higher means for photosynthetic rate (*A*), stomatal conductance (gs_w) and transpiration (*E*), indicating a better photosynthetic capacity in relation to control plants. The results obtained in this work open perspectives for future applications of these endophytes in the control of ramulose in cotton.

Keywords: Biocontrol; Phytopathogen; Fungal diseases; Endophytes; *Colletotrichum*.

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Avaliação do potencial antimicrobiano do ácido hexanóico em linhagens de *Xanthomonas citri* de diferentes regiões

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Abstract

Brazil is the world leader in sweet orange production. Despite this, the Brazilian citriculture faces constant threats such as citrus canker (CC), an infectious disease caused by the bacterium *Xanthomonas citri* subsp. *citri* (*X. citri*). The current methods to control citrus canker include recurrent sprays of copper, which is an environmental toxic metal. Hexanoic acid (HA) is a carboxylic acid derived from hexane, and several reports have suggested an antimicrobial potential for this acid. In addition, HA is water soluble, which raised the possibility of using the acid to protect plants against bacterial infection. Here, the anti-*X. citri* potential of HA was evaluated against a panel of field isolates of *X. citri.* The method used was the resazurin microtiter assay (REMA), which measures the respiratory activity of the cells. Nine different *X. citri* strains, collected from different São Paulo state regions, were exposed to different concentrations of HA ranging from 1000 ppm to 7.81 ppm. Subsequently, they were spread on NYG medium to check for their ability to resume growth and determine the minimal bactericidal concentration of the acid. Of the 9 strains tested, 2 strains resumed growth at 125 ppm, 5 at 250 ppm, and 2 at 500 ppm, setting the minimal bactericidal concentration at 1000 ppm. Therefore, HA was able to inhibit growth of different isolates of *X. citri*, and, in addition to the fact that it can potentially be used for plant protection, HA could be considered as an alternative for citrus protection in management programs.

Keywords: citrus canker; copper

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UASB reactors, in series, for the production of biogas from molasses and filter cake

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Abstract

Brazil is the world's largest producer of sugarcane, used to produce ethanol and sugar. However, the sugar-energy industry generates large amounts of molasses and filter cake, which have a high energy potential. The objective of this work was to evaluate, in two upflow anaerobic sludge blanket reactor (UASB - R1, and R2), in series, with volumes of 12 and 5.6 L, for the anaerobic co-digestion of molasses and filter cake. The molasses is rich in carbon and easily degradable. In the filter cake, there are nitrogen and phosphorus, favorable for microbial development. The hydraulic detention time applied in the reactors (R1+R2) was 34.6 h. The organic load rate (OLR) values applied in R1 and R2 ranged from 0.6 to 13.3 g total COD $(L\ d)^{-1}$ and from 1.4 to 21.3 g total COD $(L\ d)^{-1}$, respectively. The observed volumetric methane production was up to 1.11 and 0.86 L CH_4 $(L\ d)^{-1}$ in R1 and R2, respectively, during 280 days of operation. The average values of the ratio of intermediate alkalinity and partial alkalinity remained between 3.95 and 3.27 in R1 and R2, respectively. With increasing OLR, instabilities were observed in the system due to the accumulation of volatile acids. It is expected the adaptation of the inoculum sludge to the imposed conditions, and that the continuous supplementation of molasses and filter cake will enable the increase in volumetric methane production and stability in the system.

Keywords: Biogas; anaerobic digestion; methanogenic archaea; anaerobic reactors.

