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V SIMPOSIO DE MICROBIOLOGIA APLICADA

11, 12, 13 e 14 de maio de 2011
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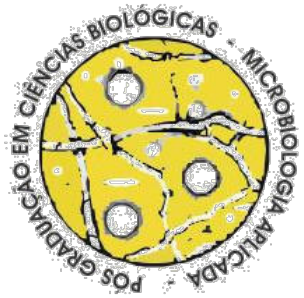

Coordenador: Profº Dr. Fernando Carlos Pagnocca

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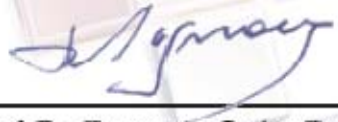
Editorial

O V Simpósio de Microbiologia Aplicada, realizado no Instituto de Biociências (UNESP – campus Rio Claro) pelo Programa de Pós-Graduação em Ciências Biológicas – Microbiologia Aplicada durante os dias 11 e 14 de maio de 2011 foi um evento aberto para pesquisadores, graduandos e pós-graduandos, em nível nacional e internacional, como oportunidade para publicar e compartilhar trabalhos e experiências, realizar contatos e parcerias com outros cientistas da área, contribuindo para divulgação de pesquisa científica no país.

O Simpósio de Microbiologia Aplicada da Universidade Estadual Paulista, campus Rio Claro, nasceu como uma nova versão do "I Curso de Extensão em Microbiologia Aplicada", realizado em 2004. Nesse evento, foram abordados as pesquisas realizadas pelo Programa de Pós-Graduação em Ciências Biológicas - Microbiologia Aplicada desta instituição. Em sua quinta edição, V SMA contou com 387 participantes nacionais e internacionais que compreenderam 13 estados brasileiros e 4 países além do Brasil (El Salvador, Espanha, França e Nigéria), superando as expectativas iniciais. Durante o evento, foram apresentados 173 trabalhos originais (170 na forma de painel e 3 na forma oral) nas três temáticas propostas: Ambiental, Biotecnologia e Sistemática. Assim, houve a apresentação de 71, 91 e 11 trabalhos nos temas: Ambiental, Biotecnologia e Sistemática, respectivamente. Além disso, um trabalho de cada área foi selecionado para apresentação oral.

Durante o Simpósio foram ministradas 8 palestras de alunos do Programa de Pós-Graduação em Ciências Biológicas - Microbiologia Aplicada, sendo 2 alunos de mestrado e 6 de doutorado. Com isso permitiu-se a disseminação do conhecimento gerado pelas dissertações e teses concluídas e em andamento pelos alunos do programa. Esta atividade tem imensa contribuição para o Programa de Pós-Graduação pela divulgação das pesquisas junto à comunidade científica do Brasil e do exterior. Em relação aos convidados para o evento, houve a presença de representantes do Departamento de Bioquímica e Microbiologia (vice-chefe), do Instituto de Biociências (vice-diretor), da Reitoria da UNESP (pró-reitora de pesquisa e assessoria), da Sociedade Brasileira de Microbiologia (presidente) e autoridades locais.

O evento foi organizado pelo corpo docente e discente do Programa de Pós-Graduação em Ciências Biológicas - Microbiologia Aplicada. Desta forma, o evento contribuiu para a divulgação e maior visibilidade do programa, além de ampliar a disseminação científica referente às pesquisas realizadas. Em nome da comissão organizadora, agradecemos a vailosa participação de todos que nos prestigiaram com sua participação e permitiram a realização do V Simpósio de Microbiologia Aplicada.



Prof. Dr. Fernando Carlos Pagnocca
Coordenador do Programa de Pós Graduação em
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RESUMOS

EM ORDEM ALFABÉTICA

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ACTIVATED SLUGDE APPLIED IN PLASTICS BIODEGRADATION

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Keywords: Biodegradable polymer; Blend; Poly (e- caprolactone); Starch.

Many synthetic materials like poly (e-caprolactone)(PCL), are not degraded by microorganism in the environment which contributes to their long life-time of hundreds of years. One of the viable alternatives to accelerate the attack of microorganisms to PCL is the addition of natural polymers like starch, to guarantee at least a partial biodegradation. Starch is being a good choice because it is an abundant and low cost raw material in the market and it is applied in many purposes area. Biological treatment in aerobic conditions by activated sludge process has been widely used to industrial effluents and the capacity of the microorganism to degrade different organic compounds suggest that activated sludge could be consume the plastic formulation. Large quantities of package composed by synthetic polymers are discharged into the environment like solid disposal, so the aim of this study is to propose a plastic blend that is able to biodegraded by microorganisms from activated sludge. The material used in this study was PCL P-787 (Union Carbide, Brazil) and maize starch SNOW-FLAKE® 064051 (Corn Products, Brazil). The films of plasticized PCL were prepared at 30°C by adding 67,5% (w/w) of PCL and 32,5 wt% of Edenol 3023 in a high mixer at 400 rpm until complete homogenization. The blend proportion was 35% (w/w) starch, 15% (w/w) plasticizer and the 50% (w/w) PCL. The process was started by adding maize starch under stirring (200 rpm) in a high mixer and heating to 100°C during 10 min. The plasticizer Edenol 3203 was slowly added at 400 rpm. Then, the system was then cooled until 30°C and the PCL was added under stirring (400rpm), until complete homogenization. The material so obtained was processed in a simple screw extruder Haake with 60 mm diameter screw and L/D = 25, with a 20 rpm speed screw with the following heating profile: 90, 80, 70, 60°C from the feed to the die. The blend and plasticized PCL were prepared as films with approximately 0.90 mm of thickness and 2 x 6 cm sizes. The sludge was collected from a municipal wastewater treatment plant (Campinas, Brazil). Aerobic biodegradation was performed in a batch reactor (capacity of 2,5 L), containing PCL and PCL/starch blend films and inoculum. The experiment was performed at room temperature under constant aeration for until 120 days of biodegradation. The biodegradation process was observed by scanning electron microscopy (SEM), atomic force microscopy (AFM) and the mass loss and elemental analysis were determined. The PCL presented low degradation by this biological system, however, when starch was added, the blend degradation increased as verified by SEM and AFM. This behaviour was expected since starch is recognized by many microorganisms in nature, facilitating the PCL biodegradation. It was observed a low mass reduction in 84 % to the blend PCL/starch and 41% to PCL film. AFM show the surface modification, especially where the starch granules was consumed by microorganism in the blend sample. SEM images corroborate with the AFM results. The elemental analysis presented an increase percentage to C (carbon), N (nitrogen) and H (hydrogen) after 120 days of biodegradation. This results show that biomass of activated sludge was adhered to film even with the significative loss mass. The procedure utilizing activated sludge was a very promising process to study the biodegradation of PCL/starch blend and PCL film studies in this work.

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ALGAE AND CYANOBACTERIA PRESENT IN ECOLOGICAL FILTER USED IN TREATMENT OF WATER SUPPLY

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Keywords: algae, cyanobacteria and ecological filter

The increase in contamination of water sources for public supply by various substances and their frequent occurrence in the aquatic environment and drinking water has raised the question about its impact on the environment and public health. These substances have high potential for bioaccumulation and low biodegradability, which can have adverse effects. The use of Eco Filter is a promising technology in water treatment, because this does not require the application of chemicals, as well as its observed ability to remove various compounds. Through this process we can offer low-cost water, tasteless, odorless and safe. Inside the tank Ecological Filtration is between the living relationship of the food chain, where there is the development of microorganisms, mainly algae, cyanobacteria and bacteria that play a fundamental role in the biodegradation of various compounds. This study aims to identify the taxa of algae and cyanobacteria present in an ecological filter that was fabricated in a box of asbestos from 1000 liters, dimensions: 1m x 1.55 m and 0.72 m deep. The water supply of the filter is derived from a natural lake - Lake of Ipê, located in Ilha Solteira - SP. Morphological characteristics and metrics of these microorganisms were taken into account and was used to identify specific references where possible to the species level. Samples were collected fortnightly from April to November 2010, at two different points, the wall surface and the sand filter, or schmutzdecke, totaling 32 samples.Sampling was done randomly by "scraping" of 100 ml of the material that was packaged in amber bottle and fixed in situ in 4% formaldehyde. We identified 92 taxa, distributed in nine classes and 58 genera. Most taxa were recorded in the classes *Chlorophyceae* (28 species, 30.4% of total) and *Zygnemaphyceae* (29 species, 31.5% of total). *Zygnema* and *Spirogyra* (classes) and cyanobacteria *Geitlerinema amphibium* occurred in 85% of samples during the study period. The algae, mainly filamentous form a "network" over the sand filter and likely contribute to increasing the efficiency of the filtration process, preventing the passage of impurities. It is noteworthy that the identification of algae and cyanobacteria that occur on ecological filter, and the change of species over time, can contribute to understanding the biofiltration capacity of this system.

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AMYLASES PRODUCTION BY RHIZOSPHERIC FUNGI ASSOCIATED WITH *Opuntia ficus-indica* Mill.

COSTA, F.E.C. [1*], PEREIRA, P. P. [1*], PEREIRA,S. G.[1*], SILVA, F. T. [1*], TEIXEIRA-LOYOLA, A.B.A. [1*], SARAIVA, G.K.V. [1*], MELO, I.S. [2*]
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Keywords: rhizosphere, starch, *Opuntia*, fungi

Roots have great influence on soil that surrounds it (rhizosphere) because it release up 40% of total dry matter produced by photosynthesis of organic carbon as root exudates. Rhizospheric microorganisms act on the nutrients cycling, including starch. Amylases are produced by bacteria, yeasts and fungi, especially filamentous fungi. Amylases include a group of enzymes that act on the starch releasing several products from dextrins to glucose. These enzymes have biotechnological applications in the pharmaceutical, textile, detergent and food industries. This work aimed to evaluate the production of amylases by rhizosphere fungi associated with *Opuntia ficus-indica* Mill's semi-arid. Strains used in this work are from the UNIVÁS Microbiological Collection, maintained with periodical sampling in Petri dishes containing Sabouraud agar media, after reactivation. The production of amylase was tested in TLE liquid medium ($CaCl_2$ 0.1 g L^{-1} KH_2PO_4 7.0 g L^{-1}, K_2HPO_4 2.0 g L^{-1}, $(NH_4)_2SO_4$ 0.1 g L^{-1} , $MgSO_4.7H_2O$ 0.1 g L^{-1} and 0.1 mL of trace elements) containing starch (1%) as carbon source. The inoculated erlenmeyer were incubated in rotary shaker at 28 ° C and 160 rpm. After 24 and 48 hours of incubation 20 mL of media were collected, centrifuged and used for the determination of amylase activity. For the determination of amylase activity were used two different methods: the dextrinifiying activity (FUWA,1954) and saccarifiying method (Miller, 1959). Others parameters evaluated were the mycelial growth and pH change. All tested strains (n = 17) were able to produce amylase dextrinifying activities after 24 h of incubation, with values up to 10 enzyme units per ml of fermented medium. Five of these strains showed increased activity at 48 hours of incubation, and three showed a significant reduction in those values. This reduction of values can be related to the fact that the induction medium not receive glucose addiction, an easily metabolizable carbon source, glucose present (released) in media was consumed. The mean values for saccarifiying activity has significantly increased at 48 hours of incubation, and the number of positive strains, which go from 8 to 10, reaching values up to 15 enzyme units per ml of fermented medium. Dextrinifying and saccarifiying activities of tested strains did not correlate with the pH change or with mycelial growth. The pH soured significantly. We conclude that some of the tested strains are able to starch degradation, with potential for biotechnological application of these, after purification and supplementary tests (times exceeding 48 hours).

Financial support: PIVIC - UNIVÁS, EMBRAPA (01/2009 PAC−EMBRAPA)

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ANALYSIS IN SEASONAL SCALE OF DENSITY OF DENITRIFYING BACTERIA PRESENTS IN ITUPARARANGA RESERVOIR

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Keywords: Denitrifying bacteria, reservoir Itupararanga, nitrate, nitrite.

The Itupararanga reservoir has a central role in the water supply system which it´s inserted in, but lately the water quality is suffering degradation due to the entry of untreated sewage, which contains nitrogen compounds such ammonia, nitrate and nitrite. This work aimed to estimate the density of denitrifying bacteria by the technique of most probable number (MPN), presents in water and sediments of this reservoir. Three sites were sampled: EC2, EC3 and Sorocaba-montante, in October (2009) and April (2010). The sampling station EC2 belongs to a transition zone, and in the vicinity of this site has a condominiums facility, farms and recreational home. The point EC3 is located in the downstream of Sorocaba river; while the station Sorocaba-montante is near from the influents: Sorocabuçu and Sorocamirim rivers, while the latter receives a pollutant load in Ibiuna; while the Sorocabuçu river has as tributary the Una river. that is qualified with low levels of water quality.

The highest concentrations of denitrifying bacteria in sediment collected in October 2009 ($3{,}21.10^{10}$ MPN/g STV) and in the water sample in April 2010 ($1{,}2 .10^{7}$ MPN/g STV) at station EC2 may indicate inadequate release of effluents from the condominiums located near the station. The station EC3 presented lowest concentrations of nitrate, 0,46 $mg.L^{-1}$ in October 2009 and 0,4 $mg.L^{-1}$ in April 2010, this may explain the lowest concentration of denitrifying bacteria in the sediment in October 2009 ($5{,}2.10^{8}$ MPN/g STV) and in the water to collect in April 2010 (4.10^{5} MPN/g STV). While the station Sorocaba-montante presented the highest concentrations of nitrate (0,57 $mg.L^{-1}$), nitrite (0,28 $\mu g.L^{-1}$), and the highest concentration of denitrifying bacteria in sediments samples in April 2010 ($3{,}3.10^{13}$ MPN/g STV).

The highest concentrations of denitrifying bacterias were found in sediments, probably, because of accumulation of organic matter and nitrogen compounds and it is emphasized that oxygen concentration in sediments was lower than in the water column, which favored the growth of denitrifying bacteria that are predominantly anaerobic.

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Analysis of adsorption isotherms in the process of azo dye biosorptive Direct Blue 71 in solution by *Aspergillus oryzae*

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Keywords: Freundlich, Langmuir, biossorption, azo dye, *Aspergillus oryzae*.

The adsorption isotherms show a certain adsorbed solute amount by an adsorbent surface as a function of solute equilibrium concentration. The Langmuir and Freundlich equations are used to evaluate these isotherms. Langmuir follows the monolayer adsorption model and Freundlich the multilayer. Many micro-organisms are used in pollutant adsorption, for example, textile dyes. This study aims to evaluate Direct Blue 71 azo dye biosorption in a pH 2.5 solution by fungus *Aspergillus oryzae* through Langmuir and Freundlich isotherm adsorption equations studies. Triplicate test solutions were prepared with a 1 mL of 1000 mg/L DB71 dye stock solution to, 7.5, 6, 4.5, 3 and 1.5 mL of pH 2.5 distilled water and 1.5, 3, 4.5, 7.5 and 6 mL of *A. oryzae* as paramorphogenic form (pellets), respectively. Each biomass mL corresponds to 11 mg / ml dry weight. Control solutions were prepared with 1 mL of dye stock solution and 9 mL of pH 2.5 distilled water. Test solutions were incubated at 30°C for 120 minutes. They were then centrifuged at 4000 rpm for 20 minutes and analyzed in a UV-VIS spectrophotometer at 583 nm. For the isotherms adsorption evaluation it was used the- Freundlich equation: Log (x / m) = log K + n.log Cf, where x / m = adsorbed solute mass per unit of adsorbent mass (mg / g), Cf = solute concentration in equilibrium (mg / L), K is x / m, where Cf = 1 and n = expressed in liters of liquid dye solution per gram of adsorbent, and Langmuir: Cf (m / x) = 1 / (k1k2) + (1/k2). Cf, where k1 = capillarity index expressed in liters of dye solution, adsorbed dye (mg) at saturation (1/mg) and k2 = amount of solute that should saturate a adsorbent mass unit with a single layer (mg / g). The remaining dye concentrations were calculated using the standard line equation : Abs^{583nm} = 0.0492 + (0.02336 x remaining dye concentration), with the results for samples with 1.5, 3, 4.5, 6 and 7.5 mL of biomass, 64, 20, 44.46, 28.16, 13.22 and 5.08 mg / L, respectively. Hence, it was determined the equation correlation coefficients equal to 0.96969 for Langmuir and 0.9342 for Freundlich. Analyzing the correlation coefficients (R) it was possible to conclude that the R Langmuir adsorption isotherm was closer to 1 when compared to Freundlich. Therefore, the biosorption process follows the principle of Langmuir, in which adsorption only occurs in one dye layer. This is due to the pellet adsorption surface homogeneity, which implies the presence of identical adsorption sites.

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Analysis of growth and exopolysaccharide production by the fungus *Lasiodiplodia theobromae* in different concentrations of carbon sources

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Keywords: *Lasiodiplodia theobromae*; carbon sources; exopolysaccharide

The biopolymers have been the subject of intense research, in view of it's high potential for application in different areas. The exopolysaccharides of microbial origin in addition to having properties similar or superior to traditional (from vegetables and seaweed), are often able to form viscous solutions. Some of them also form gels in aqueous media, even at low concentrations and, in some cases, these polymers are used as substrates for new product development, for exemple, exopolysaccharide can be used to form films able to retain and slowly release drugs into the skin in patients who require drug blood concentration always high. The production of microbial exopolysaccharides is not exposed to climate change, marine pollution or problems in crops that affect the supply and change the cost of traditional production of gum. They are also less susceptible to variability in their chemical and physical properties, maintaining the standard of quality because their production can be controlled carefully.

The objectives of this work is to verify the effect of different concentrations of sacarose on exopolyssacharide production by *Lasiodiplodia theobromae*.

The fungus was grown in Petri dishes containing culture medium with agar, minimal medium of Vogel and glucose, letting it grow for 7 days. The fungus was then placed in Erlenmeyers with culture medium with 0.05% glucose and Vogel medium, at 28°C and 180 rpm. Thereafter it was beaten in a blender for 2 min and inoculated into culture media for fermentation with different concentrations of sacarose, 3%, 4% and 5% (also with the minimal medium of Vogel) for 72h. After fermentation, the content of the Erlenmeyers was centrifuged and in the broth obtained was added ethanol 3:1 for the precipitation of exopolysaccharide. Total reducing sugars was measured by Somogyi-Nelson method (ref) and EPS and biomass were determined by gravimetric method.

For the results and discussion was obtained that increasing the concentration of sucrose in the culture medium, increased the production of EPS and biomass. We obtained 3.51, 3.52 and 3.60 g / L of EPS and 16, 18 and 21 g / L of biomass plants in 30, 40 and 50 g / L, respectively. However, using the lowest concentration of sucrose, we obtained the highest values of both biomass yield (0.73) and EPS (0.15), compared to cultures with 40 and 50 g / L (Yp / s 0.58 and 0.45 ; Yx / s 0.12 and 0.10, respectively). From the results, we conclude that the increased concentration of sucrose in the medium provides no significant increase in EPS production by *L. theobromae*, and the concentration of 30 g / L will be adopted for further study.

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ANALYSIS OF THE CAPACITY OF PRODUCING BIOFILM ON GLASS, PVC AND STAINLESS STEEL BY *Salmonella sp* ISOLATED FROM RAW POULTRY

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Introduction: Bacteria of the genus *Salmonella* are among the leading causes of foodborne-disease and those of animal origin are largely responsible for transmitting this microorganism, especially poultry, since they are asymptomatic carriers. Normative Instruction No. 70 (2003), of the Pathogen Reduction Program of the Ministry of Agriculture and Supply provides rigorous control over the slaughter process. However, the presence of microorganisms forming biofilms, a complex matrix, in the food, such as *Salmonella*, is a cause of concern, because of the failures that may occur in the sanitation process. The physicochemical properties of a surface can exert a strong influence on the adhesion of microorganisms, which adhere more readily to hydrophobic surfaces (PVCs) than to hydrophilic (glass or metal such as stainless steel). Thus, this study aims to investigate the production of biofilm by strains of *Salmonella* isolated from raw poultry, in different temperatures (16º, 20º, 28º and 35ºC) and materials (glass, PVC and stainless steel). **Methodology**: From poultry samples were isolated *Salmonella* strains, according to APHA (2001). The strains were incubated for five days at 16ºC, 20ºC, 28ºC and 35ºC, in glass, PVC and stainless steel, for further verification of biofilm production by ELISA reading. S*almonella* Typhimurium ATCC 14028 was used as a control positive and non inoculated BHI (Brain Heart Infusion) as negative. **Results**: 152 poultry samples were collected in eight establishments in the city of Botucatu-SP and 76 (48%) were positive for *Salmonella*. Along with the 62 *Salmonella* strains previously isolated from the same kind of food, were completed 138 strains. Among the three materials, PVC showed the highest production of biofilm, but was not statistically significant different from stainless steel, that showed moderate production. Glass presented a difference statistically significant when compared to the other two materials, showing a lower production of biofilm. The temperature of 35ºC demonstrated higher biofilm production than the others, showing a difference statistically significant in stainless steel that demonstrated a lower production in this temperature then the other materials. When incubated at 28ºC and 20ºC the formation of this matrix was not very different considering both temperatures, being statistically significant different only in glass, which showed a lower production when compared to the others materials. At 16ºC the formation was diminished, being glass and stainless steel statistically significant different to PVC, which showed that in the lowest temperature, analyzed the production was still high. **Discussion**: The rate of contamination of chicken carcasses was high, indicating contamination in any part of the production and retail. The use of the three materials in the industries requires attention since there are differences statistically significant among them according to the temperature that they receive the carcasses or water. According to MAPA ordinance 210 (1998), the pré-chiller process should be at 16ºC, but usually it stays at ± 20ºC, temperature that the *Salmonella* can grow better and produce biofilm as well as demonstrated, and like this microorganism was capable of producing biofilm even at 16ºC, it is still a concern and requires more studies in this area.

Keywords: *Salmonella sp*, biofilm

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ANALYSIS OF THE PROFILE OF BIODEGRADATION OF ESTERS OF FATTY ACIDS CONSTITUENTS OF SOY BIODIESEL

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Keywords: ester of fatty acids, biodiesel, biodegradation

Biodiesel is a renewable energy source promising. Biodiesel consists of methyl or ethyl ester of fatty acids of low structural complexity such as oleate, palmitate, stearate, linoleate and linolenato, derived from a variety of oilseeds such as soybeans, palm oil, castor oil, canola oil, sunflower oil and animal fat. In Brazil, soybean is one of the main oil used in biodiesel production. Although the production of biofuel is an alternative to the demand for renewable energy, is important a thorough study on the biodegradability of this compound. This work aimed to evaluate the degradation process of biodiesel through analysis of the degradation profile ester of fatty acids, and see if there is any relationship, regarding the type of fatty acid ester present in biodiesel. Microcosm systems were mounted with 100g of soil Atlantic Forest, being added to minimal medium (0.1% m/v KH_2PO_4, 0.1% m/v K_2HPO_4, 0.1% m/v NH_4NO_3, 0.05% m/v $MgSO_4$, 0.01% m/v $FeSO_4$, 0.01% m/v $CaCl_2$, pH 7.0),and contaminated with soybean biodiesel concentrations B5 (5% biodiesel/95% diesel), B50 (50% biodiesel/ 50% diesel) e B100 (100% biodiesel), microcosms containing uncontaminated soil was used as positive controls (C+). Samples were collected from these systems on days 0, 30 and 60, then fuel was extracted soil using hexane as solvent. The GC analysis was performed on a GC Shimadzu 2010, injector AOC 20i with detector of flame ionization (FID) using an RTX-WAX column (30 m, with 0.25 mm of internal diameter, film 0.25 μm) and hydrogen as carrier gas. The injector temperature was 280 ° C, the temperature of the system interface controls was 280 ° C. 1 ml of injected sample was analyzed with the program of temperature: 80 ° C for 1 min, heating rate: 15 ° C / min to 230 ° C, maintained for 24 min. Analyzing the degradation of each ester of biodiesel used for the experiment, was observed that esters with the greatest unsaturation (linoleate and linolenate) were the most degraded in all samples. The esters palmitate, stearate and oleate, in the presence of a higher concentration of diesel, had a more difficult degradation. A large number of unsaturation makes the molecules less chemically stable, suitable for oxidation, degradation and polymerization. However, little is known of the fact that there is any direct relationship between the unsaturation of the esters and the degradation process. DeMello *et al.* (2007) found that the methyl ester of C16 fatty acid was degraded faster than the methyl esters of C18, and the rate of degradation of the methyl esters of C18 fatty acids did not correspond with the degree of saturation. In contradiction, Miller and Mudge (1997) reports that several esters of C18 unsaturated fatty acids degraded more rapidily than esters of C16. Although the type ester of fatty acid (considering the type of saturation), seem to influence the degradation process of biodiesel, further studies are needed, although it is also important to consider the various types of biodiesel.

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ANTIMICROBIAL ACTIVITY OF *Paenibacillus polymyxa* CO-ISOLATED FROM NODULE OF *Ormosia fastigiata* Tul.

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Keywords: Antimicrobial; *Paenibacillus polymyxa*; polyketide synthase gene.

The discovery of antimicrobial compounds with medical application represents a revolution in infectious diseases treatments. However, the indiscriminate use of antibiotics has allowed the rise of resistant strains, characterizing a global public health problem. The development of new drugs with this potential has been very restricted. Many microorganisms produce secondary metabolic with biotechnology application. Studies have showed that bacteria of the *Paenibacillus* genus produce substances with great potential use in the treatment and prevention of some pathology. This work verified the antimicrobial activity of an isolated of *Paenibacillus* versus strains of clinical cases. For the bacteria isolation we collected nodules of *Ormosia fastigiata* Tul., follow a disinfection process of the surface of the nodule using alcohol 70%, hypochlorite 1% and successive washes with sterile water. The nodule was sectioned in the upper end region with your interior inoculated in YMA on a warm chamber at 28°C for at least 72h. Colonies was re-isolated in YMA and morphologically characterized. Isolated was maintain in YMB plus glycerol 30% at -80°C. Total DNA was extracted and amplified with the primers 27F and 1525R for the 16S rDNA region and degKS2F.i and degKS5R.i for identification of polyketide synthase genes. For the evaluation of the antimicrobial activity, the inoculum was patterned from a dilution of saline solution (NaCl 0.85%) till reach reading A_{580}=0.5. From that dilution 1 mL was inoculated in 20 mL of YMB growth under constant agitation of 100 rpm for 48h at 28°C. The supernatant was centrifugated and filtrated in a membrane of 0.22µm. Indicators microorganism was growth in TSA for 18h at 37°C, then diluted in saline solution till reach reading of 0.5 on MacFarland scale. After that they were spread in plates with Muller Hinton agar through sterile swabs. A volume of 50µL of cells free supernatant was applied on wells in the medium. Plates were incubated at 37°C for 18h. The kinetics of the production of the antimicrobial substance was evaluated from samples of the supernatant collected every 3 hours and the inhibition test done like previous descript. The procedure of isolation it's a pattern for obtaining rhizobia strains from nodule of legume. However, a co-isolation of an organism that shows an inhibition halo around it colonies has occurred. After the morphological and molecular characterization, the isolated was identified like *Paenibacillus polymyxa*, a rizhobacteria that promote growth in plants. Antimicrobials tests reveals the capacity of this isolated in produces antimicrobial substances that inhibited the growth of strains of *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Enterococcus faecallis* and *Escherichia coli*. The kinetics production reveals that from 27h has occurred production of the inhibitory substance, characterized like a secondary metabolite. The presence of PKS gene points toward the identification of the antimicrobial substance and your use on the pharmaceutical industries and the application of this strain of *P. polymyxa* as a biocontrol agent in plant cultures.

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APPLICATION OF A YEAST BIOSURFACTANT AS AN ALTERNATIVE COLLECTOR TO SYNTHETIC SURFACTANT IN THE TREATMENT OF INDUSTRIAL WASTEWATER IN A FLOTATION COLUMN

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Keywords: flotation, biosurfactant, effluent.

The flotation is an alternative for the efficient treatment of wastewater containing suspended materials of mineral or organic nature. The addition of appropriate chemicals to the flotation can induce or enhance the separation efficiency, and surfactants of chemical origin, known as collectors, are commonly used. Surfactants are substances used to promote a selective hydrophobization of the particles in the flotation pulp, allowing its adhesion to air bubbles and increasing the collection efficiency. The developments of new technologies in the production of environmentally safe surfactants lead to the production of biosurfactants, which are metabolic byproducts of bacteria, yeasts and molds. Thus, the purpose of this study was to evaluate the biosurfactant produced by the yeast Candida lipolytica (UCP0988), as an alternative collector to the synthetic surfactant sodium oleate, in treating an effluent from a synthetic acid mine drainage of a coal located in Santa Catarina State. The biosurfactant was produced in media formulated with industrial waste and subsequently isolated using organic solvents. Biosurfactant solutions at critical micelle concentration (0.03%) were prepared and applied to the flotation process. Flotation tests were carried out in batch column operating with air flow variables for a period of 60 minutes. The results revealed that the biosurfact neutralized the effluent pH naturally, without the addition of other substances, adjusting this parameter to set discharge limits by the State Decree number 14.250/81 from Santa Catarina State. The biosurfactant removed over 90% of metals iron and manganese after 15 minutes of process, suggesting that the use of biosurfactants as flotation collectors constitutes a promising alternative for the treatment of wastewater containing high levels of heavy metals.

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ASSAY FERMENTATION OF YEAST STARTER CANDIDATE ISOLATED FROM CACHAÇA DISTILLERIES IN BAHIA

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Keywords: yeast, fermentation, ethanol

The yeasts have the ability to convert sugars into ethanol and carbon dioxide in the fermentation process through the action of enzymes, being *Saccharomyces cerevisiae* the specie that most stands out for its high yield and tolerance to concentrations of ethanol. The use of selected strains in the fermentation of the grape's sugar cane favors a faster onset of the process providing less competition for nutrients, increased yield and quality of the resulting product. The ideal strain can be identified by examining the role of these yeasts in the fermentation process. Therefore, this study aimed to evaluate some parameters fermentation of a strain selected from the stress tests (ethanol tolerance, osmotolerance and thermotolerance). During the fermentation process, were analyzed the following parameters: monitoring the viability of cells by fermentation time, dry weight, reducing sugars, quantification of ethanol and ethanol yields. The *Saccharomyces cerevisiae* strains analyzed, JP1 (control) and sample 91 (isolated from distillery 3), were grown on Sabouraud agar at 28ºC for 24 h. After this period, suspensions were prepared of these micro-organisms (5 x 10^5 cells.mL^{-1}) and 5 mL of a suspension inoculated was into Erlenmeyer flasks containing 100 mL medium for fermentation (15% sucrose, 0.5% potassium dihydrogen phosphate, 0.15% ammonium chloride, 0.01% magnesium sulfate heptahydrate, 0.01% potassium chloride and 0.6% yeast extract) for 24 h. Aliquots of 62.50 mL (25% of inoculum) at a concentration of approximately 1.0 X 10^7 UFC.mL^{-1} of the yeasts were inoculated in flasks containing 187.50 mL of test medium with a total final volume of 250 mL. Every four hours, in a total of 24 hours of fermentation, aliquots were removed for analysis and quantitation of cell viability, dry weight, reducing sugar and ethanol production. The fermentation experiments were conducted in duplicate. The yeast sample showed results similar to control, indicating its potential for producing ethanol. The sample JP1 produced 22.1 mL ethanol.100 mL^{-1} while the test isolate (91) showed 21.9 mL ethanol. 100 mL^{-1} for 24 h. However, further additional testing and in higher scales tests are required to better show the action of yeast in the fermentation process.

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Assessment of water and sediment quality of Sorocaba's river: emphasis on determination of detergents and microbial characterization.

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Keywords: Coliforms, denitrifying bacteria, nitrifying bacteria, LAS

The Sorocaba's river is located in Middle Tiete's basin, one of the most important left margin of this basin with a lengh of 227Km and a flux of 13m³/s. The major pollutants that has disturbed the water and sediments quality of Sorocabas's river are domestic and industrial wastewater, industrial and pesticids. The population growth is the responsible for the increase of this contamination, damaging the recovery of the river, the local ecosystem and drinking water source, putting in risk the population health. The aim of the present research was to measure and compare the water and sediment quality of Sorocaba's river next to the cities: Votorantim, Sorocaba and Laranjal Paulista. Samplings were realized during the dry season (September 2010). The samples were submitted for physical-chemical analysis at the collection site through Multiprobe Yellow springs. At Enviromental Microbiology Laboratory of UFSCar-Sorocaba, were analyzed solids (total and total volatile) , alkalinity, acidity, nitrate, nitrite, turbidity and detergente concentration (linear alkyl benzene sulfonate - LAS). Estimation of coliforms, nitrifying and denitrifying bacterias density were performed by the technique of most probable number (MPN). Through the results can be verified that dissolved oxygen concentration (DO) in Sorocaba (4,82 mg/L) and pH (4,83) were the lowest. Also in Sorocaba, it was found that the total volatile solids concentration was higher (40,871 g/L), which shows greatest amount of organic matter. The LAS concentration was low in the water, and sediment in three cities: from 0,2 to 0,5 mg/L in water, and 0,112 to 0,204 mg/g in sediment. In Votorantim, were found the highest levels of total coliform in water ($5{,}0.10^7$ MPN/100mL) and sediment ($1{,}3.10^9$ NMP /g). The highest thermotolerant coliform concentration in water and sediment ($2{,}3.10^7$ NMP/100mL e $3{,}0.10^8$ NMP/g) was also in Votorantim. The nitrate concentration in water at Laranjal Paulista was the highest (0.7 mg/L), due to the high amount of nitrite oxidizing bacterias ($2{,}8.10^8$ MPN/100mL) and the low denitrifying bacteria concentration in water ($8{,}0.10^5$ MPN/100mL). At Sorocaba the amount of denitrifying bacteria in the water was higher ($1{,}6.10^{19}$ MPN/100mL), thus the nitrate concentration in this sample was low (0.33 mg / L). This was the only place that have ammonia oxidizing bacterias in the water ($1{,}4.10^5$ MPN/100mL), therefore the nitrite concentration was the highest (66.6 ug/L) compared with others cities.

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BACITRACIN PRODUCED BY *Bacillus licheniformis* (UCP 1014) USING ECONOMIC MEDIUM FORMULATED WITH MILK SERUM

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Bacillus are gram-positive bacteria, most saprophytic, rod shaped, aerobic or facultative anaerobic. They have a wide use and importance in many industrial sectors such as food, obtaining protein, leather, and detergents, beyond the production of antibiotics. Bacitracin is a major polypeptide antibiotics produced by *Bacillus licheniformis* and *Bacillus subtilis*, functioning as an inhibitor of cell wall biosynthesis. The increasing problems of environmental pollution have been aggravated due to high production of solids and liquids that are released into the environment, most often without any previous treatment. Whey is a byproduct of cheese manufacture of high nutritional value and is considered an extremely problematic pollutant due to its high organic load and high volume generated, and is considered a rich substrate because of its high content of lactose, protein and minerals. This work aimed to study the biotechnological potential of *B. licheniformis* (UCP1014), isolated from soil contaminated by oil, through the production of bacitracin using whey based media, replacing the glutamic acid from the standard medium described by Hedlin (1949) by the whey and using glucose as an additional source of carbon. The experiment was performed in triplicate evaluting the influence of temperature, concentrations of glucose and whey, and analyzing the diameter of the halo produced by the microorganisms. The production of antibiotic was made by using an inoculum prepared by inoculating the *Bacillus licheniformis* (UCP1014) into nutrient broth (pH 7.0) and then incubating at 46ºC for 16 h in an orbital shaker at 150 rpm. Samples were taken every 4 h up to 96 h, centrifuged to obtain cell-free culture supernatants and then sterilized through 0.2 μm filter paper. The production of antibiotic was detected by disk diffusion test using *Micrococcus flavus* (UFPEDA 323) as test microorganism . The inhibition zones were measured after 24 h of incubation at 37°C. The results showed that the production of bacitracin by *Bacillus licheniformis* occurs at alkaline pH, microbial growth after 96 hours at 46ºC, for a serum concentration of 10% (v/v) and 40g/L of glucose, with inhibition zones of 19mm in diameter. 15 mm-diameter zones were also detected for a serum concentration of 70% (v/v) and 0 g/L of glucose. Other experiment, by isolating *B. subtilis* from soil to evaluate the potential of peptide antibiotic production, achieved the best activity against *M. luteus* in microorganism test at 37ºC, and with results also obtained at 30 ºC and 50 ºC. Studies with the objective to verifity the effect of temperatures ranging from 30ºC to 55ºC on the synthesis of exoprotease and bacitracin, as well as on sporification in *B. licheniformis*, are also in progress.

Keywords: bacitracin; *Bacillus licheniformis*; milk serum.

Financial support: CAPES, CNPq, FACEPE, UNICAP

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Bacteria SOD and catalase especific responses to mesotrione herbicide

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Keywords: *Pantoea ananatis*, oxidative stress, Callisto herbicide, biodegradation.

Mesotrione is a benzoylcyclohexane-1,3-dione herbicides that functions by inhibiting 4-hydroxyphenyl pyruvate dioxygenase (HPPD) in target plants. While mesotrione was first registered for use in 1993, only a limited number of microorganisms have been reported to degrade this herbicide. In the study reported here, we investigated whether mesotrione-degrading bacteria showed differences of oxidative stress with commercial herbicide (Callisto®) and the active ingredient (mesotrione). We analyzed superoxide dismutase (SOD) and catalase activities in *Pantoea ananatis*, characterized as highly mesotrione degrading strain.It was possible to observe a decline of total protein quantities between periods of 12 and 24 hours of incubation of *P. ananatis* in mesotrione medium, when compared with Callisto® medium treatment, probably due to a decreased in bacteria metabolism, considering it does not use the herbicide as a carbon source. After 24 hours Callisto® treatment, the rate of superoxide expression was higher than mesotrione treatment, with Mn-SOD, Fe-SOD and Cu / Zn-SOD isoforms observed at gel electrophoresis, the higher expression for the first one. With mesotrione treatment, only Mn-SOD isoform was expressed, showing a very specific response to active ingredient. Catalase showed a significant increase for Callisto and mesotrione from 12 to 24 hours treatments, but higher for Callisto. The gel electrophoresis banding pattern showed five isoforms after treatments, but one isoform was highly and specific expressed in response to mesotrione treatment. We concluded that, unlike what would be expected, the commercial herbicide Callisto has a higher toxic effect over the bacteria, with no specific SOD and catalase responses from *P.ananatis*, against the different components of herbicide. Still, the active ingredient ensures a very specific SOD and catalase responses, probably because *P.ananatis* is capable to inactive the molecule by degradation, and no additional oxidative stress is produced by energy production from mesotrione.

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BIOCONVERSION OF CELLULOSE TO HYDROGEN BY DARK FERMENTATION

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Keywords: *Anaerobic bacteria, acid treatment, cellulase*

Hydrogen is known as a clean energy resource. The biological production of hydrogen has been attracting attention as an environmentally friendly processs that does not consume fossil fuels. Cellulosic plant and waste materials are potential resources for fermentative hydrogen production. Cellulose is a linear biopolymer of glucose molecules, connected by β-1,4-glycosidic bonds. Enzymatic hydrolysis of cellulose requires the presence of cellulase. The present study aimed to investigate the efficiency of acid pretreatment on ruminal fluid in order to enrich H_2 producing bacteria consortia to enhance biohydrogen rate and substrate removal efficiency. In this study, fermentative hydrogen producers were enriched on cellulose (2g/L) in a modificated Del Nery medium (DNM) at 37ºC and initial pH 7.0 using rumen fluid (10% v/v) as inoculum. To increase the hydrogen production it was added cellulose (10mL) to the medium. The gas products (mainly H_2 and CO_2) was analyzed by gas chromatography (Shimadzu GC 2010) using a thermal conductivity detector. The volatile fatty acids and ethanol were also detected by GC using a flame ionization detector. Cellulose degradation was quantified by using the phenol-sulfuric acid method. Analysis showed that the biogas produced from the anaerobic fermentation contained only hydrogen and carbon dioxide, without detectable methane after acid pretreatment test. On DNM the hydrogen production started with 4 h ($5{,}3 \times 10^5$ mmol H_2/L) of incubation, and the maximum H_2 concentration was observed with 34 h ($7{,}1 \times 10^6$ mmol H_2/L) of incubation. During the process, it was observed a predominance of acetic acid and butyric acid as well as a low production of acetone, ethanol and n-butanol in all experimental phases. Butyrate accounted for more than 77% of total. As a result of the accumulation of volatile fatty acids (VFAs), the pH value in anaerobic digestion system was reduced to 4,0. On microscopy analyses there were observed rods with endospores. The batch anaerobic fermentation assays performed on anaerobic mixed inoculum from rumen fluid demonstrated the feasibility of H_2 generation utilizing cellulose as substrate. Based on the results, it can be concluded that the acid treatment was efficient to inhibit the methanogenic archaea cells present in rumen fluid. The rumen fluid cells present a potential route in converting renewable biomass such as cellulose into hydrogen energy.

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Biodegradation of azo dyes in aerobic and anaerobic conditions utilized the *Shewanella putrefaciens* (CCT 1987).

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Keywords: biodegradation, azo dyes, bacteria, aerobic and anaerobic conditions

The production of dyes in Brazil is of 26.500 tons. Due to this amount, the rivers are being contaminated with this aromatic compound. It causes many problems, affecting the aquatic life by obstructing light penetration and limiting oxygen transfer in water bodies. In conventional active sludge treatment, the dyes resist to biodegradation, that is why the screening of microorganisms to biodegrade them is very important. The microorganism selected to this study was the bacteria *Shewanella putrefaciens* (CCT 1987), the analysis were realized with a suspension of bacterium in nutrient broth medium (concentration: meat extract 1.5 g.L-1 and peptone 2.5 g.L-1) and the dye (initial average concentration of 50 mg. L^{-1}). The dyes utilized were B31 and R34. They are azo dyes (ie. that it has one or more –N=N- groups). The initial assay was pH 8.5, temperature 25ºC, in static and aerobic condition. The degradation of the samples was observed by spectrophotometry. In anoxic conditions the degradations were 87% and 79.21% in the dyes B31 and R34 respectively, and in aerobic condition the degradation didn't occur. It probably happened because the bacteria in aerobic condition can utilize the oxygen like an acceptor of electrons and in anoxic conditions this role could be realized by the dye molecule. The degradation of azo dyes is very important because there are dyes that are mutagenic and carcinogenic.

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BIODEGRADATION OF DIESEL OIL HIDROCARBONS BY BACTERIA IN PURE CULTURES AND MIXED

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Keywords: Bacteria, biodegradation, hydrocarbons, consortium

Introduction: The bioremediation technique stands out among the available recovery for environments contaminated by petroleum derivatives It uses microorganisms capable of metabolizing pollutants compounds and transform them into less toxic forms (Vidali, 2001). Many studies were conducted using microbial consortium to bioremediation, since they allow the establishment of interactions between species, which tend to act to stimulate co-metabolic reactions. As petroleum and its derivatives are composed of molecules of different molecular weights, including some of macromolecular structure, the use of mixed cultures allows the establishment of a succession of populations that leads to a succession of attacks on polluting compounds, providing efficient degradation of these (Jacques et al., 2007).
Materials and Methods: The bacterial consortium studied belonging to a culture collection of the Laboratory of Biotechnology and Biodiversity for the Environment, Department of Microbiology – UFV. It was composed of the bacterial strains *Acinetobacter baumannii* LBBMA 04, *Pseudomonas aeruginosa* LBBMA 58, *Ochrobactrum anthropi* LBBMA 88b, *Acinetobacter baumannii* LBBMA ES11 and *Bacillus subtilis* LBBMA 155, all isolated from environments contaminated with petroleum hydrocarbons. The bacterial strains in pure cultures and mixed culture were cultured in mineral medium supplemented with diesel oil 2% (v / v) in the absence of carbon source. The catabolic activity of the bacterial strains was verified through the respirometry technique and degradation of hydrocarbons obtained by gas chromatography.
Results and Discussion: The highest CO_2 production was achieved in dealing with the consortium. GC analysis of residual hydrocarbons showed that this treatment was presented the highest degradation of hydrocarbons nonane, decane and undecane, with values higher than 99% degradation, which proves the existence of metabolic complementarity between the consortium members

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BIODETERIORATION OF SISAL FIBERS BY *Penicillium* sp.

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Keywords: Biodeterioration, *Penicillium*, sisal, natural fibers.

Introduction: Sisal fiber is usually used to produce carpets and ropes. Cement composites using natural fibers have been attracting great interest due to its low cost and high availability, as a environmental friendly and energy save material. However, environmentalphysical, chemical and biological agents reduce the durability of natural fibers cement composites. The objectives of this study were to evaluate the biodegradation of sisal fibers by *Penicillium* sp, comparing the microstructure and mechanical properties of these fibers. **Materials and Methods:** Initially, fungal strains were isolated from samples of sisal fibers obtained from a Cooperative of Bahia and submitted to classical identification by micromorphological analysis of reproductive structures. Enzymatic assays were conducted to evaluate ligninolytic and cellulolytic activities of the strains. Sisal fibers were submitted to continuous immersion (240 and 320 days) and alternate wetting and drying cycles in fungal suspension. Fibers microstructure were evaluated with thermogravimetry (TG) and scanning electron microscopy (SEM), and tensile strength was also measured. **Results and discussion:** A total of six strains were isolated of which three were micromorphologically identified as *Penicilium* sp., *Aspergillus* sp. and *Cladosporium* sp. In ligninolytic assay, only one fungal strain (*Penicillium* sp.) showed a discoloration halo, which is a indicative of ligninolytic activity. The same strain (*Penicillium* sp.) showed the largest discoloration halo for cellulolytic activity among all isolates. After a period of accelerated aging in a fungal suspension (*Penicillium sp*), there was a significant decrease of the two peaks of thermal decomposition of the fiber, mainly of the fibers subjected to cycling in the fungal suspension, suggesting that part of the chemical components (cellulose and lignin) of the fibers were degraded by the biological agent.In microscopy, morphological changes were observed on fibers surfaces subjected to aging, mainly the disappearance of parenchymal cells present in natural fiber, which may be attributed to the loss of lignin, whose biological function is to unite the fibrous cells.There was a reduction in the tensile strength of the fibers exposed to biological agents, ranging up to 53.44% reduction of the original strength when exposed to cycling (60 days). While in continuous immersion, the reduction was 44.86% and 51.62% for 240 days and 320 days respectively. Sisal fibers are susceptible to biological degradation, since microstructure abnormalities and loss in mechanical performance compared to the tensile strength assay were clearly observed and may be due to the removal of chemical compounds (cellulose, hemicellulose and lignin).