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Potential of halophilic archaea inoculation in alleviating the salt stress on maize

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Abstract

Soil salinization is one of the most destructive environmental factors responsible for a decline in crop productivity worldwide, also by desertification, and consequent decline arable lands. The application of beneficial microbes, especially plant growth-promoting microorganisms (PGPM) have been used to alleviate the detrimental effects induced by salinity stress on plant growth and development. However, although microbial interactions might potentially benefit and protect the host plants from various abiotic stresses, the contribution of the inoculation with Archaea to salt tolerance is still unknown, mainly due to their limitations in cultivation. The halophilic archaea are commonly found in salty environments. In this work, a Haloarchaea strain, CMAA 1910 was isolated from rhizosphere of the halophilic plant *Atriplex nunmularia*, cultivated under saline irrigation (5,98 mS/cm^{-1}) on the experimental field in the Brazilian semi-arid region. The halophilic Archeon was inoculate on maize seed ($CFU_{600}10^8$) to evaluate the potential of mitigate the salt stress effects on maize seedlings. The experiment was carried with NaCl irrigation (50mM 250mM). Inoculated plants was able to increase the salt tolerance index (STI) to 80%, and the dry biomass against the control. Overall, these results showed, for the first time, the great potential of the haloarchaea strain CMAA1910 for mitigating the saline stress on maize seedlings under greenhouse-controlled conditions. Furthermore, potential genes have been identified on CMAA1910 genome associated with plant hormone biosynthesis, osmotic stress response as indole-3-acetic acid (IAA), proline/glycine betaine osmolytes and K^+ uptake corroborate the functional capacity of archaea to alleviate negative effects of salinity on plants.

Keywords: Rhizosphere archaea; Soil salinity; Plant-growth promoting archaea; Salt mitigation; *Atriplex*).

Penicillic acid: Biological activity against *Xanthomonas* spp.

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Abstract

Penicillic acid is a mycotoxin naturally obtained from some filamentous fungi. The production of penicillic acid was mainly related to the genera *Aspergillus* and *Penicillium*. This compound has a well-known bacteriostatic potential, including for Gram-negative bacteria, like the genus *Xanthomonas*. Considering the phytosanitary problem caused by the *Xanthomonas* genus, this work aimed to evaluate the penicillic acid bioactivity against *Xanthomonas euvesicatoria* (*Xae*), *X. axonopodis* pv. *passiflorae* (*Xap*), *X. manihotis* (*Xam*) and *X. albilineans* (*Xalb*). Therefore, penicillic acid was evaluated using microdilution in a 96-well plate (REMA) with concentrations from 200 to 1.6µg.mL^{-1} for each one of the phytopathogens. This assay also included a positive control (Kanamycin 20µg.mL^{-1}), a vehicle control (DMSO 1% v/v), and a negative control (no treatment). The bioactivity was measured using resazurin dye fluorescence, which indicates bacterial growth. From that, the inhibitory concentration (IC) for inhibition growth of 50% was calculated, based on the dose-response curve. Furthermore, an aliquot of each well was inoculated on an agar plate and incubated for 48h to obtain the Minimum Bactericidal Concentration (MBC). The results of this work were interesting, *Xam* showed the lowest IC50 (15.6µg.mL^{-1}) followed by *Xeu* (22.5µg.mL^{-1}) and *Xap* (28.8µg.mL^{-1}). *Xalb* presented the higher IC50 (131.4µg.mL^{-1}). About the MBC assay, *Xam* and *Xeu* presented no growth on the plate at 200µg.mL^{-1}, while *Xap* and *Xalb* had no growth until 100µg.mL^{-1}. The results obtained by this work corroborate with other studies, which show the potential of penicillic acid to inhibit bacterial growth, including gram negatives phytopathogens.