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Biological hydrogen production from environmental sample in tropical countries

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Keywords: anaerobic bacteria, fermentation, sediment reservoir, glucose, xylose

The hydrogen gas is regarded as clean and renewable energy source, since it generates only water during combustion when used as fuel. It shows 2.75 times more energy content than any hydrocarbon and it can be converted into electrical, mechanical energy or heat. Inoculum sources have been successfully tested for hydrogen biological production in temperate climate countries as sludge treatment plants sewage, sludge treatment plant wastewater, landfill sample, among others. However, hydrogen biologic production with inoculum from environmental samples such as sediment reservoirs, especially in tropical countries like Brazil, is rarely investigated. Reservoirs and fresh water lake sediment may contain conditions for the survival of a wide variety of microorganisms which use different carbon sources mainly glucose and xylose, in the fermentation. Glucose is an easily biodegradable, present in most of the industrial effluents and can be obtained abundantly from agricultural wastes. A wide variety of wastewater resulting from agriculture, industry and pulp and paper processed from wood may contain xylose in its constitution. Such effluent contains glucose and xylose concentrations of about 2 g/L.
In this sense, this work verified hydrogen biological production in anaerobic batch reactor (1L), at 37 ° C, initial pH 5.5, headspace with N_2 (100%), Del Nery medium, vitamins and peptone (1 g/L), fed separately with glucose (2g/L) and xylose (2 g/L). The inoculum was taken from environmental sample (sediment reservoir Itupararanga - Ibiúna - SP-Brazil). It was previously purified in serial dilutions at H_2 generation (10^{-5}, 10^{-7}, 10^{-10}), and heat treated (90º C - 10 min) later to inhibited the H_2 consumers. The maximum H_2 generations obtained in both tests were observed at 552 h, as described below. At the reactors fed with glucose and xylose were observed, respectively, 9.1 and 8.6 mmol H_2/L, biomass growth (0.2 and 0.2 nm); consumption of sugar concentrations 53.6% (1.1 glucose g/L) and 90.5% (1.8 xylose g/L); acetic acid generation (124.7 mg/L and 82.7 mg/L), butyric acid (134.0 mg/L and 230.4 mg/L) and there wasn't methane generation in the reactors. Microscopic analysis of biomass in anaerobic reactors showed the predominance of Gram positive rods and rods with endospores, whose morphology is characteristic of H_2-generating bacteria, in both tests. These species were selected from the natural environment. In DGGE analysis performed difference were observed between populations from inoculum and in tests. This analysis confirmed that some species of bacteria were selected which remained under the conditions imposed on the experiment. The efficiency of the pre-treatment of inoculum and the imposition of pH 5.5 inhibited methane-producing microorganisms and the consumers of H_2. Therefore, the experimental conditions imposed allowed the attainment of bacterial consortium of producer H_2 taken from an environmental sample with concentration of xylose and glucose similar to the ones of the industrial effluents.

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Biomass and lipids production by *Cunnighamella elegans* **UCP 542 using glycerin and corn steep liquor**

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Keywords: Lipids, Glycerin, Oleaginous microorganisms

The lipids are found in fungi as components of membranes and reserve material.The search for new sources rich in unsaturated fatty acids has intensified. The mucorales fungi were found to be a promising lipid producer. The aim of this work was to investigate the biotechnological potential of the filamentous fungus *Cunninghamella elegans* related to biomass and lipids production using glucose and glycerin, as carbon sources. *C. elegans* was grown in the media synthetic medium for Mucorales and modified (substitution of glucose by glycerin obtained from biodiesel production and corn steep liquor), pH 6.0, and inoculums ‘of spores suspensions 10^7 /mL. The Erlenmeyers flasks were incubated at 28^0C in orbital shaker of 150 rpm, during 5 days. A factorial design 2^2 with four central points, and variable response was biomass and lipids productions. The lipids were extracted from biomass using methanol/chloroform/acetone, and the amount of lipids was determined by gravimetric method. The best condition was observed using the inexpensive medium (glycerin and corn steep liquor) for its growth of *C. elegans* developed in this study. The results showed the biomass yield of 2.5g/L, and the higher total lipids obtained were 8.0% in the same condition. From these results it was found that the glycerin and corn steep liquor supplementation in the synthetic medium did not affect the morphological and microscopic aspects of the fungus. The inexpensive medium (Synthetic medium for Mucorales supplemented with glycerin and corn steep liquor) for biomass and fungal lipids were dominated by palmitic and linoleic acids, corresponding to a 100 and 560 times comparing with the synthetic medium for Mucorales.The agro industrials residues glycerin and corn steep liquor could be indicated as alternatives sources of carbon and nitrogen in order to facilitate the biotechnological production of biomass and lipids, and contributed with the reduction of industrials residues accumulation.

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BIOSURFACTANT/BIOEMULSIFIER PRODUCTION BY Candida *lipolytica* IN ACID AND ALKALINE SEAWATER

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Key words: biosurfactant, Candida *lipolytica*, seawater

INTRODUCTION: Biosurfactants have attracted much attention because of characteristics such as toxicity; higher biodegradability; better environmental compatibility; higher foaming; high selectivity and specific activity at extreme temperatures, pH and salinity; and the ability to be synthesized from renewable feedstock. The aim of this work was to study the production of biosurfactant/bioemulsifiers in acid and alkaline seawater.

MATERIAL AND METHODS: A 2^3 full factorial design, with three center points, was carried out to investigate the effects and interactions of the pH (6,10,14), ammonium sulfate and potassium dihydrogen orthophosphate concentrations on the emulsification activity and surface tension of 96 h cell-free cultures filtrates of Candida *lipolytica* UCP 0988. The yeast grown at 28º C and 150rpm for 96 h, in seawater supplemented with nitrogen and phosphorus sources, using corn oil as only carbon source. The seawater was collected from a sampling point, at Bairro Novo beach in Olinda, Pernambuco. The emulsification activity was determined according to Cirigliano and Carman (1984). One unit of emulsification activity was defined as that amount of emulsifier that effected an emulsion with absorbance at 540 nm of 1.0. The surface tension was measured by Du Noy ring method using a digital tensiometer (SING and CAMEOTRA, 2004).

RESULTS AND DISCUSSION: The 96h cell-free filtrates produced high levels of emulsification activities for a large range of pH (6-14). Emulsification activities between 5 and 6 UAE were obtained for water-in-corn oil, water-in-canola oil and water-in-soybean oil emulsions and to motor oil-in- water and burned motor oil- in-water emulsions. Emulsification activities ranging between 0.4 and 6 UAE and between UAE 0.2 and 3.9 UAE were obtained for water-in-diesel oil and water-in-hexadecane emulsions, respectively. The increase of pH from 6 to 14 favored significantly the surface tension reduction of 96 h cell-free filtrate. On the other side, the increase of potassium phosphate concentration favored significantly the emulsification activity.

CONCLUSIONS: Candida *lipolytica* can be induced to produce emulsification activity and reduce surface tension when it is grown in acid and alkaline seawater supplemented with and phosphorus using corn oil as carbon source.

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Biosurfactant production by *Geobacillus stearothermophilus* on low cost medium using coconut oil as carbon sources

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Keywords: *G. stearothermophilus*, Biosurfactants, Industrials residues.

Introduction: Biosurfactants are amphiphilic compounds produced by microorganisms that possess surface activity, and the ability to reduce surface and interfacial tension among two immiscible liquids. The biosurfactants have low toxicity and biodegradabilidade[1]. The use of alternative substrates such as industrial wastes and vegetable oils, can contribute to the reduction costs, since the culture medium represent approximately 50% of product value final[2]. In this work the biosurfactants production by *G. stearothermophilus* was studied using a factorial design with Central Composite Rotatable Design (CCRD) of 2^2, to evaluate the influence of medium components: corn steep liquor (industrial residue), coconut oil and distilled water to reduce surface tension.

MATERIAL AND METHODS: Samples of *Geobacillus stearothermophilus* isolated from oil contaminated area at the Port of Recife - PE, held at the Bank of Cultures Center for Research in Environmental Sciences, Catholic University of Pernambuco, in nutrient agar at 5°C. *G. Stearothermophilus* was grown in Erlenmayer flasks with 200 mL of Luria Bertani medium (LB) consisting of tryptone 10g / L, yeast extract 5g / L, sodium chloride 10g / L. After 24 hours, the growth was measured by turbidity in a spectrophotometer (660nm), to form the inoculum (10^8 cells / mL / optical density DO 660nm / 0.8). Aliquots (12mL) of this suspension, were transferred to Erlenmayer flasks containing 200 mL medium with corn steep liquor and substrate and pH adjusted (7.0). The surface tension determination in cell-free metabolic liquid containing the biosurfactant after 72 hours of culture was performed on a tensiometer with automatic measurement[3].

RESULTS AND DISCUSSION: The influence of carbon and nitrogen sources on the production medium is a parameter considered to the biosurfactant production[2]. The results show that *G. stearothermophilus* was able to use as a nutrient coconut oil and corn steep liquor as a source of carbon and nitrogen, respectively, due to the presence of vitamins and amino acids. After 72 hours of culture, was observed a maximum production of biosurfactant, with a significant reduction in surface tension of water (72mN/m to 31.50mN/m). *G. stearothermophilus* showed significant biotechnological potential in using corn steep liquor as a carbon source, producing surfactants. Thus, we suggest research for application in environmental remediation processes.

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BIOSURFACTANT PRODUCTION BY ISOLATES OF *Bacillus* GENUS

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ABSTRACT

The search for biosurfactants has increased due to its numerous applications in different fields. Among its use are found the oil recovery, cleaning of oil storage tanks, metal-contaminated soils remediation, polyaromatic hydrocarbon bioremediation, marine bioremediation, therapeutics agents, agricultural uses, personal care, food industry, pulp and paper industry. Bisurfactants have important advantages compared to synthetic surfactants such as lower toxicity, higher biodegradability and stability under extreme conditions. In this sense, studies related to isolation of new producing species, novel methods of analysis, extraction, purification, production and optimization are a challenge due to numerous industrial potentials and biotechnological applications of biosurfactants. This study reports the biosurfactants production by, three strains of the genus *Bacillus* (12A, 12B and 4B) isolated from an oil contaminated soil near to Getúlio Vargas Refinery (REPAR-PR). The emulsification index (E_{24}) of the biosurfactants produced by these isolates were determined in the presence of n-dodecane and kerosene. Microorganisms were cultivated in Erlenmeyer flasks of 50 mL containing 10 mL of minimal M9 medium containing 0.2 % (w/v) of glucose incubated for 12 hours at 28 ± 2 °C under agitation (180 rpm). Thus, the supernatants were recovery and used to evaluate the emulsifying capacity and stability in the presence of hydrocarbons. Kinetics of the cell growth and biosurfactant production by the three bacterial isolates grown in minimal media was different carbon sources (glucose, olive oil, soy oil, maize oil, castor oil and residual glycerol). A qualitative test for identification of rhamnolipids, an important class of biosurfactant, was carried out using plate assay with the substrate with methylene blue and cetyltrimethylammonium bromide-CTAB. Emulsification calculated for the three isolates were on average 34.8% in the presence of n-dodecane, while in the presence of kerosene the isolates 12A and 12B presented an average of 24 % and the isolate 4B 33.7 %. The emulsion generated by biosurfactants produced by strain 4B demonstrated great stability in the presence of the hydrocarbons (E_{24} = 20.1 % with n-dodecane and E_{24} = 18.6 % in with kerosene) for more then 48 hours. The higher biosurfactant production was reached at 24 hours for isolate 12A, 18 hours for isolate 12B, after what the E_{24} decreased over the culturing time. However, for the isolate 4B a plateau ranging from 24 hours to 168 hours of culture was observed sutaining a E_{24} index of 38 % in Kerosene.
Tanking into account the carbon source, the olive oil was better carbon source for the isolate 12A (E_{24} = 37.2% in the 24 h cultures), while glucose was better for 12B (E_{24} = 33.2% in the 18 h cultures) and 4B (E_{24} = 36.6% in the 24 h cultures). Residual glycerol obtained from biodiesel facilities also proved to be an interesting carbon source for biosurfactant production, also proved to be an interesting carbon source for biosurfactant production. The qualitative test for rhamnolipids identification was positive for all isolates. Eventually, the overall results are promising and suggest continuity, aiming to optimize the biosurfactant production and develop tests related to its applications.

Keywords: biosurfactants, *Bacillus*, emulsification index, kerosene

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BIOSURFACTANT PRODUCTION BY *Mucor circinelloides* UCP 0069 USING AGROINDUSTRIAL RESIDUE

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Keywords: Tensio active, *Mucor*, Corn steep liquor

Surfactants are compounds that have properties of reducing surface and interfacial tensions by accumulating at the interface of immiscible fluids, increase the solubility and the biodegradation of hydrophobic compounds. The reduction of the overall biosurfactant production costs usually depends on the strain improvements, the use of low cost raw materials such as agricultural and industrial wastes as substrates. *Mucor circinelloides* is a fungus belonging to the class Zygomycetes and has been widely studied because they have high capacity for lipid accumulation when grown on ethanol under anaerobic or aerobic conditions. *M. circinelloides* also used as source of carotenoids and lipids, and it accumulates high levels of these compounds in the mycelium. This work describes the biosurfactant production by *M. circinelloides* in mycelial form using agroindustrial residue as a low cost substrate. The strain *Mucor circinelloides* was isolated from mangrove sediment (Rio Formoso, Pernambuco). The strain was maintained on potato dextrose in the Culture Collection Universidade Católica de Pernambuco (UCP), registered in World Federation Culture for Collection (WFCC). The medium of biosurfactant production was constituted bb corn steep liquor (CSL) and post-frying soy bean oil, and a 2^2 factorial design, and variable response was surface tension and emulsifier index. The Erlenmeyer's flasks were kept in static and agitated conditions (150 rpm) during 120 h, at 28 °C. After this period the liquid metabolic free cells was analyzed. The best results showed a surface water reduction of 72 to 26.70 mN/m assay 1 [8% of CSL and 3% of post-frying soy bean oil], under 150 rpm. However, the best results using statistic condition showed a surface water reduction of 72 to 28.24mN/m, and assay 6 [CSL 6% and 0.58 of post-frying soy bean oil]. On the other hand, the highest emulsifier index 80% and 43.47%, respectively agitate and static conditions were obtained using palm oil to assay 6 [CSL 6% and 0.58 of post-frying soy bean oil]. These results suggested higher potential of the *M. circinelloides* (mycelia form) to biosurfactant production in low cost media in dual conditions agitation or static conditions. Biosurfactant produced under the conditions established in this study possibility use in bioremediation processes.

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BIOSURFACTANT PRODUCTION BY *Rhodotorula glutinis* USING AGROINDUSTRIAL SUBSTRATES AS CARBON AND NITROGEN SOURCES

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Keywords: Biosurfactant; *Rhodotorula glutinis*; Surface tension.

Surfactants are amphipathic molecules that have both hydrophobic and hydrophilic domains and are capable of lowering the surface tension and the interfacial liquids. The biosurfactants are natural secondary metabolites produced by various microorganisms (bacteria, fungi and yeasts), and classified according to their chemical composition and microbial origin. The aim of this study was the production of biosurfactant by *Rhodotorula glutinis* using a factorial design 2^2, and with the independent variables corn steep liquor and soy bean oil post frying, and variable response was reduction of surface tension and emulsification index. The experiments were carried out using the microorganism *Rhodotorula glutinis* belongs to Culture Collection of Nucleus of Research in Environmental Sciences- NPCIAMB, and are registered in World Federation Culture for Collection (WFCC). The strain was maintained in Sabouraud dextrose agar medium at 5^0C. The production of biosurfactant was performed in 250 mL Erlenmeyer flasks of 100mL of capacity containing the medium according factorial design using corn steep liquor and oil after frying. The flasks were inoculated with 5% of the inoculums, and the pH was adjusted to 5. The flasks were kept at 30°C in orbital shaker at 150 rpm, for 96 hours. After this period the net metabolic fermentation was obtained by centrifugation during 15 min at 4500 g and filtered in Millipore membrane. The analyses were of the surface tension and emulsification index was determined. The ability of *R. glutinis* grown on agroindustrial substrates as carbon and nitrogen sources to synthesize biosurfactant under aerobic condition, at 30ºC during 96 hours of cultivation has been demonstrated. The maximum reduction of the surface tension was 28.23 mN/m and emulsification index of 96.29% using burned motor oil. However, we observed an emulsifier index of 78.12% when was used the post-frying soy bean oil as substrate. The results confirm effectiveness that agro industrials residues as a good substrates for production biosurfactant and suggest employment to enhanced solubility of hydrophobic pollutants in bioremediation applications.

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BREWING: THE MICROBIOLOGICAL CONTROL FOR THE ASSURANCE OF ORGANOLEPTICAL STABILITY.

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Beer is a beverage made from the fermentation of a sweet aqueous extract called must, which is obtained from germinated cereal, mostly barley. Alcohol and carbon dioxide are produced by yeasts from sugars and to the must hops are also added to bring flavor. Beers usually have a low alcoholic level (normally between 0 – 8.5° GL) and are made of water, malt, hops and sugar. Several raw materials are directly connected to the quality of the final product as well the presence of microbial contamination. In this context, this work aimed to evaluate the microbial contamination of the fermentation process and the beer organoleptical quality in order to verify the effect of contamination on the beer quality. From August to October 2001 daily microbiological analysis of samples collected in different steps of the process were made, after asepsis, in the brewery located in Mogi Mirim, SP, followed by the training of the staff involved in the work of asepsis and decontamination of the brewery. The samples were taken off from the fermentation and maturing tank, fermentation basin and from the can of pasteurized beer. For the microbiological analysis the following methodologies were used: filtering of samples in coating, Gram test, catalase test, plating and incubation in selective media of cultivation (WLD, Raka Ray, YM + CuSO4 and NBB-C). For the sensorial analysis of the beer samples the techniques used were OK Technique / NOT OK and Triangular Sensorial test. At the fermentation and maturing basin there was contamination only by aerobic bacteria. According to the Gram's test, catalase test and morphological analysis by microscopy, the genus *Gluconobacter sp*. was found. The results of the triangular sensorial showed a higher level of detection of off-flavors (1,0) which is related to the bacterial contamination. In the yeast basin, there was no microbiological growth in any kind of culture. This shows that there was suitable asepsis at this spot during this period. The sensorial test revealed OK score, which means no altered organoleptical characteristic. With the pasteurized canned beer there was no microbiological growth in any culture media. This shows that the pasteurization process was efficient. As above, no altered organoleptical characteristic was found. The microbiological control and a suitable frequency of the asepsis of the brewing process are important tools to guarantee the beer organoleptical stability as well as the monitoring of colony forming unit (CFU) and the detection of *off-flavors,* both decreasing during the months of the analysis, reaching lack of contamination in all used media within the period. The positive results in the beginning of the analysis show that the adherence to the rules of good practices of brewing including personal hygiene, correct handling of tools and absence of cross contamination are essential for obtaining the results of this control.

Keywords: Brewing, yeasts, Microbiological Control, Organoleptical Stability and Brewing Quality.

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Cadmium biosoption by immobilized dead yeast cells from bioethanol industries

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Keywords: cadmium, yeast, biosorption

There are many reports about effluent discharges that bring hazardous materials and metals poured into aqueous environments and soils that end-up in the food-chain threatening natural and human population health. The toxic and carcinogenic cadmium is released by industrial processes at 20,000 tons per year with no efficient removal or ameliorating system to protect from its negative effects. The first report on cadmium toxicity appeared in 1946 in Japan and was described as the "Itai-Itai Syndrome" related bone problems. Presently cadmium is wide used in the automobile industry, telecommunications, on the dying and ink processes, PVC and other plastics, phosphate fertilizers, batteries, fungicides, leather industry, etc and it was found in large amounts in urban waste and metallurgy industrial discharges. Dead yeast cells are an abundant residue of the Brazilian ethanol industry and the industries still don't have a proper or desirable destiny for all these cells, which means tons of vitamins and proteins that have not a major end. In the other hand, heavy metal residues are a problem, especially for leather industry, where Brazil is also a great producer. In this work, dead yeast cells were used to evaluate cadmium biosorption samples. Yeast cells from sugar-cane bioethanol fermentation process 80% concentrated were obtained from COSAN Group, at Piracicaba, SP, Brazil. The yeast cell suspension was spread on trays, frozen at –80 ^{0}C, 24 h and lyophilized for 48 h and then killed by heat and pressure at 120 ^{0}C 30 minutes, 1 atm. Na-alginate 0.5 % and 20% (W/V) of dead yeast cells were added to 100 mL of deionized water at 9.5 pH. This suspension was mixed homogenized and droplets poured into Calcium Chloride 4% using a peristaltic pump for the micro spheres production. The same procedure was done with alginate solution without adding dead yeast cells. Samples were collected each hour and analyzed in atomic absorption spectrophotometer Varian AA-175. Biosorption was measured using a two double draft tube fluidized acrylic bioreactors were built (15 cm height) with 40 mL of internal volume and 100 mL of external volume dimensions. Each internal column was filled with 28 g of micro spheres connected to a peristaltic pump calibrated to sustain a flow of 2 mL per minute of cadmium synthetic solution at a concentration of $CdCl_2$ of 250 mg Cd/L. Suspension desorption were measured also. Results have shown that dried dead yeast cells at 20 % (W/V) immobilized in Na-alginate beads 0.5 % can be an efficient alternative for the capture of cadmium. The average biosorption rate was 122.10 mg Cd/g of dry biomass in comparison to the control using only the Na-alginate beads 0.5 % where the biosorption rate achieved 57.29 mgCd/g of dry biomass. For countries as Brazil, the yeast cells residue from fuel industry represents large amounts of such biomass. The research represented by the use of yeast cells from ethanol industry in a stable and efficient process demands future studies with the purpose of effluent treatment other than cadmium and other heavy metals to develop large scale processes using immobilized yeast cells, in order to minimizes the heavy metal impact on environment as well as using the yeast cells residues from ethanol industry.

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CADMIUM INHIBITION IN ANAEROBIC MICRORGANISMS IN ORGANIC MATTER REMOVAL AND SULFATE REDUCTION PROCESSES

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Keywords: organic matter removal, anaerobic microorganisms, cadmium, DGGE, 16S rRNA

The present work evaluated the acute toxicicity in the methanogenic specific activity, using acetate as main carbon source (1280 mg O_2.L^{-1}, in COD terms) in both methanogenic and sulfidogenic conditions. Duran® flasks were used as batch reactors, with 1143 ml working volume (700 mL of liquid phase and 443 mL of headspace). The reactors were inoculated with granular biomass from UASB reactor treating poultry slaughterhouse wastewater. At the start of experiment, the headspace of reactor was filled with N_2 to assure the anaerobic conditions. Temperature was kept at 30°C and the mixture was performed in a shaker equipament. Total pressure and methane and carbon dioxide concentrations essays were analysed in headspace samples; in liquid phase pH, dissolved COD, sulfate, total dissolved sulfide, volatile acids contend and cadmium concentration analysis were performed. At 1st condition, the essays were performed in methanogenic condition, using 5 reactors, considering cadmium concentrations of 0 (control reactor), 5, 10, 25 and 100 mg Cd^{2+}.L^{-1}, respectively to R1 to R5 reactors. At 2nd conditions 4 reactors were analysed, operating with 0, 10, 25 and 100 mg Cd^{2+}.L^{-1}, respectively to R6 to R9 reactors. The sulfidogenic condition was assured adding sodium sulfate as sulfate source with its initial concentration corresponding at 0.67 COD/SO_4^{2-} ratio. At ending of each of this essays, samples of biomass were taken aiming the characterization of *Bacteria* and *Archaea* domains by PCR and DGGE molecular biology techniques. The methanogenic specific activity of biomass exposed to cadmium increasing concentration remained between 0.6 mmol CH_4.L/g SVT.h (R2) and 0.9 mmolCH_4.L/g SVT.h (R5), at methanogeic condition, and 1.2 mmol CH_4.L/g SVT.h (R7) to 0.7 mmol CH_4.L/g SVT.h (R9) in condition which the reactor was kept at sulfidogeic conditions. Sulfidogenic activity was also assessed by means of total dissolved sulfide amount in aqueous phase and no acute inhibition effect was observed. DGGE bands profile for *Archaea* domain was similar for all studied conditions. Although DGGE technique is not quantitative, the decrease in intensity and number of bands for *Bacteria* domain in samples from R8 and R9 reactors (sulfidogenic condition), indicated a probable selection of these microrganisms. Thus, the biomass from UASB reactor sludge is resistant to the influence of cadmium on the studied concentrations, however is important to conduct studies aiming the influence of substrate degradation in chronic cadmium contamination conditions.

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CALLISTO® DEGRADATION BY *Aspergillus* sp.

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Keywords: fungus, mesotrione, herbicide degradation

Callisto® (EPA Reg. No. 100-1131) is considered a pre emergent and post emergent herbicide, used for controlling annual broadleaf weeds in corn fields, corn seed production , and corn for silage. Callisto® was determined to be a reduced risk pesticide in 1999 by the United States Environmental Protection Agency (USEPA). The degradation metabolites are MNBA [4-(methylsulfonyl)-2-nitrobenzoic acid] and AMBA [2-amino-4-(methylsulfonyl) benzoic acid]. Mesotrione, the active ingredient of Callisto®, is a benzoylcyckohexene-1,3dione that inhibits the enzyme 4-hydroxyphenyl pyruvate dioxygenase (HPPD) in target plants. Mesotrione was first registered for use in 1993, and a limited number of microorganisms have been reported to degrade it. The goal of this work was to observe whether *Aspergillus* sp. is able to degrade mesotrione, and compare with a bacteria degrading pathway, represented by *Bacillus* sp. Fungi samples were collected from plates with rich media {Nutrient Agar, NA, pH=6.8±0.2, plus 35mM of Callisto® (15mM mesotrione), and Potato Dextrose Agar – PDA, pH=5.6±0.2, plus 35mM of Callisto®} leaving opened in the Laboratory of Microbiology, Ponta Grossa State University, Brazil. Optimal growth temperatures were tested at 18 °C, 28 °C and 37 °C. Influence of pH in the degradation process was evaluated by modifying pH of NA to 5.6, by adding hydrochloric acid 1mol.L^{-1}, and PDA to pH 6.8, by adding sodium hydroxide 1mol.L^{-1}. The fungus was identified by microscopic observation from a slide culture, after incubation in BDA, for seven days, at 28 ^{o}C. The effect of different nutrient sources over Callisto® degradation by *Aspergillus* sp. was investigated. Just in NA, pH 5.6 and 6.8, we could observe halo formation, indicating herbicide degradation. We concluded that pH has no major effect over halo formation, but media composition is relevant. Probably, the higher sugar composition of PDA medium facilitates to access energy production in fungus, inhibiting the enzymes responsible for herbicide degradation. We also evaluated the capacity of mesotrione degradation, the active ingredient of Callisto®, by HPLC analyses.

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CASSAVA WASTEWATER AS CARBON SOURCE FOR THE POLYHYDROXYBUTYRATE PRODUCTION BY *Azotobacter vinelandii* CCT 2841.

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Keywords: polyhydroxyalkanoates; cassava wastewater; *Azotobacter vinelandii*.

The polyhydroxybutyrate (PHB) is also known as bioplastic, have thermoplastic properties and performance characteristics similar to conventional plastics. However, bioplastics are easily degraded by the microorganisms action in the environment with the advantage on the conventional plastics of petrochemical origin, take decades to decompose in nature and also produce toxins during the degradation process. Therefore, there is a special interest in the plastics production from materials that can be easily eliminated from our environment. The PHB, intracellular polymer belonging to the polyesters family, can be synthesized by many bacteria in bioreactors from sugars under stress. This polymer can represent up to 80% of total dry mass of the cell and are 100% biodegradable and biocompatible with the animal tissue. Some possible PHB applications include: biodegradable carriers that have the function of drug release for a given time within the individual's body; surgical needles; sutures; bone tissue replacement; etc. The biodegradable plastics advantage is not requiring surgical removal. The *Azotobacter vinelandii* advantage is the PHB production during its growth through the use of a wide variety of carbon source like those found in molasses cane sugar, beet sugar and corn syrup, for example. Another advantage is the easy attainment of this bacterium, since *A. vinelandii* is found in soils and in freshwater. In this work was studied the PHB production by *A. vinelandii* CCT 2841 using cassava wastewater, produced by cassava processing, as a main carbon source. The optimal values for the PHB production were determined by MSR (STATISTICA software). For the PHB (Y1 = polyhydroxybutyrate) optimization production was carried out the experimental design to determine the best production area. Thus, was performed a statistical fractional factorial design 2^{3-1} and the independent variables were: X1 = incubation temperature (ºC); X2 = time (h); X3 = agitation (rpm). Each experiment consisted of: autoclave materials sterilization; pre-inoculation preparation (*Azotobacter vinelandii* CCT 2841 from the stock culture was transferred for tubes containing Plate Count Agar inclined, which were incubated in an oven at 30 ºC for 24 hours); pre-fermentation (with the pre-inoculum previously obtained, was realized the bacterial cells suspension by adding 5 mL of nutrient broth and trace metals solution mixture contained in the flask (total of 44.95 mL of nutrient broth and 0.05 mL of trace metals solution, and then moved the suspension to the same flask. The vials were incubated in an orbital shaker rotating at 30 °C for 24 h and 225 rpm) and fermentation (for each experiment was used 50 mL of cassava wastewater (pH = 7.0) added of 0.40 g yeast extract, 50 µL of trace metals solution and inoculum standardized to 0.9 absorbance at 620 nm by spectrophotometer) in rotary shaker. Then, the cells were centrifuged and performed the extraction and precipitation of intracellular polymer (PHB). It was found that the highest biomass (2.0 to 2.5 g L^{-1}) was in 35 ºC, 48h and the agitation was not an important factor for cell growth. One can also observe that the PHB yield was maximum (200-300 mg g $cell^{-1}$ h^{-1}) in the incubation temperature of 25 °C, the shortest incubation time (8h) and agitation at 225 rpm.

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CHARACTERIZATION OF AMYLASES FROM *Rhizopus oryzae* and *Rhizopus microsporus* var. *oligosporus*: OPTIMAL TEMPERATURE AND STABILITY

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Keywords: amylase, optimal temperature, stability, *Rhizopus oryzae*, *Rhizopus microsporus* var. *oligosporus*.

Amylase is one of the most important industrial enzymes, acts on the hydrolysis of starch mainly generating dextrin, maltose and glucose. Most amylase used in the processing of starch and its derivates are extracellular enzymes of microbial source. Several microorganisms produce amylases, especially the species *Rhizopus oryzae* and *Rhizopus microsporus* var. *oligosporus*, because they are considered safe for food use (GRAS) by the FAO (Food and Agriculture Organization). The thermostability characteristic for these enzymes is very desirable because it allows the conduct of processes of starch hydrolysis in high temperatures. The aim of this study was to compare the optimum temperature and stability of the enzyme produced by *Rhizopus oryzae* CCT 3763 and *Rhizopus microsporus* var. *oligosporus* CCT 3762. The extract was produced by submerged fermentation (SmF) using wheat flour type II (3%) as a carbon source. Fermentation was carried out in a rotary shaker at 160 rpm for 96h, pH 5.5, at 30°C and 37°C, for *R. oryzae* and *R. microsporus* var. *oligosporus*, respectively. The methodology for characterization of extract was the measure of amylase activity, based on hydrolysis of starch and color reaction with iodine. For this, an U (Unit Enzyme) was defined as the amount of enzyme required to hydrolyze 10 mg of starch in 30 minutes of enzymatic reaction (at 60ºC). The optimal temperature was determined by incubation of the reaction mixture at 20°C-80°C, pH 5.6. For stability, the enzyme solution was incubated at various temperatures (50, 55 and 60°C) for 9h at pH 5.6, in the absence of substrate. The results showed a maximum enzyme activity of 3.93 U/mL and 4.80 U/mL, in the optimum temperature of 60°C and 65°C for *R. oryzae* and *R. microsporus oligosporus*, respectively. Amylase from *R. microsporus oligosporus* exhibited half-life of 8h at 50°C, while amylase from *R. oryzae*, only 3h. At 55°C, the enzyme from *R. microsporus oligosporus* still showed good stability, keeping approximately 70% activity after 4h of incubation, while the enzymatic activity of *R. oryzae* decreased gradually coming around to zero after 3h. When incubated at 60°C, the enzymes from *R. oryzae* and *R. microsporus oligosporus* lost, respectively, 76% and 96.3% activity after 2h, showing that temperatures above 60°C are not recommended for this amylase in very long processes. The dates showed an expressive difference between two amylases produced by microorganisms, so it was considered enzyme of *R. microsporus oligosporus* superiorly more stable than *R. oryzae.*

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CHARACTERIZATION OF THE STRESS TOLERANCE OF THE PARENTAL STRAIN OF *Saccharomyces cerevisiae* (PE-02) AND SPORE-DERIVATIVE HAPLOIDS

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Keywords: sporulation, stress tolerance, filamentation, *S. cerevisiae*.

Yeasts are important in many biotechnological processes, with broad industrial use. The strain *Saccharomyces cerevisiae* (PE-02) is one of the most commonly used in ethanol production and known by its high sporulation capacity during fermentation processes, which may provide different descendants of parental strain. In this context, this work aimed to characterize the stress tolerance profile of the industrial strain *Saccharomyces cerevisiae* (PE-02, parental strain) compared with the haploid spores (SA-08, E-13, E-14 and E-15) dissected from a single tetrad. The stress tests were performed in Petri plate in YEPD, transferring 10µL of the suspension cell solution standardized at $1x10^8$ cells/mL. The experiment was incubated for 3 days at 30ºC with three replicates. This work revealed that all strains were resistant to lactic acid (3%), and high glucose concentration (500 g/L), except for SA-08 (400 g/L). All strains grew at 39ºC, but they did not grow at 40ºC. The spore SA-08 was not resistant to acetic acid, but it was resistant to a concentration of 12% ethanol; the other strains were resistant to 0.4% acetic acid and 16% of ethanol. The parental strain was more resistant to acidic stress (pH 1.5-1.75) and the spore derivatives showed resistance at pH 1.75. The filamentation in response to the depletion of nutrients (invasive growth) was restricted to the parental strain. The results showed that the haploid spore SA-08 was more susceptible to the stress and that the remaining spores showed resistance profile similar to the parental strain except in acid stress and invasion of agar, where PE-02 proved to be more resistant. Further tests should be performed to characterize the fermentative capacity of the parental strain and the haploid spores.

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CHARACTERIZATON AND PROPERTIES OF THE BIOSURFACTANT PRODUCED BY *Candida sphaerica* CULTIVATED IN A BIOREACTOR

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Surfactants are amphipathic compounds with hydrophilic and hydrophobic portions with application in many industrial sectors with emulsifying properties. Most of the surfactants are synthesized from petroleum derivatives; however, new environmental laws have encouraged the research of surfactants from microbial origin Due to the high production cost, agro-industrial wastes have attracted interest from researchers as an alternative use of low cost substrate for the production of biosurfactants. The biosurfactants have advantages over synthetic surfactants such as biodegradability, low toxicity, stability at different pHs, temperatures and NaCl and production from renewable sources. Given the potential for production of biosurfactants by yeasts, a biosurfactant was isolated from *Candida sphaerica* UCP 0995 grown in refinery residue of soybean oil and corn steep liquor. After producing over 144 h at 28 ° C in 1 L bioreactor, the biosurfactant was extracted from the cell-free broth with 6.0 M HCl and precipitated with methanol. After 24 hours at -15 ° C, samples were centrifuged and dried, and the total yield of the isolated product was of 15.432 g/L. The surface tension, measured in a tensiometer, was 28 mN/m and the CMC 0.08%. Biosurfactant was characterized as a glycolipid and showed no toxicity against *Intybus chicory* seeds and *Solanum gilo*. Therefore, the biosurfactant can be used in biotechnological processes, since the costs associated with the substrate are reduced, the production yield is satisfactory and the ability to reduce the tension is high, increasing the chances for competition of these compounds against their similar counterparts.

Keywords: biosurfactant, characterization, toxicity, *Candida*.

Financial Support: FACEPE, CNPq, TERMOPERNAMBUCO, UNICAP.

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CHECKING *IN VITRO* ANTIMICROBIAL ACTION OF EXTRACT OF LEAVES *Protium heptaphyllum* (Aubl.) Marchand.

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Keywords: Leaves. Extract. Antimicrobial. Almecegueira.

The attainment of antimicrobials from medicinal plants was an important development for medicine, yet there are still many medicinal species with possible antimicrobial activity that require more thorough studies and researches and research so that these substances are known and used. The effectiveness of medicinal plants has been investigated for treatment of infections as well as its effects and pharmacological properties, and its targeted use in treating various diseases. The oral cavity is one of the places where there is a greater variety of microorganisms that may, by an imbalance, become pathogenic. Scientific studies related to the evaluation of leaf extract of almecegueira are very rare; it is noteworthy that despite the widespread use was not known until then research attesting to its antimicrobial property. It is very important to scientific confirmation of the medicinal properties of a plant species for its preservation. Based on this popular knowledge and in the use widespread among people of their habitat was decided to study of the extract from the leaves of *Protium heptaphyllum* (Aubl.) Marchand (almecegueira) to evaluate its antimicrobial activity on microorganisms commonly found in the oral cavity, and in medical interest. The almecega or almecegueira (*Protium heptaphyllum* Aubl. Marchand) belongs to the Burseraceae family, which has a pantropical distribution, and covers about 20 genera and 500 species, in our country appear seven genera and 60 species being common to the Cerrado. The leaves were collected from almecegueira Biological Reserve and Experimental Station of Mogi-Guaçu, SP, Brazil, since they were chosen to be a completely renewable plant and that there would cause major disruptions to it. Prepared its 70% ethanol extract, which was later passed by rotary evaporator to total extraction of ethanol. The microorganisms tested were: *Streptococcus mutans* (ATCC 25175), *Lactobacillus casei* (ATCC 7469) involved in formation of oral biofilm, and *Staphylococcus aureus* (ATCC 25923) part of the group of microorganisms normal human, but still can produce significant opportunistic infections when found appropriate conditions. The plates were divided into four parts plus the center. In each of the five parts were placed in sterile paper discs 6 mm in diameter soaked in solutions with the following concentrations: Pure Extract (250 uL), 1:1 (250 uL extract + 250 uL of DMSO), 1:2 (Extract + 500 uL 250 uL of DMSO), 1:3 (250 uL extract + 750 uL of DMSO) and Chlorhexidine 0.12% (control - in the center of the plate). The tests were performed in duplicate against each strain tested. For the microorganisms tested, there was no inhibition zone in any assay. In antimicrobial assessment performed by Kataoka et al. (2008), the leaves of *Campomanesia adamantium* (guabiroba flat), also common to the Cerrado, the extract showed no inhibition zone for (*Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*), however, the extract from the bark had inhibition zone of *Staphylococcus* aureus (0.7 mm), *Pseudomonas aeruginosa* (4.0 mm), *Candida albicans* (2.7 mm) and *Streptococcus pyogenes* (2.0 mm), a similar method of extraction of active ingredients and antimicrobial activity were used.

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CHEMICAL AND MICROBIOLOGICAL CHARACTERIZATION OF SEWAGE SLUDGE, PRIOR AND AFTER NATURAL BIODEGRADATION PROCESS.

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Keywords: solid residues, soil microbiota, biodegrader microorganisms, mineralization.

One problem that has been dragging on the last decades is the adequate final disposal of the solid residues (sewage sludge - SS) generated by the Sewage Treatment Stations. A viable alternative for the disposal of the SS is its application in agriculture, since it is rich in organic matter and nutrients. However, the SS may constitute a contamination source of toxic agents of organic nature and heavy metals. Therefore, its application in agriculture involves special care in order to avoid damages to the exposed organisms as well as to the environment. The use of biodegradation by microorganisms seems to be a promising tool to diminish the toxicity of the SS. It was assessed the potentiality of biodegradation of SS by endemic microorganisms of the soil where they are disposed and by the own biota of the sludge. For this, SS samples (domestic sewage treatment stations from the city of Rio Claro - SP/ Brazil) were buried, for a period of 0, 2 and 6 months, in pits made on the Experimental Garden of UNESP, Rio Claro. After each period, it was carried out chemical analyses and an evaluation of the microbial population of the SS sampled. The parameters chosen for the chemical analyses, as well as the methods used, were based on the established by the CONAMA Resolution nº 375, of August 29, 2006, which determines the maximum limits of more than 50 pollutants for the use of SS in agricultural soils. Microorganisms were identified by the VITEK II® of Biomerieux. Chemical characterization of SS, at 0 and 2 months, showed that the organic substance m,p-cresol and the chlorinated dioxins and furan were above the limits allowed by legislation. However, in the sample of 6 months, m,p-cresol was no longer present and dioxins and furan had their values reduced, being that 2,3,7,8-tetrachlorodibenzo-p-dioxin and 2,3,7,8-tetrachloro-dibenzo-furan, considered the most toxic substances among this class of compounds, were not detected. The microbial analysis showed the presence of 13 different genera of bacteria. *Enterobacter cloacae* and *Bacillus* sp were present in all samples. *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus fecalis* and *Staphylococcus* sp were identified only in the SS time 0 and *Providencia rettgeri* only in the sample of 2 months. *Proteus mirabilis* and *Morganella morganii* were found in the samples of 0 and 2 months. *Pseudomonas aeruginosa* and *Serratia marcescens* were identified in the sample of time 0 and 6. In the sample of 6 months it was identified two other bacteria (*Citrobacter freundii* and *Ochrobactrum anthropi)*. With the assays, it was possible to observe an alternation in the microbial composition, involving enterobacteria and non-fermenting bacteria, during the periods in which the SS remained buried, possibly due to the fact that different groups of microorganisms acted in the several stages of biodegradation. This can be explained by the degradation ability of specific bacteria in each step of the biodegradation. Thus, metabolites resulting from breaks of large molecules may serve as substrate for other degrading microorganisms. We emphasize that, although the bacteria found in this study are frequently associated to human pathologies, they also may be associated to bioremediation and a consequent elimination of toxic substances of the environment.
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CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF *Eucalyptus staigeriana* ESSENTIAL OIL.

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KEYWORDS: antimicrobials, essential oils, *Eucalyptus staigeriana*, *Salmonella* Enteritidis, *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*.

INTRODUCTION: Antimicrobial potential of plant species essential oils have been widely studied in the last decades and it is related to its chemical composition. *Eucalyptus* gender, which belongs to Myrtaceae family, comprehends about 900 species and subspecies traditionally utilized for medical purposes by native populations. Recent findings of its biological activities reinforce the possibility of its utilization by food and beverage industry as a potent sanitizing. **MATERIAL AND METHODS:** *Extraction procedure: Eucalyptus staigeriana* fresh leaves (1:10 w/v) collected from Esalq/USP forest garden (Itatinga-SP) were utilized for hydrodistillation in a Clevenger equipment. The oil acquired after 4 hours of extraction was measured for yield characterization, dehydrated with anhydrous sodium sulfate for residual water elimination and packed in an amber flask refrigerated until the moment of use. *Antimicrobial activity:* the determination of the Minimun Inhibitory Concentration (MIC) was performed through analysis of *Salmonella* Enteritidis ATCC 13076, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Listeria monocytogenes* ATCC 07644 growth curve front of essential oil, utilizing 96-wells microplates which essential oil final concentration ranged from 12.8 to 0.1 μL of oil by ml of Tryptic Soy Broth (TSB) (Oxoid®) inoculated (1-2x10^5 CFU.mL^{-1}). Controls: inoculated TSB (–), chlorhexidine added inoculated TSB (0.12% v/v) (+) and essential oil added sterile TSB (white). Tween 80 (5% v/v) (Sigma®) was utilized as emulsifier. Microplates were incubated in spectrophotometer (Victor™X3, PerkinElmer®) at 35°C for 18 hours and the absorbance readings were performed in 1-hour intervals at 600 nm. *Essential oil chemical characterization:* Through Shimadzu® (QP 5000), gas chromatography coupled to mass spectrometry (CG-MS) was performed in a capillary column Atm 54 ms. Helio was utilized as carrier gas, with injection temperature of 280°C and flow of 1 $ml.min^{-1}$ *split* mode. Chromatographic conditions: 50ºC initial temperature (3.5 minutes), heating until 100ºC at 7ºC.min^{-1} (3.5 minutes), heating until 250°C at 10°C.min^{-1} (3.5 minutes). Mass spectrum was obtained in 70 eV and the mass/load relation interval (*m/z*) between 50 and 500. Tolerance band among the calculated and tabulated Index of Kovats (IK) values was ± 10 units. **RESULTS AND DISCUSSION:** *E. staigeriana* essential oil showed activity in all evaluated microorganisms. The following MIC were detected (in μL oil.ml $broth^{-1}$): *S.* Enteritidis (6.4), *E. coli* (6.4), *S. aureus* (3.2) and *L. monocytogenes* (3.2). Major compounds founds were limonene (17.66%), geranial (10.84%), nerol (9.34%), p-mint-2,4(8)-diene (6.68%), methyl geranato (5.62%), beta-pinene (5,56%), alpha-terpineol (5.39%), geranyl acetate (5.05%), alpha-phellandrene (4.37%) and geraniol (4.31%). These compounds of related abundance in *E. staigeriana* essential oil with antimicrobial, antifungal and anthelmintic activity showed to be effective against microorganisms of food importance, confirming its potential use by food industry as a natural sanitizing. **FINANCIAL SUPPORT:** FAPESP 2007/59905-1.

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CHEMICAL PROFILE ANALYSES OF MIXED AND SINGLE ACTINOBACTERIA CULTURES

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Keywords: actinobacteria, secondary metabolites, single culture, mixed culture, HPLC.

Introduction: the increase in the levels of multidrug-resistant pathogenic microorganisms and tumor cells shows the need for discovering new antibacterial and antitumor drugs. Endophytic actinobacteria are an underexplored natural source that shows a great potential in the search for bioactive molecules. Traditional approach using single cultures are usually employed to culture microorganisms. A recent strategy in the attempt for the production of small molecules is the use of mixed cultures. **Experimental:** two endophytic actinobacteria strains previously isolated from the roots of *Tithonia diversifolia* (Asteraceae) and indentified as *Streptomyces platensis* (RTd22) and *Streptomyces* sp. (RTd31) were grown under single and mixed conditions. Each strain was previously grown in ISP2 agar. Two plugs of 0.5 cm each were transferred to 3 mL of liquid ISP1 medium and incubated for 72 h (30ºC at 120 rpm). An aliquot of the culture broth was spread into Petri dishes containing ISP1-agar and incubated at 30ºC for seven days. The actinobacteria RTd22 and RTd31 were separately cultured using a two-step fermentation process. First, 20 plugs were transferred into 100 mL of ISP1 seed medium and incubated for 48 hours at 30 ^{o}C, 120 rpm. Second, 10 mL from the seed medium were inoculated into 90 mL of ISP1 fermentative medium and incubated for additional 21 days at 30 ^{o}C, 120 rpm. Mixed cultures were cultured using the same procedures described above, excepted that was used 5 mL of ISP1 seed medium from each strain culture to be transferred to 90 mL of ISP1 fermentative medium. The culture broths were filtered under vacuum and the liquid broth was submitted to liquid-liquid partition with ethyl acetate (EtOAc) and n-butanol (BuOH) (3 x 50 mL each). The crude EtOAc and BuOH extracts and remanescent aqueous culture broth were concentrated and submitted to high efficiency liquid chromatography analyses (HPLC-DAD using reverse phase analytical column in gradiente mode increasing from 10% to 100% of acetonitrile in water for 30 min at flow rate of 1 mL/min.). **Results and discussion:** EtOAc extracts did not show any difference between the single and mixed cultures. Among BuOH extracts was observed a peak at 14.23 min in RTd31 single culture that was not observed in RTd22 single culture and mixed culture. The aqueous extract obtained from mixed culture showed a peak at 35.94 min that was not observed for both single cultures. These results suggest that secondary metabolites production in mixed culture is influenced by the interaction of the strains. Further experiments will be performed in order to verify the antibacterial and antitumoral activities for EtOAc, BuOH and aqueous extracts.

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CHITOSAN PRODUCTION by *Absidia corymbifera* AND APPLICATION IN DECOLORIZATION OF SOFT DRINK INDUSTRY EFFLUENT

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Keywords: *Absidia corymbifera*, Chitosan, Decolorization.

Abstract

Color is the first contaminant to be recognized in wastewater and the presence of very small amounts of dyes in water is highly visible and undesirable. Chitosan has been investigated by several researchers as a biosorbent for the capture of dissolved dyes from aqueous solutions. In this work, the production of chitosan by *Absidia corymbifera* and its application for decolorization of soft drink industry effluent were studied. A 2^3 full factorial design was carried out to investigate the effects and interactions of the concentrations of corn steep liquor, glycerin and urea on the biomass productivity and chitosan yield on the biomass. The sporangioles of *A. corymbifera* were harvested from cultures grown for 12 h at 37ºC on Petri dishes containing PDA medium. For development of young culture, mycelium discs of 6mm were transferred to Erlenmeyer flasks of 250mL containing 100 mL of the culture medium (6% of glycerin , 6% corn steep liquor and 0.25% of urea). and the flasks were incubated at 37ºC in an orbital shaker at 150 rpm, during 12 h. For fungal submerse cultivation, after 24 h, 50 mL of young culture medium were transferred to flask of 1000 mL containing 550 mL of culture medium,with the same composition, being the flasks incubated at 37ºC in an orbital shaker at 150 rpm, during 72 h. Only the increase of urea concentration presented significant negative effects on biomass productivity and yield of chitosan. The chitosan obtained by biomass extraction, using an alkali-acid soluble treatment was characterized by infrared spectroscopy. The degree of deacetylation (DD) for microbial chitosan was 90.3%, considered high, when compared to commercial chitosan Polymar. The chitosan was applied in effluent with initial Chemical Oxygen Demand (COD) equal to 5800mg/L. The best color and COD removal efficiencies were 100% and 99.8%, respectively. The results showed values similar to those suggested by the literature, with regard to the rate of decolorization , which reports a rate of 99% of color removal under optimal conditions, using a combination of coagulant agent with chitosan. The biopolymer showed high adsorption capacity compared to activated carbon and it can be used in various types of industrial effluents treatments. Therefore, investigations conducted by adsorption with microbiological chitosan indicate this biopolymer as a promissory sorbent for soft drink industry effluent treatment.

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CITRIC ACID PRODUCTION IN VARIOUS STRAINS OF *Yarrowia lipolytica* USING GLYCEROL AS CARBON SOURCE

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Keywords: Citric Acid; *Yarrowia lipolytica*; Glycerol.

In Brazil, it is expected an availability of 200 thousand tons of biodiesel glycerol with the mixture of 5% biodiesel into mineral biodiesel. Nevertheless, the purification and clarification of glycerol is a very costly process, and the use of raw glycerol to generate products with high added value, may reduce the price of biodiesel in the market. Raw glycerol can be used as carbon source by some yeast to produce citric acid. The aim of the present study was to determine citric acid production by five strains of *Yarrowia lipolytica* (NRRL Y-1095, CCT 6770, NRRL Y-109, YLI and YLII) using glycerol as the sole carbon source. Fermentations were performed in 500 ml capacity baffled flasks with 100 ml of mineral medium made of KH_2PO_4 0.2 $g.l^{-1}$, $MgSO_4.7H_2O$ 0.4 $g.l^{-1}$, $CaCO_3$ 6.0 $g.l^{-1}$, $FeCl_3$ 0.65$mg.l^{-1}$, $ZnSO_4$ 1.2 $mg.l^{-1}$, CuSO4.5H2O 0.31 $mg.l^{-1}$, $MnSO_4$ 0.27 $mg.l^{-1}$ e thiamine 5 $mg.l^{-1}$ and incubated at 28ºC and 150 $rev.min^{-1}$ using shaker incubator. Were performed three different experiments: A - Glycerol 75 $g.l^{-1}$ and NH_2SO_4 2 $g.l^{-1}$ incubated for 96 h; B – Glycerol 100 $g.l^{-1}$ and Urea 2 $g.l^{-1}$ incubated for 96 h; C – Glycerol 75 $g.l^{-1}$ and NH_2SO_4 2 $g.l^{-1}$ incubated for growth 72 h, then more glycerol 100 $g.l^{-1}$ added at 72 h and incubated until 144 h. The fermented broth was analyzed to evaluate citrate and glycerol consumption using HPLC equipped with a UV and RI detector and a Phenomenex – Rezex Organic Acids column (300 mm x 7.8 nm) at 60°C, and mobile phase H_2SO_4 0.005M water solution at 0.4 $ml.min^{-1}$ flow. Experiment A resulted in a best production by *Yarrowia lipolytica* CCT 6770 and NRRL Y-1095, reaching a citrate production of 18.16 $g.l^{-1}$ and 17.67$g.l^{-1}$ respectively, and a yield of 0.32 g_{cit}/g_{glol} and 0.33 g_{cit}/g_{glol}. Experiment B showed lower results compared to experiment A, indicating that urea is not a good nitrogen source. Best results in this experiment reached 10.26 $g.l^{-1}$ and a 0.1 g_{cit}/g_{glol} yield by *Yarrowia lipolytica* NRRL Y-1095. Experiment C showed the best results among all, where *Yarrowia lipolytica* NRRL Y-1095 produced 30.06 $g.l^{-1}$ of citrate after 144 h fermentation, with and 0.30 g_{cit}/g_{glol} yield. According to Costa (2002) and Papanikolaou and Aggelis (2002), citric acid fermentation by *Y. lipolytica* best occurs in two phases, one phase of cell growth, and another production of citrate, after depletion of extracellular nitrogen. The statement collaborates with the result, where were produced more acid when production was performed in two stages, and done with more time and with a higher amount of glycerol. Therefore, *Yarrowia lipolytica* NRRL Y-1095 were the best citric acid producer using one and two stage fermentation among the tested.

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Cladosporium AND RELATIVES (CAPNODIALES, DAVIDIELLACEAE) ISOLATED FROM LEAF-CUTTING ANTS (FORMICIDAE, ATTINI).

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Keywords: microbial ecology, gynes, dematiaceous fungi, Davidiellaceae, taxonomy

Ants from the tribe Attini cut leaves into pieces that they use as substrate for growing a symbiotic fungus, which on they feed. Recent studies found that a large diversity of microorganisms could be present in the nests, including dematiaceous fungi. This study aimed to isolate *Cladosporium* and related black fungi from gynes of *Atta laevigata* and *A. capiguara*, in order to increase the knowledge on the natural distribution of these microbes of great agricultural and medical importance. The body and infra-bucal pellet of the gynes were submitted to the oil flotation technique. A total of 92 isolates, was preserved in slants at 10^0C and by ultra-freezing at -80^0C. Through morphological and molecular data, 15.5% of the isolates were identified as *Cladosporium* and *Davidiella* species (Davidiellaceae, Capnodiales). *Cladosporium* is a dematiaceous hyphomycete which comprises more than 772 current names. Species of *Cladosporium* are widespread and usually encountered on plant and other kind of debris. They are also isolated from air, soil, food, paint, textiles, and interact with humans causing health problems like allergic lung mycoses. Some representatives are phyto or enthomopathogenic, and are used in biological control. *Davidiella* is the teleomorph, recently created. The results showed a predominance of *C. perangustum, C. xylophilum* and *C.* cf. *tenuissimum* associated to ants, reported for the first time in this investigation. *Cladosporium/ Davidiella* in ant nests are still poorly known. Although some insights can be preliminarily discussed, further studies will be necessary to understand their microbial importance in this niche.

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COMPARATIVE STUDIES OF *Bacillus thuringiensis* var. *israelensis* METABOLISM IN DIFFERENT CONCENTRATIONS OF CASSAVA FLOUR PROCESSING WASTE BASED MEDIA

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Keywords: Bioinsecticides; agroindustrial wastes; *Bacillus thuringiensis* var. *israelensis*; fermentation

Techniques of production of entomopathogenic bacteria are developed looking for to increase the productivity and to reduce the costs of the fermentative process. *Bacillus thuringiensis* var. *israelensis* (*Bti*), an aerobic bacterium, generates certain toxins with pesticide action, which can be used on the control of transmissible diseases by culicides, specially *Aedes aegypti*, the dengue´s vector. This biopesticide has been produced by submerged fermentation and, in Brazil, this production has been made by very little research centers. For the implementation of a viable vectors control program through biopesticides, some studies about culture media are essential in order to join efficiency and low costs. It has been using agro industrial wastes or by-products as nutrient sources in culture medium, having been used, in this study, the "manipueira", a by-product of the processing of the cassava flour. The fermentations were accomplished in Erlenmeyer flasks of 500 mL containing 250 mL of culture media, conditioned in shaker at 180 r.p.m. and 28ºC, and the media were composed by "manipueira", in concentrations that varied between 25 and 1000 mL/L, that´s say: 25, 50, 75, 125, 175, 250, 325, 400, 500, 600, 700, 800, 900 and 1000 mL/L. The time of the process varied between 48 and 120 hours. They were appraised the following parameters: cellular growth, pH, spores production, organic matter reduction (COD analysis), sugar reduction and media lethal concentration (LC_{50}) against *Aedes aegypti* larvae. Although there was a proportional cellular growth to the manipueira concentration, the production of spores was similar in all the cases, at the end of the process, in spite of the smallest speed of production of the same ones in the highest concentrations. In relation to COD variation, it exists, also, a percentile minor of reduction in the highest concentrations. In the analysis of variation of sugars reducers, the concentrations more discharges are the ones that present larger slowness in the reduction of this. When analyzing the bioassays results, it was verified that the best concentrations are of 500 and 600 mL/L, with a LC_{50} of 3,7 ppm for 500 mL/L and 4,2 ppm for 600 mL/L concentration. The other concentrations, that´s say, 400 mL/L, 700 mL/L, 800 mL/L, 900 mL/L e 1000 mL/L had a LC_{50} of 6,5, 7,2, 8,0, 7,5 e 8,1 ppm, respectively. The 800, 900 e 1000 mL/L concentrations had, therefore, low efficiency. It´s possible conclude that "manipueira" is a viable alternative in fermentative process for *Bti* biopesticides production.

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COMPARATIVE STUDY OF THE EFFICACIES OF NINE ASSAY METHODS FOR THE DEXTRANSUCRASE SYNTHESIS OF DEXTRAN

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Keywords: Dextransucrase assays; Direct measurement of dextrans; Ferricyanide/arsenomolybdate; 3,5-Dinitrosalicylate; Reducing values; Over-oxidation.

In the present study, nine different assay methods for the determination of the amount of dextran synthesized by dextransucrase have been examined. We show that two diverse methods that are widely used: (1) the reducing value methods, especially the DNS method and the ferricyanide/arsenomolybdate method, and (2) the ^{14}C-sucrose rapid filter paper method, gave erroneous results. The reducing value methods gave extremely high values due to over-oxidation of D-fructose, D-glucose, leucrose, and dextran; and the ^{14}C-sucrose filter paper method, gave low values due to the removal of some of the ^{14}C-dextran from the filter papers by the methanol washes. We have found the direct measurement of dextran by its precipitation with three volumes of ethanol from the sucrose/dextransucrase digests, followed by dehydration and determination by weighing, or when ^{14}C-sucrose is used, determination by liquid scintillation counting and the specific activity of D-glucos: these latter two methods gave identical number of μmoles of D-glucose incorporated into dextran/min.

Nine different assay methods for dextransucrase and related enzymes (glucansucrases) have been compared and evaluated for their efficacy. They can be divided into two distinct types: (1) a direct method by isolating the synthesized dextran and the determination of its amount, and (2) an indirect method, by measuring the D-fructose that is released from sucrose when the D-glucose moiety of sucrose is polymerized into dextran. The first group consists of four methods that give the amount of dextran produced at any given time; the second group consists of four different colorimetric methods measuring, the amount of D-fructose produced by measuring the reducing value equivalent of D-fructose, and a fifth method that measures the amount of D-fructose enzymatically.

The direct measurement of dextran, after precipitation from the dextransucrase digest and re-precipitation two more times, followed by dehydration give the most accurate measurement of the amount of dextran synthesized by dextransucrase and, therefore, are the methods to be used to obtain an accurate and useful activity of dextransucrase for assays and kinetic studies. These methods can be used for any glucansucrase that synthesizes glucan from sucrose, such as mutan, alternan, comb-dextrans, and so forth. The use of the reducing value methods to determine the activity of dextransucrase and related enzymes by measuring the putative reducing value of D-fructose gives highly inflated and unacceptable values, due to over-oxidation of D-fructose and other reducing sugars in the digest, and dextran if allowed to remain in the assay solution. This is especially so for DNS and the ferricyanide/arsenomolybdate methods, both in the presence of dextran and in its absence. The ferricyanide/arsenomolybdate method gave an even higher value for dextransucrase, in the absence of dextran, than did the DNS method.

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COMPARISON BETWEEN SUGARCANE BAGASSE AND WHEAT BRAN AS SUBSTRATE IN BIOSURFACTANT PRODUCTION.

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Keywords: bioremediation, biosurfactants, agro-industrial wastes.

Increased environmental awareness, with the recurrent oil contamination events, had made the development of ecological, compatible and inexpensive techniques becomes a priority[1,2]. The biosurfactants are amphiphilic compounds with effective surface-active and biological properties applicable in the bioremediation of water and soil[3]. The utilization of agro-industrial wastes in the biosurfactant production can reduce the production price as well as the environmental pollution[4]. Therefore, the purposes of this study were to compare the biosurfactants produced by solid-state fermentation using sugarcane bagasse and wheat bran. First of all, the microorganism *Aspergillus ochraceus* have been propagated in PDA tubes and incubated at 25°C during seven days. After that, the spores have been suspended in nutrient solution and incubated in Erlenmeyer flasks containing five grams of the substrate to be used, sugarcane bagasse or wheat bran, 30 mL of nutrient solution containing the inoculum at the concentration of $1{,}7.10^6$ spores/mL and 1% of glucose as carbon source. The experiments have been made at 25°C and pH 5,3, during 72, 120 and 168 hours. The comparison between the two experiments has been made using the parameters surface tension and the emulsion index, which were able to determine the presence of the biosurfactant in the fermentation broth. In the test of surface tension, the experiment made with sugarcane bagasse has presented results of 61,43, 59,17 and 63,27 mN/m for 72, 120 and 168 hours, respectively. Using the wheat bran the results were 40,5, 40,0 and 39,5 mN/m. These results can show that the biosurfactant produced by sugarcane bagasse were not as efficient as the one produced by wheat bran to decrease the surface tension and the biosurfactants produced by wheat bran in 72 hours were better than that produced by sugarcane bagasse in 168 hours. The emulsion index after 24h using the sugarcane bagasse was 6,35%, 5,59% and 27,77% while using wheat bran we had 29,36%, 35,23% and 29,41%. Here we can see that the biosurfactant produced by sugarcane bagasse were able to produce an stable emulsion only in 168h while the wheat bran´s biosurfactant in 72 hours had already produced an stable emulsion. In conclusion, the biosurfactants produced both were able to reduce the surface tension and produce an stable emulsion. Taking into account that the time is an important parameter in high scale production, the wheat bran´s biosurfactant had a better potentiality because it is able to do this only in 72h.

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COMPARISON OF METHODS FOR ISOLATION OF MYCOBACTERIA FROM ENVIRONMENTAL WATER.

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Keywords: Isolation, Mycobacteria.

The treatment of water used for supply, usually done by chlorination, has as indicators of microbiological quality the number of total and fecal coliforms, but these indicators have limitations, particularly in prediction of disease risk. The chlorine treatment controls contamination by more susceptible microorganisms as the coliforms and the elimination of these microorganisms in treated water reduces competition and may promote the growth of chlorine resistant bacteria as mycobacteria and the survival of protozoan cysts. Infections caused by environmental mycobacteria, also known as nontuberculous mycobacteria (NTM) may be acquired by ingestion, inhalation and inoculation from environmental sources. The continuous appearance of new invasive medical and nonmedical techniques, the increasing number of procedures performed and also the growing number of cases of patients with diseases that lead to immunosuppression, have increased the number of cases of infections caused by NTM. Therefore, this project has as main objective the standardization and validation of methods for isolation of mycobacteria in environmental waters. The development of studies to improve the conditions of isolation or specific tests is support for a better diagnostic and epidemiologic technology, being the main point for the understanding and controlling the spread of environmental mycobacteria. To compare methods for isolation of mycobacteria in water it was performed the adaptation of the Thomson et al protocol, with the species *M. abscessus*, *M. massiliense* and *M. fortuitum* fast-growing, and *M. lentiflavum* slow-growing, testing decontamination methods with cetylpyridinium chloride (CPC) and acid treatment with H2SO4, and the use of sodium thiosulfate to neutralize chlorine, and different culture medium of inoculation such as Löwenstein-Jensen (LJ), Middlebrook 7H10 agar supplemented with OADC and Medium Tryptone Soy (TSA). The results showed no difference in growth in different culture medium, in exception of the species *M. lentiflavum* and *M. abscessus*, which had less growth on TSA. The LJ initially presented itself more sensitive, with slower growth, but at the end of 2 weeks of growing, showed small difference in the 7H10 medium. Among treatments for decontamination, the CPC proved to be the least aggressive in the recovery of mycobacteria in relation to the acid treatment which recovered only 8.3% in *M. massiliensis*, compared with the CPC that has recovered 100% of the same species. But the CPC is also less aggressive to contaminants and recommended therefore, in water with low contamination.

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COMPARISON OF SUGAR METABOLIZATION KINETICS BY INDUSTRIAL YEASTS BG-1, CAT-1 AND PE-2

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Keywords: yeast, sugar metabolization, alcoholic fermentation.

INTRODUCTION: *Saccharomyces cerevisiae* BG-1, CAT-1 and PE-2 are the most widely used yeast strains for fuel ethanol production in Brazilian distilleries. These strains have different extracellular invertase activities and, as sucrose is the main sugar in sugarcane based substrates, a different profile of sucrose, glucose and fructose could be expected during fermentation. The present work was performed aiming at the study of the metabolization profile of sucrose, glucose and fructose during fermentation.

MATERIAL E METHODS: The fermentative assay was carried out in 250 ml Erlenmeyer flasks with the inoculum (8 g wet biomass, 5 ml wine and 15 ml distilled water) of the strains BG-1, CAT-1 and PE-2. Each flask was incubated in a shaker (100 rpm, 30ºC) and fermentation was performed simulating the industrial fed-batch process, by means of a peristaltic pump, feeding 60 ml of mixed must (19% TRS) during 5 hours. During fermentation, samples from each flask were collected at 0, 1, 2, 4, 6 and 8 hour intervals, centrifuged (5000 rpm, 5 min) and the supernatants were analyzed for sucrose, glucose and fructose by HPLC.

RESULTS AND DISCUSSION: The results showed that the strains BG-1, CAT-1 and PE-2 presented a distinct kinetic profile of metabolization and consumption of sucrose, glucose and fructose. It was observed that BG-1 strain readily hydrolysed sucrose throughout the feeding , resulting in a low concentration (<0,06%) of this sugar during the fermentation. That behaviour was consistent with Parazzi (2006), demonstrating a high invertase activity for BG-1 strain, and therefore a rapidly hydrolysis of sucrose in comparison with the two others strains. The sucrose hydrolysis was slower for CAT-1, with an accumulation of this sugar reaching a maximum at 2 hours of fermentation (3,79%), declining afterwards. Also the low levels of glucose and fructose (the hydrolysis products) observed, suggests that sucrose hydrolysis rate equals the hexoses uptake rates for this strain. This is justified by low invertase activity of CAT-1, as described by Parazzi (2006). As PE-2 strain has an intermediate invertase activity (Parazzi, 2006), the sucrose hydrolysis was faster than that of CAT-1, leading to a maximum sucrose accumulation of 2,21% at 1 hour of fermentation. The glucose and fructose uptake occurred more slowly for PE-2 and BG-1 strains. This indicates that sucrose hydrolysis rate exhibited by PE-2 and BG-1 strains exceeds glucose and fructose uptake rates, leading to an accumulation of hexoses that could impose differential osmotic stress towards different yeast strains.

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CORN STEEP LIQUOR AND CANA MOLASSES AS SUBSTRATES FOR BIOSURFACTANT PRODUCTION

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Keywords: biosurfactants; *Pseudomonas sp.*; Wastes; Bioremediation.

Surfactants are powerful amphipathic agents widely used in the petroleum, food and pharmaceutical industries, among others. The compounds of microbial origin that exhibit surfactive properties, i.e., reduce the surface tension, are called biosurfactants and constitute metabolites from bacteria, yeasts and filamentous fungi. Many biosurfactants have been produced but few have been commercialized due to the high process costs, especially as a function of the use of expensive substrates and the purification processes. In this sense, a biosurfactant was produced from industrial residues as low-cost substrates and characterized regarding the efficiency (surface tension reduction capacity) and emulsification capacity. Fermentations were conducted with the bacterium *Pseudomonas* sp. in mineral medium supplemented with 3% corn steep liquor and 3% cane molasses at 200 rpm during 144 hours. During cultivation, samples were withdrawn for surface tension measurements, pH and biomass quantification. The biomolocule obtained was able to reduce the water surface tension from 70 mN/m to 29 mN/m after the first 24 hours of cultivation. The pH increased along fermentation from 6.6 to values around 8.5 and maximum biomass was obtained after 48 hours. The emulsification activity was determined against different hydrophobic substrates, including hydrocarbons and vegetable oils under different pH, temperatures and NaCl addition. It was observed high emulsification indexes, depending on the environmental condition and substrate tested, showing the specificity of the surfactant. The biosurfactant produced has attractive properties as reduced surface tension and emulsification capacity, showing potential to be used in the environmental area as it can reduce the surface tension between the oil-water phase allowing the removal of the hydrophobic compound from the contaminated site.

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CULTIVATION OF *Aphanothece microscopica Nägeli* ON GAS-OUTLET FROM ETHANOL FERMENTATION

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Keywords: cyanobacterium, *Aphanothece microscopica Nägeli*, carbon dioxide, ethanol fermentation

Carbon dioxide is a byproduct of ethanol fermentation produced in large quantities and it is the main cause of greenhouse effect. For this reason it is essential to developing technologies that allow the use of this gas so that it is not released into the atmosphere. Cyanobacteria are photosynthetic microorganisms, capable of using CO_2 with O_2 production. *Aphanothece microscopica Nägeli* is a cyanobacterium that has been studied for the purpose of increasing the value of the agro industrial residues in the production of single cell and oil in addition to wastewater treatment and CO_2 sequestration. In this context, the aim of this study was evaluated the possibility of using carbon dioxide from gas-off ethanol fermentation to support of cyanobacterium *Aphanothece microscopica Nägeli*. Ethanol fermentation was set up with sugarcane syrup diluted (25°Brix) and a yeast inoculum of 25g/L. Gases produced in the fermentation process were bubbled in the BGN medium cultivation with cyanobacteria (10^6cell/mL) with monitoring of pH, cell concentration and chlorophyll, during 50 hours. There were no differences between growth profile and chlorophyll, compared with the air-feed flasks containing cyanobacteria. However, pH of the BGN medium that received the fermentation gases decreased to 4.7, while with aeration the pH decreased slightly maintained in the range 7.1 - 7.9. Despite of this great difference, there was no influence of pH on the growth parameters. Thus, autotrophic production of cyanobacteria biomass could be an alternative for use of gases from ethanol fermentation.

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CULTURABILITY AND MOLECULAR DETECTION OF HITHERTO-UNCULTURED BACTERIA FROM SOIL.

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Keywords: *Acidobacteria*, *Verrucomicrobia*, Amazonian soils, PCR-based detection method

Soils are inhabited by many bacteria from phylogenetic groups that are poorly studied because representatives are rarely isolated in cultivation studies. Part of the reason for the failure to cultivate these bacteria is the low frequency with which bacterial cells in soil form visible colonies when inoculated onto standard microbiological media, resulting in low viable counts. The *Acidobacteria* and *Verrucomicrobia* divisions are among those divisions of the domain Bacteria represented by a large diversity of 16S rRNA genes, which occur in particular abundance in soils, but contain few cultured members. Hence, our appreciation of the physiological diversity of *Acidobacteria* and *Verrucomicrobia* is limited, as is our knowledge of their role in global biogeochemical cycles. Clearly, a better understanding of these divisions would be attained by having a greater diversity of their members available in pure culture for detailed study. In this study we investigated the culturability of *Acidobacteria* and *Verrucomicrobia* from Amazonian tropical soils. PCR-based surveillance method (plate wash PCR) was used for molecular detection of these two bacterial groups. Some elements of the cultivation procedure included the following: the use of agar media with little or no added nutrients, 10^{-1} to 10^{-9} dilutions from aliquot of soil (30 g fresh weight), relatively long period of incubation (more than 30 days) and incubation under hypoxic (2% O_2 [vol/vol] and 93% N_2 [vol/vol]) atmosphere with elevated concentration of CO_2 (5% [vol/vol]). A simple, high-throughput, phylum-specific PCR-based method was used in control reaction in the presence of *Acidobacterium capsulatum* (DSM 11244) and *Verrucomicrobium spinosum* (DSM 4136) DNA. This method greatly facilitated detection of target bacteria from as many as 500 colonies of non-target microbes growing on the same agar plates. When phylum-specific primers were used, *Acidobacteria* and *Verrucomicrobia* were detected in 10^{-4} dilutions after a period of fifteen days under hypoxic atmosphere. PCR based on *Acidobacteria*-specific primers revealed a similar amplicon and positive control band position in cells suspensions 10^{-1} to 10^{-3} from agar media maintained under incubation during twenty one days. Bacterial colonies grown separately in the agar media after re-incubation of suspended colony material (10^{-4} to 10^{-9} dilutions) from replicates agar plate did not subject to plate wash PCR. Long inoculation time (more than 30 days) associated to re-incubation of suspended colony material benefited the detection of amplicons with same band position than acidobacterial and verrucomicrobial positive control in PCR reaction. Our results illustrate the power of integrating culture methods with molecular techniques for detection of *Acidobacteria* and *Verrucomicrobia* in culture media.

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DENSITY OF ARBUSCULAR MYCORRHIZAL FUNGI IN RHIZOSPHERE SOIL FROM SUGARCANE.

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The arbuscular mycorrhizal fungi (AMFs) are important members of the soil-plant system, since the high diversity of these fungi is intimately linked to the diversity and productivity of plant communities. The AMFs form mutualistic symbiosis, called arbuscular mycorrhizae (AM), in this symbiosis, the plant supplies the fungus with energy for growth and reproduction by photosynthates, and the fungus provides a range of services to the plant and to the soil. These fungi bring benefits to the plant because their hyphae spread into the soil, working as an extension of the root system, so, the fungus assists in the absorption of water and nutrients, mainly phosphoros. Based on these data, this project aims to determine the spore density of arbuscular mycorrhizal fungi in the rhizosphere of sugarcane cultivated among plant species in alley cropping systems. Three arboreal species were used: Cedar (*Cedrela fissilis*) Guapuruvu (*Schizolobium parahiba*) and Ipe (*Tabebuia serratifolia*), they were planted in rows far apart at 30, 45 and 60 meters between themselves, and between the rows were grown sugarcane variety RB935744. For analysis, samples of soil were collected from the rhizosphere of trees, from 5 meters away from the alley cropping of the rhizosphere of sugarcane, and from between rows of sugarcane, totaling 15 samples. In the laboratory, the samples were dried, sieved and processed associating the techniques of wet sieving and centrifugation in 50% sucrose. Spore quantification has been made under a stereomicroscope in Syracuse. The number of spores founded in the samples varied between 45 and 263 spores in 250 grams of soil. There were no significant differences between the areas, which may be explained by the short implementation time of the experiment.

Keywords: arbuscular mycorrhizal fungi, sugarcane, Cedar, Guapuruvu, Ipe.

Financial support: Universidade Federal de São Carlos (UFSCar)

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DETECTION AND CHARACTERIZATION OF THE VIRULENCE FACTORS OF *Escherichia coli* ISOLATED FROM DRINKING WATER SUPPLIES IN THE STATE OF MARANHÃO, BRAZIL.

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Keywords: *E. coli*, drinking water, virulence.

INTRODUCTION: *Escherichia coli* is the predominant bacterium in the normal human intestinal flora. However, there are certain strains of *E. coli* associated with virulence mechanisms that render the bacteria enteropathogenic for humans. The World Health Organisation (WHO) estimates that about 1.1 billion people globally drink unsafe water and the vast majority of diarrheal disease in the world (88%) is attributable to contaminated water, due to lack of sanitation and hygiene. *E. coli* is one of the microorganisms used as a parameter for verifying the quality of water used for consumption. According to Portaria 518, 2004, of the Brazilian Ministry of Health, under no circumstances water used for human consumption may contain *E. coli* or thermo-tolerant coliform bacilli; in which case *E. coli* is the indicator of first choice.
The general aim of this study is to detect and characterize the virulence factors of 75 *E. coli* strains isolated from water samples of drinking water supplies in municipalities and Indian tribes in the interior of the state of Maranhão, Brazil. The specific aims are: the obtainment of isolated *E. coli* strains from drinking water; the detection of hemolytic activity (hemolysis) and enzymatic activity (lipase) of *E. coli* strains; the detection of the production of biofilm; the detection of curli fimbriae; and determining a pattern of microbial resistance and sensibility towards antibiotics.
METHODS & MATERIAL: The detection of coliform bacilli and *E. coli*, done using the Colilert (Quanti-Tray/2002^{TM}) method, was performed according to the manufacturer's instructions, approved by the *Standard Methods for the Examination of Water and Wastewater* and by Portaria 518 of the Brazilian Ministry of Health. Afterwards the test of hemolytic activity (hemolysis) was performed in sheep blood agar 5%, for the detection of enzymatic activity (lipase) samples were cultured in Mullen Hinton agar, with the addition of olive-oil 2%. For detecting the production of biofilm the samples were cultured in Congo Red agar. For the curli fimbriae detection, samples were cultured in Luria Bertani agar. The antimicrobial resistance test was performed in accordance with the Kirby Bauer Method of agar diffusion.
RESULTS & DISCUSSION: Of the 75 samples, 57 (76%) tested positives for lipolysis, 62 (83%) tested positive for hemolysis in sheep's blood, 38 (51%) tested positive for the production of biofilm and 58 (77%) tested positive for the detection of curly fímbriae. The antibiotics that encountered the largest percentage of resistance were AML and AMC (resp. 32% and 100%) and those with the largest sensibility were ATM and CAZ (both 100%). This research has shown a high level of hemolysin and lipolytical enzyme production, which are both important virulence factors, as they allow the bacteria to cause cellular and tissue damage. Also high levels of curli fimbriae and biofilm production were detected, which assist in the adhesion and fixation of bacteria to the host cells and thus facilitate the development of infectious processes. Our results show the need for the general population to receive better quality treated water, as determined by law, in order to avoid diarrhea and other diseases caused by pathogenic microorganisms in drinking water.

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DETECTION AND TEST SUSCEPTIBILITY SANITIZERS OF DIARRHEAGENIC *Escherichia coli* STRAINS ISOLATED BEACHES OF SÃO LUÍS – MARANHÃO

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ABSTRACT
The marine environment is composed of major importance for society because the uses that this ecosystem provides. Thus, the water quality of this environment is key to its uses are not harmful to health. The city of São Luís located in the state of Maranhão - Brazil, has no effective treatment of wastewater, which are released directly into rivers or on beaches. This is strong evidence that the coastal environment can be indicators of fecal contamination (FIB). Samples contaminated with fecal coliform from the main beaches of São Luís, such as Ponta d'Areia, São Marcos, Calhau and Olho d'Água, were analyzed with the objective of determining which pathogens and which diarrheagenic strains of *Escherichia coli* are present on beaches and in addition, be tested for susceptibility to sanitizers strain of *E. coli*. The isolation and identification of pathogenic bacterial strains of the samples were performed by means of selective and EnteroKit B, whereas the identification of diarrheagenic strains and toxin production of *Escherichia coli* were performed by PCR using specific primers. To test the susceptibility of strains of *E. coli* sanitizers was performed by the method of successive dilutions, which were made in five different dilutions. In all sites were identified species of Enterobacteria such as *E. coli*, *Serratia liquefaciens*, *Hafnia alvei*, *Salmonella spp*., *Serratia sp*., and the predominance of the first kind. Strains of *E. coli* subjected to PCR were identified mostly as ETEC, equivalent to 82%, and only on the beaches of Ponta d'Areia and Olho d'Água subtypes were identified EHEC and EAEC. The sanitizers were tested two brands of detergents, alcohol and two brands of household bleach. The latter presented a higher efficiency in presenting five dilutions tested bactericidal for all strains of *E. coli*. The detergent brand II showed greater efficiency in comparison with detergent brand I, since the first one presented a more bactericidal and bacteriostatic against the second. But the domestic alcohol for all strains of *Escherichia coli* tested, inhibited the growth of microorganisms, and their bactericidal action at much lower compared to other sanitizers.
Keywords: Enterobacteria; Diarrheagenic *Escherichia coli*; Sanitizers; beaches of São Luís – MA.

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DETECTION OF 1,3-PROPANEDIOL BIOSYNTHESIS GENES IN *Klebsiella pneumoniae* GLC29

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Keywords: 1,3-propanediol, glycerol, biodiesel, metabolic engineering

Introduction:
Nowadays, researchers are focusing on new ways to produce energy, due to the limited sources of petroleum available on the planet. Biodiesel, derived from vegetable oils, is a new kind of green fuel that is being introduced in the energy market and its production process generates large amounts of glycerol as by-product. Glycerol can be used as carbon source in microbial bioprocesses, such as the production of 1,3-propanediol. The 1,3-propanediol is used in the manufacture of plastic materials, fibres and it is easily recycled.
Klebsiella pneumoniae GLC29 was showed a good performance as 1,3-propanediol producer since it does not depend on vitamin B12 addition in the culture medium and its growth is not restricted under anaerobic conditions. The biosynthesis of 1,3-propanediol from glycerol is performed in two enzymatic steps. First glycerol is dehydrated to 3-hydroxypropionaldehyde by glycerol dehydratase encoded by *dhaB*, *dhaC* and *dhaE*. 3-Hydroxypropionaldehyde is reduced to 1,3-propanediol by 1,3-propanediol dehydrogenase (encoded by *dhaT*). The gene *dhaF* encodes a glycerol dehydratase reactivation factor and DhaR is a transcriptional regulator of the operon *dhaTBCEF*. In this study genomic data from *Klebsiella* were used to design primers for the detection of *dhaB* and *dhaT*. These primers were successfully used to detect such genes in *K. pneumoniae* GLC29.

Material and Methods:
K. pneumoniae cells were cultivated in LB medium and its DNA was extracted using the QIAprep Spin Miniprep Kit (QIAgen USA). The sequences of *dhaB* (glycerol dehydratase, large subunit) and *dhaT* (1,3-propenodiol oxireductase) were retrieved from sequenced genomes at GenBank. The primers were designed considering conserved regions using the Fast PCR software. The genes were amplified by PCR and the amplicons were separated by agarose gel electrophoresis and detected after staining with ethidium bromide.

Results and Discussion:
The primers to detect *dhaB* (dhaBF and dhaBR) were designed from genome sequences from nine *Klebsiella* spp. and two *Citrobacter* spp. Only one amplicon of 619 bp was obtained and the sequence of this fragment showed about 98% similarity to part of *dhaB* genes from other *Klebsiella*. For the amplification of *dhaT*, primers (dhaTF and dhaTR) were designed based on genome sequences of six *Klebsiella* spp. and one *Citrobacter* sp. Only one amplicon of 420 bp was obtained and its sequence presented about 98% similarity to part of *dhaT* orthologs from other *Klebsiella*. Thus, the primers designed were successfully used to detect *dhaB* and *dhaT*. These primers are now being used to detect plasmids harbouring the complete *dha* operon in a genomic library.

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DETECTION OF ROTAVIRUS AND ADENOVIRUS IN FECAL SAMPLES FROM CHILDREN HOSPITALIZED IN SÃO LUÍS, MARANHÃO, BRAZIL.

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Rotavirus has been considered a major agent of intestinal infection worldwide, especially in children residing in less developed countries. These children are generally infected in the first 5 years of life. Besides them, other viruses have also been associated with diarrhea, including the enteric adenoviruses. Thus, this study was aimed at detecting the presence of rotavirus and adenovirus in fecal samples from children with and without acute diarrhea under five years old, hospitalized in São Luís - MA. The study was conducted from May 2009 to November 2010, and evaluated the incidence and seasonality of infections in the region. The detection of rotavirus was performed by enzyme immunoassay (ELISA) and RT-PCR. Adenoviruses were detected by PCR. The χ^2 test was used in the comparison of data, with a significance level of 95% ($P < 0.05$), using the Epi Info software. Of the 105 samples analyzed, 9 (8.6%) were positive for rotavirus and 3 (2.9%) for adenovirus. The majority of rotavirus cases (66.6%, 6 / 9) were detected from May-June, i.e., during the rainy season in our region. The cases of rotavirus infection were diagnosed in all age groups, especially in children aged 6 to 11 months (33.3%, 3 / 9) and female (66.6%). The adenovirus infection showed no seasonally defined, because they were detected in months with distinct climatic characteristics. The results of this study further demonstrate the involvement of these viruses in childhood diarrhea, pointing to the need of a continuous monitoring. Additional studies can identify rotavirus groups in vaccinated children.

Keywords: Rotavirus, adenovirus, childhood diarrhea, ELISA, RT-PCR.

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DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC) OF 3,4,4' TRICHLOROCARBANILIDE AGAINST *Lactobacillus fermentum* AND *Saccharomyces cerevisiae.*

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Keywords: MIC; 3,4,4' Trichlorocarbanilide (TCC); *Lactobacillus fermentum*

Introduction: *Lactobacillus fermentum* is an important contaminant on industrial process for fuel ethanol production. TCC showed antibacterial properties against this bacteria. In this study the association of TCC with the surfactants benzalkonium chloride (BC) and sodium dodecyl sulfate (SDS) were tested in *L. fermentum´*s control.
Materials and Methods: Two strains of *L. fermentum* were used (CCT 1396 and ATTC 9338) as well as two of *S. cerevisiae* (ATCC 9763 and FCLA M26). MIC´s test used was the one described by Jones *et al* (1985) and nine different formulations of TCC were studied in association with BC and/or SDS. MIC was taken at the times 24 h and 48 h.
Results and discussion: The MIC is calculated using the TCC's dosage as reference. There weren´t difference between 24 h and 48 h. This study revealed that there is a maximum concentration of SDS and BC that could be combined with TCC aiming the selective inhibition of *L. fermentum* without affecting *S. cerevisiae*. The BC is much more lethal to *S. cerevisiae* than the SDS when combined with TCC. For the proportions of 1:0.1 up to 1:8 mixture of TCC and SDS, the lowest MIC for *S. cerevisiae* were 10 to 20 μg/mL. The combination of TCC and BC above 1:2 showed a MIC of only 2.5 μg/mL for the yeast, or 4 to 8 times lower than the SDS. In previous studies it was found that the BC has a similar MIC (8 μg/mL) to both *S. cerevisiae* and *L. fermentum* (OLIVA-NETO & YOKOYA, 1998), which explains why increasing the concentration of BC in this chemical formulation approximated the MIC between them. Moreover, whereas the formulation with TCC alone showed a MIC for *L. fermentum* of 10 - 20 μg/mL, the best combination to inhibit this bacterium was TCC and BC (1:1) with a MIC 4 to 8 times lower (MIC = 2.5 μg/mL) than only TCC. These results were higher than found by Oliva-Neto & Yokoyama (1998) who obtained a MIC of 0.5 μg/mL of TCC and SDS (1:0.1) against *L. fermentum* and more than 200 μg/mL to the *S. cerevisiae*. This difference may be due to the difficult of solubilizing the TCC at the used solvent, where the SDS did not increase the TCC's solubility, which would rise the power and configure the synergism between molecules. In conclusion, the most interesting formulation for studies for industrial application is TCC and BC (1:1). As the BC is used in cane mills in a concentration of 10 – 20 μg/mL, using the combination found in this study may decrease the quantity of BC in 4 to 8 times.

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DEVELOPMENT OF TECHNIQUE FOR DETENTION OF BIOFILM FORMED UNDER WELD SEAMS, AIMING THE STUDY OF CORROSION INDUCED MICROBIOLOGY

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Keywords: Biofilm, Microbiologically Induced Corrosion (MIC), Welding. overcomeis, ofaltering

Since the beginning of the 19th century, scientific knowledge embraces the correlation between microbial adhesion on the surface of biologically inert materials (ferrous, metallic and polymeric), biofilm formation and corrosion-inducing microorganisms. The scientific literature cites the presence of microorganisms and enzyme activity stimulates the processing of pitting corrosion, as well as, the emergence of differential aeration cells. These favor an increase in erosion and galvanic corrosion of metallic materials. Moreover, the microbial biofilms are significantly capable of altering the behavior of the phenomenon of corrosion in metal structures, holding tanks and pipelines of industrial food, pharmaceutical and other, increasing the localized changes in the type and concentration of ions, pH levels and oxygen transfer between the surface and phase Buck metal. However, this correlation is not so clear and obvious, on metal surfaces subject to metallurgical modification, which is promoted by the engineering and welding processes. Since the heat input generated on the material surface during the weld bead formation changes the metallurgical features (structure and geometry of the grain metallurgical), it is necessary to establish a correlation between the heat input from the welding process, microbial adhesion, biofilm formation and microbiologically induced corrosion. One first obstacle that must be initially overcome is related to the fact that the weakness/resistance of biofilm under a direct mechanical action brings difficult for the removal of this biofilm, which, in this case, is attached to the welded specimens. Thus, this situation would prevent the realization of microbiological analysis and metallurgical phenomena corrosion. An alternative proposed by the present work is the containment of the biofilm on acrylic resin polymethylmethacrylate, where microbial biofilm were collected from the tanks to a dairy pasteurizer Cristal in the town of Barra do Garças - MT and then stored in Petri dishes with cooling temperature until its transfer to the acrylic resin, which was prepared by adding methyl methacrylate monomer, with the biofilm and waiting about 15 minutes until the hardening resin. Once the biofilm is immobilized, it was possible to view and store the same in its original position (formation). After complete drying of the resin at room temperature showed that the biofilm remained immobile and kept their original characteristics unchanged, allowing its possible visualization by an optical microscope in the next stages of work. Therefore, it is now possible of applying this described technique on the welded metal surface (heat-affected zone and weld metal) study.

Financial Support: Thanks to the dairy Cristal / MT for access to its facilities and professional donation by Dr. Antonio acrylic resin.