Keywords: Penicillic Acid; *Xanthomonas*; in vitro; growth inhibition;

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Chemical and biological controls for the management of foliar diseases in the soybean (*Glycine max* (L.) Merrill)

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Abstract

Agricultural producers have adopted the practice of associating biological products with chemicals in the same application. However, the benefits of this practice are not known experimentally. Therefore, the objective of this work is to evaluate the agronomic efficacy of chemical and biological agents, applied alone and in mixture, in the control of Asian soybean rust (*Phakopsora pachyrhizi*) and target spot (*Corynespora cassiicola*). The experiment was installed at Fazenda Areão (Piracicaba, São Paulo), with 5 repetitions of each one of the 10 treatments, applied every 10 days. As chemical control, two commercial fungicides registered for the crop were used alternately: trifloxystrobin + tebuconazole (commercial product Nativo, 0.5 L/ha) and epoxiconal + fluxapyroxad + pyraclostrobin (commercial product Ativum, 0.8 L/ha). As biological control, two different products were evaluated, one already registered for the culture, composed of *Bacillus subtilis* (commercial product Bio-Imune, 0.5L/ha) and the other still in the registration phase, composed of a mixture of acids and organic salts and essential oil compounds in three different dosages (0.47; 0.94 and 1.88 L/ha). Another four treatments correspond to joint applications (tank mixtures) of chemical and biological fungicides and the tenth treatment (control or witness) corresponds to the absence of spraying. The severity of both diseases was evaluated with the help of existing diagrammatic scales. Preliminary results do not indicate a greater effectiveness of control in the mixtures, the most efficient treatment being the one constituted only by the use of chemical fungicides.

Keywords: Soybean; foliar diseases; tank mixtures; biocontrol.

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Reuse of annatto agro-industrial waste and spent mushroom substrate: a circular economy model

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Abstract

Circular economy systems can be applied to mushroom cultivation, using waste materials to create sustainable farms. In addition, annatto agro-industrial residue reusing can generate solutions, since its disposal is a problem for annatto farms. For the first time, we aim to develop the reusing waste from mushroom and annatto production in a circular system. Two experimental phases were conducted. Phase I evaluated annatto agro-industrial residue proportions (0, 20, 40, 60, 80, and 100%) in two oyster mushroom strains production (16/01 and 20/01). Bags with 1.8 kg containing eight replicates were made. The bags were brought to a climate chamber. Later, the bags were opened and the mushrooms were harvested. The yield mushroom was evaluated. Phase II measured spent mushroom substrate proportions (0, 20, 40, 60, 80, and 100%) for the annatto seedlings cultivation. Five annatto seeds were sown in tubes and thinned out after 18 days of cultivation, leaving one plant per tube. After 60 days of cultivation, height and root dry matter were evaluated. The t and Tukey tests (0.05) were applied to the data set. Phase I results demonstrated that annatto residues high proportions (>40%) reduced the mushroom yield. However, the 16/01 strain showed a greater yield. Phase II results showed that 60% spent mushroom substrate replacing increased height and dry matter by about 35 and 95%, respectively. It concluded that the mushroom strain 16/01 and 60% of spent mushroom substrate reuse on the annatto seedlings production would be the best sustainable way for a circular economy model.

Keywords: *Bixa orellana*, oyster mushroom, residue, seedlings production, sustainable system.

Financial support: Coordination for the Improvement of Higher Education Personnel–CAPES (Finance Code 001) and São Paulo Research Foundation – FAPESP (#2021/09034-1).

Exploring the biosynthetic genes cluster involved in the production of mycosporine-like amino acids in the cyanobacterium *Capilliphycus salinus* ALCB114379

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Abstract

Cyanobacteria are a highly diverse group of prokaryotic organisms, comprising unicellular and filamentous forms with remarkable adaptability to different environments. They are capable of performing photosynthesis and nitrogen fixation, and of producing a wide range of specialized metabolites. Identifying the genes responsible for synthesizing these metabolites is essential to understand the ecosystem behavior of these microorganisms and for bioprospecting biomolecules of biotechnological interest. This work aimed to analyze the genome of the cyanobacterium *Capilliphycus salinus* ALCB114379 to explore its potential for synthesizing bioactive molecules. The genome was assembled using HiFiASM and its quality and completeness were assessed using Quast and CheckM, respectively. Potential functional genes were annotated using the tool Prokka and classified into 23 subsystems with BlastKOALA. The mycosporine-like amino acid biosynthetic gene cluster was predicted in the "Biosynthesis of Other Secondary Metabolites" subsystem. Synteny analysis revealed that the four genes responsible for mycosporine-like amino acid biosynthesis are colocalized in the same way as in strains known to produce this biomolecule. Identifying the conserved domain using InterPro demonstrated that despite having moderate identity (between 50% and 70%) compared to known producing strains, they all share the same domain. Absorbance analysis showed a peak wavelength at 330 nm, suggesting the presence of mycosporine-like amino acid. However, to confirm its actual production, liquid chromatography coupled with mass spectrometry needs to be performing in cell extracts of *Capilliphycus salinus* ALCB114379.