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DIVERSITY AND PREVALENCE OF AIRBORNE FUNGI ISOLATED FROM SÃO LUÍS, NORTHEAST BRAZIL

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Airborne fungi are considered the main agents related to allergy in patients that suffer from asthma and rhinitis, besides being related to appearance of many mycoses. The knowledge about the prevalence of these microorganisms and their seasonal variation in a specific region can be useful for human health, by improving the diagnosis and treatment of respiratory allergic disease provoked by their inhalation. In São Luís there are no epidemiological studies related to the isolation and identification of fungi species from urban areas of the city. In the present study we investigated the prevalence, diversity and seasonal variations of airborne fungi in São Luís, MA. Air samples were taken by the Petri plate gravitational method in six urban areas, in both dry and rainy seasons of the year. Ágar Sabouraud Dextrose onto Petri dishes was used for isolation of fungi through air exposition during 15 minutes, at 1.5 meters above the ground. The colonies developed after a period of 24 hours were counted and their concentrations were calculated as CFU (*C*olony *F*orming *U*nits). The evaluation of the proportion of spores in different periods was made using the chi-square test; the average number of spores for the different seasonal periods was made by Student´s t test; the level of significance (α) used in the tests was 0.05; for the assessment of fungal diversity we calculated the diversity index of Shanon-Wiener. A total of 96 (ninety six) Petri dishes were exposed in six different urban areas São Luís, from which 2993 colonies were counted. Half of the plates were exposed in the dry period and remaining in the rainy period. During dry season 2031 (67.9%) colonies was counted and in the rainy season, 962 (32.1%). The chi-square test of those data has shown a significant difference in fungi spores concentration in those different periods. The Student´s t test of the data also showed significant differences between averages of spores in dry and rainy periods, nevertheless fungi diversity was higher in rainy period than in the dry season. Relative humidity, rainfall precipitation and wind speed can have influenced the diversity and concentration of fungal spores presents in the air. Isolated fungal genera were: *Aspergillus* (38.37%), *Fusarium* (13.90%), *Curvularia* (11.18%), *Penicillium* (10.88%), *Neurospora* (3.32%), *Acremonium* (2.72%), *Rhizopus (*2.42%), *Fonsecaea* (2.11%), *Syncephalastrum* (2.11%), *Alternaria* (1.81*%), Nigrospora* (1.81*%), Chaetomiun* (1.51%), *Scedosporium* (1.21%), *Cladosporium* (1.21%*), Mycelia sterilia* (1.21*%), Scopulariopsis* (0.91%), *Drechslera* (0.60%), *Ulocladium* (0.60%), *Mucor* (0.60%), *Aureobasidium* (0.30%), *Wangiella* (0.30%), *Paecilomyces* (0.30%), *Trichoderma* (0.30%) and *Verticillium* (0.30%). Overall such fungi are considered major triggers of allergic processes. In general the data show the presence of a large number of airborne fungi spores in São Luís and all filamentous fungi genera found in this research are opportunistic and they may cause disease in susceptible patients.

Keywords: Airborne fungi; respiratory allergies; *Aspergillus*; mycoses; seasonal variation.

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DRINKING WATER QUALITY IN TWO STATE SCHOOLS IN CUIABÁ/MT

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Keywords: drinking water quality, State School, water treatment station

The waters for human consumption are subject to direct or indirect contamination by toxic chemicals and pathogenic microorganisms, representing a risk to human health. According to World Health Organization, hundreds of thousands of people worldwide suffer from diseases transmitted by contaminated water. The State and Municipalities are responsible for providing good quality water for the population and all the operation process and supply should be accomplished with maximum accuracy, which often does not happen. The school environment is the place that requires a rigorous monitoring of the drinking water quality, allowing interventions, when necessary. Thus, the quality of water consumed in two public schools in Cuiabá/MT was monitored: Presidente Médici State School, supplied by the São Sebastião Water Treatment Station and Raimundo Pinheiro State School, supplied by the Tijucal Water Treatment Station. Water samples were collected once a week for five consecutive weeks, in the period April 28 to May 25, 2010, in five different sampling points in each school: water metering residence point (point of connection to public supply systems), entry point of water tank, exit point of water tank, drinking fountain and main kitchen faucet. The parameters analyzed were: total coliforms and *Escherichia coli* (chromogenic/fluorogenic substrates); heterotrophic bacteria (spread plate); turbidity (turbidimetry); apparent color (colorimetry); pH (potentiometry) and residual chlorine (Gen Kit). The results were compared with values established by Decree 518/2004 of the Brazilian Health Ministry. At Presidente Médici State School, total coliforms and *E. coli* were not detected. The values of heterotrophic bacteria were higher than the limit established by legislation (500 CFU/mL) in the drinking fountain in the first week (520 CFU/mL) and entry point of water tank in the fourth week (1875 CFU/mL). The turbidity values ranged from 0.23 to 2.69 NTU and were not above the maximum (5.0 NTU) allowed by Decree 518/2004, while 41.7% of the samples to apparent color exceed the permissible values (15 uH). The pH values ranged from 6.12 to 6.98. By analyzing the residual chlorine, 66.7% samples were above 2.0 mg/L, maximum value allowed by law, and may be related to the proximity of the school with the water treatment station. At Raimundo Pinheiro State School, total coliforms was detected at the water metering residence point in the first week of collection (14.6 NMP/100mL), and the values of heterotrophic bacteria were above the limit set by legislation (500 CFU/mL) in the second, third, fourth and fifth week in the drinking fountain (520; 825; 1570 and 860 CFC/mL respectively) and in the fourth week in the water metering residence point (4785 CFU/mL). The turbidity values ranging from 0.33 to 1.76 NTU and two samples to apparent color were above 15 uH (17 uH in the entry point of water tank and 18 uH in the main kitchen faucet). The pH values ranged from 6.22 to 7.16. According to the values obtained to residual chlorine, 68.0% of the samples were below 0.2 mg/L. It is recommended periodic cleaning and replacement of the filters from fountain drinking and kitchen faucet because they can be sources of heterotrophic bacteria in water as noted in the Raimundo Pinheiro State School. It is necessary interventions at the Tijucal WTP to ensure that water supply to the same school contains minimal residual chlorine indicated by the legislation.

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ECO-FRIENDLY PRODUCTION OF ASTAXANTHIN BY *Mucor circinelloides* USING AGROINDUSTRIAL RESIDUES

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Keywords: cassava wastewater, corn steep liquor, carotenoids.

With the growing production of industrial residues, also increases the concern about the more efficient use of the same. Therefore, several processes that use these wastes as substrates are being developed for the production of chemicals and byproducts of higher commercial value. Thus, many industries have invested in the use of microorganisms as agents reducing these wastes for the production of various substances (enzymes, carotenoids, antibiotics). Carotenoids can be biosynthesized by photosynthetic microorganisms (algae and cyanobacteria) and non-photosynthetic (bacteria, yeasts and fungi). Astaxanthin is a carotenoid employed in the aviculture and aquaculture of fish and crustaceans, added to feeds in order to improve the colour, increasing the quality and consumer acceptance in the marketplace. By having the chemical synthesis of complex and costly, there is great interest in using the same biological sources of astaxanthin, using microalgae and fungi as producers. This study aimed to investigate the use of industrial residues in different concentrations on astaxanthin production by *Mucor circinelloides* with the aim of formulating a medium low cost. The production of astaxanthin was carried out using *Mucor circinelloides* grown on Pedri dishes containing PDA (Potato Dextrose Agar) incubated at 28 °C, during 5 days. After this period spores was collected and was used as inoculum to Erlenmeyrs flasks containing 100 mL of medium using industrial residues cassava wastewater and corn steep liquor in different concentrations (4%, 7% and 10%), and pH 6.5. The flasks were incubated for 96 hours, in orbital shaker at 120 rpm, at 25 ° C. The flasks were submitted to exposition of lights, no lights (dark), and under the influence of white light. The pigment astaxanthin was extracted using DMSO and acetone solvents, and the yields were determined by spectrophotometer UV-Vis (470 nm). From the cultures performed, it was found that there was no production of astaxanthin by *Mucor circinelloides* in all conditions, with the maximum content of astaxanthin (27.49 μg/g) obtained from cassava wastewater with corn steep liquor 4%, under the influence of white light. The results show that both the low concentrations of industrial residues as the incidence of white light were favorably to the production of astaxanthin. Thus, the industrial residues cassava wastewater and corn steep liquor has the potential to be used as medium of low cost, on astaxanthin production by *Mucor circinellides*.

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Economic production of astaxanthin by *Mucor circinelloides* (ucp-69) using sugarcane molasses

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Keywords: *Mucor javanicus*, astaxanthin, light blue.

Carotenoids are the most widely distributed class of pigments in nature and have essential biological functions in animals. Astaxanthin (3.3'-dihydroxy-beta, beta-carotene-4,4'-dione) is the principal carotenoid pigment of salmonids and gives attractive pigmentation in eggs, flesh and skin. Additionally, a beneficial role of astaxanthin as food supplement for humans has been suggested. However, each natural pigment source has its limitations and they currently cannot compete economically with the synthetic additive. A brief exposure to light results in a substantial synthesis of carotenoids by microorganisms. In this way, the aim of this work was to analyze the potential of sugarcane molasses in the production of astaxanthin by *Mucor circinelloides* (UCP-69). The fungus *M. circinelloides* (UCP-69) belongs to the Culture Collection of the Universidade Católica de Pernambuco (UCP), registered in the World Federation Culture for Collection-WFCC. For the production of astaxanthin spores were inoculated in 100mL of the medium of molasses (molasses and distilled water in the concentrations of 4%, 7%, and 10%), pH 6.5, during 96h, using blue LED´s. After this period of the fermentation the biomass was separated by centrifugation and submitted to the extraction of astaxanthin. The cells were disrupted using DMSO and acetone solvents, and the astaxanthin obtained by centrifugation at 2000 rpm/10 min. The content of astaxanthin was measured in spectrophotometer and absorbance of 470 nm. The best results of astaxathin were obtained in 10% molasses corresponding to 23.7 μg/g of biomass. These results corroborated with the literature, which utilized molasses for the production of carotenoids by *Xanthophyllomyces dendrorhous*, and indicate it is an excellent medium for carotenoid production. The economic medium using molasses as carbon source showed excellent production of astaxanthin by *M. circinelloides*, additionally with cost reduction.

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ECOTOXICOLOGICAL ASSAY AND MICROBIOLOGICAL ANALYSIS OF SEWAGE SLUDGE OF TWO WASTEWATER TREATMENT PLANTS FROM CAMPINAS

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Keywords: coliforms, composting, *Salmonella*, sludge, *Vibrio fischeri*.

An alternative to the final disposal of sewage sludge is its use in agriculture that allows the recycling of nutrients, besides facilitating the improvement of soil physical conditions and the reduction of the use of space in landfills. For these purposes, composting can be used as a post treatment. In order to achieve further composting, sludge samples were collected in two wastewater treatment plants from Campinas/SP: Samambaia and Piçarrão. Piçarrão WWTP's flow is 556Ls^{-1} and the quantity of sludge produced by anaerobic reactor is 10 tons per week. Samambaia WWTP's flow is 204 Ls^{-1} and 70 tons are generated per week by an aerobic reactor. Samples were collected after centrifugation and dehydration. In order to have the sludge microbiological characteristics, the parameters analyzed were *Salmonella* and Total and Thermotolerant coliforms. In addition, it was used the acute toxicity assay in *Vibrio fischeri* bacteria to characterize the sludge´s toxicity. The methodologies used were 1-2 Test Salmonella (AOAC Official Method nº 989.13), Standard Methods 9223 B (Enzyme Substrate Test) for coliforms test and Brazilian technical standard L5.227 for acute toxicity test in *Vibrio fischeri*, whose value is expressed as EC 50 (%), which represents the concentration of sludge that cause inhibition or decrease in bacteria's fluorescence by 50%. After that, the sludge was classified according to Brazilian law CONAMA 375/2006, using as parameters coliforms and *Salmonella*. Results showed there is no *Salmonella* bacteria in both sludge analyzed and the concentrations of total coliforms were 4,2 x 10^9 NMP / g de ST and 1,1x10^9 NMP / g de ST for Piçarrão's and Samambaia's samples, correspondingly; thermotolerant concentrations were 6,5 x 10^7 NMP / g de ST for Piçarrão's sludge and 1,4 x 10^7 NMP / g de ST for Samambaia's. Therefore, according to these results and CONAMA 375/06, both sludges can't be used in crops because of the concentrations of coliforms. Regarding the acute toxicity test in *Vibrio fischeri*, the mean and standard deviation of the EC 50´s values were 10.81 ± 5.58 (n = 5) for Samambaia´s sewage sludge and 15.69 ± 19.75 (n = 5) for Piçarrão´s sewage sludge. The fact that the standard deviation was larger than the mean value in Piçarrão's sludge samples demonstrates the great variability of the values found. In addition, the mean values found in the samples of both sludges are very low, which sets very toxic sludge samples. So it's necessary to have another treatment, for example composting, to decrease the concentration of coliforms and toxicity for later use in solo.

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EFFECT OF CADMIUM ON THE EXPRESSION OF ENZYMES OF *Rhizopus arrhizus*.

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Keywords: Cadmium; *Rhizopus arrhizus;* enzymes.

Cadmium is an of heavy metals most dangerous to living systems, its toxicity depends on its ability to change the dynamic life processes in biological systems by combining the macromolecules and metabolites, resulting in changes in growth and activity of enzymes (JARUP & AKESSON, 2009). The correlation between the expression of enzymes and ability to tolerate / withstand harsh environments contaminated with heavy metals has been presented (GADD 2010). This study aimed to evaluate the effect of cadmium on the activity of phenoloxidases, tannase, cellulase and amylase of *Rhizopus arrhizus.*
Culture discs grown in medium Synthetic for Mucorales was inoculated in nutrient agar medium containing cadmium at concentrations of 0.5 mM, 1mM and 2mM, the indicator for phenoloxidase was gallic acid, tannic acid for tannase, cellulose for cellulase and starch for amylase. Cultures were incubated at 28 ° C for 5 days. After this period the activities were revealed by the formation of halos. The results are expressed as the mean diameter of halos (in millimeters) of three experiments.
The strain was capable of expressing all the enzymes tested. The enzyme activity was detected in all treatments. Exposure to cadmium induced reduction in the expression of enzymes in relation to control culture. However, the activity is related to the concentration of cadmium and enzyme type. The literature shows that heavy metals influence the activity of extracellular enzymes of microorganisms. However, the data presented are the first to report such activities in *R. arrhizus* in response to cadmium exposure.
The strain showed positive results for enzyme activity. In the presence of cadmium, the expression of enzymes is reduced as a function of concentration of the metal.

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EFFECT OF CARBON AND NITROGEN CONTENT IN THE EXPRESSION OF ENZYMES PARTICIPATING IN THE BIODEGRADATION OF TEXTILE DYES IN TWO LIGNINOLYTIC FUNGI.

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CEP: 81530-900

Keywords: *Lepista sordida*, *Lentinus crinitus*, textile dyes, biodegradation, laccase, expression profile.

Successful bioremediation of soil and water ecosystems polluted by toxic recalcitrant compounds depends on the availability of appropriate microorganisms and their enzymes. Lignolytic basidiomycetes exhibit an exceptional ability to degrade lignin and many structurally-related pollutants, this ability is related to the production of lignin-modifying enzymes. A key role in the quantity and kind of enzyme produced is the source and concentration of carbon and nitrogen present in the culture medium. In this work we assessed the effect of carbon and nitrogen content of the culture media on the expression of destaining activity, protein profile, and laccase activity by *Lepista sordida* and *Lentinus crinitus*. There were tested six carbon (glucose, fructose, starch, maltose, sucrose and glycerol) and nitrogen sources (sodium nitrate, ammonium tartrate, ammonium carbonate, ammonium oxalate, peptone and urea). The evaluated concentrations were 5, 10 and 15 $g.L^{-1}$. Cultures were incubated for 15 days at 28°C in the dark and the supernatants collected for analysis. Lac activity was determined by monitoring the oxidation of ABTS at 420nm. The destaining activity assay measured the consumption of the dye, over a 90-min period, when samples were added to a RB220 solution, at final concentration $0.1g.L^{-1}$. In order to monitored enzyme activity in gel, samples were subjected to electrophoresis on 12% SDS-PAGE gels, soaked in renaturation buffer overnight and then rinsed in 50mM acetate buffer pH 5.0 containing either 0.05mM ABTS or 0.01% guaiacol and incubated overnight at 28^oC. Replicate gels were developed by silver staining to verify the protein expression profile. In *L. crinitus* the highest levels of destaining activity were obtained when grown in 10 $g.L^{-1}$ glucose (85%). With some carbon sources, like as starch and glycerol 15 $g.L^{-1}$, the yield of destaining activity was very low. When nitrogen sources were assessed using 10 $g.L^{-1}$ glucose as carbon source, again some substrates promoted the production of destaining activity and some of them, such as urea and ammonium chloride, were inhibitory. For *L. sordida* drastic differences in the production of destaining activity were observed. The best production of destaining activity (57%) was promoted in cultures containing 5 $g.L^{-1}$ sodium nitrate with maltose 5 $g.L^{-1}$, as carbon source. In gel activity assays and silver stained SDS-PAGE gels revealed, in *L. crinitus*, a unique band of 41 kDa displaying phenol oxidase activity, suggesting that only one enzyme is involved in the process. Differently, *L. sordida* zymograms revealed that two polypeptides of 48.5 and 36.5 kDa were responsible for lac activity. Zimograms also revealed that dye destaining and phenol oxidase activity are related to the same polypeptide. Protein secretion profile also suffered drastic changes according to culture content. These results follow the general assumption that there is not a unique standard culture condition to enhance the production of lignolytic enzymes and it must be experimentally established for each kind of enzyme and fungal isolate.

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EFFECT OF DIFFERENT PLANT EXTRACTS ON MYCELIAL GROWTH, SPORULATION AND GERMINATION OF MANGO *Colletotrichum gloeosporiodes* ISOLATES (M*angifera indica* L.).

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Keywords: experiments, *Colletotrichum gloeosporioides*, mango.

The mango tree culture (*Mangifera indica L.*) has a broad adaptation to climatic conditions in Northeastern Brazillian region, becoming one of the symbols of economic development. However, many are the problems found in this culture`s production chain, one of the most important being the attack of phytopathogenic microorganisms. Among them, the anamorphic *Colletotrichum gloeosporioides* of *Glomerella cingulata* stands out.
The experiments were performed on UFAL`s (Universidade Federal de Alagoas) CECA (Centro de Ciências Agrárias) Lab of Phytopathology and Food Analysis. The *Colletotrichum gloeosporioides* isolates were obtained from mango fruits that featured symptoms of anthracnose. The fruits were washed with soap and water and then fragments of the lesions were removed, treated with alcohol 50%, sodium hypochlorite 25% and two portions of sterile distilled water. The fragments were tagged on a potato-dextrose-agar environment and incubated at ±2 °C until the fungus growth. To prepare the extracts, 65g of each plant "in natura" were shredded and immersed in ethanol for 48 hours, filtered in gauze and concentrated on a rotating evaporator until dryness. Over the obtained residue, 30mL of a Tween solution were added on 20 to 10%. The plants that were used in the extract preparation were the *Solanum paniculatum* (Jurubeba), *Fleurya aestuans* L. (Nettle), *Azadirachta indica* (Neem) and *Momordica charantia* L. (Bitter Melon). In order to determine the concentration to be used on the "*in vitro*" experiment, a Tukey test was applied for the last measurement`s averages. On the "*in vivo*" experiment, the data was submitted to analysis of variance and the Duncan test was applied for the 5% probability averages, utilizing the SAEG program.
On isolating *C. gloeosporioides*, pathogenicity about the mango fruit was obtained, confirming the Koch postulates. Mycelial growth showed a significant reduction at different concentrations of the plant extracts. All the extracts inhibited the germination, without the occurrence of significant variations relating to the different tested concentrations. On the 5% Duncan test it was observed that the Bitter Melon extract had a greater effect on the pathogen inhibition *in vivo*. The rest didn`t statistically differ from each other.

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EFFECT OF INOCULUM IN THE EMULSIFICATION INDEX OF BIOSURFACTANTS PRODUCED BY *BACILLUS VELEZENSIS* USING WHEAT BRAN AS SUBSTRATE IN SOLID STATE FERMENTATION.

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Keywords: *Bacillus velezensis*, biosurfactant, solid state fermentation.

Some compounds of microbial origin exhibit surfactant properties and are known as biosurfactants, which are byproducts of bacteria, fungi and yeasts metabolism. The present study aims to evaluate the effect of the pre-inoculum in emulsifying activity and biosurfactants production by *Bacillus Velezensis* in solid state fermentation using wheat bran as substrate. three types of inoculum were used in the fermentation: pre-inoculum prepared with wheat bran as substrate, submerged pre-inoculum prepared with nutrient broth and direct addition of bacteria suspended in slant nutrient agar. Microorganisms were grown in tubes with nutrient agar at 37°C for 24 hours. After this period, a cellular suspension was made, and 2 ml of this served as inoculum to the wheat bran without pre-inoculation medium and 1 ml for the preparation of liquid pre-inoculum (50 ml of nutrient broth, kept in agitation at 37°C for 24 hours) and solid pre-inoculum (10g of wheat bran with buffer and glycerol, incubated in Erlenmeyer flask at 37°C for 24 hours). From the pre-inoculum broth, it was transferred 2 ml of cell suspension to plastic bags containing 10g wheat bran moistened to a relative humidity of 80% with phosphate buffer solution and glycerol as inducing production of biosurfactant and incubated for 96 hours at 37°C. Extraction of cell-free broth of fermentation media was carried out with 100 ml of water heated to 90°C and agitation at 200 rpm in Shaker for 60 min. Then, the sample was vacuum filtered and the extract used for the evaluation of the emulsifying activity. The emulsifying activity was determined by adding toluene (2 ml each) in the culture broth free of cells (3.5 ml) in test tubes followed by stirring at high speed in a vortex for 2 min, subsequently performing the optical spectrophotometer at 620 nm. The extract which had the direct addition of the microorganism obtained absorbance of 2.1, that with the submerged pre-inoculum in nutrient broth obtained 2.4 and with fermented wheat bran inoculum an absorbance value of 2.1. The results indicated that there was no significant difference in the biosurfactant production in different types of pre-inoculum. It was concluded that there is no need to carry out pre-inoculum for the tested conditions..

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EFFECT OF MICROCYSTIN TOXICITY IN BIOASSAYS WITH PLANARIANS *Dugesia tigrina*

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Water bodies are subject to processes of pollution through effluent discharges untreated domestic and industrial, whose combination is hard to control and to manage. Several water sources are subject to eutrophication processes due to sources of pollution, whose main consequence is the dominance of potentially toxic cyanobacteria, affecting the potability of water and can damage the environment and human health. The detection of toxic agents in water bodies is difficult and expensive, and determining the potential of a water sample can be made indirectly, using indicator organisms for contamination. The planarians (*Dugesia tigrina*), due to its ease of cultivation, regenerative activity and sensitivity to environmental variations, are organisms with potential for ecotoxicological tests. Potassium dichromate ($K_2Cr_2O_7$) is an inorganic salt soluble in water, used as an oxidizing agent in chemical industry. May be carcinogenic and causing several damages to human health and the environment, small concentrations can disturb different organisms. Cyanobacteria are photosynthetic organisms and prokaryotes, with about 40 known species that produce toxins. The species *Microcystis aeruginosa* is one of the hepatotoxin microcystin producing genera, since there are reports of liver tumors promoted by frequent ingestion of this toxin. The release of toxins in the environment occurs after cell lysis, which may occur during the water treatment system, justifying the adoption of appropriate techniques for detection, treatment and management of these microorganisms. Therefore, the objective is to investigate the potential use of planarians as a biological model in ecotoxicological bioassays using known reference substance (potassium dichromate) and toxic strains of *Microcystis aeruginosa*. Bioassays animals were used 8 to 12 mm (adults), were maintained under constant temperature (22 º C) and without food 72 hours before and during testing to standardize the physiological conditions, animals are separated and is made removing the region cephalic animals in the posterior portion of the auricles with the aid of a scalpel and magnifying glass. Preliminary testing of standardization of cephalic regeneration of the animals under standard conditions, with the endpoint of the test in the formation of eyespots and auricles, determined that the average time of regeneration was 6 days. The planarians subjected to chronic tests (9 days) indicated that the presence of the reference substance (potassium dichromate) leads to a delayed regeneration time. In sensitivity tests (acute) with potassium dichromate at different concentrations, the LC50 value obtained for this kind of flatworm was 27.49 $mg.L^{-1}$ at pretest and 34.16 $mg.L^{-1}$ in repetition, indicating that planarians are more sensitive than some species of fish and aquatic insects widely used in ecotoxicology testing. Tests with toxic strains of *M. aeruginosa*, subjected to cell lysis, indicate that the presence of these toxins compromise the head development of the planarians, as well as anomalous behavior, such as hypersensitivity. The results indicate that bioassays with planarians may be an alternative for drug testing and an indication of the presence of cyanotoxins.

Keywords: planarian, cyanotoxin, biological model, bioassays, ecotoxicology.

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EFFECT OF MICROORGANISMS CO-INOCULATION IN CULTURE MEDIUM ON THE CALCIUM PHOSPHATE SOLUBILIZATION

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Keywords: phosphorus, acidity, acid phosphatase, glucose.

Some microorganisms species are presumed to be efficient in inorganic phosphate solubilization, due the organic acids production of low molecular weight, chelation, ion exchange and enzymes action such as acid phosphatases (Chaiharn & Lumyong, 2009, Rodríguez et al, 2006). Aspergillus niger has demonstrated a high ability to calcium phosphate and aluminum solubilize, as well the bacterium *Burkholderia cepacia* (Barroso & Nahas, Oliveira et al, 2009). Several studies have been performed to quantify the individual skill sets and direct inoculation with these microorganisms in vegetables (Afzal, 2008; El-Azon, 2008). Considering the fact that microorganisms live associated and that harmony can interfere with each species behavior, this work aimed to study the effect microbial co-inoculation on solubilization of calcium phosphate in vitro, to verify the potential of these organisms together. The microorganisms were grown for nine days in liquid culture medium with insoluble calcium phosphate and glucose as carbon source. Four treatments with three replicates were incubated: No Inoculum, Co-inoculation (A. niger + *B. cepacia*), *Aspergillus niger* and *Burkholderia cepacia* and withdrawn four times (day of inoculation, third, sixth and ninth days). During the nine days incubation, co-inoculation and the fungus solubilized the same amount of Pi, the growth was similar in both with equal reduction in medium culture pH. Several studies have shown the greatest ability of *Aspergillus niger* in releasing inorganic phosphate compared to other fungi and bacteria (Saber, 2009; Souchier, 2007). The acid phosphatase activity was higher in *B. cepacia* until the sixth day of growth, although this enzyme activity is correlated to phosphate solubilization exclusively on co-inoculation. Both, co-inoculation and bacteria consumed the greatest amount of glucose, so that there was a negative correlation between glucose consumption and increased dry weight. On the other hand, correlation wasn't found between soluble P and the growth of microorganisms co-inoculated, suggesting that in conditions where there is readily available sugar, calcium phosphate isn't used for the microorganisms development. This result was also found by Xie et al (2009). This study demonstrated that co-direct inoculation of different microbial species in the plant in order to potentiate the insoluble phosphate solubilization can't be effective, so that the microorganisms have specific requirements for their growth and performance of their skills.

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EFFECT OF SURFACTIN AND CYCLODEXTRIN ON PHENANTHRENE SOLUBILITY.

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The degradation of polycyclic aromatic hydrocarbons (PAH) in soil and water is hindered by their low bioavailability, which is related to their high soil sorption and low solubility in water. Surfactin is a biosufactant produced by some species of the genus *Bacillus* that decreases the surface tension of water and increases the solubility of hydrophobic organic compounds (HOC). The ciclodextrins (CD) are cyclic oligosaccharides with a hydrophobic cavity and a hydrophilic shell. They are water-soluble and have the ability to form inclusion complexes, incorporating hydrophobic molecules in their hydrophobic cavities. These two compounds may be used to enhance the biodegradation of PAH by mean of the increase bioavailability of these contaminants. The aim of this work was to evaluate the effect of a biosurfactant and a synthetic solubilizer on the solubility of phenanthrene.

Briefly, 5 mL of phenanthrene in acetone were added in triplicate to 15 mL glass tubes for each treatment. The acetone was evaporated leaving a mass of 0,25 mg of phenanthrene. The CD utilized on this experiment was Hydroxy propil β-cyclodextrin (HPCD). A 10 mL aliquot of water containing no surfactin or HPCD was added to three tubes as the controls. To evaluate the activity of HPCD, we used concentrations based on its solubility (18g/L) (10, 50, 100, and 150%). The surfactin was produced by the *Bacillus subtillis* strain 155 from the LBBMA collection. The concentrations of surfactin were chose based on its critical micellar concentration (CMC) nominally 0,5x, 1x, 5x and 10x CMC. The tubes were shaken for three days at 30^{o} C, and allowed to settle (4 hours). 1 mL of each sample was quantified by high performance liquid chromatography (HPLC).

The solubility of phenanthrene in the control was 0,004 mg/L. In the treatments with HPCD the solubility were, in crescent order from 10 to 150%, 0,04 mg/L, 0,86 mg/L, 4,29 mg/L and 5,31 mg/L. The solubility in the treatments with surfactin were, in crescent order from 0,5 to 10x CMC, 0,009 mg/L, 0,01 mg/L, 0,07 mg/L and 0,17 mg/L. The effect of HPCD was more expressive than that obtained with by the surfactin, although, the concentration of HPCD was much higher. The increase obtained in the treatment with HPCD shows a sigmoidal pattern which represents a reduction of its activity, while the results of surfactin showed a linear regression with r^2 of 0,989. It indicates that the increase of surfactin above 10x its CMC may increase phenanthrene solubility beyond the value obtained in this study.

Gao *et* al. (2003) tested the biodegradation of HPCD by bacteria isolated from soil. The author pointed out that HPCD may thus become another pollutant when used for HOC washing. Lima *et* al. (2011) evaluated the toxicity of biosurfactants and synthetic surfactant (SDS) on Vibrio fishery, and observed that the biosurfactants were significantly less toxic than the SDS.

Keywords: Biosurfactant, HPCD and PAH.

Gao, Y. S. S.; Cai, H. Z. B.; Wang, L. Influence of nonionic surfactants and hydroxypropyl-b-cyclodextrin on the biodegradation of nitrobenzene. **World Journal of Microbiology & Biotechnology**. N. 19. Pag. 783–790, 2003.

Lima, T. M. S.; Procópio, L. C.; Brandão, F. D.; Leão, B. A.; Tótola, M. R.; Borges, A. C. Evaluation of bacterial surfactant toxicity towards petroleum degrading microorganisms. **Bioresource Technology.** N. 102. Pag. 2957–2964, 2011.

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ENUMERATION OF EPIPHYTIC BACTERIA GROUPS ASSOCIATED WITH FRUIT OF BRAZILIAN SAVANNA-LIKE CERRADO

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Keywords: Bacterial group, Fruit, Savanna-like, Cerrado

The Brazilian Cerrado is a savanna-like vegetation and is the second largest biome in Brazil covering an area of approximately 2,000,000 km^2 corresponding to 24% of the country's area. It is estimated that there are 160,000 species of plants, fruits and microorganisms, many of which are endemic to this biome. Within this context, our aim was to enumerate the epiphytic bacteria groups associated with fruits of Brazilian savanna-like Cerrado of Minas Gerais State. Fruits samples were collected from three cities (Arcos, Passos and Luminárias) in Minas Gerais state of Brazil. The fruits sample were placed in sterile Nasco® plastic bags and stored at 4°C until further use. The bacterial groups were enumerated by the surface spread and pour plate techniques, plating in triplicate 100 µl of each diluted sample. Enumeration of microorganisms was carried out using three different culture media, Nutrient Agar medium (Oxoid, S/P, Brazil) and Eosin Methylene Blue Agar (EMB) (Oxoid, S/P, Brazil) and De Man, Rogosa and Sharpe Agar (MRS) (Oxoid, S/P, Brazil), supplemented with 0.4 mg/ml nystatin (Sigma, St. Louis, USA). After spreading, plates were incubated at 28°C-30°C for 48 h for mesophilic bacteria, and 37°C for 48 h for *Enterobacter*. Colony forming units (log c.f.u./g) were quantified. For each type of medium containing isolated colonies, the square root of the number of colonies was taken at random for identification. Mesophilic bacteria was the most frequently found microorganism group, showing an population of 12.41 log c.f.u./g. *Enterobacter* also showed growth of 7.72 log c.f.u./g. A total of 1749 isolates were obtained from 33 samples of fruits analyzed. Our data indicated that the fruits of Cerrado contained bacterial groups different. The present study is an initial knowledge of the epiphytic bacteria groups of the fruits of Brazilian savanna-like Cerrado of Minas Gerais State, which have not been previously elucidated. Future work involving the identification of these isolated for species level and the evaluation of the biotechnological potential of this bacterial community as well the construction of Bacterial Metagenomic Library will be accomplished.

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ENZYMATIC EXPRESSION OF *Mucor javanicus* AS RESPONSE TO EXPOSURE TO RED REMAZOL.

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Keywords: Reactive Red; Dye; *Mucor javanicus*; enzymes.

The textile effluents induce acute toxicity. Substances like dyes modify the metabolic and enzymatic activities of living systems. The presence of azo dyes has been widely studied in order to understanding its potential of pollution. The use for microorganisms has been promoted as a treatment for textile wastewater. Recently there has been a growing interest in studying the enzymes of fungi due to their potential biotechnological applications, including bioremediation, where the bioremediation process is related to the presence of enzyme complexes, which can degrade a large variety of compounds. This study aimed to evaluate the effect of Remazol Red on the growth and activity of phenoloxidases, tannase, amylase and cellulase from *Mucor javanicus*. Discs of cultures obtained from Synthetic Medium for Mucorales were inoculated onto the center of Petri dishes containing nutrient agar and Remazol Red in concentrations of 0,01%, 0,001% and 0,0001%. The plates were added of gallic acid, tannic acid and carboxymethylcellulose as specific substrates for phenoloxidase, tannase and cellulose. Cultures were incubated at 28 ºC for 5 days. After this period the activities were revealed and measured by the formation of inhibition halos around colonies. The results are expressed as the mean diameter of halos (in millimeters) of three experiments. The enzymes activities corresponded to halos, clear zones, which means the reduction of color and that the dye was degraded. The results revealed that the isolate was able to grown in remazol red presence and expressed all enzymes tested. In response to exposure to the dye, the strain showed enzymes activities significantly higher than the control culture. Indeed, the activity varied with the concentration of Red Remazol and type enzyme. The isolate was able to grow in the presence of different concentrations of azo dyes, and additionally, the expression of the enzymes was directly influenced by their concentration. These results are corroborated buy the literature, which shows that the exposure to dyes influences the enzymatic activity of microbial cells, suggesting its potential activity in the bioremediation of xenobiotics. The data presented are the first to report such activities in *Mucor javanicus.*

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ENZYMATIC HYDROLISIS OF CELLULOSE COUPLED WITH BIOHYDROGEN PRODUCTION BY ANAEROBIC MICROBIAL CONSORTIUM

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Keywords: *hydrogen, cellulase, fermentation, rumem fluid, DGGE*

Hydrogen (H_2) is a clean energy source with high energy yield (122 KJ/g) compared with fossil fuels. Biohydrogen holds the promise for a substantial contribution to the future renewable energy demands. Among various hydrogen production processes, biological method is known to be less energy intensive, since they are carried out at ambient temperature and pressure. As cellulose is the most abundant renewable natural resource and substrate, the conversion of cellulose to hydrogen has attracted attention as a means of biological hydrogen production. In this study, we investigated the microbial population in hydrogen-producing microflora that was enriched from rumen fluid by using cellulose as substrate. The rumem fluid cells were submitted to acid treatment and were incubated at 37^o C in modificated Del Nery medium added of cellulose (4g/L) + cellulase. It was also made a control without cellulase. The microbial community in the microflora was investigated by denaturing gradient gel electrophoresis (DGGE) of the PCR amplified 16S rDNA fragment with set primer to Domain Bacteria (968FGC - 1392-R). The biogas and the organics subproducts generated were determined by gas chromatograph (Shimadzu GC). The consumption of cellulose was analyzed by using the phenol-sulfuric acid method. The maximum cumulative hydrogen production was detected after 74 h of incubation (8,7 x 10^6). Hydrogen gas was evolved with the formation of acetate (12%), butyrate (63,7%), ethanol (11,4%), acetone (8,3%) and n-butanol (11%) by decomposition of cellulose powder. Microscopy analysis showed a predominance of rods with endospores, both Gram-positive and Gram-negative. The analysis using PCR-DGGE technique based on 16S rDNA sequences show that the microbial community in the biohydrogen production process from inoculum, reactor sample and control sample is significantly different. The similarity coefficient was 30% between the inoculum and the reactor sample, and 79% between the inoculum and the control sample. It was noted the predominance of some bands in the samples, which indicates different dominant populations among inoculum, reactor and control samples. The results demonstrated that cellulose is a potencial substrate for hydrogen production, especially when combined with cellulase. In addition, the rumen fluid was satisfactory to enhance anaerobic microbial community capable of produce H2 through cellulose degradation.

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ETHANOL PRODUCTION BY *ZYMOMONAS MOBILIS* DURING SUCROSE FERMENTATION: OPTIMIZATION OF CULTURE CONDITIONS USING FACTORIAL DESIGN

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Current interest in ethanol as a potential fuel has stimulated research on various aspects of the fermentation process. Different techniques for increasing productivity, such as continuous culture, cell recycle amd vacuum distillation, have been evaluted, but another important consideration is the improvement of the fermenting organism to give maximum productivity. One of the most promising ethanol-producing organisms is the bacterium *Zymomonas mobilis*. *Zymomonas mobilis* is a unique bacteria among the microbial world, with peculiar growth, energy production and responde to culture conditions, causing a great interest in scientific, biotechnological and industrial fields. The bacteria`s ability to make possible energy production in favor of product formation, respond to physical and chemical environmental manipulation as well as its limited product formation make it an ideal microorganism for the study and development of microbial processes for ethanol production. The aim of this work was analyse the optimum operational conditions for the ethanol production by *Zymomonas mobilis* CCT 4494. Inoculum was prepared from activated culture using Erlenmeyer flasks containing 50 mL of fermentation medium. The flasks were placed on orbital shaker (model MA 830) under controlled temperature (30°C), 200 rpm, during a period of 24 hours. The pre-fermentation medium was composed in gL^{-1} by: yeast extract 5.0, KH_2PO_4 1.0, $(NH_4)_2SO_4$ 1.0, $MgSO_4.7\ H_2O$ 1.0 and sucrose 1.0. The medium for ethanol production had the same composition of pre-fermentation medium, differing only in concentration of sucrose and nutrientes. The fermentations were carried out batchwise in Erlenmeyer flasks containing 50 mL of fermentation medium, placed on orbital shaker (model MA 830) under controlled temperature, 200 rpm, during a period of 24 hours. The *cellular biomass* was determined in a spectrophotometer (model Cintra 5 UV-VIS "DoubleBeam") based on 660 nm an calibration curve (Calazans *et al*., 1997). *Ethanol* was determined by gas chromatography using Chromatograph - HP-5890 Series II - detector FID (Flame Ionization Detector).The experiments were performed under the principles of statistical methodology of response surfaces (Box; Hunter, 1978). For the experimental design, we used the software *Statistica 6.0*, from a factorial design 2^{7-2}. The independent variables were:pH; temperature, KCl, K_2SO_4, $MgSO_4$, $CaCl_2$ and Sucrose. The bacterium *Zymomonas mobilis* CCT 4494 well adapted in fermentation medium containing high concentrations of sucrose and tolerated pH and temperature variations. The optimum conditions were pH 8; 40°C; KCl (18 $g.L^{-1}$); K_2SO_4 (5 $g.L^{-1}$); $MgSO_4$ (5 $g.L^{-1}$); $CaCl_2$ (1 $g.L^{-1}$) and sucrose (250 $g.L^{-1}$), resulting in a maximum ethanol concentration of 76.6 $g.L^{-1}$. Observed in the analysis of variance (ANOVA) that the independent variables significant (p<0.05) were: temperature, KCl and sucrose, with a positive (coefficient>*0*) and K_2SO_4 and $CaCl_2$, with negative influence (coefficient <*0*) on the ethanol production.

Keywords: ethanol, *Zymomonas mobilis*, factorial design, sucrose, fermentation

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EVALUATION OF ANAEROBIC MICROORGANISMS IN MEDIUM WITH SULFATE

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This study compared two sulfate medium by characterization of anaerobic microorganisms by the use of traditional techniques and molecular biology. The study of these organisms is of great interest in the treatment of wastewater, domestic wastewater, bioremediation of areas contaminated by oil, mainly due to their metabolic versatility. MPN values of sulphate reducing bacteria (SRB) were 1.0E+08 and 4.9E +07 for Postgate C and Zinder medium respectively. However, for methane-producing archaea (MPA), the values were of the same magnitude of 2.8E+07 and 3.5E+07 for Postgate C and Zinder medium, respectively. In the quantification of total anaerobic microorganisms (TAM) values were 2.2E+10 for medium Postgate C and 1.8E+11 for medium Zinder. Bacteria identified by sequencing 16S rRNA gene in the experiment of MPN from the medium Zinder (dilution 10^{-7}) were related to Acinetobacter sp. (69%), Dechloromonas sp. (17%), Desulfovibrio sp. (6%), Clostridium sp.(6%), Sulfurospirillum sp. (3%). The medium Postgate C (dilution 10^{-5}) was Veillonella sp. uncultivated (64%), Desulfovibrio sp. uncultivated (21%), Clostridium sp. uncultivated (10%) and Citrobacter freundii (5%). Clostridium sp. may have been responsible for the conversion of lactate to butyrate, propionate and/or ethanol; Veillonella may have been responsible for the conversion of lactate to butyrate, propionate, acetate, CO_2, and H_2; whereas Desulfovibrio sp. probably were responsible for the sulfate reduction to sulfide using lactate, propionate, butyrate as carbon source. The Dechloromonas sp. can be metabolized butyrate, propionate and acetate. The Acinetobacter sp. and Sulfurospirillum sp. possibly used the lactate and acetate. Citrobacter sp. was possibly responsible for the sulfate reduction with lactate as electron donor. The methanogenic microorganisms were probably responsible for the conversion of acetate to carbon dioxide and methane in the biogas verified by gas chromatography analysis. The presence of Veillonella sp. and Citrobacter sp. only in the middle Postgate C may be related to higher concentrations of sodium lactate (7.5g/L) and presence of yeast extract (3.0g/L) in this environment.

Keywords: anaerobic microorganisms, phylogeny, most probable number

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EVALUATION of ANTIBACTERIAL ACTIVITY of ESSENTIAL FRUITS OILS MIXTURE OF *dioecious* Lindl *Pepper* AND *Aniba duckei* Kostermans STEM.

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Keywords: Mixture of Oils. *dioecious* Lindl *Pepper*. *Aniba duckei* Kostermans. Antibacterial activity.

The plants are important sources of biologically active products, many of which are models for the synthesis of new medicines that can act effectively, because with the increase in indiscriminate use of antibiotics, bacteria resistant to the conventional antimicrobial therapy have arisen and essential oils are singled out as an alternative to the problem, thus arousing the interest of the scientific community as a source of natural antimicrobial substances, and, in this context, the essential oil of the *dioecious* Lindl *Pepper* added to the *Aniba duckei* Kostermans have proven very promising, due to its low cost, ease of getting through the planting and mainly by its effectiveness. When we carried out the research, we aimed at evaluating the antibacterial activity of the essential oils mixture of the *dioecious* Lindl *Pepper* (fruit) and the *Aniba duckei* Kostermans (stem) species. It was performed the extraction of essential oils under consideration, through the hydrodistillation method, using a Clevenger system and soon after it was made the combination of such oils in equal parts. In applying the mixture of essential oils and patterns of eugenol and linalool as antibacterial agents, it was used the Bauer- Kirby method for *Chromobacterium violaceum* bacteria, *Enterococcus faecalis*, *Salmonella Thyphi*; the antibiotics Tetracycline, Erythromycin, Cefoxitin, Cefotaxime, Ampicillin, and Penicillin to act as a comparison. In this research, it was noted that the mixture of essential oils was characterized as more efficient than the Eugenol and Linalool standards and the antibiotics tested for *Enterococcus faecalis and Chromobacterium violaceum* microorganisms. For *Salmonella Thyphi*, the mixture just was not more efficient than antibiotics: Erythromycin, Tetracycline, Cefoxitin, Cefotaxime, Ampicillin and Penicillin. Based on those facts, the antibacterial activity study revealed that the essential oils mixture presented excellent activity against all bacteria under consideration, being eugenol added to linalool the main responsible for that effectiveness.

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EVALUATION OF *Clostridium perfringens* SPORES IN SEWAGE TREATMENT PILOT PLANT SEEKING REUSE

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Keywords: *Clostridium perfringens*, sanitary sewage, pilot plant

INTRODUCTION: *C. perfringens* is usually found in the intestinal tract of humans and other animals as a regular symbiote and its presence in water may sign fecal contamination. It can cause gastrointestinal distress, food poisoning and gas gangrene, among other effects. An important aspect of this species is the ability to sporulate and remain in the environment for long periods, working as a remote indicator of pollution. *C. perfringens* spores have been suggested as a safe surrogate indicator of disinfectant activity for *Cryptosporidium parvum* and other hardy pathogens in water.
MATERIALS AND METHODS: The applied system consisted of an anaerobic baffled reactor, followed by vegetated "constructed wetlands" (using crushed stone as medium), slow filtration and disinfection by chlorination using sodium hypochlorite. 48 analyses were conducted during a period of 12 months, obtaining 12 samples of raw sewage, 24 treated sewage and 12 samples of treated sewage submitted to chlorination. Microorganisms were monitored and quantified by the multiple tubes method. The culture medium DRCM (Differential Reinforced Clostridium Medium) was used for the presumptive test. In the confirmation test, Litmus milk was used as the culture medium. The results were calculated and expressed in MPN/100 mL.
RESULTS AND DISCUSSION: Regarding the results for *C. perfringens* spores quantification, the reduction values between the system input and output ranged from 1 to 6 log. Due to the obtained outcome, the treatment system chosen, associated as an alternative to chlorination disinfection is effective in reducing this species level. Total spores elimination did not occur in any of the measurements performed, however the obtained values for reduction was significant and important considering the alternative for disinfection and to the fact that these spores are more resistant to the sodium hypochlorite action.
CONCLUSIONS: The anaerobic baffled reactor itself was not effective for removal of the microorganism studied, but the combination of treatment systems was efficient for the removal of spores associated with disinfection by sodium hypochlorite.
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EVALUATION OF DIFFERENT INCUBATION TIMES OF XTT TETRAZOLIUM ASSAY TO ASSESS THE METABOLIC ACTIVITY OF PLANKTONIC YEASTS.