Keywords: Bioprospecting; Genomics; Photoprotective pigments; Specialized Metabolites.

An in silico study involving Mangiferin, 7-Epiclusianone, Fukugetin, Lapachol, Plumbagin and Guttiferone-A against C. albicans proteins

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Abstract

The *Candida albicans* is an opportunist fungus that lives symbiotically in the human body and is present in regions such as vagina, intestine, and mouth, in which it assists in the natural process of good functioning in these certain places. However, the microorganism can develop an invasive characteristic and proliferate in an exacerbated way. Furthermore, this fungus is becoming a big problem, because as treatments are used for a long time in a repeated way, the fungus will become resistant to mutations and become more resilient, and the search for the new drugs with the better efficiency becomes constant, but for faster tests and without waste use *in* silico methodology which was used with the secundary metabolites guttiferone-A, fukugetin, plumbagin, lapachol, mangiferin and 7-epiclusianone against proteins generated by the gens: CLN3, CPH1, FAS2, SKN7, SLN1, TUP1, TEM1, DBF4, ERG5 and LTE1, through the Blinding docking methodology used the AutoDock Vina software for known the energy of interaction, but for comparison it was done the same methodology with the controls drugs of each gene. From the results, it is possible to observe that the fukugetin and guttiferone-A shown the potential to act as multi-targets because have better bindings energy results with the gens than another secondary metabolites and the controls compounds. Thereby, it was possible to observe the natural drugs showed excellent antifungal performance in the *in silico* research and now is necessary tests *in vitro* or *in vivo* for better confirmation of the results.

Keywords: *Candida albicans*; resistance; natural products; molecular docking.

Financial support: CNPq

ß-glucosidase activity in soils under Integrated Production Systems

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Abstract

Integrated Agricultural Production Systems (IAPS) are alternatives capable of recovering degraded areas for agricultural production. The increase in diversity in IAPS, above and below ground, can alter microbial activity, nutrient cycling and carbon (C). The C is one of the main components of organic matter and its cycling can be achieved by ß-glucosidase activity. In this study, the objective was to determine the activity of the enzyme ß-glucosidase in areas of IAPSs. Soil samples were collected in (1) Livestock-forest – ILF, (2) Crop-livestock – ICL, (3) Native Forest (NF) and (4) Pasture area (PP). In each area, 9 repetitions per layer were performed: 0-5, 5-10, 10-20 e 20-30 cm. The ß-glucosidase enzyme activity was determined by Tabatabai methodology, and Duncan test was used as a statistical parameter. In the 0-5 cm layer, ILF presented a value of 56.29 (± 11.90), ICL 57.07 (± 17.54) e PP 53.45 (± 7.03) did not differ and were greater than NF (38.84 ± 13.76). At a depth of 5-10 cm, the ICL with a value 26.97 (± 5.86) did not differ PP (29.73 ± 5.27) and ILF (25.31 ± 6.60), however NF (20.48 ± 4.90) did not differ ILF. In the 10-20 cm layer, ICL with a value 23.65 (± 4.51) differed from NF (14.80 ± 4.71) and did not differ from PP (19.92 ± 3.23). Finally, in the 20-30 cm layer, there was no statistical difference. The results suggest that IAPS can be able to change the microbiological conditions of the soil.