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Keywords: *Candida*, Metabolism, Quantitative Analysis

Introduction: Yeast infections are an increasingly important problem, particularly in immunocompromised patients. Colorimetric assays of cellular viability are important tools in the study of eukaryotic cell activity and antifungal resistance. Some tetrazolium salts are widely used as indicators of cellular metabolism. XTT, 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide has been used to determine the viability of *Candida* biofilms after antifungal therapies. This method seems to be useful for comparisons involving one strain, but its use may be difficult in attempts to compare different fungal species, especially in the planktonic form. This study performed a comparative assessment of XTT metabolism among differents *Candida* species in the planktonic form. In addition, different times of XTT incubation were also evaluated. Methods: The microorganism used in this study was an American Type Culture Collection (ATCC; Rockville, Md, USA) strain of *C. albicans* (90028), *C. glabrata* (2001), *C. dubliniensis* (7987), *C. tropicalis* (4563) and *C. krusei* (6258). To obtain the standardized planktonic suspensions of *Candida*, the yeasts were individually inoculated in 5 mL of Tryptic Soy Broth and incubated (24h/37°C). Each culture was harvested after centrifugation at 2000 rpm for 10 min, washed twice with sterile distilled water and resuspended in PBS to a turbidity 10^6 cells ml^{-1} (MicroScan Turbidity Meter, Siemens). Aliquots of 200µl of each *Candida* standardized suspension were individually transferred to separate wells of a 96-well microtiter plates. The XTT solution was prepared instantly before use by adding 1.5ml of XTT (1mg/ml in sterile saline; Sigma Chemicals, St.Louis, MO) in 300µL of menadione solution (0.4M Minacetone; Sigma Chemicals). Then, 12µL of XTT was added to each well of the microtiter plate. The plates were incubated in dark at 37°C for 1, 2, 3, 4 and 5h. Then 100µL of the reacted XTT salt solution was transferred to a new 96-wells microtiter plate and the cell viability was analyzed by proportional colorimetric changes and light absorbance measured by a microtiter plate reader (Thermo Plate—TP Reader) at 492 nm. The experiments were performed with ten replicates for each experimental condition and data were statistically analyzed using ANOVA and Tukey's test (α=5%). Results: For all strains evaluated, a relationship between incubation time and color development was noted when the incubation duration is no longer than 3h. The incubation times of 2 and 3h promoted the highest values of absorbance for *C. albicans*, with the lowest variation coefficient (approximately 8%). For *C. glabrata*, *C. dubliniensis*, *C. tropicalis* and *C. krusei* the incubation during 3h was the most effective in promoting colorimetric changes without an elevated variation coefficient. Discussion: Several investigations already showed that the XTT salt produces color changes that can reflect the number of viable cells in the yeast sample. In addition, some studies suggested a linear relationship between incubation time and color development. Our results are in accordance with the findings from these previous studies. In conclusion, the XTT assay can be considered a valuable tool for examining the behavior of *Candida* species in planktonic form.

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Evaluation of Helminth Eggs and Protozoan Cysts from effluent in a System of Sewage Treatment in Cuiabá / MT.

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Keywords: Helminths, Protozoa, Effluent, Reuse

The treated effluent of stabilization ponds can be reused for various purposes, including landscape irrigation and fish farming. However, for the reuse not offer a risk to public health it is necessary that the effluent produce certain microbiological standards. This study analyzed samples of the effluent from the Sewage Treatment named Lagoa Encantada (Cuiabá - Mato Grosso) in order to evaluate the efficiency of the removal of helminth eggs and protozoan cysts; determining the predominant parasite species and also final effluent quality for reuse in landscape irrigation and fish farming, according to the standards analyzed. The samples were collected at Sewage Treatment named Lagoa Encantada (Cuiabá – MT) in the period from november 2009 to november 2010. The samples were analyzed from the effluent entry (stabilization pond), the effluent treatment and treated effluent (station exit). The materials used were: 10 ml pipettes, Pasteur pipettes, 1000 ml beakers, buckets, hose thin, Vortex, centrifuge tubes 50ml, optical microscope, 5l bottles, plates, coverslips, McMaster chamber, solution of formaldehyde, solution aceto acetic acid, ether solution, Lugol solution and zinc sulfate solution. The laboratory technique used for the identification of protozoan cysts was the method of Ritchie (1948) modified (AYRES & MARA, 1996). For the quantification of helminth eggs were used the method Bailenger (1979) modified (WHO, 1989, 2006). We found 06 (six) eggs per liter in the effluent input. Output in the effluent (treated wastewater) were not found helminth eggs or protozoan cysts, indicating that 100% removal of these parasites in the effluent of sewage treatment studied. The helminth eggs found in raw wastewater were predominantly of the genera *Ancylostoma, Ascaris, Trichuris and Hymenolepis.* The cysts of protozoa were identified as the genera *Entamoeba* and *Giardia*. According to WHO, the treated effluent to be used in fish farming, landscape irrigation should not have helminth eggs and protozoan cysts. Thus, the results show that the effluent from the Sewage Treatment named Lagoa Encantada is suited to this kind of reuse with respect to their efficiency in removal of helminth eggs and protozoan cysts, however it is important to consider other microbiological parameters, physicists and chemists.

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EVALUATION OF *Melaleuca* sp. OIL IN THE CONTROL OF *Pestalotiopsis longisetula*. A PHYTOPATOTHOGENIC FUNGUS IN STRAWBERRY CULTIVATION ON FIELD CONDITIONS

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Keywords: *Melaleuca* oil, *Pestalotiopsis longisetula*, strawberry

Pestalotiopsis longisetula phytopathogenic fungus has been studied in order to understand the most efficient way of controlling this microorganism in strawberry cultivation. It has be found in plantations in Minas Gerais and Espírito Santo states since 2004. In the last three years, it has become a severe and expressive pathogen causing injury. This research aimed to evaluate the potential of *Melaleuca* sp oil on the *Pestalotiopsis longisetula* in field conditions. Strawberry mother plants, cv. Oso grande, was acquired from a specialized company in tissue culture. The disinfection of leaves were in alcohol (70%) for 30 seconds, in sodium hypochlorite with 0,5% of activate chloride, for 1 minute, and finally they were washed up in sterilized distilled water. Pieces of tissue were placed in Batata Dextrose Agar (BDA) medium. After proving the sanity of the material, the plants were transplantated for three aisle carried out inside the botanic laboratory of Universidade do Vale do Sapucai. In each aisle 128 plants were planted and grown for thirty days before receiving the following treatments: the treatment one received only water on the plants leaves; the second one was treated with *Melaleuca* sp. oil on the concentration of 1% and the third one, the plants were treated with Frowncide 500Sc and Amistar fungicide solution, also on the concentration of 1%. After treating the leaves with the above products they were injured and inoculated with *Pestalotiopsis longisetula* propagules. The inoculum was obtained through addition of 10 ml of distilled and sterilized water in the fungus colony growing in dish Petri. The suspension was adjusted to $1x10^6$ conidius ml^{-1}. The disease evaluation of was performed every three days during one month. The results showed that the disease symptoms appeared at the first evaluation in the control treatment, while the treated leaves with *Melaleuca* sp. oil and with the fungicides the symptoms only were observed after 5 and 8 days, respectively. The control treatment presented a percentage of *Pestalotiopsis longisetula* higher than 65%, while in the oil treatment the incidence occurred in 43% of the plants. For fungicides treatment the percentage was 25%. This result shows that it was not possible to use the *Melaleuca* sp. oil as a control for the *Pestalotiopsis longisetula* fungus, contrary to the results obtained by Oliveira and Fraga (2007) that had good results *in vitro* conditions. It is already known that not always the *in vitro* acquired results are reproducible in *in vivo* conditions.

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EVALUATION OF PARAMETERS FOR THE PRODUCTION OF LIPASE IN SOLID-STATE FERMENTATION BY *Fusarium sp*.

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Keywords: Agroindustrial residues, Fusarium sp., lipase, Solid-state fermentation

Lipases (EC 3.1.1.3) are enzymes belonging to the group of serine hydrolases that act on long chain glycerides releasing fatty acids and glycerol. They are also able to catalyze reverse reactions such as esterification and transesterification. Although lipase is traditionally produced by submerged fermentation (SF), the solid-state fermentation (SSF) has been studied as an alternative production technology due to advantages it provides in relation to SF, as the use of agricultural wastes as substrate for microbial growth and enzyme production, reducing production cost. The fermentations were carried out in 250mL Erlenmeyer flasks with 10g of dry substrate, moistened with phosphate buffer 0.1 mol/L pH 7.0, to obtain the required 60% humidity in the experiment and 1% v/w olive oil. The flasks were inoculated with 1mL of spore suspension (10^8) and then incubated at 28°C with samples removed every 24h for up to 120h of cultivation. The tested substrates were: soybean bran (*Glycine max*), corn bran (*Zea mays*), sugarcane bagasse (*Saccharum sp.*), castor bean cake (*Ricinus communis*) and crambe cake (*Cambre abyssinica*). After determining what the best substrate for lipase production, different oils were tested (corn, olive, soybean and crambe) and concentrations (1 and 3% v/w), and different solutions to moisten the substrate (water, Czapeck mineral solution and phosphate buffer 0.1 mol/L pH 7.0) to verify its influence on the production of lipase. The crude enzyme extract was obtained after addition of 1% saline solution on the solid fermented, followed by agitation in a shaker (180rpm, 40min), filtration and centrifugation (3000rpm, 10min). The determination of lipase activity was done by the method of hydrolysis of *p*NPP, accompanied by the 410nm reading. One unit of enzyme activity was defined as the release of 1µmol *p*NP (*p*-nitrophenol) per minute and expressed in units per gram of dry substrate (U/gds). It was observed that the best substrate for lipase production was the crambe cake (5.08±0.25 U/gds). The use of soybean and corn bran resulted in similar activities (2.00 ±0.42 and 2.04 ±1.03 U/gds, respectively) after 72h of fermentation, and the castor bean showed maximum activity (1.88±0.59 U/gds) after 120h of fermentation. The sugar cane bagasse resulted in no significant activity. The results are due probably to high-fat in the residual crambe cake (22.3%). The literature reports that fats induce the production of lipases, while high levels of carbohydrates inhibits. Corn, soy brans and castor bean cake present, respectively, 1.75, 2.2 and 1.15% of fat content in its composition. The sugarcane bagasse, despite a similar fat content (2.44%), did not result in meaningful activities probably due to the difficulty that the fungus had to access the nutrients of the substrate, which contains high amounts of lignocellulose, as well as due to the high carbohydrates content (60.1%). The addition of oil did not influence the enzyme production ($p=0.99$), suggesting that the nutrients in the crambe cake were sufficient to supply the nutritional needs of the fungus. The best solution to moisten the substrate was phosphate buffer ($p<0.10$), possibly by keeping the pH stable during the fermentation time, protecting both the enzyme and the fungus.

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EVALUATION OF POLYCROSS YEAST HYBRIDS GROWING IN HIGH SUGAR CONCENTRATION INDUSTRIAL SUBSTRATE

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Keywords: *Saccharomyces cerevisiae*, hybrid, polycross, high sugar concentration

INTRODUCTION

Industrial fuel ethanol production process imposes several stressing condition upon fermenting yeasts. Many of such stresses are applied simultaneously or sequentially in industrial fermentations, as high osmotic pressure, high ethanol titers and high acidity. Tolerant yeast strains are desirable in order to cope with such stressful conditions. Due to the polygenic nature of most of these traits, as ethanol tolerance, a rational approach for yeast improvement is a difficult task. Hybridization is a conventional and easy procedure not yet exploited for strain improvement to be used in fuel ethanol production in Brazil.
The present work aimed at yeast strain improvement by means of hybridization of the best parents available.

MATERIAL AND METHODS

Saccharomyces cerevisiae PE-2, CAT-1 and SA-1 were sporulated and micromanipulated to obtain haploid cells that were separately grown in YPD liquid medium. After 24 h, cultures were inoculated into 100 ml flasks with YPD liquid medium to obtain diploid cells, both by poly- and directed crosses. Seven crosses were performed: PE-2 vs. PE-2, CAT-1 vs. CAT-1, SA-1 vs. SA-1, PE-2 vs. CAT-1, PE-2 vs. SA-1, CAT-1 vs. SA-1 and PE-2 vs. CAT-1 vs. SA-1. Samples were plated onto YPD solid medium, then 15 colonies from each sample were inoculated separately in YPD liquid media for 48 h pre-growth. After pre-growth, 96-well plates were filled with 10 µl inoculums and 90 µl of 27 % (w/w) mixed must (sugarcane juice and molasses) and incubated at 30ºC on microplate reader TECAN, measuring optical density (OD_{570}) at 2 h intervals during 24 h.

RESULTS AND DISCUSSION

Polycross generated strains 56 (O.D. 1,103) (CAT-1 vs. CAT-1), 61 (O.D. 1,06) (PE-2 vs. CAT-1) and 109 (O.D 1,09) (PE-2 vs. PE-2), presented respectively, 12, 8 and 11 % biomass gain when compared to the parents (O.D. 0,98). Hybrids derived from directed crosses, as 3 (O.D. 1,0) (PE-2 vs. PE-2), 13 (O.D. 1,0) (PE-2 vs. PE-2), 32 (O.D. 1,03) (PE-2 vs. CAT-1), 38 (O.D. 1,02) (PE-2 vs. CAT-1) and 42 (O.D. 1,04) (PE-2 vs. CAT-1), showed 2-6 % of gain over the parental PE-2 strain. These results allowed to conclude that both poly and directed-crosses methodologies are effective in generating more vigorous strains, even from high performing industrial yeasts as PE-2 and CAT-1. Hybridization could be an interesting approach for strain improvement for very-high-gravity fermentation, were several stresses are imposed simultaneously towards yeast.

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EVALUATION OF SOLANUM LYCOPERSICUM CRUDE EXTRACT CONCENTRATION ON THE INHIBITION OF *MONILIOPHTHORA PERNICIOSA* GROWTH, WITCHES´ BROOM DISEASE OF COCOA.

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Keywords: Cocoa, α–tomatine, *Moniliophthora perniciosa*

Cocoa culture is one of the most important tropical cultures in the world and its production is highly compromised by the witches broom disease, caused by *Moniliophthora perniciosa* fungi. Due to this pathogen, cocoa Brazilian production decreased 75 % in ten years since it was first identified in 1989, in Bahia State. Nowadays, Brazil contributes with only 4 % of world production. In attempt to fight this disease, sanitary pruning is recommended, resistance variety grafting and fungicide application. The last one not recommended for its misbalancing environmental damage on tropical systems. *M. perniciosa* fungi showed growth inhibition in culture media with crude tomato leaves extract, due to α-tomatine compound, a glycoalkaloid present in those extracts. This work aimed to evaluate the crude extract potential, from *Solanum lycopersicum*, as a natural fungicide for witches´ broom disease control, caused by *Moniliophthora perniciosa*. Tomato leaves were collected, dried at forced circulation oven at 50 ^{0}C, for 48 hs. Dried leaves were crushed and homogenized. Infusions from different leaves concentrations were tested (from 0.2 to 8%) added to the WDA medium composition (wheat 1%, dextrose 1% and agar 2%). Media were poured into Petri dishes and *M. perniciosa* CP44 was inoculated and incubated in BOD incubator for 20 days at 27 ^{0}C.This first assay, fungi did not grow on the plates containing 8,7,6,5,4,3,2 and 1 % of crude extract. A second assay was carried out using 15 to 0.2 % of crude extract and after 20 days, fungi did not grow in 1 to 0.6 % of crude extract added to WDA. For concentrations of 0.4% and 0.2% fungi presented grow of 75 and 50 % respectively in comparison to control (WDA without leaves extract).Such results indicates crude extracts from tomato dry leaves at 0.6 % are efficient against *M. perniciosa* in laboratory conditions.

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EVALUATION OF THE PRE-INOCULUM UTILIZATION IN BIOSURFACTANT PRODUCTION BY SOLID STATE FERMENTATION

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Keywords: biosurfactant, *Bacillus subtilis*, solid state fermentation

Biosurfactants are one of the mainly class of natural surfactants and are classified according to their chemical composition and microbial origin. The production of biosurfactants by microorganisms is intimately linked to the environmental and nutritional conditions provided and the factors that influence microbial growth also affect their production. Currently, most of the compounds are synthetic surfactants and the main factor restricting the widespread use of biosurfactants is their production cost when compared to the ones with synthetic origin. The solid-state and submerged fermentation can be used for the production of biosurfactants, but the solid-state fermentation is considered a simple technology for production of compounds with interest and an alternative to avoid foaming, a limiting factor in obtaining these compounds by submerged fermentation. This study aimed to evaluate the influence from the pre-inoculum use in biosurfactant production by *Bacillus subtilis* in solid state fermentation. The fermentation was carried out in polyethylene bags containing 10 g of wheat bran and phosphate buffer solution and glycerol concentration to obtain 60% moisture and incubated in a chamber at 37°C for 96 hours. The fermentation was conducted in three ways: without pre-inoculum, with liquid pre-inoculum and solid pre-inoculum. *Bacillus subtilis* was grown in tubes with nutrient agar and incubated in a chamber at 37°C for 12 hours. After this period a cellular suspension was prepared from the tube where 2 ml of this suspension served as inoculum in the bags of wheat bran without pre-inoculation. The same cellular suspention were used in 1 ml to prepare the pre-liquid inoculum, which consisted of 50 ml of nutrient broth, kept under stirring at 37°C for 12 hours. This cellular suspention was also aplied at the solid pre-inoculum containing 10g of wheat bran with buffer and glycerol, incubated at 37°C for 12 hours in an erlenmeyer flask. 2 ml of liquid and solid pre-inoculum were removed after 12 hours and inoculated in the bags. The surface tension and emulsifying activity were analyzed, the surface tension was performed in free-cell extracts, using a tensiometer and the emulsifying activity of the medium was analyzed using 3.5 mL of free-cell extract and 2 mL of toluene. This mixture was stirred in vortex for 2 minutes and the absorbance was read in spectrophotometer at 620 nm. The results showed that there was a decrease in surface tension in the three fermentations, with similar results, around 55 mN/m^{-1}, and the emulsifying activity values were also close, although the fermentation used as liquid pre-inoculum showed better results. The use of pre-inoculum, both the liquid and solid, didn´t influence the surface tension and emulsifying activity, compared to the fermentation without pre-inoculum.

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EVALUATION OF YEASTS AS POTENTIAL PLANT GROWTH PROMOTERS

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The plant growth-promoting microorganisms (PGPM) comprise a group of soil organisms capable of improving plant development through different mechanisms. Among them, the mycorrhizal fungi, rhizobacteria, diazotrophic bacteria (nitrogen-fixing bacteria), phosphate-solubilizing organisms and those able to produce plant hormones are the best studied. Yeasts are unicellular fungi, employed in various biotechnological processes and naturally present in the rhizosphere of plants, but in lower densities if compared with bacteria and fungi; due to this fact, little is known about the function of this microbial group in soil and its potential in promoting plant growth. Considering these aspects, the objective of this work was the characterization of yeasts isolated from agricultural areas (leaves surface and rhizosphere of maize and sugar cane) as promoters of plant growth by evaluating the ability of phosphate solubilization and production of the phytohormone indole acetic acid (IAA). About one hundred yeast isolates were evaluated. The selection of strains was realized by the use of solubilizing solid medium (PDYA) with insoluble phosphate and formation of translucent halo around colonies capable of solubilization; to detect strains producing IAA, yeasts were grown in liquid potato dextrose medium with the addition of tryptophan (5 mM), and the reaction of the culture filtrate with Salkowski reagent, which produces pink color in the medium from culture of the strains able to produce. The results indicate that the strains 3F81, 3C105, 3C79, 3C101, 3F35, 3C102, 3F189, 3C84 e 3C72 were able to solubilize phosphates and the strains 3C114, 3C59, 3C73, 3C67, 3C92, 3C91, 3C62, 3F173, 3C66, 3F177, 2F20, 2S04 and 1S111 were effective in the production of IAA in the presence of tryptophan. Some yeast isolates as 3C122, 3F157, 2S01 and 1C109 showed multifunctionality being able to solubilize phosphate and produce IAA *in vitro*. These strains will be identified and more detailed studies on the role of these strains on plant-microbial system will be realized, especially in promoting plant growth.

Keywords: yeasts, plant growth promoting, phosphate solubilization, indole acetic acid

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FACTORIAL DESIGN FOR COPOLYMERS PRODUCTION BY *Cunninghamella elegans* UCP 542

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Keywords: biopolymers, chitin, chitosan, microrganism.

INTRODUCTION: Biopolymers are macromolecules consisting by monosaccharide residues linked by glycosidic bonds. Chitin and its derivative chitosan by deacetylation, are structural polymers present in the exoskeleton of invertebrates and cell walls of some microorganisms among which the fungi of the Zygomycetes Class[1]. Traditional sources to obtaining chitin and chitosan use exoskeletons of crustaceans, lobster, shrimp and crabs shell. However problems with the places of confinement, the viability of the products, seasonality and processing on large-scale associated with chemical conversion of chitin into chitosan, limit industrial polymers potentials[2,3].The filamentous fungus *Cunninghamella elegans* species has demonstrated the ability to produce these copolymers when subjected to a satisfactory cultivation and fermentation conditions[2]. In this study was investigated the chitin and chitosan production by *C. elegans* using a factorial design with Central Composite Rotatable Design (CCRD).

MATERIAL AND METHODS: Spores of *C. elegans* maintained on Potato-Dextrose-Agar (PDA) at the Bank of Cultures Center for Research in Environmental Sciences, Catholic University of Pernambuco – UNICAP, maintained to 5°C, were transferred to form a suspension of 10^7 spores/mL. 1mL aliquot this suspension was transferred to center of Petri dishes containing PDA medium for 24 hours. Inoculum discs form was transferred to Erlenmayer flasks containing 400mL of the culture media (Corn steep liquor+Asparagine+Sucrose) for fermentation, during 96 hours on rotation shaker (150rpm/28°C).After this period, flasks were removed for biomass estimation and chitin and chitosan production. These experiments were accomplished by factorial design.

RESULTS AND DISCUSSION: The experiments results demonstrated the adaptation of *C. elegans* in the medium employed, since the yields of polymers produced were 414.1mg/g and 93.1mg/g of chitin and chitosan, respectively. These yields suggest that microrganism study, used the components present on the medium as carbon sources (sucrose) and nitrogen (corn steep liquor/asparagine) on metabolic steps for the mycelial growth, expressing a yield of 1.63g/ L biomass. On the other hand, the significant income of biopolymers is indicative of the ability of *C. elegans* to metabolize the essential components present on the culture medium for synthesis of specific enzymes in the formation of chitin and chitosan. Studies have demonstrated the use of alternative and low cost cultivation medium to obtaining fungal biomass and production of substances within and extracellular[2,3], confirming the results obtained in this work. The use of alternative medium enable the rapid microrganism growth and obtaining, a low cost/benefit the biopolymers chitin and chitosan, compared to those produced on synthetic culture media, traditionally used.

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FERMENTATION OF GLUCOSE/ XYLOSE MIXTURE BY *Kluyveromyces marxianus* UFV-3 - NEW PERSPECTIVES

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Keywords: fermentation; xylose; glucose; lignocellulosic biomass; *Kluyveromyces marxianus* UFV-3

Lignocellulosic is the most abundant biomass in the earth and has been investigated all over the world as a source of substrate for ethanol production by fermentative process. It comprises cellulose (35–50% w/w) and hemicelluloses (20–35% w/w). Hemicelluloses, unlike cellulose, are heterogeneous polymers of primarily pentoses (xylose, arabinose) and hexoses (mannose, glucose, galactose). Xylose is the most abundant monosaccharide in renewable biomass after glucose. The ability of microorganisms to convert both glucose and xylose to ethanol is ideal for an economically feasible process. In practice, the hydrolysate of lignocellulosic biomass may have different ratios of glucose/ xylose because of different hydrolysis methods and different substrates. Here, the effects of different ratio of glucose/ xylose mixtures on cell growth, sugar consumption and ethanol yield were studied in a fermentation process by *Kluyveromyces marxianus* UFV-3. That yeast came from the collection of the Microbiology Department of Universidade Federal de Viçosa (Viçosa, Minas Gerais, Brazil) and has been proved to ferment lactose with high yield. Bioconversions were investigated in a defined medium YNB (Yeast Nitrogen Base) supplemented with 0.06 % (w/v) yeast extract and glucose or xylose as carbon source. Carbon sources and end metabolites were determined by HPLC (Hewlett Packard 1050), with a refractive index detector HP 1047A, and an Aminex HPX-87H (BIO-RAD) column. Cell growth was monitored by optical density (OD) at 600 nm. The results indicated that the highest ethanol yield (0.44 gg^{-1}) was obtained after 24 hours fermentation on 100% glucose, while no ethanol was detected on 100% xylose. Just xylitol was detected in medium containing xylose with a yield of 0.40 gg^{-1}. The biomass yield was twice higher on xylose than on glucose. This result reflects the predominance of an oxidoreductive metabolism on glucose in contrast with an oxidative metabolism on xylose. Although *K. marxianus* harbors the genes for xylose assimilation, this pentose is not apparently converted into ethanol. The ethanol production from glucose in the presence of xylose was then evaluated. Glucose was the preferred substrate in fermentation on mixed sugars. Xylose assimilation started only after glucose being exhausted. All the ethanol produced was obtained from glucose since during the ethanol formation there was no xylose consumption. Additionally, the ethanol production was halted when glucose was exhausted. When an inhibitor of electron transport chain, antimycin A, was added to the medium, xylose and glucose were simultaneously consumed. In this case, ethanol formation was twice higher while xylitol was twice lower. Surprisingly, ethanol was formed from xylose. These results suggest that there is a condition in which xylose can be converted to ethanol. These facts bring a new perspective to use *K. marxianus* as an ethanol producer in a glucose/ xylose substrate mixture.

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FILAMENTOUS TO YEAST-LIKE FORM OF *Mucor circinelloides* (UCP – 0069) TRANSFORMATION BY ETHANOL: EFFECTS ON BIOSURFACTANT PRODUCTION

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Keywords: dimorphism, mucorales, biosurfactant, ethanol

The dimorphism phenomenon is exhibited by various species of *Mucor* and is an elementary example of morphological differentiation in response to environment condition and under anaerobiosis, where the fungus can change the between filamentous and yeast-like forms. Thus the dimorphism can be controlled experimentally by changes in environmental factors and is not restricted to any taxonomic group. The study of the phenomenon could enhance the knowledge of the biology of the fungus. There is significant interest in the production of biosurfactant for application in various industrial areas such as oil, food, pharmaceutical and cosmetical, once they are natural compounds, biodegradable and less toxic than synthetic ones. The search for microorganisms producing biosurfactant, which can be cultivated on industrial scale for the process cost reduction, remains a current goal. Studies were carried out to analyze the transformation of the mycelium to yeast-like cells of the fungus *Mucor circinelloides* and its production of biosurfactant. *M. circinelloides* was initially cultivated in Petri dishes containing potato dextrose agar (PDA), during 4 days. The spores were transferred to 500mL Erlenmeyers flasks containing 200mL of Yeast Nitrogen Base (YNB) medium and 2.5%, 3.5% and 5.0% of ethanol. Cultures were incubated at 150 rpm, at 28°C during 120 hours. The morphological modifications were followed by light microscopy at 1000x magnification. The metabolic liquid free of cells was used to determine the surface tension and the emulsification index. The results showed the presence of arthrospores, and yeast-like cells, which appeared in 72 hours of incubation in all ethanol concentrations. The water surface tension of 72 mN/m was reduced to 27.94 mN/m in 48 hours in the presence of YNB added 5.0% ethanol, indicating the presence of tensoactive products in yeast-like fungal culture. However, in supernatant cultures exposed to 2.5% and 3.5% of ethanol the surface tension reached 40mN/m. This investigation showed the filamentous to yeast-like transformation induced by ethanol and the production of surfactant by *Mucor circinelloides* yeast-like cells. Thus, the data suggest the potential of the dimorphic phase of the fungus in biotechnological processes for the biopolymer production.

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FRUCTOOLIGOSACCHARIDES PRODUCTION (FOS) BY YEAST ISOLATES FROM SUGAR AND ALCOHOL PLANT

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Keywords: Fructooligosaccharides, yeast, biotransformation.

INTRODUCTION: Fructooligosaccharides (FOS) are natural oligosaccharides commonly found in many vegetables such as chicory, beets, garlic, onion, asparagus, yacon, tomato, banana, barley and rye. The most comom FOS pertaining to the inulin type that are are fructose oligomers bonded at β-2-1 linkage between fructosil group and sucrose in a terminal position of the molecule as 1-kestose (GF_2), nystose (GF_3) and fructofuranosyl-nystose (GF_4). Chain with less than 10 residues are considered FOS, while 10 - 50 residues are considered inulin (Yun, 1996). They belong to the prebiotics group of carbohydrates that are ‘‘nondigestible food ingredient(s) that beneficialy affect host health by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon’’(Gibson & Roberfroid,1995).
They are produced from natural sources extraction, by sucrose transfructosylation through microbial and vegetal enzymes, and by sucrose biotransformation through various microorganisms. Thus, this study aimed at isolating yeasts from sugar cane bagasse, vinasse and filter cake capable of producing FOS in culture medium with high sucrose concentration.
MATERIAL AND METHODS: 29 yeasts isolates from sugar cane bagasse, vinasse and filter cake coming from a sugar and alcohol plant. They were cultivated and isolated in YEPD medium. The pre-inoculum culture from a loopful of cells was inoculated in tubes with 5mL YEDP and incubated at 30°C for 48h. Two milliter of this culture was transferred to 20 mL of culture medium containing 40% sucrose, 0,5% yeast extract and 0,1% urea for 72h, 150 rpm at 30°C. The suspension was centrifuged at 10.000 rpm for 15 minutes and the supernatant was analyzed by HPLC with the column Shodex KS 801, 75°C, with water as mobile phase at 1 mL/min.
RESULTS AND DISCUSSION: Only 10, out of 29 evaluated yeast isolates showed production of FOS GF2, as shown in Figure 1. The concentration of GF_2 ranged from 1.2 to 3.6%.

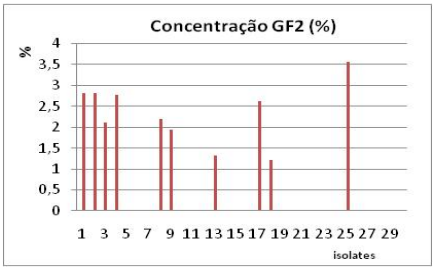

Figure 1: GF_2 production by isolates.

With the objective of FOS production by yeasts, it was concluded that some isolates from sugar and alcohol plants are able to produce 1-kestose (GF_2).
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GENETIC VARIABILITY OF *Bacillus subtilis* BASED ON RAPD MARKER ANALYSIS.

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Keywords: *Bacillus subtilis*, RAPD, variability, biotechnology,

The genetic variability among individuals was already described by morphology and enzyme analysis, however, with the progress of biotechnology, new techniques have emerged based on DNA. One of technique applicable to see the genetic variability is the Randon Amplified Polymorphism DNA (RAPD), this technique, in this work was to evaluate the genetic diversity of *Bacillus subtilis.* The bacillus in study is a species of gram-positive bacteria, saprophyte, common in soil and also described as rhizobacteria, which may be crucial in development of a plant through the synthesis of antibiotics, enzymes and phytohormones, and is often quoted for its high potential for controlling biological. For the experiment were used four strains of *Bacillus subtilis* obtained in different regions of the interior of São Paulo state, which were named: B1, B2, B3 and B4. The DNA extraction was made using the method CTAB in Molecular Biology. Genomic DNA was used in the reaction of RAPD - PCR. We selected four primers and the final product was subjected to electrophoresis on agarose gel 2% and visualized in ethidium bromide. The primers had an average of seven bands and even taking into account the low number of primers was observed genetic variability in samples submitted to analysis. However, there is a need for more research, because molecular characterization of *Bacillus subtilis* assists in selection of strains with desirable agronomic traits such as increased potential in full or partial control of diseases.

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GLUCOSE ADDITION AND FUNGI INOCULATION IN SOIL, COMPARED WITH MULTIVARIATE STATISTICAL

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Keywords: multivariate analysis, soil fungi, soil microbiology

In search of soil nutrients, strong competition occurs between the microorganisms saprophytic and pathogenic species (Grodnitskaya and Sorokin, 2007). Among the fungi community, influencing several pathogenic agents that cause plants and seeds diseases (Long, 2002; Felix, 2007). Due to these diseases, significant losses occur in agriculture and may even limit the cultivation of different plant species of economic interest. The aim of this study was to introduce in the soil, fungi isolated from the seeds, *Aspergillus flavus*, *Fusarium verticillioides*, *Penicillium* sp. and these development in two soil types. Were selected soils from two areas located in Jaboticabal city, SP: native forest, where the soil was classified as Eutrophic Oxisol – LVE, and an annual crop area, where the soil was classified as Dystrophic Oxisol – LVD, classification according to Embrapa (2006). The fungi obtaining and cultivation were by BDYA medium (Kucey, 1983), distributed in test tubes, for multiplication of these, isolated from corn seeds. The inoculum was suspended in distilled water and counted in a Neubauer chamber, with a total of 1,175 x 10^6 spores / g dry soil. For the Petri dishes assembly, 62 g soil were weighed and transferred to each plate and moisture balanced to 60% of Water Holding Capacity. The solutions were prepared by adding 2.32 mL of the fungus concentrate, plus 27.28 g of glucose, when treatment involved the addition of glucose, and then turned up the volume with distilled water to 660 ml (LVD) and 748 mL (LVE). On each plate, were added 15 and 17 mL respectively for LVD and LVE. After this procedure, the plates were incubated for a period of 21 days, with all treatments separated by incubator without light and temperature controlled at 25°C. Separately for each soil, the treatments were obtained through a factorial 4 x 2. Was considered the multivariate statistical analysis, because of complex attributes. Used the Euclidean distance as a measure of similarity between sampling units and cluster analysis was performed by connecting simple. Clear difference was noted in three groups at multivariate analysis, observed in which, the soil distinction and glucose addition. In group one, fertile soil and natural vegetation (LVE), all with the glucose addition, *Penicillium* sp. showed a very close resemblance of *Aspergillus flavus* than *Fusarium verticillioides*, and the control seems a little off the fungi inoculated. The other groups (two and three) showed the same behavior described in one group. The glucose addition influenced the fungi development in most cases. However, the fungus *Fusarium verticillioides* doesn't seem to be influenced by the glucose addition.

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GLUCOSE PROFILE ON CULTIVATION OF *Aphanothece microscopica Nägeli* IMMOBILIZED IN CALCIUM ALGINATE BEADS

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Keywords: cyanobacterium, *Aphanothece microscopica Nägeli*, alginate beads, glucose profile

The use of cyanobacteria in biotechnology has been increased in recent years in food, cosmetic, aquaculture and pharmaceutical industries. Moreover, several strains of have been used on wastewater treatment, mainly for nutrient removal. Since the size of single cells implies a problem in the application of these organisms on biotechnology processes, cell immobilization techniques have been developed in order to solve those questions. Indeed, the use of immobilized cyanobacteria cells in water purification processes has been reported from long ago. Most of the immobilization techniques devised for microorganisms in general can be applied to microalgae, with the limitation of light transmission to living cells. *Aphanothece microscopica Nägeli* is a cyanobacteria that has been studied with a view to the valorization of agro-industrial wastewater, production of single-cell protein and CO_2 removal in tubular photobioreactors. However, there is a little previous study about immobilization of these cyanobacteria. Thus, the aim of this research was evaluate the glucose profile at cultivation of *Aphanothece microscopica Nägeli* immobilized on calcium-alginate beads by gel entrapment technique. Particles with average diameter 4mm containing 10^6cell/mL were submerged in vinasse and BGN medium with glucose. Data suggests glucose *quasi*-depletion in vinasse after ten hours, with a slight decrease of particle size during the experiments. Results indicate the feasibility of immobilization of *Aphanothece microscopica Nägeli* and heterotrophic growth with glucose uptake, consisting an alternative for continuous organic removal by cyanobacteria.

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GROWTH OF *Aphanothece microscopica Nägeli* AND C*hlorella vulgaris* AT VARIOUS GLUCOSE CONCENTRATIONS IN MIXOTROPHIC CULTURES

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Keywords: microalgae, cyanobacteria, *Aphanothece microscopica Nägeli*, *Chlorella vulgaris*, mixotrophic cultures.

Microalgae are eukaryotic, as the green algae, or prokaryotic photosynthetic microorganisms, as the cyanobacteria. These microorganisms have attracted interest due to ability to remove organic matter and nutrients by incorporation into the biomass, suggesting its application in wastewater treatment. Microalgae are preferentially grown photoautotrophical systems, using solar energy with CO_2 fixing. Alternatively, these microorganisms can be cultivated on heterotrophic or mixotrophic growth, using organic compounds as energy and carbon source, such as organic acids, acetate and sugars. Although glucose is the most commonly used carbon source for heterotrophic and mixotrophic cultures of microalgae, information on the concentration required for optimal metabolic growth is too scattered to reach a definite conclusion. Cyanobacterium *Aphanothece microscopica* Nägeli has great application in the valorization of organic residues in the south of Brazil, being recognized for its potential to remove organic matter from agro-industrial effluents into heterotrophic cultures, i.e. the absence of light. Genus *Chlorella* is one of the most studied among the microalgae, due to biomass composition in terms of protein and lipids. Moreover, this microalga is frequently used to wastewaters treatment. In this context, this work aims to evaluate the effect of different concentrations of glucose in the growth of microalgae *Aphanothece microscopica Nägeli* and *Chlorella vulgaris* in mixotrophic conditions. The experiment were conducted at the Laboratory of Applied Microbiology (LABMAC/CCA/UFSCar), in Erlenmeyer flasks containing 150 mL of inoculum (10% v/v; 10^6 cell/mL) in BGN and WC media (for *Aphanothece* and *Chlorella*, respectively), supplemented with different concentrations of glucose (0, 12.5, 25, 37.5 and 50g/L). Experiments were set up at 25ºC, photoperiod of 12 hours (light-dark with photon flux density of 30µmol/m^2.s) and air-flow rate of 1VVM. *Aphanothece microscopica Nägeli* presented the maximum specific growth rate (μ_{max}) at the intermediate concentration of glucose, 25g/L (μ_{max} = 0.051 h^{-1}), reflecting a duplication time (t_d) of 13.5h. For *Chlorella vulgaris*, the concentration of 12.5g/L of glucose led to better growth, with μ_{max} = 0.024 h^{-1} and t_d = 28h. For both species, the smallest μ_{max}, consequently, higher t_g, were observed when glucose were not added to the medium. Although cultures are mixotrophic, both BGN and WC medium shows no organic source, so that the addition of glucose is interesting to keep the metabolism during the dark period. Additionally, the addition of organic substrate results in increases growth rate. The second lowest μ_{max} was observed at higher concentration of glucose (50g/L), which was due, probably, to the inhibitory effect of the substrate. Preliminary results suggest that the mixotrophic system using an organic substrate constitutes an interesting alternative, since the presence of the substrate could contribute to the maintenance of metabolism in the dark period, in addition to supporting the attainment of high cell concentrations.

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HYGIENIC QUALITY OF CHEESE PRODUCED IN SOUTHERN RIO GRANDE DO SUL

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Keywords: cheese, microorganisms, contamination

During the production of cheeses, several factors may change their characteristics, among which stand out the bad manufacturing practices, milk contamination after heat processing, storage temperature and inadequate raw material with high initial microbial load. Microbiological contamination is a serious danger to the health of consumers, particularly high-risk groups such as children and the elderly, and causes great economic losses, and dairy products, the raw material itself and by using high moisture content in local production are particularly susceptible to such contamination (Perry, 2004). The aim of this study was to assess the hygienic quality of cheese produced in southern Rio Grande do Sul. were analyzed thirty samples of cheese, industrially manufactured in southern Rio Grande do Sul during the years 2008 to 2010. The samples obtained were sent to the laboratory of food microbiology in the Department of Agroindustrial Science and Technology, Federal University of Pelotas, to perform the microbiological testing of *Listeria monocytogenes*, coliforms at 45°C, *Salmonella* spp. and Staphylococcus coagulase positive. The results were analyzed according to current standards of the Agência Nacional de Vigilância Sanitária(ANVISA), according to Resolution RDC N^{o}. 12 of January 02, 2001, and the samples were found unfit for human consumption when it exceeded the limit. Of the 30 samples of cheese analyzed, 21 (70%) were within the standard established by law. Seven samples (30%) had coliform count at 45°C beyond that allowed (5x10^{3} CFU/g). With regard to analysis of *Listeria monocytogenes*, Staphylococcus coagulase positive, and *Salmonella* spp. showed results within the limit set at 100% of samples. Therefore, it is concluded that 30% of the samples consisted unfit for consumption may have been contaminated in some stage of processing, directly or indirectly with fecal material, or even in cold storage.

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IDENTIFICATION OF LACTIC ACID BACTERIA BY PHENOTYPIC AND GENOTYPIC CHARACTERIZATION

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Keywords: Lactic Acid bacteria, phenotypic and genotypic , *Lactobacillus*

1. Introduction

Lactic acid has a wide range of applications in the pharmaceutical, food, leather, textile and cosmetic industries. One of the most important applications of lactic acid is in biodegradable markets, such as polylactic acid, which can be used to improve physical properties in the production of garbage bags, agricultural plastic sheeting and computer parts. It can also be applied in sutures and surgical implants owing to its biocompatible and bioabsorbable characteristics.

Lactic acid is industrially produced either through chemical synthesis or microbial fermentation. The advantage of the biological method is that an optically pure lactic acid can be obtained by choosing a strain of lactic acid bacteria, whereas chemical synthesis always results in a racemic mixture of lactic acid. The optical purity of lactic acid is very important to the physical properties of PLA and obtaining a more stable crystalline polymer than that achieved with a racemic lactic acid.

2. Materials and Methods

Nineteen bacteria was selected and characterized phenotypic and genotypic. For the characterization phenotypic was tested at different conditions (growing in pH 9.6, pH 4,4, NaCl 6.5%, 18%, 45ºC, 10ºC and production of CO_2). Also, was used the kit API 50 CH. Concerning by characterization genotypic, the extraction of DNA was made by TNES method. The amplification was made by PCR using kit Pure Taq Ready-To-Go PCR Beads (GE Healthcare) and the primers 27 FA e 27 FC as 10 pmol/µL concentration. For the purification of PCR product was employed the kit PCR DNA and Gel Band Purification Kit (GE Healthcare). For the sequencing was used the kit BigDye Terminator v3.1 Cycle Sequencing Kit (applied Biosystems) following instructions of manufacturer and the samples was applied in automatic sequencer ABI 3500. The sequences forward and reverse was aligned using BioEdit. The bacteria was identified in level of specie by comparison with sequences known on Gen Bank, using BLAST (www.ncbi.nlm.nih.gov/blast).

3. Results and Discussion

The results for characterization phenotypic showed the same results, in level of genus, of characterization genotypic. Thereby the characterization revealed: 21.0% of lactic acid bacteria was *Weissella paramesenteroides*, 15,7% *Lactobacillus casei*, 15.7% *Pediococcus pentasaceus*, 15.7% *Leuconostoc lactis*, 15.7 % *Enterococcus faecium* 5.2% *Lactobacillus rhamnosus*, e 5.2% *Leuconostoc mesenteroides* subsp. *Mesenteroides* and 5.2% *Streptococcus* sp. Abdel-Rahman, *et al.*, 2011, Cho *et al.*, 2006 and Yousif *et al.*,2010 was made isolation and identification (genotypic and phenotypic) of lactic acid bacteria and found similarity between the results of both methods.

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IDENTIFICATION OF RECOMMENDED STRAINS FOR SOYBEAN INOCULANTS BASED ON FINGERPRINTS PATTERNS ACCORDING TO BRAZILIAN LEGISLATION

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Keywords: Bradyrhizobia, molecular profiles, BOX- ERIC-PCR, ARDRA.