Keywords: soil; enzyme activity; microbiology activity.

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Essential Oils from *Cymbopogon* species inhibit growth of the bacteria *Xanthomonas citri* subsp. c*itri*

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Abstract

Essential oils (EOs) obtained from *Cymbopogon* species are well-known for their wide range of properties, such as anti-parasitic, antimicrobial, anti-inflammatory among others. As other EOs, *Cymbopogon* oils are Generally Recognized as Safe (GRAS) and to be non-toxic to humans. These characteristics point to such oils as viable candidates to sanitization agents against bacterias such as *Xanthomonas citri* subsp. *citri*, the causative agent of the citrus canker. In this work, essential oils from three *Cymbopogon* species, *C. flexuosus* (lemongrass), *C. winterianus* (citronella) and *C. martini* (palmrose) were assessed as to their antibacterial action against the bacteria *Xanthomonas citri* subsp. c*itri*. A microdilution assay (REMA) was performed in a 96- well plate, using the resazurin dye as growth indicator, with a range of concentrations from 10.000 to 78.1 ug.ml-1. Inhibition percentages obtained were used to elaborate a dose-response curve and the values for 90% and 50% of growth inhibition were estimated. Concurrently, aliquots from the 96-well plate were inoculated on solid NYG plates and incubated for 48h when then the lowest concentration were bacterial growth was not visible was considered to be the Minimum Bacterial Concentration (MBC). Tests revealed that lemongrass had the lowest IC90 (156.76 ug.ml-1), followed by Palmrose (202.75 ug.mL-1) and Citronella (256 ug.mL-1). Lemongrass also had the lowest MBC (312.5 ug.mL-1). Such differences may be related to different concentrations of each main components in the EOs, such as citral and geraniol.

Keywords: Xanthomonas; Essential Oils; Lemongrass; Antibacterial.

Financial support: CNPq (The National Council for Scientific and Technological - 142367/2019-2)

Phosphate Solubilizing Microorganisms: From Plant Growth Promotion to Soil Bacterial Community Modulation- the case of *P. agglomerans* 33.1 and arbuscular mycorrhizal fungi

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Abstract

Although phosphorus is abundant in the soil, it is often inaccessible to plants. However, some microorganisms can supply phosphate to host plant by solubilization and/or mineralization of phosphorus from inorganic and organic sources. Among these microorganisms, *Pantoea agglomerans* 33.1, known for promoting plant growth, plays a significant role in phosphate solubilization, while arbuscular mycorrhizal fungi (AMF) enhance plant growth by forming a mutually beneficial relationship with plant roots in phosphorus-deficient conditions. This study aimed to assess the effects of co-inoculations of phosphate solubilizing strain 33.1 and AMFs on plant growth, phosphorus accumulation in plants, and the bacterial community of the soil. Sugarcane plants were co-inoculated with 33.1 and the mycorrhiza *Rhizophagus clarus*, *Rhizophagus intraradices*, or *Dentiscutata heterogama*, and after 30 days at a greenhouse, plants were analyzed for dry weight and phosphorus accumulation. For bacterial community investigation, 16S amplicons from the soil samples were sequenced by Illumina Miseq and analyzed using R packages dada2, phyloseq, and vegan. The co-inoculation was able to increase the root dry weight of sugarcane plants by at least 325%. Phosphorus accumulation by roots was favored by most treatments. Furthermore, no differences in diversity from the bacterial soil community were observed, but the inoculation altered the composition and abundance of some specific groups inside it. Our findings suggest that co-inoculation of 33.1 and AMFs could be beneficial to agriculture, improving phosphorus availability and plant growth. As a result, this study provides a starting point for further and comprehensive investigations into the promising synergy between these microorganisms.

Keywords: Phosphorus; *P. agglomerans* 33.1; Arbuscular mycorrhizal fungi; bacterial soil community.
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