Introduction: Inoculation in soybean is very important in Brazil's agriculture since the fifty's. However, the first legislation about the fabrication of commercial product was created only in the eighties. The Ministry of Agriculture, Livestock and Food Supply (MAPA-BR) required that the inoculants should have only recommended strains; especially soybean inoculants must have two different recommended strains. For soybean there are four reference strains, representing two bradyrhizobia species - they are *Brazyrhizobium elkanii* strain SEMIA 587 and SEMIA 5019; and *Bradyrhizobium japonicum* strain SEMIA 5080 and 5079. The actual legislation predicts the utilization of rep-PCR to identify the different strains found in inoculants; however, this molecular tool doesn´t seems to be enough. The main goal of this work is show the fingerprints patterns of the combination of the strains, simulating the commercial product, with the molecular markers rep-PCR and ARDRA (Amplified rDNA restriction analyses).
Material and methods: This work studied four reference strains that were grown in LM media, for 48h at 37°C, and were mixed (1:1 vol), simulating the commercial product. Furthermore, three commercial products were also used. DNA extraction was conducted with Kit (Wizard DNA Purification, PROMEGA). rep-PCR were performed with primer BOX A1 (5´-CTACGGCAAGGCGACGCTGACG-3´) and ERIC1 (5´- ATGTAAGCT-CCTGGGGATTCAC-3´)/ ERIC2 (5´- AAGTAAGTGACTGGGGTGAGCG-3´). ARDRA-PCR was made with primers of 16S rDNA, subsequently 10µL of the purified amplification products were digested with *Mbo*I, *Hae*III, *Hind*III, *Eco*RI and *Alu*I restriction enzymes (Invitrogen by Life Technologies). The amplification and the digested fragments were resolved by 1,5% agarose gel in horizon electrophoresis for 2,5 hours at 60V and stained with Blue Green (LGC Biotecnologia). Fingerprints patterns were visualized under UV illuminator G2200 (Kodak).
Results and discussion: The fragments length obtained with BOX- and ERIC-PCR ranged from 400 to 3000 bp and has an average 15 fragments per strain. This technique is considered powerful and simple to separate the strains, meanwhile, the combination from two strains fingerprints patterns of was not similar to the combination from two separated profiles, and when the commercial product was tested, the patterns were different, missing some bands. All the PCRs were made in triplicates, which showed that the technique was not reliable. ARDRA showed that *Hind*III and *Eco*RI did not exhibit patterns that identify the strains, meanwhile the other enzymes showed some polymorphisms between the strains. This kind of polymorphisms could be useful to identify the different strains inside the commercial inoculants, although, the technique is much more complex, which does not seem to be suitable to be proposed to be incorporate in the legislation. Further studies of these polymorphisms are being undertaken in order to achieve an easy and accurate technique for identification of strains.

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IDENTIFICATION OF THE BIODEGRADATION POTENTIAL GENUS *Burkholderia*

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Introduction

The genus *Burkholderia* comprises a very diverse group of bacteria that occupy different ecological niches, with species that cause diseases and other important skills to have the branches of agriculture, biotechnology and the environment, as biodegradation. These bacteria are morphologically similar and the genus is divided into seventeen genomovars, where genetically similar species are grouped together, forming the *Burkholderia cepacia* complex (BCC). Hydrocarbons are organic compounds resulting from incomplete combustion of organic matter and petroleum derivatives are used as indicators of pollution in the environment. Some of these hydrocarbons may cause damage to living beings, carcinogenic and mutagenic, and accumulate in the food chain. Because they are poorly soluble in water and very toxic to cells, these compounds are not metabolized by many microorganisms to contaminate and render environments. By rapid biodegradation containing the redox indicator 2,6-dichlorophenol-indophenol (DCPIP) is possible to select bacterial strains with potential to remediate areas contaminated with these hydrocarbons. The test is based on the discoloration of DCPIP (from blue to colorless), since the bacteria will metabolize the hydrocarbon releasing electrons that reduce the indicator, confirming the bioremediation of contaminated environment.

Material and Methods

Through molecular studies of 16S rRNA and *rec*A gene was possible to make the appropriate classification of species in 450 isolates of Terra Preta Antropogênica (TPA) and adjacent at four sites (TPA Calderão-Cultivado, TPA Caldeirão-Capoeira, TPA Hatahara and TPA Mina-I), and obtained 177 isolates of the genus *Burkholderia*. With sequences of 16S rRNA and *rec*A phylogenetic trees were constructed. These strains were used in biodegradation test in 96-well plates where each well contained medium BH, the indicator DCPIP, one of the substrates: naphthalene, phenanthrene or diesel and inoculum. For each plate, 15 isolates were tested, along with a standard DSMZ - *Pseudomonas fluorescens*, positive and negative control, always in triplicate.

Results and Discussion

By molecular analysis of the 16S rRNA phylogenetic grouping was efficiently by grouping pathogenic species. By analysis of the *rec*A gene, the sequences of the isolates were grouped with reference sequences, since the isolates are of tropical origin, which require more specific studies. The degradation test, 19 isolates degraded naphthalene, 16 degraded phenanthrene and 126 degraded diesel, generating a total of 132 isolates of the genus *Burkholderia* that have the ability to degrade at least one of the three substrates. Highlights are the isolates BCM 48 (*B. pyrrocinia* - ADJ Cal-Cult), BCM 49 (*B. unamae* - ADJ Cal-Cult), BCM 59 (*B. glumae* - ADJ Cal-Cult), BCM 67 (*B. cepacia* - Cal-ADJ Cap), BCM 244 (*B. glumae* - TPA Mine-I), BCM 254 (*B. sartisoli* - TPA Mine-I) and BCM 291 (*B. gladioli*) which degraded the three substrates simultaneously. Of the 132 isolates with the potential for biodegradation, 61 isolates were obtained from TPA, since the horizon is a little disturbed by farming, but rich in organic matter from human activities in the past.

Key words: TPA; Diesel; polyaromatic hydrocarbons; 16S rRNA; DCPIP.
Financial support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

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IMPACT OF THE TREATED EFFLUENT FROM SEWAGE TREATMENT STATION - SABESP ON WATER QUALITY OF ITAPETININGA RIVER, SP

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Keywords: Itapetininga River, water quality, sewage treatment plant

Since antiquity, water bodies have been used as a vehicle for dispersion of effluents generated by various human activities. As a result, the physical, chemical and biological properties of water are altered causing harm to aquatic life and humans. The treatment of these effluents aims to reduce pollution and allow the reestablishment of the receiving water body, reducing the risks to the environment. The Itapetininga River, located in the southwestern state of São Paulo, is responsible for the public supply of Itapetininga city which has approximately 138 000 inhabitants. In this municipality 94% of households have sewage collection and 100% of this is treated. This study analyzed the impact of effluent discharge from sewage treatment station (STS) - SABESP on the water quality of Itapetininga River since this effluent is disposed at the Ponte Alta Stream, a tributary of the river studied. Four sampling points were established in the Itapetininga River: P1 - Porto Velho Neighborhood, Itapetininga city, before it receives waters from the Ponte Alta Stream; P2 - Curuçá Neighborhood, Itapetininga city, after receiving the waters from the Ponte Alta Stream; P3 - SP 270 Rechan municipal road, an important point to verify the autodepuration of Itapetininga River; P4 - three kilometers before the Itapetininga River mouth, where it empties into the Paranapanema River. A sampling point in the Ponte Alta Stream (P5) was also established, about 400 meters from its stream mouth and has already received the STP effluent. The parameters measured in months Nov/2009 and Jan, Mar, May, Aug and Set/2010 were *Escherichia coli* (MPN/100mL), biochemical oxygen demand (BOD) (mg/L), dissolved oxygen (DO) (mg/L), total phosphorus (TP) (mg/L) and total nitrogen (TN) (mg/L), according to the methodologies established by the Standard Methods for the Examination of Water and Wastewater. The P5 had the highest average for *E. coli* (50650 ± 27477) while P1 had the lowest average (385 ± 411). After receiving the waters of the Ponte Alta Stream, values of *E. coli* increased in the Itapetininga River and there is a decay later: P2 (8298 ± 4265), P3 (3108 ± 2821), P4 (1464 ± 1398). For DBO, the highest average was also found in P5 (19.9 ± 8.4) followed by P4 (13.5 ± 5.3), P3 (12.3 ± 3.9), P1 (11.5 ± 4.7) and P2 (10.4 ± 5.0). OD values were: P1 (6.0 ± 1.5), P2 (6.0 ± 1.5), P3 (6.2 ± 1.7), P4 (6.2 ± 1.6) and P5 (5.6 ± 1.0). It was not possible to determine PT and NT in the months Nov/2009 and Set/2010. For the PT, the highest average was found in P5 (286 ± 106) followed by the average found in P2 (224 ± 123). P5 also had the highest average for NT (2.03 ± 1.56) followed by the average found in P1 (0.95 ± 0.24). The STS - SABESP has no specific system for final disinfection and this contributes to increase the rate of *E. coli* in the Itapetininga River. No major changes were observed in the Itapetininga River when the other parameters were analyzed, emphasizing the importance of treatment of domestic sewage in water conservation.

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IMPROVING CELLULOLYTIC ENZYME PRODUCTION USING SUGARCANE BAGASSE IN A SOLID-STATE FERMENTATION BIOREACTOR

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Keywords: endoglucanase, xylanase, cellulase, enzymes, bioethanol, solid-state fermentation.

Solid-state fermentation (SSF) represents a promising alternative process for reducing the costs of cellulase enzymes and contributes to the viability of cellulosic ethanol production. Among the factors that affect the enzyme production by SSF are the substrate and the nutritional medium used for microorganism cultivation. In this context, this study evaluates the use of sugarcane bagasse as a substrate for cellulases and xylanases production by a selected strain of *Aspergillus niger* cultivated under SSF, using a instrumented lab-scale bioreactor. Fermentations were carried out using a substrate with 70% initial moisture at 32ºC, air flow rate of 24 mL/min with 70% relative humidity, during 72 h. Different concentrations of CMC (0 to 5g/L), soy peptone (0.75 to 24 g/L) and yeast extract (0.25 to 12 g/L), and the addition of wheat bran was used to evaluate enzyme productivity. By using sugarcane bagasse and wheat bran in proportion of 1:1 resulted in a 6.5 times increase in enzyme production. Endoglucanase activity up to 64 U/g was achieved using higher soy peptone concentration, while xylanase production (118 U/g) was favored using lower soy peptone and yeast extract concentrations. These results show that sugarcane bagasse nutritional deficiencies as SSF substrate can be suppressed by the addition of wheat bran and the right choice of the medium components and their concentrations, thus improving enzyme productivity.

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INDUSTRIAL WASTES AS ALTERNATIVE SUBSTRATES FOR BIOSURFACTANT PRODUCTION WITH APPLICATION IN THE ENVIRONMENTAL AREA

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Biosurfactants are emulsifiers of hydrocarbons produced by bacteria, fungi and yeasts. They are polymers forming micelles that accumulate at the interface between liquids of different polarities. The interest of the industry in surfactants of microbial origin has been increasing due to the ability of these biomolecules in reducing the surface tension, giving emulsification, solubilization and phase dispersion. The environmental compatibility and the low toxicity are factors for the advancement of the research in this biotechnological area. Despite the advantages of biosurfactants over their synthetic counterparts, they are not produced in large scale due to high production costs. The aim of this work was to use two industrial wastes as low-cost substrates for biosurfactant production by the bacterium *Pseudomonas cepacia*. The microorganism was grown in mineral medium supplemented with 2.0% corn steep liquor and 2.0% soybean frying oil for 144 hours at 30ºC under 200rpm. The growth kinetics and production showed that the biosurfactant was able to reduce the water surface from 70.0 mN/m to 27.57 mN/m. The maximum biomass production was 11.97 g/L and the biosurfactant yield was 5.2 g/L after 144 hours of cultivation. The results show the great potential of the biosurfactant for environmental and industrial applications, especially in sectors of the petrochemical industry like the bioremediation of soils contaminated with hydrophobic pollutants.

Keywords: biosurfactants; *Pseudomonas cepacia*; Wastes; Bioremediation.

Financial support: Conselho Nacional de Pesquisa e Desenvolvimento (CNPq), Fundação de Amparo à Pesquisa e Tecnologia do Estado de Pernambuco (FACEPE) and TERMOPE/ANEEL.

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INFLUENCE OF CULTURE MEDIUM FOR THE FORMATION OF BIOFILM *Pseudomonas aeruginosa* ATCC 25853 ON THE SURFACE OF STAINLESS STEEL

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Keywords: Biofilm; Milk; TSB; *Pseudomonas aeruginosa*

Introduction

Introduction

Since the time of Antonie van Leuwenhoek, scientists from various areas have done work on biofilms, which correspond to structured microbial communities adhered to abiotic surfaces and / or biotic, consisting mainly of water and exopolysaccharides (EPS). The process of biofilm formation occurs through a series of sequential events, which can be governed, in part, by physical-chemical factors, while its development depends on strain and environmental conditions. The aim of this study to assess the ability of biofilm formation by *Pseudomonas aeruginosa* on stainless steel in the presence of reconstituted skimmed milk and TSB (Tryptone Soy Broth).

Materials and Methods

Coupons of stainless steel AISI 304 were placed in Petri dishes of 140 mm in size. On each plate, were immersed in 60 mL TSB and reconstituted skim milk and inoculated 10^8 CFU/ mL of *P. aeruginosa* ATCC 27853, incubated at 37 ° C and shaken at 50 rpm. Every two days of incubation, one coupon was removed from each petri dish. These were washed with peptone water, and biofilm was removed using a sterile swab. It promoted serial dilution and the number of viable cells determined in TSA, using the technique of surface plating. The plates were incubated at 37 ° C for 24 hours, being accomplished, the standard plate count. All the research was conducted in three replicates (10 days each) and analysis in duplicate.

Results and Discussion

Pseudomonas aeruginosa biofilm formed on stainless steel when grown in reconstituted skimmed milk (7.6 log CFU/cm^2) and TSB (5.80 log CFU/cm^2) at 37 ° C. Most biofilm formation of *P. aeruginosa*, when grown in milk, may be related to heterogeneity of substances found in it. Several authors mention that the properties of milk support the growth of pathogenic and spoilage microorganisms, bacteria are found predominantly Pseudomonads.

Carbon sources and micronutrients as well as influencing the quantification of the biofilm, can also influence the type of biofilm formed by *Pseudomonas aeruginosa*, a biofilm that varies from flat to a highly structured biofilm.

The medium has great importance in biofilm formation, since it can affect all segments of industry.

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INFLUENCE OF NITROGEN SOURCE ON THE PRODUCTION OF RED PIGMENTS BY *Monascus ruber* USING GLYCERINE AS SUBSTRATE.

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Keywords: *Monascus ruber*, glycerine, pigments, nitrogen sources.

Monascus ruber whose main feature is the ability to produce secondary metabolites with polyketide structures with yellow, orange and red pigmentation can produce pigments that can be used for coloring foods, replacing the synthetic dyes. The high cost of manufacturing of natural dyes can be minimized by using low cost organic waste. An alternative substrate for pigment production is glycerine, obtained as the main byproduct of the biodiesel industry. The use of amino acids in the culture medium has been used as a stimulant of extracellular accumulation of pigments contributing to the increased productivity of red pigments.The pigments react with the amino group forming water-soluble pigment.

The microorganism used in the glycerine fermentation was the filamentous fungus *Monascus ruber* CCT 3802 maintained in tubes with potato dextrose agar at 4 ºC. Pigment production was evaluated in submerged cultivation using Erlenmeyers flasks, in the following medium ($g.L^{-1}$ of distilled water): glycerine, 20; K_2HPO_4, 5; KH_2PO_4, 5; $CaCl_2$, 0.1; $MgSO_4.7H_2O$, 0.5; $FeSO_4.7H_2O$, 0.01, $ZnSO_4.7H_2O$, 0.01; $MnSO_4.H_2O$, 0.03 and nitrogen sources (glycine 5, and 9; peptone 5 and monosodium glutamate (MSG) 5). The pigments were quantified by spectrophotometer at 510 nm, until 192 h and a sample of 5 mL were taken every 24 hours.

There was greater pigment production in cultures containing glycine as nitrogen source. In flask containing 9 $g.L^{-1}$ of glycine production was 5.8 UA (Units of Absorbance) of red pigments. While in flasks with 5 $g.L^{-1}$ was produced 5.2 UA. Cultive with MSG the production was 4 UA and in the flask containing peptone, only 1 UA of red pigments was observed. Was also observed increased growth in cultives with glycine. It is know that the *Monascus* pigment production is associated with growth, wich was observed in this study. The nitrogen source is a very important factor in growth and pigment production. The pigments are mainly produced in the cell (intracellular). The replacement of oxygen in the pigments structures by the nitrogen of the amino groups of compounds such as amino acids, peptides and proteins, increases the production of red pigments. It was found that there was production of water-soluble red pigments in cultures containing glycine and MSG as nitrogen sources. Thus, it is possible to obtain products with higher added value, such as *Monascus* pigments, using co-products of biodiesel, as crude glycerine using a suitable nitrogen source.

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INFLUENCE OF pH, SUGAR AND ETHANOL STRESSES IN THE GROWTH OF *Dekkera bruxellensis*

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Keywords: *Dekkera bruxellensis*, ethanol, sugar concentration, pH

The yeasts *Dekkera bruxellensis* are important contaminants of the fermentation for fuel alcohol and beverage production. A little is known about their role in the fermentative process and what conditions may influence their growth. In this work the growth of three strains of *D. bruxellensis*, isolated from distilleries in São Paulo State (CCA059, CCA077 and CCA155), were evaluated in conditions of stress like low pH (1.0, 1.5, 2.0 and 2.5), high ethanol concentration (9%, 10% and 11%) and high sugar concentration (300g/L, 350g/L and 400g/L). The growth curves were obtained using 125-mL Erlenmeyers flasks with 50 mL of liquid YEPD, at 30ºC, 160 rpm, for 64 hours. The growth was measured by absorbance at 600 nm. The strains of *D. bruxellensis* had different responses to the stresses: CCA155 was more sensitive to the low values of pH, but grew well with high ethanol concentrations; however, the strains CCA059 and CCA077 were more sensitive to the concentrations of 10 and 11% of ethanol, but more resistant to the low pH. The strain CCA077 was very sensitive to 9% of ethanol, which can facilitate its control in the distillery. Concerning the sugar concentrations, all strains were resistant and presented vigorous growth in the highest concentrations. The strains displayed different responses to the conditions of stress here studied, which show the variability existing among strains of the same species. Studies concerning factors which can influence the growth of these yeasts are demanded, especially in combination, because negative effects of their contamination in the fermentation have been related, with significant impacts in the alcohol yield.

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INFLUENCE OF THE ACID TREATMENT IN THE GROWTH OF *Saccharomyces cerevisiae* STRAINS WITH DIFFERENT COLONY PHENOTYPES

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Keywords: contaminants, fermentation, wild yeast

Saccharomyces cerevisiae yeasts are the unique agents of the alcoholic fermentation, displaying colony phenotype that may vary from mucous to wrinkled colonies, and cell morphology from dispersed cells to pseudohyphal growth, respectively. Many reports about the harmful effects of the biotype "wrinkled colony" are found but a little is known about its characteristics and response to stress conditions. In this work the effect of the acid treatment upon the cells of *S. cerevisiae* were analyzed using both colony phenotypes, in order to find a condition to inhibit the growth of the biotype "wrinkled colony". The cells (strain PE-02, mucous colony and dispersed cells; strain 52, wrinkled colony and pseudohyphal growth) were pre-grown in sugar cane juice at 4° Brix overnight at 30°C and following an acid treatment was applied to the cells recovered by centrifugation in conditions of pH 1.0, 1.5 and 2.0, under agitation, at 30°C, for 2 hours. Following they were inoculated in sugar cane at the same concentration and cultivated with agitation of 160 rpm, at 30°C for 36 hours. Samples of the cells before the acid treatment, right after the acid treatment and after 18 and 36 hours of cultivation were taken from the flasks, serially diluted and plated in YEPD medium. The plates were incubated at 30°C for 3 days and the number of colony forming units was counted. The results showed that for all the treatments the wrinkled colony strain was mostly affected, being more efficient in the pH 1 and 1.5. For the mucous colony, there was a decrease in the growth after the acid treatment, more relevant at pH 1, but its recovery was quite significant, because after 36 hours it had increased its population over the initial amount. We can conclude that the acid treatment at values of pH 1.5 would be effective to control the growth of the biotype "wrinkled colony" without affecting substantially the growth of the main yeast, the starter ferment.

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INFLUENCE OF TIME AND TEMPERATURE ON FERMENTATION FOR THE PRODUCTION OF AMYLASE BY *Rhizopus microsporus* var. *oligosporus*

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Keywords: amylase, temperature, time of fermentation, *Rhizopus microsporus* var. *oligosporus*

Amylases are a complex of enzymes that together can catalyze the complete hydrolysis of starch resulting glucose. Have great biotechnological important due to its large spectrum of applications, such as in food, detergents, paper, textiles, chemical and pharmaceutical industry. Can be obtained from various sources such as plants, animals and microorganisms, however, microbial enzymes are usually higher demand for large scale production. The fungus *Rhizopus microsporus* var. *oligosporus* is considered efficient in the production of amylase, and safe for food use (GRAS) by the FAO (Food and Agriculture Organization). The aim of this study was to select the best condition of submerged fermentation, as to time and temperature for the production of amylases by *R. microsporus* var. *oligosporus* CCT 3762. *microsporus* var. *oligosporus* CCT 3762. The characterization of the enzymatic extract was a measure of amylase activity, based on the hydrolysis of starch and color reaction with iodine. For this, an U (Enzyme Unit) was defined as the amount of enzyme required to hydrolyze 10 mg of starch in 30 minutes of enzymatic reaction at 60°C. The fermentation media was composed of ammonium sulfate, monobasic potassium phosphate, urea and 3% of wheat flour type II, which is a secondary product with low value added of processing industry of wheat, at pH 5.5 and 160 rpm. The microorganism was initially inoculated through the cell suspension at a concentration of $2x10^7$ spores per gram of starch substrate for 48 hours and the biomass obtained was used as inoculum for the fermentation step. Initially the cultivation was 30°C varying the fermentation time in 72h, 96h, 120h and 144h. It was observed a maximum activity of 4.43 U/mL in 96 hours becoming significantly constant after this period. As this time was more favorable, it was chosen to the test of comparison between the temperatures of 30°C and 37°C. It was found that the amylase production at 37°C (3.88 U/mL) was statistically similar at 30°C (p=0.07). Thus, was conclude that the best condition for the production of amylase by *Rhizopus microsporus* var. *oligosporus* was cultivation in 96 hours and there was no influence of temperatures studied in this study on fermentation.

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INTERFERENCE OF CARBENDAZIM AND TIOPHANATE-METHIL IN THE ACTION OF *Trichoderma viride* AND *Trichoderma harzianum* IN PLANT DISEASES INTEGRATED CONTROL.

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Keywords: fungicides, integrated control, compatibility, *Trichoderma* spp.

Introduction: The use of fungicides is one of the main methods of plant disease control, however, these chemicals cause impacts in the environment and in human health. Integrated control is an alternative to reduce the use of these products. The implementation of this practice requires caution considering that biological control agents could be affected by the chemical products. The aim of this work was evaluate the effects of active ingredients carbendazim and thiophanate-methyl, both benzimidazoles fungicides, on *Trichoderma viride* isolate TV21 and *Trichoderma harzianum*, isolate TH11.
Material and Methods: Samples were analyzed for vegetative growth, sporulation, direct antagonism and volatile metabolites production in culture medium supplemented with each active ingredient in the recommended concentration for use (500 g/L and 700 g/kg, respectively). To analysis of vegetative growth, the isolates were cultivated in Potato-dextrose agar medium (PDA) and the diameter colonies were measured after five days. Concluded the analysis of vegetative growth, the colonies were used to prepare a spore suspension that was used to count in Neubauer chamber. To analyze the direct antagonism assay, was tested *Fusarium oxysporum* f.sp. *phaseoli* like a pathogen in the fungicide supplemented medium with each *Trichoderma* spp. isolate. After five days the pathogen colony was measured. To the experiment with volatile metabolites production, the *Trichoderma* sp. isolated were inoculated on PDA supplemented with fungicides and the pathogen was inoculated on PDA. After, both Petri dishes were overlapping and incubated by five days, and the pathogen colony was measured. The results expressed in continuous values were analyzed by ANOVA followed by Tukey post-test (5%) for media comparison. Other results were evaluated by regression and by orthogonal contrast method.
Result and Discussion: The active ingredient carbendazim inhibited the vegetative growth and sporulation of *Trichoderma viride* (TV21) and *Trichoderma harzianum* (TH11); because of that, was not possible realize the other tests and carbendazim showed a fungicide effect in both tested isolate. The active ingredient tiophanate-methil not interferes on vegetative growth or sporulation of *Trichoderma* isolateds tested. Neither the antagonistic capacity nor the volatile metabolites production was affected, and the integrated control (use of tiophanate-methil and *T. viride* or *T. harzianum*) was more effective than the biological or chemical control alone, in the *in vitro* assays. These results shows that carbendazim was toxic to *T. harzianum* and *T. viride* and is not recommended to integrated control, while tiophanate-methil was considered compatible with *T. viride* and *T. harzianum* and for some mechanisms, like volatile metabolites, acts potentiating the effect of biological control.

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ISOESTERASE: PRESENCE AS INDICATORS OF RESISTANCE IN PLANTS TREATED WITH BARLEY AFTER ELICITOR XANTHAN GUM OR ASSOCIATED WITH FUNGICIDE OPERA®.

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Keywords: Xanthan gum, isoesterase, barley.

Introduction: For control leaf spot in barley plants (Embrapa 195) caused by *Bipolaris sorokiniana*, the measure most used by producers has been treated with fungicides that can be cause risks to the environment and human health. To eliminate these drawbacks, one of the methods considered has been the use induced of resistance. Thus, the purpose of this study was to evaluate the effect of xanthan gum elicitor associated with fungicide for control leaf lesions and the results correlated with the presence of esterase isozymes.

Materials and Methods: Experiments were conducted by spraying barley plants is organized as follows: a) healthy; b) treated with xanthan gum; c) inoculated with the pathogen; d) treated with inducer and 24 h after inoculated with the pathogen; e) ditto the group d, however, after 48 h; f) ditto the group d, however, after 72 h; g) xanthan gum + pathogen and Opera®; h) plants only with Opera®. During the first 24 hours after pathogen inoculation, the plants were kept in a moist chamber (100% RH), ambient temperature and dark. Then the material was transferred to a greenhouse and evaluated after 7 days.

Results and Discussion: The results showed that plants treated with elicitor xanthan gum (XG) at different times (24, 48 and 72h) showed protection of 94 to 98% and lesions from 0.015 to 0.35 mm^2. When treated with Opera® protection was 77% and lesion from 0.098 to 2.80 mm^2. The treatment of plants with Opera® and XG promoted a protection of 92.6% and lesions between 0.018 to 0.052 mm^2. In relation to the esterase isozymes of barley plants healthy, only treated with elicitor XG, and XG + pathogen, demonstrated the presence of four bands of esterase isozymes with Rf 0.213, 0.69, 0.717 and 0.856 but with higher enzymatic activity that can be correlated with greater induction of resistance both locally and systemic. Using Opera® fungicide, plant extracts showed three bands (Rf = 0.39, 0.69 and 0.856), whereas the latter two had an increase in activity compared with healthy plants. Barley plants when treated with elicitor-fungicide (Opera® and XG) were observed two bands with Rf = 0.69 and Rf = 0.856 and 92.6% protection.

Conclusion: We conclude that when the esterase Rf = 0.856 was active between 5504 and 7544 the largest lesions were inhibited whereas when the activity was increased to 13,942, the smaller size of lesions were inhibited.

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ISOLATION AND CHARACTERIZATION OF BACTERIAL STRAIN CAPABLE OF LIMONENE BIOTRANSFORMATION.

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Keywords: bacteria, biotransformation, characterization, isolation, limonene.

The citrus industry has a key role in the Brazilian agribusiness, with the production of frozen concentrated orange juice as the main activity and having the State of Sao Paulo as the main producer, processor, and exporter. The orange essential oil is a byproduct of the juice production, which makes Brazil the world's largest producer of this kind of essential oil. Limonene is the main component of citrus essential oil, accounting for nearly 95% of its composition. Because of the high production of essential oil from sweet orange in Brazil, limonene has a very low commercial value. One way of obtaining other products from limonene with higher commercial value is through the use of microorganisms capable of biotransforming this terpene. Thus this work aimed to isolate and identify bacterial strains from sweet orange fruits that can grow in presence of limonene and therefore metabolize it. Using a fruit at the beginning of decomposition stage we isolated some bacteria from the peel, the main source for essential oil in citrus. This was done by adding the extract to a medium containing $(NH_4)_2SO_4$ 37.83mM; $(NH_4)_2HPO_4$ 12.34mM; NaCl 8.55mM; $MgSO_4.7H_2O$ 1.62mM; $CaCl_2.2H_2O$ 4.08mM; KCl 28.83mM; $FeSO_4.7H_2O$ 0.03mM; $ZnSO_4.7H_20$ 0.03mM; and $CuSO_4.5H_2O$ 0.04mM. The suspension was incubated for one day at 37ºC with rotation at 250rpm and from that we isolated bacteria on plates. The isolated bacteria were grown in LB medium and had their 16S-23S intergenic region amplified for identification using universal primers. The amplicons were purified using the Geneclean kit (BIO 101) and cloned into pJet1.2 (Fermentas). The plasmidial DNA was extracted through alkaline mini preparations using 100μL of Solution I (glucose 50mM, Tris-HCl pH 8.0 25mM, EDTA pH 8.0 10mM), 150μL of Solution II (NaOH 0.2 N, SDS 1%), and 200μL of Solution III (60mL of potassium acetate 5M, 11.5mL of glacial acetic acid, in a total of 100mL). The cell debris was removed by centrifugation, and the DNA was purified with Geneclean kit (BIO 101). The sequencing was done with BigDye 3.1 (Applied Biosystems) using primers specific for pJET1.2. The sequences were obtained in an ABI 3730 automatic sequencer (Applied Biosystems) and processed in the LASERGENE 99 (DNASTAR), resulting in contigs that were then used for comparison with sequences from the public database Genbank, using the blastn tool. We observed similarity with sequences from citrus chloroplast as well as uncultured bacteria with the different sides of the cloned insert.

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ISOLATION LACTIC-ACID BACTERIA OF FRESH MILK FROM PELOTAS,RS

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Keywords: Lactic-Acid Bacteria, fresh milk.

The Lactic Acid Bacteria (LAB) are a group of microorganisms naturally found in foods, including milk and derivates. The microorganims in this group have several morphological, physiological and metabolic characteristics in commom: They are Gram positive, non-spore forming, catalase negative, fastidious, anaerobic, aero-tolerants and acid-tolerants. Several studies describe the participation of LAB as a constituent of the microflora of milk and its derivates, especially artisan cheeses. However, in Brazil the studies for the identification and characterization of lactic microflora in these products are scarce.The isolation and identification of microorganisms from natural sources has been a way widely used for obtaining useful and genetically stable strains (ADNNA and TAN, 2007). The objective this study was to isolate LAB of samples of fresh milk. The LAB were isolated of 03 samples of Milk fresh from different farms localizated in Pelotas, Rio Grande do Sul. For the isolation was used ágar and broth MRS (Man, Rugosa and Sharpe). From the pure cultures held tests of Gram, catalase, morphology as well as tests of their growth in MRS broth under the following conditions: 3.0 and 4.0% NaCl, 0.3 and 1.0 % bile salts and pH 3.0 and 4.0. In this study were obtained 12 isolates of lactic acid bacteria, all were characterized as Gram-positive and catalase negative. All isolates showed resistance to pH 3 and 4. This information is extremely important to predict the resistence of culture at pH values that the bacteria will be submitted after being ingested with the food that contains. In the same way, the isolated microorganisms showed resistant in media added 3-4% NaCl. This information is important to evaluate the possible applications of bacteria isolated. However, was possible to identify that there was no growth of any microorganism isolated in medium containing 1% bile salts, while 100% of them were resistant microorganisms in the medium containing 3% of salt. The LAB acid-tolerants and bile tolerants can be isolated in conditions of stress. The LAB are used for conservation of foods through fermentation promoting beneficial effects to health, preventing contamination caused by pathogenic microorganisms. These isolates can be tested in dairy products for checks its beneficial properties replacing the use of commercial cultures. The importance to use LAB tolerants to several concentrations of NaCl, bile salts and several pH ranges is in to diversification of foods products with the same benefits offered by these microorganisms.

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ISOLATION OF BLACK YEASTS (CHAETOTHYRIALES) FROM LEAF-CUTTING GYNES FOR GENOMIC STUDIES

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Keywords: microbial ecology, dematiaceous fungi, taxonomy, Attini, genome sequencing

The Attini, also known as leaf-cutting ants, live in obligatory symbiosis with basidiomycetes fungi (Agaricales: Lepiotaceae), which they propagate in order to start new colonies. This symbiosis was reviewed under a molecular phylogenetic point of view and the analyses showed the occurrence of black yeasts (Chaetothyreales) forming a derived monophyletic lineage, in the *Apterostigma* ant-microbe association. Although the species of chaetothyrialean fungi are recurrently observed on humans, their environmental niches are not well clarified yet. Their presence in natural substrates has been considered uncommon. The purpose of this study is to select a black yeast strain associated with leaf-cutting ants in Brazil so they can be used in genome sequencing by the Broad Institute, Cambridge, Massachusetts State. In order to study black yeasts in gynes, the oil flotation isolation technique was used. Representatives of *Atta laevigata* and *A. capiguara* were collected during two mating flights: in 2008 and 2009. Bodies and infra-bucal pellets were submitted to the method. The isolates were preserved in slants at 10°C and by ultra-freezing at -80°C. Based on morphological and molecular characteristics, 9 strains were recognized as black yeasts, five from *Atta capiguara* bodies, like *Exophiala spinifera*, *E. dermatitidis* and *E. salmonis;* and four strains of *E. dermatitidis* recovered from *Atta laevigata* pellets. The most abundant species was *E. dermatitidis* (66,6%). Among the species found, *E. dermatitidis* could be indicated to genome sequencing because it is known as the causative agent of serious infections and often isolated from rather extreme environments polluted with aromatic hydrocarbons, such as tropical creosoted railway ties. The genome study will provide an enormous boost to the study of medical and ecological aspects of black yeasts. The isolation of fungi from the body and pellet gynes revealed the possibility of spreading these fungi by winged forms. Further studies are needed to better define the role played by these micro-organisms in the ant nests, as well as the side effects of the dispersion of potential pathogens in the environment.

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Kinetics of extracellular polysaccharide (EPS) production by *Azospirillum brasilense* Ab-V5

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Keywords: Plant growth-promoting bacteria, fructose, glucose, agregation rate

Endophytic bacteria are known as a microbial group living inside plant tissues, without trigger any plant defense response. Indeed, several endophytic bacteria are considered plant growth-promoters, increasing plant biomass and yield of some agriculturally important crops. In this sense, some isolates of the Azospirilla group has been exploited as inoculant for grasses, e.g. maize, wheat and rice. Plant growth-promoting Azospirilla needs to colonize external tissues and/or endophytic plant tissues in order to gain intimal association with the plant host and then express the benefic traits. The colonization of the host plant by growth-promoting bacteria has been shown to be associated with the production of extracellular polysaccharides (EPS), differently from the symbiotic Rhizobia that carry the nod genes responsible to the expression of lipo-chito-oligo-saccharides involved on the nodules formation. Nevertheless, studies regarding the EPS produced by *Azospirillum* sp. are scarce on the literature. This work aimed to evaluate the EPS kinetics and the cellular aggregation of an *Azospirillum brasilense* strain Ab-V5 in response to carbon sources. To reach these objectives, a pre-inoculum of *A. brasilense* Ab-V5 was grown for 24-h in 5 mL of Dyg´s liquid medium at 28 ^{o}C. After the normalization of cells density to a $O.D._{540nm}$ of 0.05, corresponding to a 10^7 cells mL^{-1}, an aliquot of 1 mL of this suspension was transferred to 24 mL of aggregation media with different carbon sources, incubated at 28 ^{o}C with 150 rpm shaking, and sampled at intervals of 12-h incubation. Cellular aggregation tests and EPS precipitation were performed on these samples. Total carbohydrates were determined by the phenol-sulfuric method, and total protein concentrations were measured using the Bradford assay. The EPS production by *A. amazonense* Ab-V5 under fructose as carbon source (medium I) reached a peak of 0.135 g l^{-1} after 72-h of growth. The use of sucrose as carbon source (medium II) resulted a much lower EPS production with the peak reached in a short incubation period (0.063 g EPS L^{-1} after 36-h incubation). On both media, the EPS concentration decreased after the peak been reached. Bacterial cellular growth was inferred from total protein in the cultures. On both media, the EPS peaks were correlate with the stationary phase of the growth cycle, with values up to 0.06 g of protein L^{-1} on the medium I and up to 0.03 g of protein L^{-1} on medium II. Cellular aggregation rate was found to be 41.8% on the medium I, and 25.1% on the medium II. These results support the finding that EPS is related to the secondary metabolism due to the EPS peak had been coincident with the stationary growth phase. The use of fructose or glucose as carbon sources had influence on the bacterial growth, and hence differences on EPS production and cell aggregation. The effect of others carbon sources on EPS production and quality in under way.

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Lactobacillus delbrueckii MUTANTS WITH TOLERANCE ENHANCED TO FREEZING

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Keywords: Probiotic; Acridine orange; Freezing; *Lactobacillus* mutants

Lactobacillus delbrueckii UFV H2b20 is a lactic acid bacteria that displays probiotic properties. Like other lactic acid bacteria, its tolerance to preservation methods, such as freezing and freeze-drying, needs to be increased. Otimization of freezing conditions has not been enough to maintain a high the number of stable viable cells during long storage periods. In this context, this work aimed at generating mutant strains of *L. delbrueckii* UFV H2b20 with increased tolerance to the freezing. *L. delbrueckii* UFV H2b20 was cultured in MRS broth at 37 ºC overnight and sub - cultured in MRS broth containing 50 µg mL^{-1} of acridine orange at 37 ºC overnight. After the incubation period the suspension of cells the was frozen -20 ºC for 10 days for accomplishment of selection of mutants. With 10 days of storage, the frozen culture was diluted in PBS buffer (0,02% KCl, 0,144 % Na_2HPO4, 0,8 % NaCl, 0,024 % KH_2PO4, pH 7.0) and spread plated on MRS agar determined by drop plating on MRS agar and incubated at 37 ºC overnight . Selection of mutants was accomplished based on the tolerance to three freezing -thawing (-20 ºC/25°C) cycles. Cell numbers UFC were determined by drop plating on MRS agar at 37 °C for 24 hours. Adaptative responses to cold shock (10 °C pre- incubation), and heat shock (50 °C pre – incubation), for 30 minutes, before freezing and storage for 70 days at –20 °C, was compared to those of the wild type. Statistical analysis was performed using SAEG (Version 9.1, 2007). The experiments were repeated three times and media of values were compared at $P < 0.05$ using the Tukey test. The ten isolated mutants presented significantly ($P < 0,05$) higher survival to conditions of freezing-thawing cycles while the wild type strain did not survive those conditions. Mutants Lb M4, Lb M6, and Lb M10 were the ones that presented increased tolerance to the conditions. The mutant Lb M6 presented the highest survival percentage, and for that reason was selected to be further characterized. Incubation at 10 ºC accentuated significantly ($P < 0,05$) the survival of the mutant strain Lb M6 during 70 days storage at -20 ºC. This might probably have happened due to modifications in genes involved in the adaptative response to cold stress. When they were pre- incubated at 50 ºC the survival was increased, however, the mutant strain and wild strain didn't present significant differences ($P > 0,05$). The probiotic properties were not tested yet, but resistance to processing conditions is a variable trait in probiotic and starter cultures.

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LIPASE PRODUCTION BY *Bacillus licheniformis* (UCP 1014) USING ICE CREAM INDUSTRY RESIDUES BY FACTORIAL DESIGNER

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Keywords: lipase, *Bacillus licheniformis*, factorial designer

Lipases, triacylglycerol hydrolases, are an important group of biotechnological relevant enzymes ubiquitous in nature and produced by various plants, animals and microorganisms. Microbial lipases have gained special industrial attention due to their stability, selectivity and broad substrate specificity. Lipases are by and large produced from microbes and specifically bacterial lipases play a vital role in commercial ventures. Members of the *Bacillus* genus are generally potential candidates for biotechnological applications, including antibiotics and enzymes production. Microbial lipases are mostly extracellular and their production is greatly influenced by medium composition, and physical and chemical factors such as temperature, pH, and dissolved oxygen. These enzymes are generally produced in the presence of lipid such as oil or any other inducer, such as triacylglycerols, fatty acids, hydrolysable esters, Tweens, bile salts, and glycerols. The nutritional requirements for microbial growth are fulfilled by several alternative media as those based on defined compounds (synthetic medium). The use of industrial residues as components of culture media has emerged as an alternative to minimize the disposal of tailings from various manufacturing industries. The factorial design is often used by scientists to understand the effect of two or more independent variables upon a single dependent variable. This work was carried out to select an alternative medium for lipase production by *B. licheniformis* (UCP 1014) and to formulate a new medium using residue from ice cream industry through a factorial design 2^4 in variable conditions. The cellular growth occurred during 96h, 150 rpm, at 37^{o}C. The results showed that the medium C (glucose 1.0%, peptone 2.0%, 0.5% yeast extract, olive oil, 1.0%, $NaNO_3$ 0.1%, 0.1% KH_2PO4, $MgSO_4$. $7H_2O$ 0.05%), induced a lipase activity of 256 UI/mL. By using the factorial design, the condition 11 (peptone 2.0%, 0.5% yeast extract, $NaNO_3$ 0.1%, 0.1% KH_2PO4, $MgSO_4$. $7H_2O$ 0.05% and ice cream residue 5%) induced the highest enzyme activity, corresponding to 480 UI/mL. An increase of 46.8% was obtained by the use of the ice cream residue. The results suggest an alternative to reusing oil residues from the ice cream industry, commercially worthless raw material, as substrate for biotechnological purposes, in particular, as component of culture medium for the induction and increase the production of microbial lipase.

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MEDIUM OPTIMIZATION FOR THE CYCLODEXTRIN GLICOSILTRANSFERASE PRODUCTION USING SORGHUM STARCH

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Keywords: Cyclodextrin glycosyltransferase; enzyme activity and sorghum

The enzyme cyclodextrin glycosyltransferase (CGTase, EC 2.4.1.19) catalyze the reaction of formation of cyclodextrins (CDs) from starch as carbon source. The CDs form inclusion complexes with host-molecules due to their truncated conical shape, changing the physical and chemical characteristics of them.

Sorghum is one of the Brazil's agricultural crops. The CGTase production by *Bacillus circulans*, strain obtained from the culture collection of the industrial microbiology laboratory of the Biological Sciences Institute of Rio Claro (SP, Brazil), under ideal conditions in sorghum starch medium was developed in this study.

The production has been achieved by carefully monitoring the medium, with variable concentration of sorghum starch, nitrogen sources (yeast extract; tryptone) at 35°C in flasks under controlled agitation. The enzymatic activity was measured, based on the discoloration of phenolphthalein solutions at 550 nm, which occurs after complexation with CD, following the method of Makela.

The main goal of the present study was to determine the optimum medium condition for CGTase production by *Bacillus circulans*. In order to this, various experiments were performed to estimate the effect of carbon and nitrogen sources (0.29, 0.5, 1.0, 1.5 and 1.71 %) on enzyme production. The effect of important parameters were optimized using surface response methodology (RSM) based on central composite design with fourteen experiments. The proposed model equation using RSM has shown good accord with the experimental data, with a correlation coefficient (R^2) of 0.87, which ensures adequate confidence of the model.

The highest enzyme production of 48.2 $U.mL^{-1}$ was obtained with concentration of sorghum 0.929, tryptone 0.476 and yeast 0.476. Observing figure 1 and 2 it is possible to see that exists an optimum activity region. The agreement of sorghum starch and nitrogen sources (0.9 until 1.1 %) can compose this higher enzyme yield region.

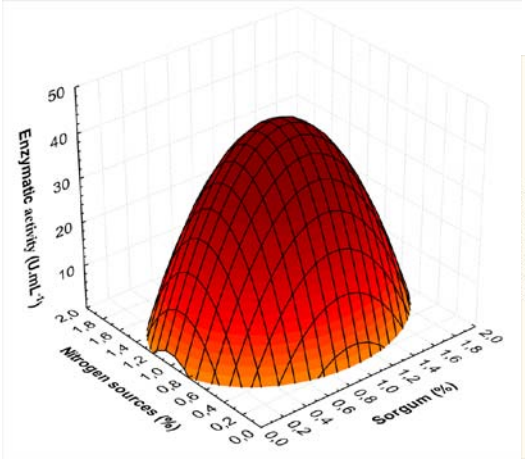

Figure 1: Surface Plots of CGTase activity ($U.mL^{-1}$) in function of nitrogen sources (%) and sorghum starch (%).

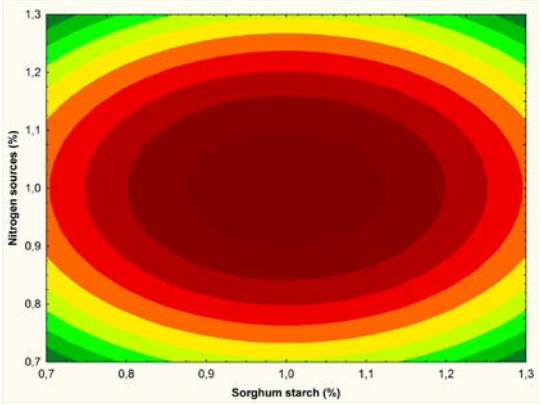

Figure 2: Contour Plots of CGTase activity ($U.mL^{-1}$) in function of nitrogen sources (%) and sorghum starch (%).

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Mesotrione Degradation Localization Genes Determined by Plasmid Cure

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Keywords: herbicide degradation, Callisto®, *Pantoea ananatis*, acridine orange.

Recent studies about mesotrione (Callisto®) biodegradation revealed a bacterial strain, *Bacillus* sp., producing 2-amino-4-methylsulfonyl benzoic acid (AMBA) or 4-methylsulfonyl-2-nitrobenzoic acid (MNBA) as secondary metabolites. We identified a different degradation pathway (Pileggi et al., data not shown) in *Pantoea ananatis*, by which these metabolites were not produced. Other authors describe the role of large plasmids in the degradation of different toxic compounds, but not mesotrione. We are interested in the possibility of mesotrione degradation be codified by plasmids harboring genes in *P.ananatis*. The strain was treated with acridine orange (curing agent) using 0 $\mu g.mL^{-1}$, 100 $\mu g.mL^{-1}$, and 150 $\mu g.mL^{-1}$ concentrations in 100 mL flasks, after 48 hours of incubation, at 30°C. Isolated colonies were transferred to 96 wells plates with BMM (2 mM potassium phosphate buffer, pH 7.0; 3 $g.L^{-1}$ $NaNO_3$; 0.5 $g.L^{-1}$ $MgSO_4$; 0.5 $g.L^{-1}$ KCl; 0.01 $g.L^{-1}$ $FeSO_4$; 0.04 $g.L^{-1}$ $CaCl_2$; 0.001 $g.L^{-1}$ $MnSO_4$) plus 0.4 g/L glucose, plus 0.03 mM mesotrione. We are developing a new screening technique for mesotrione degradation, based on low buffer strength medium and bromothymol blue pH indicator. After 48 hours, 10 µL of bromothymol blue were added in each well, and the color of media was observed. Blue media meant basic pH and positive degradation, and yellow color indicated acid pH, and therefore no degradation. From 315 colonies of *P. ananatis* tested, we obtained yellow color in two wells, representing non degrading colonies, one from 100 $\mu g.mL^{-1}$ acridine orange treatment and another one from 150 $\mu g.mL^{-1}$. The results suggest that plasmid curing occurred in a rate of 0.63%, lower than found in literature data. We used lower cure temperature than cited in literature (40°C), because *P. ananatis* is temperature sensitive. Degradation lost must be confirmed further by HPLC analysis.

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MICROBIAL CAPACITY TO DEGRADE ACENAPHTENE AND 2,4-DICHLOROPHENOXYACETIC ACID

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Keywords: contaminant, herbicide, polycyclic hydrocarbon derived, bioremediation, bacterial consortium.

ABSTRACT

Biodegradation of soil depend mainly on the metabolic activities of microorganisms to accelerate the mineralization of the contaminants or their transformation to less harmful chemicals. Here we present the capacity of indigenous bacteria to degrade the herbicide 2,4-Dichlorophenoxyacetic acid (2,4-D) and the polycyclic aromatic hydrocarbon Acenaphtene (Ace). *Methylobacterium sp., Xanthobacter autotrophicus, Burkholderia cenocepacia, Aurantimonas sp., Campylobacter jejuni, Methylobacterium chloromethanicum e Klebsiella pneumoniae* were isolated from 2,4-D contaminated soils. *Klebsiella pneumoniae* was isolated from residual water contaminated with fuel. After inoculation the growth was monitored through spectrophotometer at 600 nm (Shimadzu UV 2450). Both matrices were sampled in Porto Velho City (Rondônia, Brazilian Amazon) For the herbicide biodegradation experiment, soil bacterial consortium were incubated in a 2,4-D concentration of 10 $mg.l^{-1}$ during 22 days. To evaluate the 2,4-D bacterial biodegradative capacity we measured the bacterial activity (^{3}HLeucine incorporation) in different 2,4-D concentrations ranging from 100-1600 $mg.l^{-1}$. For the petroleum-derived biodegradation experiment, bacterial isolates and consortium were incubated in several Ace concentrations from 5-200 $mg.l^{-1}$ during 7 days. Bacterial growth were monitored through 2,6-Dichlorophenol indophenol (DCPIP), a colorimetric degradative indicator. The results from 2,4-D experiments showed that bacterial consortium presented high resistance decreasing the bacterial activity only at 2,4-D concentrations higher than 800 $mg.l^{-1}$. The results from petroleum-derived experiments showed that *Acinetobacter Baumannii* isolate and bacterial consortium were able to degrade Acenaphtene after 48h, while others bacterial isolates remains 206h for total DCPIP discoloration. Notwithstanding these single results, this work indicates that indigenous bacteria from Brazilian Amazon may be used as biodegradative tool for bioremediation studies.

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MICROBIAL CHARACTERIZATION IN EXTENDED AERATION ACTIVATED-SLUDGE PLANT

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Keywords: denitrifying bacteria, nitrifying bacteria, microscopy, sewage..

The activated sludge process is the most widely used biological wastewater treatment more effective and has been widely used in several counties. It consists in enabling the growth of permanent and acclimated microbial community through continuous aeration with recycle of biomass, which held by the degradation or stabilization of organic matter from domestic wastewater. The principle and effectiveness of treatment is the formation of flakes, as well as containing the microorganisms decomposing organic matter. This microrganisms are able to settle easily in the secondary settling tank and clarification of effluents could be made more efficiently, become suitable to be sent to the bodies water receivers. This study aimed to microbial characterization in extende aeration actived sludge plant. We took samples for three months (November to January). In each one we sampled four points of the wastewater treatment plant: a) Influent; 2) Effluent; 3) aeration tank, and 4) settling tank. In this period the system had removal of BOD from 72 to 81% and COD from 78 to 79%. The samples showed mean values of pH 6.5 (± 0.26; SD), turbidity of 138.6 ± 33.42 NTU and temperature 24.5 ± 2.76 °C. The sample of December had higher nitrate concentration in the influent (2.15 mg/L), while on effluent, the highest concentration was found on November (4.05 mg/L). Already the nitrite influent was higher concentration in January, with a value of 78.9 µg/L, and effluent in December showed 396.2 µg/L. Higher levels were found of nitrifying bacteria in aeration tank of December demonstrated ammonia-oxidizers bacteria concentrations of 8×10^5 MPN/100 mL and for nitrite-oxidizers bacteria concentration of 9×10^8 MPN/100 mL. Denitrifying bacteria showed higher value in December (1.4×10^{10} MPN/100mL) in the aeration tank. This higher concentration of ammonia-oxidizing bacteria is responsible for the high concentrated nitrite effluent of December. The largest concentration of denitrifiers bacteria is responsible for the lower concentrated nitrate effluent when compared to other months. In microscopy examinations was observed in all samples high occurrence of Rotifera Class, high occurrence of testate amoebaes of the Class Rhizopoda, for example *Arcella* sp. and *Euglypha* sp. Also observed in smaller quantities, ciliated protozoa of the genus *Aspidisca* sp. and *Opercularia* sp., flagellate protozoa Euglenophyta class and algae *Desmidium* sp. and *Tetramorus* sp., plus annelid *Aelosoma* sp. This results showed the high effectiveness of the activated sludge process, however the maximum removal that the system could offer was not achieve.

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MICROBIAL CHARACTERIZATION OF LANDFILLS LEACHATE IN SÃO CARLOS AND ARARAQUARA/SP

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Keywords: Coliforms, denitrifying bacteria, nitrifying bacteria, landfills, leachate

In final disposal of solid waste, improperly storing can cause environmental contamination due to migration of substances originated during decomposition. This study aimed evaluating physic-chemical and microbiological parameters of landfills-leachate samples in São Carlos and Araraquara/SP, and samples from anaerobic filter. This filter was inoculated with sludge from the leachate lagoon treatment in São Carlos. Four samples were analyzed: three samples were from São Carlos, sludge from the lagoon, effluent of lagoon, and effluent from anaerobic filter feeding with effluent of lagoon, after two months; and one sample of leachate from the Araraquara landfill. Microbiological tests were performed measurements of populations of coliform bacteria, nitrifying and denitrifying bacteria and total heterotrophic bacteria in order toidentify which of these interfere with the biological treatment. The parameter of COD was used to monitor the treatment efficiency of leachate in biological reactors. Lagoon sludge showed total solids 161.9 g/L and total volatile solids 44.1 g/L. Microbial community estimation presented total coliforms 2.2 x 10^7 MPN/gTVS; negative for thermotolerant coliform; denitrifying bacteria 8 x 10^9 MPN/gTVS; and total heterotrophic bacteria 6.6 x 10^4 CFU/mL. Anaerobic filter showed lower COD removal (14%), losses of total solids in effluent, and removal of log 4 in thermotolerant coliforms (influent value 8 x 10^4 MPN/100 mL). Denitrifying bacteria of anaerobic filter suggested low significative variance (1.3 x10^{10} to 1.3 x10^{11} MPN/100mL). For nitrifying bacteria, ammonia oxidizers indicated slight decreasing (2.3 x10^6 to 8 x 10^4 MPN/100mL) and nitrite oxidizers showed low significative variance (2.3 x 10^5 to 4 x 10^4MPN/100mL). Leachate sample from Araraquara showed total solids 6.3 g/L, total volatile solids 1.9 g/L, and COD of 1709 mg/L. This sample suggested total coliforms (2.3 x 10^6 MPN/100mL), thermotolerant coliforms (5 x 10^5 MPN/100mL) and denitrifying bacteria (2.8 x 10^{11} MPN/100mL) higher than the lagoon effluent. About nitrifying bacteria, ammonia oxidizers showed similar value (2.6 x 10^6 MPN/100mL) when compared to lagoon effluent of São Carlos and nitrite oxidizers was negative.

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MICROBIAL DIVERSITY OF SLUDGE FROM A PILOT-SCALE TREATING REAL SEWAGE

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Keywords: anaerobic wastewater treatment; autotrophic denitrification; DGGE; 16S rRNA

The reduction of nitrogen levels from wastewater can be accomplished by a variety of physical, chemical and biological processes. Biological nitrogen removal advantages include higher efficiency and lower costs. Sulfide can be oxidized to elemental sulfur or sulfate by communities of sulfide oxidizing bacteria using nitrate or nitrite as electron acceptor. This autotrophic denitrification reduces global carbon requirements for removal of nutrients and produces high concentrations of sulfate. The microbial communities established in denitrifying reactors treating real sewage with nitrate as electron acceptor and sulfide as electron donor were analyzed using 16S rRNA targeted molecular methods, including denaturing gradient gel electrophoresis (DGGE) and phylogenetic analysis. In addition, sludge samples were also characterized by microscopy and enumeration by MPN (Most Probable Number). Morphologies detected include: rods, cocci, filaments and sulfate-reducing bacteria-like. Predominant phylotypes of the different samples were monitored using DGGE of PCR-amplified 16S rRNA gene fragments and sequencing. Fingerprinting analysis showed changes in bacterial communities in two 30-day period conditions following a change in substrate composition: condition C1, feeding with 100% non-nitrified effluent anaerobic; condition C2, feeding with a mixture composed of 20 - 30% of the anaerobic effluent and 70 - 80% nitrified effluent. Most bacterial sequences were clustered within the *Pseudomonas*, *Aeromonas*, *Acidobacteria*, *Chlroroflexi*, *Clostridium*, *Ralstonia* and *Cupriavidus* genera. Quantification by MPN showed that the relative abundance of bacteria increased during operation period. Denitrifying bacteria were estimated at the end of conditions C1 and C2: 9.2×10^{21} and 1.6×10^{22} cells/100 mL of culture, respectively. These results provide insight into the importance and dynamics of balanced communities of bacteria in denitrification process.

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MICROBIOLOGICAL MODIFICATION TO OBTAIN FOOD WITH BETTER NUTRITIONAL VALUES.

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Keywords: Babassu mesocarp, Manihot leaves, fermentation.

According to WHO, malnutrition is a global problem affecting a large portion of population. Considering only children, some 300 million worldwide are affected. For optimal development, feeding should proceed according to the physiological demands of each organism, derived from functions that have their nutrients on the organic system. Thus, the balanced consumption of nutrients ensures the proper functioning of the biological processes of organism. In Brazil, there are still many low-income communities that lack easy access to quality foods, mainly in the North and Northeast. The babassu palm, native from these regions, has its fruits used in the diets of these poor communities by the incorporation of babassu mesocarp flour [BMF] in school lunches, however, despite presents good levels of carbohydrates, has no satisfactory amounts of protein. To supply the nutritional deficits the food fortification has been encouraged, principally when aimed high-protein, for use in regions where the access to nutritional quality of foods is still limited. Hence, dried cassava leaves [DCL] were used as a supplement to the concentration of proteins in the BMF, as an essential nutrient for growth of the fungus. These materials must have, in addition to production facility and good sensory characteristics, satisfactory nutritional characteristics, and can present desirable functional characteristics, such as a high nutritional value and health benefits by reducing the risk of chronic degenerative diseases. The objective of this project was measure the increase protein in the samples of BMF with different concentrations of dried cassava leaves (2; 4 and 8%) through the solid-state fermentation (28,0 ± 2,0°C) by the fungus *Rhizopus microsporus* var. *oligosporus.* After fermentation (168 hours) the samples showed gains of proteins with respect to BMF being that, the sample without supplementation presents an increase of 0,11%, while the samples containing 2% of dried cassava leaves obtained an increase of 1,01%, the samples containing 4% showed an increase 2,33% and the samples containing 8% in increased from 3,50%. These results proved to be even better after the experiment of protein quality, which involves the assessment of growth of the bacterium *Streptococcus zymogenes*, which requires essential amino acids available for growth. That might be viewed as a solution of the fermented product (8% DCL), indicating that fermentation provided the availability of essential amino acids. These results show the potential of using agricultural products and especially in solid substrate fermentations in obtaining more digestible food as well as providing better nutritional balance.

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MICROBIOLOGY WATER QUALITY FOR ANIMAL CONSUMPTION

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Keywords: animal consumption, *E.coli*, heterotrophic bacteria, water

Water designed for human or animal consumption must attend to minimal quality standards in order to guarantee the ideal consumption, without injuries due to bad quality, such as microbiology or chemical contaminants. This work aimed to evaluate microbiology quality of water offered in the Institute of Animal Science and Pastures (IZ), focused on animal consumption. Three areas were chosen for the 25 sampling analysis: Maracanã area, destinated for Bovine growing and fattening, IZ main area and Environmental preserved area Armelindo Nindo Budini, called Manancial, passing by "Isidoro Bordon Grove". In these samples, pH , total coliforms, *E.coli* and heterotrophic bacteria were measured, using Colitest Commercial Kit for total and *E.coli* and, R2A and TSA media for heterotrophic bacteria counting. The pH from all samples showed results according to legislation standards for animal consumption, around 5.8 for natural sources and from 6 to 7 at other sites (lagoon, pipes, etc). Total coliforms were presented at the Lake, at both sampling sites (boarder and inside the Lake) and in the paddock no. 6, from Maracanã area. At Institute of Animal Science and Pasture (IZ) main area, total coliforms were observed at Planalto Stream, at both sampling sites, at the Grove Isidoro Bordon and in the small lake, by the Guesthouse. In the Manancial area, no total coliforms were found. From such areas, *E.coli* were presented at the lake edge, from Maracanã area and at the Planalto Stream, inside the IZ. From all 25 sampling sites, 28 % showed Total Coliforms and 8 % presented *E.coli*. For heterotrophic bacteria counting to animal consumption, maximum counting must be 1000 CFU .mL^{-1} according to CETESB standards and 68 % of the samples showed superior results superior for R2A medium and 32 % for TSA medium. Comparison of both culture media, R2A presented results 293 % higher than TSA medium, showing the high nutrient medium can fade the microbiological variability present at some ecosystems. R2A medium, nutrient poor, is very similar to the water environment and it is the most indicated medium for it is purpose. Based upon this work, periodically management must be done at animal drinking spots at the paddocks and there is a need for better preservation at water sources inside the Research Institute of Animal Science and Pastures to guarantee water quality for animal production. This work attempt to the lack of studies for better water quality given to livestock, which reverses in human health quality.

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MOLECULAR PHYLOGENY AND EXPRESSION ANALYSIS OF *recA* GENE IN *Lactobacillus delbrueckii* UFV H2b20

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Keywords: *Lactobacillus*, probiotics, acid stress

Probiotics are living microorganisms which when ingested in adequate amounts confer health benefits for the host. *Lactobacillus* strains used as probiotics are exposed to several environmental stresses, such as the low pH in the stomach and the presence of bile salts. Stress response in probiotic bacteria is among the topics that may impact the food industry, also for technological reasons. Tolerance to acidic conditions is one of the desirable characteristics of potential probiotic cultures. Lowering of the intracellular pH leads to the protonation of DNA bases followed by the cleavage of glycosyl bonds and consequently to the depurination and depyrimidation of DNA. The RecA dependent DNA repair system is one of the mechanisms activated during acid stress response in bacteria. *Lactobacillus delbrueckii* UFV H2b20 exhibits probiotic characteristics and can also be used in fermented foods. Thus, the objectives of this work were to characterize *recA* of *L. delbrueckii* UFV H2b20 and to study its expression in different times periods when the cells are subject to acidic medium treatament. Quantitative real-time PCR (RT-qPCR) assay was performed to establish a relationship between *recA* mRNA accumulation and the exposure of the culture to acidic conditions. Total DNA from *L. delbrueckii* UFV H2b20 was extracted according to the protocol adapted for lactic acid bacteria in the laboratory of Industrial Microbiology. To obtain the *L. delbrueckii* UFV H2b20 *recA* nucleotide sequence, *primers* were constructed based on the sequence of the gene encoding the protein RecA in *L. delbrueckii subsp. bulgaricus* ATCC 11842 (access number: NC_008054). The amplified DNA was cloned in pGEM-T Easy Vector, transformed into *Escherichia coli* JM109, and sequenced. The *recA* nucleotide sequence of *L. delbrueckii* UFV H2b20 exhibited 97 % identity with the *recA* nucleotide sequence of *L. delbrueckii subsp. bulgaricus* ATCC 11842. Phylogenetic analysis performed using Bayesian Inference grouped the sequence with the *recA* gene sequences of *L. delbrueckii subsp. bulgaricus* ATCC 11842 and *L. delbrueckii subsp. bulgaricus* BAA 365. RNA extracted from *L. delbrueckii* UFV H2b20 was transcribed into cDNA using the ImProm-II™ Reverse Transcription System (Promega®) and random *primers*. Relative expression of the *recA* gene in *L. delbrueckii* UFV H2b20 at pH 3,5 for 30 and 60 minutes was analyzed by RT-qPCR through the comparative $2^{-\Delta\Delta Ct}$ method using the *sigA* housekeeping gene in order to normalizing the expression of *recA*. The primers were constructed based on *L. delbrueckii subsp. bulgaricus* ATCC 11842 genome sequence. The results showed 3,4 and 1,9 fold increases in the expression of the gene when cells were subjected to pH 3.5 for 30 minutes and for 60 minutes, respectively, indicating a response to acid stress. In view of the diversity of stress response mechanisms in different species and between different strains of *Lactobacillus*, the knowledge of this mechanism in *L. delbrueckii* UFV H2b20 is important for the development of better probiotic products.

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Morphological and molecular weight changes of Poly (ε-caprolactone) /starch /soy protein isolate blends during soil biodegradation

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Keywords: Biodegradation, soil, blend, poly (ε-caprolactone), soy.

Introduction

The development of biodegradable polymers came to reduce the volume of plastic waste discarded in the environment. As a result, the use of natural polymers in the manufacture of blends has provided the use of renewable resources such as starch and soy. In this work, the biodegradability of the four different compositions of poly (ε-caprolactone)/modified starch and soy protein isolate (SPI) were evaluated in two soils with different texture. Morphologic and molecular weight characterization were done before and after 180 days of soil incubation.

Material and Methods

-Blends with various compositions of Poly (ε-caprolactone) (PCL), modified corn starch, soy protein isolate (SPI) and sorbitol were prepared in a single-screw extruder (Vortex).
-The biodegradability of the PCL/Starch/SPI blends has been determined by respirometric tests according to ASTM D 5988-03 and methodology described by Anderson (1982).
-Molecular weights (Mw) of the blends were evaluated before and after biodegradation using a size exclusion chromatography (SEC) Viscotek, TDA 302.
-The morphology of the sample surfaces was analyzed in a scanning electron microscope LEO 440i.

Results and discussion

Regardless of soil texture, the molecular weight of the pure PCL sample (54,600 g/mol) decreased with time of incubation, reaching 26,000 g/mol in 180 days of incubation.
On the other hand, the other compositions of PCL/Starch and PCL/Starch/SPI presented an increase in the molecular weight, which is exemplified by the increase in Mw from 51,000 g/mol to 78,000 g/mol. These findings are different from the literature where it is shown that the reduction in molecular weight of polymers is often taken as an indicator of biodegradation (Jayasekara et al., 2005; Shah et al., 2008).
However, Pometto et al. (1993) noticed an increase in the molecular weight of the low density polyethylene, starch and Mater-Bi® blends submitted to aerobic biodegradation, and they suggested that the formation of a microbial biofilm is the cause of this increase.
In this work, the morphology of the sample surfaces of pure PCL showed only surface degradation while the other blends showed the presence of structures similar to what may be a biofilm on the surface. It is suggested that the increase in molecular weight may have been caused by formation of this biofilm.

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NUTRITIONAL PARAMETERS ON PRODUCTION OF A NEW GLUCAN FROM LASIODIPLODIA THEOBROMAE USING RESPONSE SURFACE METHODOLOGY.

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Abstract: Microbial polysaccharides represent a real alternative to the ones now in use that are mainly of plant and seaweed origin. Fermentative production of exopolysaccharide (EPS) offers unique advantages such as high concentrations of pure product, process reproducibility and stable costs, independently of seasonal variations. The noticeable number of recent papers reporting studies of selection of new EPS producers and their characterization, as well as the optimization of the production processes, is evidence of the wide interest. It is now well recognized that β (1-3)-D-glucans, including those of mixed β (1-3, 1-6) glucosidic linkages, are biological response modifiers (BRMs) as they are able to stimulate the nonspecific (innate) immune system of animals. Although there are no descriptions in the literature regarding the EPS produced by *L. theobromae*, a β-glucan has been isolated from *Botriosphaeria rhodina*.

Botryosphaeran is a water-soluble glucan produced by *Botryosphaeria rhodina* MAMB-05 when cultured on different carbohydrate substrates with highest yields when produced on sucrose.

The cumulative effect of the nutritional parameters for glucan production by *Lasiodiplodia theobromae*, isolated from eggplant (*Solanum melongena*), were investigated. The individual and interactive effects of the independent variables ($NaNO_3$ and sucrose concentration) on responses (culture medium viscosity, concentration and yield of exopolysaccharide and biomass) were studied using a 11-trial Centered Composite Design of Experiments.

Sucrose was found highly significant in influencing the glucan concentration ($p<0,01$) but in levels higher than 65g/L, which is not economically practible. Viscosity was, also, influenced by sucrose concentration in the same way. Y x/s and Y p/s yields were not influenced by the factors or its interaction.

Keywords: exopolisaccharide, glucan, nitrogen source, carbon source, response surface methodology.

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ON THE PHENOTYPIC PLASTICITY OF *ESCOVOPSIS* SP. ASSOCIATED WITH LEAF-CUTTING ANT GARDENS

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Keywords: microfungi, taxonomy, parasite, *Atta*, *Acromyrmex*

Microfungi in the genus *Escovopsis* are specialized parasites of the fungus cultivated by leaf-cutting ants. Previous studies based on macromorphological characteristics revealed that different *Escovopsis* sp. morphotypes may infect nests of leafcutter ants. To confirm that such morphotypes are also defined at a microscopic scale, we surveyed for phenotypic markers on the micromorphology on several of the parasite. We analyzed the microscopic characteristics of 36 *Escovopsis* sp. strains isolated from the leaf-cutting ant genera *Atta* sp. and *Acromyrmex* sp. of different geographical regions in Brazil. Wet mount microscope slides were prepared following incubation of strains on PDA at 25° C for seven days. Size, growth pattern and shape of conidiophores hypha, vesicles and conidia were analyzed after ten observations of each structure. Regardless of ant genera or geographic regions, microscopic observations revealed that asexual reproductive structures of all isolates exhibited similar morphology. Thus, general morphological pattern among strains included conidiophores with both sympodial and dichotomous branching; cylindrical vesicles and ellipsoid conidia. On the other hand, the overall size of conidiophore structures varied among isolates with hypha reaching 3.3 - 10.6 µm in diameter; vesicles reaching 3.2 - 10.8 and 19.5 - 79.3 µm in length and width, respectively; and conidia reaching 1.6 - 6.3 µm in length. No correlation was found between size measurements and the origin of the parasite strains (both ant genera and geographic regions). Previous studies using the same *Escovopsis* sp. strains identified two morphotypes based on colony characteristics and growth rate. Here, we demonstrate that the macro phenotypic plasticity of *Escovopsis* sp. associated with *Atta* sp. and *Acromyrmex* sp. ants does not correlate at the microscopic level. This result adds more information on the species definition regarding this complex group of fungi. Comparisons between data derived from morphology and preliminary results on the phylogenetic analyses of these strains are presented.

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OPTIMIZATION OF CULTURE CONDITIONS FOR XYLANASE AND β-XYLOSIDASE PRODUCTION BY *Penicillium janczewskii* IN SOLID STATE FERMENTATION WITH BREWER'S SPENT GRAIN.

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Keywords: Xylanolytic enzymes, enzyme production, solid state fermentation, *Penicillium janczewskii*

The production of xylanase, β-xylosidase and α-arabinofuranosidase by a *Penicillium janczewskii* strain, isolated from soil of an Atlantic Rainforest region (Estação Ecológica Juréia-Itatins, SP), was investigated in solid cultures. Period and temperature of cultivation were the parameters investigated in order to optimize the production of the enzymes by this fungal strain. The cultures were performed in 250 mL Erlenmeyer flasks containing 5g of brewer's spent grain (BSG) as substrate. The 100% (v/m) moisture was established by the addition of Vogel's salt solution. The cultures were inoculated with 1 mL of a 10^7 conidia mL^{-1} suspension. In the first step, the xylanase and the β-xylosidase production was evaluated during 15 days. In the second step the enzyme production was investigated after the cultivation of the cultures for seven days at 20, 25, 30 and 35 °C. The xylanase activity was determined by quantifying reducing sugars and the β-xylosidase activity was determined from the hydrolysis of p-nitrophenyl-β-D-xylopiranoside. Proteins were determined by the modified Bradford method. All experiments were carried out in triplicate and the results were analyzed by Mann-Whitney test. Highest xylanase production was observed in seven days-old cultures (238.18 U/g BSG) and the β-xylosidase production was elevated from the sixth to the eighth day, with the peak of production in the eighth day of culture (0.28 U/g BSG). After the peaks of production, enzyme activities decreased. In relation to the temperature of cultivation, it was observed higher production of xylanase and β-xylosidase at 25 and 20 °C (264.5 and 0.25 U/g BSG), respectively. No β-xylosidase production was observed at 35 °C. The results demonstrated that *Penicillium janczewskii* is a promisor strain for xylanolytic enzymes production in solid state fermentation with BSG, although other parameters should be investigated in order to optimize the enzyme production.

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OPTIMIZATION OF ENZYMATIC PRODUCTION OF *Fomitella supina* (Basidiomycota)

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Keywords: *Fomitella supina*, Basidiomycete, Laccase, Ezymatic Activity

The fungi are one of the most diverse groups of organisms on Earth. There are about 105,000 species of known fungi, approx. 7% of the estimated 1.5 million species (Hawksworth, 2004). Due to their diversity and distribution, they occupy virtually all terrestrial biomes, playing a prominent ecological importance. These organisms are responsible for recycling various substances in the environment, mainly wood and its compounds. Among the components of wood, lignin is that is the most resistant to biodegradation. Thus, lignin biodegradation is one of the most important factors determining the degradation of wood and, therefore, the carbon cycle in the biosphere (Campbell, 2003). This ability to degrade the wood components is almost exclusively associated to the production of extracellular lignocellulolytic enzymes (Blanchette et al. 1997; Kullmann, Matsumura, 1997, Reddy et al., 1998, Mason et al. 2001; Gelpke et al ., 2002). The best characterized of these enzymes are laccases (Guillén et al., 2000), lignin peroxidase (LiP), manganese peroxidase (MnP) (Conesa et al. 2002; Martínez, 2002), and cellulases. One selected strain of *Fomitella supina* was grown in selected medium for enzyme induction with nitrogen and carbon sources previously established in the experimental design (nitrogen source - 3g K_2HPO_4-1g, KCl-0.5g; $MgSO_4.7H_2O$-0.5g; $FeSO_4.7H_2O$-0.01g; carbon source - 5g distilled H_2O-1000mL)(Czapek's Agar modified solution). The stabilization of laccases, produced by *Fomitella supina*, was tested. The influence of temperature and pH was carried out with application of two different assays. First assay was the influence of temperature in the range of 25-94°C, measured by a spectrophotometer coupled to a termic stabilized bath. The second assay was the influence of pH in the range of 3,0-9,0 using a spectrophotometer to obtain a activity. Enzymatic activity was read with the application of a standard protocol in both assays (Szklarz, 1984). After seven days of incubation, the high enzymatic activities were found for pH between 5.0 and 7.0. The temperature range with best enzymatic activity was between 35ºC and 70ºC. The statistical analysis (Tukey test) showed that the results are significantly different. The genetic characteristics of species and strains as well as the physical and chemical parameters as pH, nitrogen, temperature, oxygenation and presence of minerals interfere with the production and action of enzymes (Vyas et al. 1994; Tuor et al. 1995; Kamida et al. 2005; Zhao et al., 2008). Succession activity of different enzymes at different times during the degradation of substrate in the incubation, or incubation time for the induction of enzymatic activity may have favored the laccase activity (the highest activity). This enzyme is usually the first one to access the lignocellulolytic substrate.

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PETROLEUM PRODUCTS AND VEGETABLE OILS TOXICITY TO *Bacillus subtilis*

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Keywords: toxtrak, bioremediation, inhibition, oil, phenol

Exploitation, transportation and consumption of petroleum and its products causes the release of hydrocarbons into the environment. Large catastrophic accidents involving oil tankers have attracted public attention to petroleum hydrocarbons fate in marine environments. Also, refineries generated toxic wastes present high concentrations of polycyclic aromatic hydrocarbons. The environmental persistence of these compounds is known from many studies. Waste oil refineries have a vast selection of hazardous substances and high toxicity. Oil components such as nitrogen and sulfur are highly toxic, especially in form of ammonia and hydrogen sulfide, respectively. *Bacillus subtilis* is a bacterium commonly found in environments contaminated by hydrocarbons. The inhibition percentage indicating cellular toxicity was determined using the commercial kit "ToxTrack Toxicity Test" manufactured by Hach Company using a spectrophotometer. This method is based on resazurin reduction (a redox indicator dye) by bacterial respiration. In test tubes containing the samples, resazurin and *B. subtilis* cells there was also a chemical catalyst added to reduce reaction time, allowing rapid analysis of the toxicity of the substance, rather than the monitoring biodegradation. When the resazurin is reduced, its color changes from blue to red. However, toxic substances may impede the rate of reduction of this dye through the cellular inhibition. Thus, variations in the color of the mixture by the reduction of resazurin were measured with a spectrophotometer at 603 nm after 60 min. The percentage of inhibition for samples and petroleum derivatives was measured through these readings. Hence, substances such as: diesel, gasoline, kerosene, phenol and used vegetable oil, are toxic to *B. subtilis*, by presenting values higher than 10.0% inhibition. Synthetic lubricant, lubricant and used vegetable oil are slightly toxic (2.0 to 6.0% inhibition). Biodiesel also showed low toxicity towards *B. subtilis* (1.0%). Crude petroleum, however, showed mixed results in the range from -10.0% to 10.0%. According to the methodology should not be considered toxic. You can associate the toxicity of used vegetable oil to *B. subtilis* due to chemical compounds resulting from its use. Even though it does not contain the same substances present in petroleum and its products, vegetable oils may have toxic effects on sensitive organisms. Studies found that long vegetable oils chains showed more toxicity in microorganisms. Thus, the transformation of vegetable oils after used when preparing foods can cause toxicity of these substances to rise. Intermediate compounds of vegetable oils may also have been formed throughout the duration of the test and thereby increasing the toxicity to *B. subtilis*. The findings of this study corroborate methodologies for assessing acute toxicity, which have shown that aromatic hydrocarbons and volatile compounds such as that most influence the toxicity. Moreover, low molecular weight hydrocarbons such as those present in gasoline and diesel have severe acute toxic effects, mainly due to their high solubility and the presence of more volatile molecules with the ability to penetrate cells and alter cell structures.

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POLYHYDROXYALCANOATES PRODUCTION FROM BIODIESEL GLYCEROL BY *Pseudomonas aeruginosa*

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Keywords: polyhydroxyalkanoates, PHAs, biodiesel, glycerol, pseudomonas

Actually, there is an interest to develop biocompatible replacements for petroleum and petrochemical-based polymers. One such outlet that is steadily gaining momentum around the world is the biodiesel production. The crude glycerol is the main byproduct of biodiesel production, generated from the transesterification of vegetable or animal fats and oils. Although pure glycerol is an important industrial feedstock with applications found in food, cosmetics and drug industries, the biodiesel glycerol has a relative low value due to the presence of impurities. A solution for this problem has been proposed with the implementation of biorefineries that co-produce additional value-added products along with biodiesel, such as bacterial biopolymers production utilizing waste glycerol as a carbon source. The polyhydroxyalkanoates are bacterial polyesters that are synthesized under unbalanced growth conditions as intracellular granules and energy storage compounds. The aim of this work was produce PHAs from *Pseudomonas aeruginosa* LMI 6c utilizing biodiesel glycerol as a sole carbon source. The crude glycerol was obtained from Candeias Petrobrás plant (Candeias, BA, Brazil) and was synthesized from the alkaline-catalyzed transesterification of soybean oil. The *P. aeruginosa* strain was isolated from hydrocarbon-contaminated soil and obtained from the culture collection of LMI, IB, UNESP (Rio Claro, SP, Brazil). Bacterial growth from nutrient agar incubated for 72h at 30°C was used to inoculate 100 ml of nutrient broth (3 $g.l^{-1}$ meat extract, 5 $g.l^{-1}$ peptone, pH 7.0 ± 0.1) at 30°C on a rotatory shaker for 24h. The polymer production experiments were carried out by inoculating each test flask with 3.0ml of the nutrient broth culture in 200 ml of mineral salts medium, containing $(NH_4)_2SO_4$ and biodiesel glycerol as sole carbon source at concentrations between 1% and 5% (w/v). The erlenmeyers flasks were incubated at 30°C with shaking at 150rpm for 72h. After incubation, the cells were harvested by centrifugation (10600 x g, 10min, 4°C), washed twice with distilled water and lyophilized to a constant weight for PHAs determination. The cell dry weight was determined gravimetrically. The glycerol concentration was determined by liquid chromatography with Prominence UFLC apparatus (Shimadzu®). Samples of about 10mg of freeze-dried cells were subjected to propanolysis for PHAs determination.The propyl esters were assayed by gas chromatography with HP-5890-II GC. In the presented study, *P. aeruginosa* showed maximum cell growth of 2.11$g.l^{-1}$ at 1% biodiesel glycerol, which decreased slightly to 1.57$g.l^{-1}$ as the crude glycerol was increased to 5%. The polymer yields stabilized at 0.1$g.l^{-1}$ between 1% and 3% biodiesel glycerol and were decreased at 0.05$g.l^{-1}$ from 4% to 5% waste glycerol concentrations. The PHA composition analysis showed a polymer composed of monomers 3-hydroxydecanoate and 3-hydroxydodecanoate at the biodiesel glycerol concentrations between 1% and 3%. The PHA production experiments carried out at crude glycerol concentrations higher than 3% were obtained a polymer composed exclusively of monomer 3-hydroxydecanoate.

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POLYHYDROXYALCANOATES PRODUCTION FROM SOYBEAN OIL BY *Pseudomonas aeruginosa*

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Keywords: polyhydroxyalkanoates, PHAs, soybean oil, pseudomonas

The polyhydroxyalkanoates (PHAs) are biodegradable and environmentally friendly thermoplastics. They are attractive substitute for conventional petrochemical plastics due to their similar material properties to various thermoplastics and elastomers. PHAs are synthesized intracellularly and deposited as granules in bacterial cultures in the presence of excess carbon source and a growth limiting nutrient. These biopolymers have several applications in disposable items, bone and blood vessel replacements, scaffold material in tissue engineering, etc. Many species of *Pseudomonas* accumulate PHAs that are composed of either saturated or unsaturated 3-hydroxy fatty acid monomer units ranging in length from six (C6) up to fourteen (C14) carbon atoms. These polymers are called medium-chain-length (mcl) PHAs and generally exhibit properties that range from elastomeric to adhesive, depending on the specific side-chain length and the degree of unsaturation. The increase in animal fat and vegetable oil production has necessitated the discovery of additional outlets for these materials in nonfood applications. This study utilized the strain *Pseudomonas aeruginosa* LMI 6c to convert soybean oil into mcl-PHAs. The microorganism used was obtained from the culture collection of LMI, IB, UNESP (Rio Claro, SP, Brazil). Colonies from nutrient agar incubated for 72h at 30°C was scraped to inoculate 100 ml of nutrient broth (3 $g.l^{-1}$ meat extract, 5 $g.l^{-1}$ peptone, pH 7.0 ± 0.1) at 30°C on a rotatory shaker for 24h. The mcl-PHAs production experiments were carried out in 200 ml of mineral salts medium, containing $(NH_4)_2SO_4$ and soybean oil as a carbon source at concentrations between 1% and 5% (w/v). The shaker flasks were inoculated with 3.0ml of the nutrient broth culture and incubated at 30°C with shaking at 150rpm for 72h. After incubation, the cells were harvested by centrifugation (10600 x g, 10min, 4°C), washed twice with distilled water and lyophilized to a constant weight for PHAs determination. The cell dry weight was determined gravimetrically. The soybean oil concentration and residual oil was determined gravimetrically after hexan extraction of the acidified culture samples. For PHAs determination, samples of about 10mg of freeze-dried cells were subjected to propanolysis and the propyl esters were assayed by gas chromatography with HP-5890-II GC. In this work, *P. aeruginosa* showed maximum polymer yield (1.71$g.l^{-1}$) and PHA cellular productivity (54.39%) at 3% soybean oil. The composition analysis of the mcl-PHA showed a polymer composed primarily of monomers 3-hydroxydecanoate and 3-hydroxydodecanoate with low incidence of monomers 3-hydroxyhexanoate and 3-hydroxyoctanoate. Interestingly, increasing concentrations of soybean oil had an effect in the polymer composition with a decrease of the monomer 3-hydroxydecanoate and increase of the monomer 3-hydroxydodecanoate.

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POTENTIAL FOR PHOSPHATE SOLUBILIZATION OF ENDOPHYTIC FUNGI FROM ROOTS OF *Opuntia ficus-indica* Mill.

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Keywords: endophyte, phosphate solubilization, *Opuntia*, fungi

Soil characteristics and climatic factors reduce water availability throughout the year in the Brazilian semi-arid regions and when the rains arrive, torrential and concentrated in a short space of time, bleach the soil coverage and remove nutrients from this system. One of these is the phosphorus (P) present in organic soluble form and the insoluble fraction, unavailable to most of the biotic components of the system, present in the soil. P solubilization may occur by microbial action, especially by fungi. They make it readily available to plants, or accumulate it and then release it gradually when cells die. Phosphate solubilizing fungi can be isolated from inside plant tissues or organs healthy and are called endophytic microorganisms. This study aims to isolate and identify mesophilic fungal endophytic diversity associated with cactus (*Opuntia ficus-indica*) roots from semi-arid areas and to assess strains ability for phosphate solubilization. For isolation of endophytic microorganisms from the roots of *Opuntia ficus indica*, were analyzed four locations, with three plants per local and three roots of each plant. Roots were washed in tap water and surface disinfected by dipping in 70% ethanol for 1 minute in 2.5% sodium hypochlorite for 20 minutes, again in 70% ethanol for 30 seconds and three successive washes in sterile distilled water for 10 minutes in each wash. The edges were disregarded and discarded, and samples were fragmented into pieces of 0.5 cm long. Five explants were added to each plate of different media: Potato Dextrose Agar (PDA), nutrient agar (NA), King´s B (KB), Starch Casein Agar (AACK) and nitrogen-free medium (NFB) supplemented with antibiotics. Plates were incubated at 28^{o}C. Plating was done in triplicate and colonies were isolated during the period of one week to fifteen days. Strain identification was realized by morphological characteristics of colonies and microscopically analysis. Solubilization of P was tested in liquid medium supplemented with Ca_3PO_4, after one, two, three and four days of cultivation under agitation at 150 rpm and 28 °C. It was also analyzed pH change and mycelial growth. Thirty-one culturable fungi were isolated, purified and preserved by Castelani method. Many of the explants did not develop fungi, reaching dry after 45 days of incubation. The PDA was the most effective media for fungi isolation, followed by AACK and NFB media respectively. We identified eight strains distributed in the genus *Penicillium*, *Pythium* and *Microsporium*. Others strains will be subjected to stress factors in the expectation of forcing sporulation. Only ten strains present the ability to continuing to growth after replicates, eight showed higher phosphorus levels at the end of 48 hours of incubation, declining by the fourth day, probably due to bioaccumulation. Only one strain showed maximum solubilization at the end of the fourth day. Soluble phosphorus values ranged from 10 to 150 µg/mL. Few strains showed a correlation between P solubilization and pH changes. Mycelial growth not shows correlation with P solubilization. We conclude that these fungi are promising plant growth promoters.

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POTENTIAL NITRIFICATION OF RHODIC HAPLORTHOX IN FOREST ECOSYSTEMS, PASTURE AND ANNUAL CULTURE.

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The potential nitrification (PN) determines the ability of soil microorganisms in the conversion of ammonia nitrogen to nitrate. With the soil incubation under controlled conditions has the potential idea of different soil vegetation or managements about ammonification processes, with production of NH_4 and its nitrification to NO_3. This study aimed to evaluate the potential nitrification of rhodic haplorthox, under forest, pasture and annual crops in winter and summer periods. In each ecosystem, were randomly bounded four sub-areas with 100 m^2, which were collected 20 sub-samples to obtain a composite sample, depths from 0.0 to 1.0, 0.10 to 0.20 and 0.20 to 0.40 m, resulting in four soil samples (repetitions) for each ecosystem and collection depth. Soil samples were packed in plastic bags to preserve moisture and transported to the laboratory less than three hours. The data interpretation followed a statistical model of split-plot (factor 1: ecosystems and factor 2: sampling depth) with four replications in each period evaluated. The analysis method followed two steps: extraction with KCl 1 mol L^{-1} as SCHMIDT & BELLS (1994), determination of NH_4 and NO_3 by distillation according to Keeney & Nelson (1982). The results showed that on summer, the PN in soil forest was 29% higher in soil pasture and 40% in soil of annual crops, on layer 0.0 to 0.10 m, this can be understood by the major input of organic material on the soil surface in forest ecosystems, resulting higher microorganisms activity in mineralize nitrogen present on the organic waste, or this mineralization may be related to better ratio C/N in forest soils, which interferes in the N mineralization. In winter, however, the PN was than 17 and 12% in soils forest and pasture, respectively, when compared with soils under annual crop. It can be concluded that, besides the amount of nitrogen, organic matter resulting from different vegetation cover, may influence the nitrification potential. Probably, the animal excretions, richer in nitrogen, have an influence in higher PN found in soil under pasture, or the implantation of grasses with greater biomass production causes increased storage of N total in the soil. Both, summer and winter, the higher PN was observed in the soil layer from 0.00 to 0.10 m in comparison with others, demonstrating greater microorganisms activity in the surface soil region. With this study, we conclude that the forest ecosystem in the summer, presented greatest potential for nitrification, compared to pasture ecosystems and annual crops. In winter, the pasture soil showed PN similar to forest soil, but both above the soil under annual crop.

Keywords: mineralization, microorganisms, nitrogen.

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PRODIGIOSIN PRODUCTION BY A NEW STRAIN OF *Serratia marcescens* UCP1549 USING SOLID STATE FERMENTATION

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Keywords: *Serratia marcescens*, Mannitol Agar, Prodigiosin

INTRODUCTION: Prodigiosin, is a natural red pigment characterized by a common pyrrolylpyrromethane skeleton, and are produced by various bacteria in special *Serratia marcescens*. The bacterium was grown in alternatives culture media. This pigment is a promising drug owing to its reported activities to antifungal, antibacterial, immunosuppressive and anti-proliferative.

MATERIAL AND METHODS: *Serratia marcescens* UCP1549 used as a producer of prodigiosin. The bacterium was isolated from soil of the semi-arid region of Pernambuco, Brazil, and belongs to the Culture Collection of the Nucleus of Research in Environmental Sciences, Catholic University of Pernambuco. The Culture Collection is registered in the World Federation Culture for Collection-WFCC and maintained in nutrient agar at 5°C. The Erlenmeyers flasks containing peptone glycerol agar and mannitol agar and were inoculated with *S. marcescens*, incubated at 28°C during 48 hours. Then the pigment was extracted from biomass obtained from mannitol agar and glycerol peptone agar, then was submitted to purification using TLC, and was characterized by mass spectrometry- GCMS.

RESULTS AND DISCUSSION: *S. marcescens* was produced 48.50 g / L of biomass from mannitol agar medium and 17.50 g / L of glycerol peptone agar, respectively. The purification using thin layer chromatography showed the reference values (*Rf)* of 0.59 and 0.22, respectively for red and blue fractions, and these results are corroborated by Nakashima *et al* (2005). The fraction of the red pigment produced in glycerol peptone agar and mannitol agar was characterized by mass spectrometry-GCMS and showed a molecular weight of 392.3Da suggesting to undecylprodigiosin belongs to prodigiosin family.

CONCLUSION: A new strain of *Serratia marcescens* (UCP1549) isolated from soil in semi-arid showed higher biotechnological potential to produce of red pigment similar to undecylprodigiosin [2-((3-methoxy-5-(1H-pyrrol-2-yl)-2H-pyrrol-2-ylidene)methyl)-5 undecyl-1H-pyrrole.

REFERENCES: Nakashima T, Kurachi M, Kato Y, Yamaguchi K, Oda T. Characterization of bacterium isolated from the sedimenta t Coastal área of Omura Bay in Japan and several biological activities of pigment produced by this isolated. *Microbiol. Immunol*. 2005; 49: 407-415

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PRODUCTION KINETICS OF L(+)-LATIC ACID BY *Lactobacillus rhamnosus* B103, ON CHEESE WHEY AND CORN STEEP LIQUOR

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Keywords: lactic acid, growth kinetics

The lactic acid is an organic acid, widely employed in many industrial sectors. In pharmaceutical industry, the lactic acid can be used in cosmetics production, creams and lotions, in chemical industry it has been used in production of solvents and in the food industry as acidulant, flavoring and emulsifier (WEE *et al*., 2004 ALTAF *et al*., 2005). Other lactic acid interest field is on production of renewable and biodegradable plastics from poly latic acid (PLA), such as food's packaging and variable plastics utensils, which can replace products made from raw petroleum-based (DATTA *et al*., 1995). Moreover, the PLA biopolymers can be used in the medicine, on the tissues regeneration, sutures, fracture fixation, bone reposition, cartilage repairs, meniscus repairs, ligaments and implants fixation (SAKATA *et al*., 2004). The growth kinetics, the consume of substrate and the lactic acid production by *Lactobacillus rhamnosus* B103 were checked by using cheese whey and corn steep liquor, in the optimal concentrations. The lactic acid production, as well as, the sugar quantification was done by High Performance Liquid Chromatography (HPLC), equipped with ultraviolet detector at 210 nm and refractive index detector. A Rezex ROA column (300 x 7,8 mm) of phenomenex was used, it was eluted with H_2SO_4 5mM as mobile phase, the flow was 0,6 mL/min and the temperature was 65ºC. Biomass was determined by optical density at wavelength of 650 nm. The highest concentration of lactic acid was achieved in shaker (142 g/L) after 48 hours of fermentation and in the reactor (140 g/L) after 92 hours of fermentation. The maximum specific growth rate μ_{max} of *Lactobacillus rhamnosus* B103 in the reactor and in the shaker were 0,28 h^{-1} and 0,33 h^{-1} respectively, the maximum specific lactic acid production rate in the reactor and in the shaker were 0,97 h^{-1} and 1,096 h^{-1} respectively and the maximum specific sugar utilization rate in the reactor and in the shaker were 1,41 and 1,44 respectively.

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PRODUCTION OF A BIOSURFACTANT FROM *CANDIDA LIPOLYTICA* CULTIVATED IN INDUSTRIAL WASTES USING A BIOREACTOR

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Keywords: Biosurfactants, *Candida*, industrial residues, bioreactor.

Surfactants constitute an important class of chemical compounds widely used in various industrial sectors. These compounds are formed by molecular structures containing hydrophilic and hydrophobic portions that tend to distribute at the interfaces between fluid phases with different degrees of polarity (oil / water and water / oil). The hydrophilic portion consists of anionic groups, cationic, or amphoteric, whereas the hydrophobic part is usually a linear or branched hydrocarbon or showing no double bonds and / or aromatic groups. Biosurfactants are microbial metabolites produced specially by bacteria and yeasts, although some fungi also produce them. These compounds, due to its biodegradability and compatibility with the environment, unlike similar petrochemicals (synthetic), have been increasingly studied. Thus, this paper describes the production of biosurfactants in bioreactor by *Candida lipolytica* grown in low-cost waste in order to apply the biomolecule in the environmental area. The following wastes were used as substrates: 5% animal fat and 2.5%. corn steep liquor. Fermentations were performed in a bioreactor of 2.0 L capacity, which operated at a temperature of 28 º C with values of agitation, aeration and at intervals set according to a factorial design. A 2^3 factorial design with four repetitions and eight tests at the central point was used to evaluate the influence of the independent variables aeration (0,1 and 2 vvm), agitation (200, 300 and 400 rpm) and time of cultivation (48, 96 and 144hours) on the response variables surface tension, yield of biosurfactant and biomass. According to the Pareto charts of standardized effects it could be observed for a confidence level of 95%, that the variable agitation has shown a negative effect on both surface tension reduction, yield and biomass. Moreover, the cultivation time showed a positive and statistically significant influence on surface tension reduction, on the yield and on the biomass. For the variable aeration, it showed a negative influence on surface tension reduction and yield, although not statistically significant, and a positive and statistically significant effect on the biomass increase. The biosurfactant produced by C. *lipolytica* grown in a low cost medium showed potential to be applied in the environmental area once it was able to reduce the water surface tension at 28 mN/m after 144 hours at 200 rpm with an yield of 7 g/L.

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PRODUCTION OF CHITIN AND CHITOSAN BY *Rhizopus oryzae* USING, CASSAVA WASTEWATER SUPPLEMENTED WITH CORN STEEP LIQUOR

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Keywords: chitin, chitosan, *Rhizopus oryzae*, biopolymers, agroindustrial residues

Chitin is a natural linear polymer found in the shells of crustaceans, insects, fungi and yeasts. Chitosan is a natural polysaccharide originated from the deacetylation of chitin. It is soluble in acidic due to the presence of amino groups in the form of free radicals along the polymer chain. The fungal class Zygomycetes, in particular *Rhizopus oryzae* present in their cell walls as chitin and chitosan. This work was analyzed the production of chitin and chitosan by *R. oryzae* using low cost substrate, such as cassava wastewater, supplemented with corn steep liquor. The strain of *Rhizopus oryzae* (UCP 1506), was kindly supplied to the Culture Collection Universidade Católica de Pernambuco (UCP), and maintained on Potato Dextrose Agar, at 5 ° C. The experiments were carried out using cassava wastewater (10%, 7.5% and 5%), supplemented with corn steep liquor (6%, 4% and 2%), at pH 5.5 to 6.0, incubated in orbital shaker at 150 rpm, at 38, 33 C and 28°C for 96. After this period the cultures were collected using nylon filter (Silkscreen, 120F). The biomass was washed with distilled water, dried and maintained in desiccator until constant weight. The extraction of chitin and chitosan were carried out by deproteinization of biomass with 2% NaOH (30:1 v / g) at 60 ° C for 2 hours. Then was separated alkali-insoluble fraction by centrifugation. The residue obtained was treated with 1% sulfuric acid (40:1 v / g) in an autoclave at 121 ° C for 15min. Chitosan was obtained by neutralization to pH 10 and precipitation overnight at 5°C. The copolymers obtained chitin and chitosan, were washed with distilled water, dried and kept in a desiccator until constant weight. Chitin after 96h of culture showed higher in amount in 5% and 2% of cassava and corn steep liquor, at 38°C showed a yield of 51.90 mg / g and 22.01 mg/g dry of dry biomass, respectively. The best chitosan production was found in the condition 10% cassava supplemented with 2% corn steep liquor at 28°C showing a yield of 44.67 mg/g and 24.49 mg/g dry weight of chitin. The agro-industrials substrates used in these studies indicated the biotechnological potential of manipueira supplemented with corn steep liquor as rich components for mirobiological production of chitin and chitosan by *R. oryzae*.
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PRODUCTION OF CHITINASE, LIPASE AND PROTEASE OF *Beauveria bassiana* GROWN IN THE PRESENCE AND ABSENCE OF THE PULVERIZED *Alphitobius diaperinus* (LESSER MEALWORM)

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ABSTRACT

Alphitobius diaperinus (Panzer), also known as lesser mealworm, is considered an important world poultry plague. This beetle is known as a potential reservoir for poultry pathogens causing several diseases due to transmission of bacteria, virus, fungi and protozoa, resulting in serious veterinary and economical problems. Usually its control is performed using chemical insecticides (organophosphates and pyrethroids). Laboratory tests demonstrated that the use of these insecticides cause a temporary reduction of the insect population, further this control practice becomes ineffective due to habits of the beetle and the continuing presence of birds in the poultry houses. Extracellular enzymes such as chitinases, lipases, and proteases as well secondary metabolites with insecticide capability were already extracted from *Beauveria bassiana* and demonstrated potential for use in biological control. The objective of this work was to evaluate the production capacity of chitinases, lipases and proteases from four strains of *B. bassiana* (UNI-4, UNI-40, CG-71 e CG-152). The cultures were performed in 125 mL Erlenmeyer flasks with 25 mL of Minimal Vogel´s media containing 2% (w/v) of pulverized *A. diaperinus* or 2% (w/v) of glucose as carbon source. All cultures were carried out in triplicate and incubated under agitation (180 rpm) for 7 days at 28 ± 2 °C. The supernatants (enzyme extracts) were used to determine the activities of chitinases, lipases and proteases. Moreover, the content of total sugars, reducing sugars, protein, initial pH and biomass, were also determined, contributing for a better assessment about microorganism growth under these conditions. All strains were able to produce chitinases, lipases and proteases when grown in presence of pulverized *A. diaperinus* as sole carbon source and in this condition showed greater activity compared to those strains that grown in Vogel's medium containing glucose as carbon source. This result demonstrates that the presence of pulverized *A. diaperinus* in the medium induced the production of extracellular enzymes. It was also observed that the increasing order of enzyme activity produced by four strains studied was always chitinases < lipases < proteases under these conditions studied. The strain CG-152 was the best enzyme producer, which showed chitinase activity corresponding to 0.05 U/mL, lipase 3.36 U/mL and protease 346 U/mL. The four strains showed a higher capacity to produce proteases when cultured in presence of pulverized *A. diaperinus* making them potential biological control agents, since these enzymes are essential in the process of pathogenicity.

Keywords: biological control, *Beauveria bassiana, Alphitobius diaperinus*, extracellular enzymes
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PRODUCTION OF LIPASE FROM *Fusarium* sp. USING CORN OIL AND SURFACTANTS THROUGH EXPERIMENTAL DESIGN AND SURFACE RESPONSE METHODOLOGY.

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Keywords: lipase; *Fusarium* sp.; experimental design; surface response.

Introduction. Lipases (E.C. 3.1.1.3) catalyse the hydrolysis of triacylglycerols of long chain in free fatty acids, mono and diacylglicerols and glycerol. These enzymes are important for presenting versatile character; since, beyond the hydrolysis they participate of synthesis reactions in aquo-restricted environments. Filamentous fungi are the preferred ones as source of lipases, because they produce extracellular enzymes, facilitating the extraction after the fermentative process. When developing industrial fermentation, designing media and optimising fermentation conditions are of critical importance because these factors could strongly interfere with the yield of lipase production. Experimental design techniques present a more balanced alternative to the one-factor-at-a-time approach for fermentation improvement. In the present study, the effect of carbon source and concentration of surfactants on lipase production by *Fusarium* sp. in batch fermentation was determined using experimental design and surface response methodology.

Material and methods. The concentration of carbon source and presence of surfactants was evaluated by two experimental designs 2^2 independents, with 4 trials (-1 and +1), two central points and 4 axial points (-1.41 and +1.41), totaling 10 experiments each, using a computer program STATISTICA 8. The independent variables were corn oil (CO) and Tween 80 (TW) or Triton X-100 (TR) using yeast extract (0.5%) as nitrogen source. The results were evaluated by experimental design and surface response methodology.

Results and discussion. First Experimental Design (corn oil and Tween 80): The highest lipolytic activities (2.79 U/mL) were achieved in test 9 (0, -1.41). These results indicated that the addition of Tween 80 is not advantageous to increase the lipolytic activity. From the responses obtained in the first-order 2^2 experimental design (assays 1 to 10), the main effects from the interaction of CO and TW was not statistically significant at a confidence limit of $p<0.05$. However, the main effect of TW isolated was statistically significant. Thus, an antagonistic effect was observed, causing an average loss of 1.08 U/mL of activity. Second Experimental Design (corn oil and Triton X-100): The highest lipase activity was obtained in trials 1 (-1, -1), 2 (-1, +1) and 9 (0, -1.41), where values of 4.89, 4.34 and 5.59 U/mL were observed, respectively. From the responses obtained in the first-order 2^2 experimental design (assays 1 to 10), the main effects from the interaction effect of CO and TR was not statistically significant at a confidence limit of $p<0.05$. However, the main effect of CO and TR isolated was statistically significant. Thus, a synergetic effect of CO and TR was observed, causing an average increase of 1.65 and 1.64 U/mL of activity, respectively.

With these results it is possible to conclude that the surfactants Tween 80 and Triton X-100 have different effects on lipase production by *Fusarium* sp., while the first acts as an inhibitor and the second induces the production of the enzyme. Through the estimate was best defined as medium composition corn oil 2.0% (w/v) and Triton X-100 1.7% (w/v).

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PRODUCTION OF α-AMYLASE BY *Bacillus subtilis* (UCP 0999) USING MEDIUM CONTAINING ALTERNATIVE SUBSTRATES

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Keywords: amylase, *Bacillus subtillis*, alternatives substrates.

α-Amylases (E.C. 3.2.1.1.) are starch-degrading enzymes that catalyze the hydrolysis of internal α-1.4-*O*-glycosidic bonds in polysaccharides with the retention of α-anomeric configuration in the products. Most of the α-amylases are metalloenzymes, which require calcium ions (Ca^{2+}) for their activity, structural integrity and stability. Amylases find potential application in a number of industrial processes such as in the food, fermentation, textile and paper industries. The amylolytic enzymes are responsible for 25 to 33% of world production of enzymes. *Bacillus* species continue to be dominant bacterial workhorses in microbial fermentations. *Bacillus subtilis* (natto) is the key microbial participant in the ongoing production of the Soya-based traditional natto fermentation, and some *Bacillus* species are on the Food and Drug Administration's GRAS (generally regarded as safe) list. The capacity of selected *Bacillus* strains to produce and secrete large quantities (20–25 g/L) of extracellular enzymes has placed them among the most important industrial enzyme producers. The use of alternative means of production have been considered a great economic challenge for the industries that use and produce these enzymes and the synthetic media and the most expensive used in submerged fermentation of amylase. The objective was to evaluate the use of alternative carbon sources of low cost to produce a high value industrial enzyme by *B. subtilis*. The production by of amylase by *Bacillus subtilis* (UCP 0999) replacing the source of starch for water and rice flour banana peel. The medium of production of amylase: 0.65% peptone, 3.0% yeast extract, 0.5% KH_2PO_4, 0.25% $MgSO_4.7H_2O$, 1.5% $NaNO_3$; pH 7.0. Economic means the soluble starch was replaced by water of banana peel flour at a concentration of 10 g / L. The production occurred in flasks containing 250 mL of culture medium, 37 °C, orbital shaker, 150 rpm during 96h. The samples were subjected to quantitative assessment of amylase production using the dosage of reducing sugars (DNS). The results revealed that the use of alternative sources of starch in water and rice flour in the banana skin, produced the enzyme when compared to control medium containing soluble starch in 47 UI/mL is the best growth was between 40 and 70 hours in neutral pH, during fermentation kinetics. The showed that half the flour with the banana peel had a high significative score. The results suggest that formation of the enzyme is slow during the logarithmic growth, followed by an increased rate of synthesis to the extent that the growth rate decreases and reaches the stationary phase.

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QUANTIFICATION OF TOTAL AND FECAL COLIFORMS AS TOOL FOR QUALITY ASSESSEMENT OF WATERCOURSES

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Keywords: Atibaia river, coliforms, depuration.

Water is essential to survival and development of living beings, being the most abundant inorganic constituent in living matter. The main sources of supply of this resource are the rivers, however, human activities pollute and degrade these resources through the dumping of waste, whether industrial, urban or promoting illegal losses in quality and quantity of water causing huge environmental impacts. The Atibaia River supplies most municipalities crossing. Throughout their journey, the river flows through areas of intense population and industrial development, getting more pollutants into their waters. To perform the quantification of these impacts, microorganisms are most commonly used as biological indicators in watercourses, being the main the coliform bacteria because of resistance slightly higher than most pathogenic enterobacteria allowing its use as an indicator agent. The study monitored the microbiological quality during the period May 2005 to December 2009, carrying out fortnightly collections in Atibaia and Jaguarí rivers. Sampling points were: (1) Jaguarí river (Latitude: 22°41'48"; Longitude: 47°08'59"); (2) Atibaia river – upstream (Latitude: 22°41'28"; Longitude: 47°07'22"), (3) Atibaia river – 400m downstream (Latitude: 22°44'25"; Longitude: 47°07'33") and (4) Atibaia river – 900m downstream (Latitude: 22°44'22.3"; Longitude: 47°07'40.8"). The multiple tube method (MPN/100mL) was used to quality the total and fecal coliforms. In the presumptive test was used lactose broth (35°C/48h) for the enrichment of microorganisms fermenting lactose. Positive tubes from the presumptive test were transferred to bright green bile broth 2% medium (35°C/48h) for confirmatory testing of total coliform. For the differentiation of fecal coliform it was used the EC medium (44.5°C/24h). Regarding the results obtained, there was considerable decrease in the amount of total and fecal coliforms along the points of Atibaia river, showing its ability to self-purification. However it can be verified in 2008 that point (3) showed an increase in concentration of total coliforms (29.8%), when compared to point (2), changing the natural order of self-purification of the river, but the total reduction of the course was 50.7%. The year 2007 also showed a significant increase in the concentration of fecal coliform (50.5%) between points 2 and 3, however, the decrease along the course was 62.2%. The resulting data indicate that this parameter is a great tool for application in the investigation of water quality. Thus, by the results analyzed, it was found that the Atibaia river had the ability to self-purification, however, rates of coliforms found did not reach the results of Jaguarí river also class II.

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RADIAL GROWTH AND MORPHOLOGICAL ASPECTS OF *Rhizopus arrhizus* USING ALTERNATIVE MEDIUM

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Keywords: *R. arrhizus*, Radial growth, Corn step liquor.

The survival of microorganisms depends on their ability to grow under certain chemical and physical conditions, and the maintenance in laboratory. Media used in the laboratory for the cultivation of *R. arrhizus* include carbon and nitrogen sources must supply all of the necessary the nutritional requirements as enzymes, inorganic substances, and water. Recent studies described the use of alternative and low cost medium in substitution to the traditional synthetic medium. The aim of this work was investigation of the mycelia growth and the morphological aspects of *Rhizopus arrhizus* on corn step liquor and honey bee medium (CSL+HB) and Synthetic Medium for Mucorales (SMM). *R. arrhizus* was kindly supplied from Culture Collection of the Nucleus of Research in Environmental Sciences – NPCIAMB, Catholic University of Pernambuco – UNICAP. Spores (10^4 spores/mL) suspensions were transferred to Petri dishes containing PDA (Potato Dextrose Agar) medium. Discs of 6 mm were used as inoculums in Petri dishes containing the media CSL+HB and SMM. The radial growth was evaluated by measurement of diameter of the colony at 24h during 96 hours, in triplicate. The morphological aspects of the *R. arrhizus* growth was accompanied by light microscopy**.** The radial growth of *R. arrhizus* on CSL+HB showed a higher diameter of the colony than on SMM at the same period. The results showed an increase of the diameter of the colony in alternative medium (CSL+HB) corresponding to 16mm in comparison with SMM medium. In addition, the increase of the growth it is related to the amount of carbon and nitrogen sources in the alternative medium. Corn steep liquor is considered excellent amino acids and vitamins source required for *R. arrhizus* growth. The microscopic observations showed the presence of reproductive structures (sporangiospores) at the first 48 hours on SMM medium. However, different microscopy behavior was observed in the medium corn step liquor. In that medium the structures appeared rarely, the hyphae are hyaline, and higher sporulation was observed probably due rich sources of nutrients.

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REDUCTION OF TOXICITY OF SUGARCANE RESIDUE FOR USE IN RUMINANT FEEDING

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Keywords: toxicity, sugar cane bagasse, ruminant, lignin.

The sugar cane (*Saccharum oficinalis*) produces a lignocellulic residual waste called bagasse after broth extraction. This waste is basically constituted by cellulose (40%), hemicellulose (35%) and lignin (15%). Among many possibilities, its use can result in: energy production, cardboard production, ruminant feeding, and aggregates for different purposes including manufacturing of furniture and plastics. Even when fragmented, the bagasse presents resistant fibers that may injure the digestive tract of ruminants, when used as animal food. Sugarcane industry introduced a heat treatment under pressure/decompression of the bagasse which is placed in reactors and subjected to pressure about 153 atmospheres (equivalent to 200°C) for approximately 7-10 minutes. Then, the reactors are opened to liberate the pressure when the expulsion of the bagasse occurs, being directed to a place generating heaps. With the expansion, the fibers that are involved in lignin are broken exposing the cellulose and hemicellulose making them easily accessible by the microorganism. However, the heating produces phenols, 5-hidroximetilfurfural and others compounds that are toxic to many microbes including those contained in the rumen. To minimize the problem of toxicity, the term expanded bagasse [TEB] was treated with the fungus *Lentinula edodes* (Shiitake). Two portions of 500g of TEB were separated .One was used as control and the other was inoculated with *L.edodes*. After fungal growth [BFG] the solubilization of solutes was made and parameters were analyzed as: pH, chemical oxygen demand (COD), biochemical oxygen demand (BOD) and acute toxicity tests with *Daphnia similis.* The results showed a reduction of acidity (3.14 in TEB to 3.93 in BFG), an increase of the solubilized material quantified in COD of 59,360.00 mg/O^2/L and in BOD of 60,600.00 mg/O^2/L , 5 days in 20 °C. The toxicity decreased 3.8 toxic units 100/CE(50)48h. The preliminary studies indicated the possibility of improving the quality of TEB in order to compose bulky on feed animals.

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REMOVAL OF BACTERIA IN A SYSTEM OF PONDS STABILIZATION, THE CITY OF CUIABÁ-MT

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Keywords: waste stabilization ponds, coliforms, heterotrophic bacteria, stream Caju.

The treatment of sewage by means of stabilization ponds has as main objective the removal of pathogens, which is confirmed by the bacterial decay, being of fundamental importance to estimate the concentration of coliform to be launched in the receptor that does not change quality, above the limits set by environmental standards, thus ensuring their total number of uses such as legislation for its various uses such as irrigation, recreation, water supply among others. The literature shows that the maturation ponds are used to give the final polishing of the effluent, and thus are responsible for high levels of efficiency in the removal of fecal coliforms. This study aims to estimate the removal efficiency of total coliforms and *Escherichia coli (E. coli)*, the system of stabilization ponds CPA III in Cuiabá/ MT. Bacteriological analysis by the method of chromogenic and fluorogenic substrates to estimate the density of total coliforms and *E.coli* and *Spread Plate* technique for enumeration of heterotrophic bacteria. Samples were collected with monthly frequency at six sites from March 2010 to February 2011. Through these results it was possible to calculate the removal efficiency of total coliform and *E.coli.*After obtaining greater efficiency in the removal of total coliform in March with 99.933% and lower in September with 94.627% and for *E. coli* was the most efficient in October with 99,999% and lowest in August with 99.997%. It was observed that for both total coliform and *E. coli*, obtained higher efficiencies in the rainy season due to dilution of the effluent, making it less concentrated, the opposite occurred in the dry season, which had lower efficiencies, because the effluent is high concentration. The effluent stream at the launch of Caju had a higher concentration of *E.coli* in the month of June (dry season) of 294 MPN/100mL. The largest number of heterotrophic bacteria occurred in March with 3.0 x 10^6 CFU/ mL, and the lowest was observed in January with 3.1 x 10^3 CFU/ mL. The system is in agreement with its main purpose, namely, the removal of high amounts of coliform. The total number of *E.coli* present in the effluent stream is released in accordance with the limit set by CONAMA 357/2005 to water bodies of class 2. The number of heterotrophic bacteria has a maximum value allowed by law, but its determination is important to determine possible causes that affect the quality of water.

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REMOVAL OF COLIFORM BACTERIA FROM SLAUGHTERHOUSE POULTRY WASTEWATER BY CONSTRUCTED WETLANDS SYSTEM

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Keywords: coliforms bacteria, slaughterhouse poultry wastewater, constructed wetlands system

The constructed wetlands system (CWS) are being increasingly investigated and used to be a simple technology and easy operation to remove pollutants and microorganisms from water in general, wastewater especially. These systems are based on the synergistic interaction between macrophytes, microorganisms and physic-chemical properties of some solid substrate. Through natural processes, such as filtration, sedimentation, adsorption, absorption and biodegradation, occurred significant improvements in the quality of the water treated by this system. The wastewaters generated in poultry slaughterhouses are characterized by large amount of organic content and microorganisms, including pathogens, and resulting contamination of water resources. This study investigated the feasibility of CWS, at a pilot scale, for the removal of coliform bacteria from poultry slaughterhouse wastewater pretreated in aerobic reactor. Eight prototypes of the system were constructed using 55 liters plastic boxes containing filter material and macrophytes (*Eichhornia crassipes*); four were operated by upflow, and the other four by downflow. Two prototypes for each type of flow have not received the macrophyte. The filter material consisted of 3.0 cm of broken tile, 4.5 cm of pebbles (12.8 to 25 mm), 6.0 cm of soil plus pebbles (3.2 to 6.8 mm) and 3.0 cm mixed granilite (5.2 to 13.6 mm). The quantification of total coliforms (TC) and *Escherichia coli* (MPN/100mL) occurred during T_2, T_7, T_{21}, T_{35}, T_{56}, T_{77} and T_{100}, which represent the number of days after the start of the experiment. The microorganisms' determination occurred by the method chromogenic/fluorogenic (Colilert). The amount of CT in raw wastewater ranged from $1{,}3 \times 10^6$ to $2{,}4 \times 10^7$ and there was an average removal of 97.90% ± 1.87 and 96.99 ± 2.85% of these bacteria in upflow and downflow systems with macrophytes, respectively. In systems without macrophytes the removal values ranged from 95.72% ± 5.11 in the upflow system and 96.48% ± 4.30 in the downstream system. For *E. coli*, the amount ranged from 2.1×10^5 to $2{,}4 \times 10^6$ in raw wastewater. The average removal of these bacteria in upflow system was 98.97% ± 1.13 in macrophytes systems and 98.72% ± 1.25 in no macrophytes system. In the other treatments, the removal was 98.03% ± 2.82 (macrophytes downflow system) and 97.19% ± 1.52 (no macrophytes downflow system). The variance analysis not revealed differences statistically significant between the different flow types, with or without macrophytes. We conclude that the use of CWS is efficient to reduce coliforms bacteria and other microorganisms potentially pathogenic not analyzed here. This study demonstrated the positive influence of the clean technologies to improve the quality of water resources, since these effluents, in most cases, are disposed in surface water bodies.

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RESEARCH OF *INTEINS* (*INTERNAL PROTEINS*) VMA AND THRRS IN *CANDIDA* GENUS YEASTS

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Introduction: *Inteins* or "*internal proteins"* are coding sequences that are transcribed and translated with flanking sequences (exteins). After translation, the *inteins* are excised by an autocatalytic process (*self splicing*) and the host protein assumes its normal conformation and develops its expected function. These parasitic genetic elements have been found in important conserved proteins in all three domains of life. Besides the importance of *inteins* as molecular markers and as potential therapeutic targets, these elements have not yet been studied in *Candida* species, responsible for most of opportunistic fungal infections in hospitals.

Methodology: According to InBase (*intein* database) some important clinical species of *Candida* such as *C. tropicalis* has the VMA *inteins* (Vacuolar ATPase) and ThrRS (threonyl-tRNA synthetase) while *C. glabrata* only the VMA *intein*. These *inteins* have been here investigated by gene amplification in 05 strains of *C. glabrata* and 10 strains of *C. tropicalis*. For the *intein* thrRS of *C.tropicalis* the primer was designed in extein N and C terminal of thrRS gene, and the VMA *intein* of *C. glabrata* and *C. tropicalis* were designed degenerate primers in the extein of VMA gene.

Results: Expected fragments of 1020bp for thrRS and 1500bp for VMA were obtained and the identity was confirmed by sequencing and compared by BLAST tool. Polymorphism was observed in both *inteins*.

Discussion: Fragment length polymorphism was observed in both *inteins*, which indicates the potential of these elements as molecular markers. Phylogenetic analysis of the sequences will now be evaluated to better understand the dynamics of these elements in these yeast species.

Keywords: *Intein, Candida spp*
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Rhamnolipids production by *Pseudomonas aeruginosa* mutants using n-paraffin as carbon source.

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Keywords: rhamnolipids, mutants, *Pseudomonas aeruginosa* LBI.

Introduction

The biosurfactants are metabolites that exhibit surface activity and are synthesized by a variety of microorganisms, and show high structural diversity, low toxicity and high biodegradability. Among the most promising biosurfactants are rhamnolipids, which are glycolipids consisting of one or two rhamnose molecules attached to one or two fatty acid chain, the composition of these glycolipids varies according to the producer strain and carbon source used.

Materials and methods

Therefore the aim of this study was to evaluate *Pseudomonas aeruginosa* LBI mutants and paraffin, an unusual carbon source, for rhamnolipids production. The mutants' bank was made by transposon insertion in *Pseudomonas aeruginosa* LBI and stored at -20 º C. Rhamnolipid production was carried out in 125 mL Erlenmeyer flask with 50 ml of mineral salt medium and 2% (v/v) of n-parafina as carbon source; the inoculum was added at a concentration of 4% (v / v). The experiment was incubated for 120 h at 30 °C and 200 rpm in a rotary shaker. The mutants evaluated were *P. aeruginosa* LBI 1A1, 1A2, 1A3, 1A4, 1A5, 1A6, 1A7, 1A8, 1A9, 1A10, 1A11, 1B10 and 2A1. Rhamnolipids were measured in the cell-free broth as rhamnose (Chandrasekaran and Bemiller, 1980) and growth by dry biomass.

Results and discussion

The mutants demonstrated ability to grow and produce rhamnolipids with n-paraffin as sole carbon source. The highest rhamnose concentrations were achieved by *P. aeruginosa* LBI 1A11, 293.514 mg / L. This study makes clear the ability of the mutants in consume n-paraffin and synthesize rhamnolipids.

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SCREENING OF FUNGAL AMYLASE PRODUCERS FROM CERRADO AREA

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Keywords: microbial enzymes, solid state fermentation, production

Starch is an important reserve carbohydrate with great application on food industries. The enzymatic hydrolyses this carbohydrate can be applied in different sectors as food, paper, textile, and chemical industries. The α-amylase is an enzyme capable of hydrolyze α-1,4 the glycosidic bonds present in the inner part of the starch chains. The starch polymers show two molecules: amylose and amylopectin and the syrups starch (or hydrolyzed starch) can be obtained by action of amylase complex obtained from bacteria, yeasts and filamentous fungi. The commercial production of α-amylase can be carried out by submerged or solid state fermentation. This study focused on select fungi amylase producer, isolated in Cerrado area. Shaded soil sample from wood and organic material in decomposition from Cerrado area were added to flasks with 5 mL screening medium (1.0% soluble starch; 0.20% peptone; 0.20% yeast extract; 0.14% $(NH_4)_2SO_4$; 0.20% K_2HPO_4; 0.02% $MgSO_4.7H_2O$; 1.60 $mg.L^{-1}$ $MnSO4.H_2O$; 1.40 $mg.L^{-1}$ $ZnSO_4.7H_2O$; 2.0 $mg.L^{-1}$ $CoCl_2$; pH 5.0). The samples were incubated at 35 °C for 24 hours and then inoculated on Petri dishes containing solid screening medium and streptomycin. The plates were incubated at 35 °C for a period of 144 hours. Isolated colonies were transferred to tubes containing maintenance medium. Where collected about 30 fungi strains. The strains were cultured under solid state fermentation using wheat bran as substrate. Two methods were used to determine amylase production level. One is the iodine method that was used to determine dextrinization or the ratio of hydrolysis of the starch. One unit of enzyme activity was defined as the quantity of enzyme that reduces the blue color of the starch-iodine complex by 10% per minute. The other method was Somogyi-Nelson method that was used to determine reducing sugar. One unit of amylase activity was defined as the amount of enzyme required to release 1 µg of glucose per minute under the above assay conditions, using a glucose standard curve. Between cultivated strains, the strains A-1.4, A-2.6, AF-3, AF-11, A-2.5 showed the highest activities for dextrinizant activity (270 U/mL to 90 U/mL) and reducing sugar (727,0 U/mL - 202.0 U/mL). It can be concluded with these results, these strains have potential for production of α amylase.

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Resumos apresentados no V Simpósio de Microbiologia Aplicada

SECONDARY METABOLITES PRODUCTION BY YEAST AND LACTIC ACID BACTERIA COCULTURE

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Key words: alcoholic fermentation, bacterial contamination, ethanol and secondary metabolites

INTRODUCTION: Bacterial contamination in the fermentation process unfeasible the production of ethanol at high yields. These contaminants cause problems such as consumption of alcohol produced (in the case of acetic bacteria), decrease yeast viability, yeast flocculation, gum formation and especially the diversion of sugar for the production of secondary metabolites (Amorim & Oliveira, 1982). In this context, the aim of the present study was to evaluate the organic acid, glycerol and mannitol production, when fermentation takes place in a contaminated environment by lactic acid bacteria.

MATERIAL AND METHODS: it was tested a strain of *Saccharomyces cerevisiae* (PE-2), in co-culture with the bacterium strain of *Lactobaculis fructosus* (FT432B), using as control the same yeast strain without contamination. Yeasts, that remains preserved in solid YPD medium, were renewed in liquid YPD and incubated in BOD at 32±1ºC/24h. The bacterium strain (preserved in frozen skim milk), was re-activated in MRS liquid at 32°C/24h. After activation, it was prepared an initial inoculum with approximately 12% of yeast (w/v) and 10^8 bacterial cells/ml at 32°C in sterile medium MBL (0.5% yeast extract; 0.5% peptone; 1% glucose; 0,2% K_2HPO_4; 0,02% $MgSO_4.7H_2O$; $MnSO_4.H_2O$ 0.001%). After 24h, 1ml samples were collected and centrifuged for analysis of parameters. Cell viability and cell count (cell/ml) were also determinate. The formation of glycerol and mannitol was estimated by anion exchange chromatography (HPAEC), and organic acids by liquid chromatography (HPLC).

RESULTS AND DISCUSSION: Results showed that it was not possible to detect the acetate when fermentation occurred in the absence of bacterial contamination; however, in the presence of bacteria, we could notice the increased production of lactate, acetate and succinate. In both cases there was a prevalence of lactate, especially in the case of fermentation in co-culture with bacteria. Succinic acid was present almost equally in both treatments, since it is exclusively produced by yeasts. All organic acids showed a decline between 1 and 2 hours, probably due to dilution of the medium for the inoculum preparation (yeast, distilled water and wine) however, the lactic and acetic acid concentrations increased again after a period, suggesting bacterial activity. Additionally, the presence of mannitol was not significant in both cases, while glycerol showed higher levels, especially in the case of fermentation with bacterial contamination. In both cases, the yeast viability remained high until the end of the experiment (mean 82%). The diversion of sugar for the yeast and bacteria production of secondary metabolites, caused an alcoholic production decrease when the fermentation took place in the contaminated medium.

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SELECTION OF ALTERNATIVE MEDIUM FOR PRODUCTION OF PROTEASE BY *Bacillus subtilis* (UCP 0999).

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Keywords: protease, *Bacillus subtilis*, selection medium

Proteases (EC 3.4), are enzymes that catalyze the cleavage of peptide bonds in other proteins, resulting in total hydrolysis of protein and are essential constituents of all forms of life on earth, including prokaryotes, fungi, plants and animals. Microbial proteases are among the most important hydrolytic enzymes and have been studied extensively since the advent of enzymology. There is renewed interest in the study of proteolytic enzymes, mainly due to the recognition that these enzymes not only play an important role in the cellular metabolic processes but have also gained considerable attention in the industrial community. It is a well-known fact that extracellular protease production in microorganisms is greatly influenced by media components, especially carbon and nitrogen sources, metal ions, and physical factors such as pH, temperature, inoculum density, dissolved oxygen, and incubation time. Today, proteases account for approximately 40% of the total enzyme sales in various industrial market sectors, such as detergent, food, pharmaceutical, leather, diagnostics, waste management and silver recovery. Microbial proteases are classified into various groups, dependent on whether they are active under acidic, neutral, or alkaline conditions and on the characteristics of the active site group of the enzyme, metallo (EC.3.4.24), aspartic (EC.3.4.23), cysteine or sulphydryl (EC.3.4.22), or serine-type (EC.3.4.21). The genus *Bacillus* is one of the most ubiquitous and diverse, with representatives being found in the soil and associated water sources such as rivers, coastal waters and estuaries. The metabolic diversity of these organisms has led to members of this group being used for a wide range of industrial processes, including the. antibiotics, fine biochemicals insecticides and production hydrolytic (and other) enzymes. The utilization in medium of production protease by several studies have been made to replace components of traditional means of production for substrates with low cost and high added value. In this sense, the objective of this work was to produce protease by *Bacillus* species using alternative substrates and low cost. The medium of production used as control was called Horikoshi-I, and the alternative media had the following substitutions: Medium "A" nitrogen sources replaced by corn steep liquor, "B" medium carbon sources replaced by soybean oil, medium " C "Carbon and nitrogen sources replaced by corn steep liquor and soybean oil respectively. The assays occurred in 150 rpm, 37^{o}C, 96 hours. The enzyme assay used was described in the method of Lowry, with the modifications proposed by Petterson, The results indicate that the combination of C and N sources in the middle C, enhanced the production of protease in the middle alternative, obtaining values of alkaline pH (10.9) in 56 hours of growth and enzyme activity of 62 IU/mL. These values show that the association of substrates in alternative means of production, may increase the yield of production in enzymatic processes and reduce production costs of fermentation processes.

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SELECTION OF YEASTS IN ORDER TO PRODUCE FUEL ETHANOL BY SMALL PRODUCERS FROM BAHIA

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Keywords: yeast selection, ethanol, cachaça

Artisanal production of ethanol fuel in a small distillery of *cachaça* in Brazil is becoming a popular solution within the country, and contributes to sustainable agriculture for small farmers, mainly in Bahia state. This kind of alcohol differs from the ethanol contained in alcoholic beverages only by the type of fuel required for standardization. The starter yeasts have been used with excellent results in the production of ethanol, making it possible to improve the process and the fermentation conditions. Some features are considered desirable in this selection such as tolerance to high concentrations of carbohydrates, high-speed fermentation, high alcohol tolerance, high temperature tolerance and stability. The selection of strains adapted to each type of process is very important to ensure a complete fermentation, and consequently a high production of ethanol. This study selected yeast strains targeting a high yield of ethanol fuel. The yeasts were isolated from two small distilleries in the interior of Bahia and are preserved in the Coleção de Culturas de Microrganismos da Bahia (CCMB / UEFS). 44 isolates were inoculated in modified Sabouraud agar (2% glucose, 1% peptone, 0.5% yeast extract and 2% agar). For stress testing (ethanol tolerance, osmotolerance and thermotolerance), were used a rate of 0.1 mL at a concentration of approximately 1.0 X 10^7 UFC.mL^{-1}. Counting of viable cells was performed using a Neubauer chamber and methylene blue dye 0.01%. The strain used as control was the *Saccharomyces cerevisiae* JP1. All tests were performed in triplicate. Analysis of the yeasts, in most cases, the results were similar or superior to the control, indicating a high potential of these isolates to produce ethanol. Of the 44 isolates tested, 13 showed potential as a yeast starter of the process, but other parameters such as ethanol production and growth speed of yeasts are important parameters for selection of strains with desired characteristics for the production of ethanol fuel.

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Soil Biological attributes and Quality of a Oxisol under Integrated Crop-Livestock System

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Keywords: mixing systems of agricultural production; microbial biomass; enzymatic activity of the soil.

Conventional monoculture agricultural systems can reduce the quality of soils by loss of organic matter and structure because of low levels of organic inputs and regular disturbance from tillage practices. Recent efforts are focused on developing integrated cropping and livestock production systems that can provide more conservative sustainable agricultural practices. The present work has for objective to evaluate the quality of a Oxisol in the region of the Piauienses Open pasture by means of biological pointers submitted the different systems of handling. Three systems of soil management were studied: an area under conventional tillage (CT) with disk plowi and heavy harrow and soybean crop; an area under no-tillage with soybean-maize rotation and millet as cover crop (NT + M); two areas under Integrated Crop-Livestock System, with five-month pasture grazing and soybean cultivation and then continuous pasture grazing (ICL + S and ICL + P, respectively). Also, an area under Native Forest (NF) was studied. The soil depths studied were 0.00-0.05, 0.05-0.10 and 0.10-0.20 m. The anthropic intervention in the closed native forest of (NF) did not provide significant difference ($p<0,05$) in the basal breath of soil (C-CO_2) and in the metabolic quotient (qCO_2), but, they had significantly influenced ($p<0,05$) carbon of the microbial biomass (Cmic) and the nitrogen of the microbial biomass (Nmic), providing reduction of the Cmic when in comparison the NF. The FDA hydrolysis showed differences between sites. The system of conventional plantation (CT) presented the biggest values of FDA (0,140 μg FDA g of soil^{-1} h^{-1}). The values for FDA hydrolysis decreased in the order of CT (> ICL + S > ICL + P > NF and NT + M). The systems of handling of the soil change the quality of the soil, in relation the areas under native vegetation. The basal breath and the metabolic quotient, had not been enough sensible to the occured alterations in function of the different types of handling.

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Specific CAT Response to Mesotrione Herbicide in Bacteria

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Keywords: Catalase, Superoxide dismutase, Callisto®, *Acinetobacter* sp.

Mesotrione is an herbicide that inhibits 4-hydroxyphenyl pyruvate dioxygenase (HPPD) in target plants, and stops carotene production. This herbicide was registered for use in 1993, and only a limited number of bacteria have been reported to degrade it. We investigated whether *Acinetobacter* sp., a non mesotrione degrading strain, but tolerant to 500 X field rates (FR), showed differences of oxidative stress in time, type and concentration of herbicide. We compared the responses of these bacteria to commercial herbicide (Callisto®) and the active ingredient (mesotrione). Evaluation of total protein in *Acinetobacter* sp., in mesotrione (1 and 500 X FR) treatment after 12 hours of incubation showed higher protein quantities than 24 hours and Callisto® treatments. This strain is not using mesotrione as energy source, so it is possible that in a 24 hours period the metabolic rate is slower. Total protein gel electrophoresis profile demonstrated no significant differences in band pattern to mesotrione and Callisto®, after 12 hours of incubation. But for Callisto 1 X FR, at 24 hours treatment, the band pattern show higher expression for specific proteins, probably due to a response from the herbicide. At 500 X FR, 24 hours treatment, mesotrione concentration was probably too high to permit specific responses. In mesotrione treatments, we observed specific bands overexpressed at 24 hours treatment, probably in response of a longer exposition time to the active ingredient mesotrione. One specific band was found in mesotrione 12 hours treatment, 1 X FR, probably relative to a low concentration and shorter exposition time to mesotrione. No different SOD isoforms were found and no differences in expression were observed in response to mesotrione or Callisto®,. Regarding CAT treatments, Callisto® did not show significant differences in isoforms expression, but we found one specific isoform in mesotrione treatments. We concluded that Callisto®, more toxic than mesotrione, is not suitable for specific CAT responses by *Acinetobacter* sp., just the active ingredient allowed this, probably due to the interference of other components of commercial formula.

Financial support: Fundação Araucária, CAPES, CNPq.

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STRESS RESPONSE TO TAURODEOXYCHOLIC ACID IN *Lactobacillus delbrueckii* UFV H2b20

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Keywords: *Lactobacillus*, probiotics, bile salts

Probiotic bacteria find several environmental stress conditions in the human gut such the presence of bile salts. Bile salts can alter the bacterial cell wall causing damages to DNA, to RNA and the proteins and their presence induces the expression of general stress proteins. The Clp proteolitic complex consists in a proteolitic nucleus (ClpP) flanked by Clp ATPases that presents indispensable function in the protein quality control system. The objective of this work was to elucidate the molecular mechanisms involved in the stress response to the taurodexycholic acid presence in *Lactobacillus delbrueckii* UFV H2b20. Growth of *L. delbrueckii* UFV H2b20 in the presence of different concentrations of taurodeoxycholic acid was monitored by measuring optical density and the survival of the cells exposed to the same conditions, for determined periods, evaluated by the drop plate method. The results showed reduction of the growth rate in the presence of 0,05 % and 0,1 % of this bile acid, however, no growth was observed in presence of 0,5 %. Although the presence of taurodexycholic acid alters the growth rate, after four hours exposure to 0,5 % there was a decrease of only two log cycles, indicating the resistance of *L. delbrueckii* UFV H2b20 to this condition. Cell morfology after exposure to the taurodeoxycholic acid was evaluated by Atomic Force Microscopy. The presence of taurodeoxicolic acid caused cell wall alterations and reduction of the height of the cells. Total DNA extracted from *L. delbrueckii* UFV H2b20 was submitted to PCR with primers based on the *clpL* sequence from *L. delbrueckii* subsp. *bulgaricus* ATCC 11842. The resulting amplicon was cloned in pGEM-T Easy Vector, transformed into *Escherichia coli* JM109, and sequenced. The 1686 base pairs sequence revealed a ClpL homolog, confirming its presence in *L. delbrueckii* UFV H2b20. This sequence displayed 97 % identity with the gene encoding the ClpL in *L. delbrueckii subsp. bulgaricus* ATCC 11842. Phylogenetic analysis was accomplished by Bayesian Inference. The *clpL* gene from *L. delbrueckii* UFV H2b20 grouped with the gene from *L. delbrueckii subsp. bulgaricus* ATCC 11842. The expression analysis of the *clpL* gene in *L. delbrueckii* UFV H2b20 in response to taurodeoxycholic acid presence, after 30 and 60 minutes of exposure, were performed by real time PCR. There was an increase of 1,5 and 1,2 fold in the expression of this gene when the cells was exposed to 0,5 % of taurodeoxycholic acid for 30 and 60 minutes, respectively, suggesting a transitory response. The results demonstrate that *L. delbrueckii* UFV H2b20 is able to survive and growth in the taurodeoxycholic acid presence and the *clpL* gene is involved in the response to taurodeoxycholic acid stress.

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STRUCTURAL CHARACTERIZATION OF NOVEL DEXTRAN FROM *Leuconostoc mesenteroides* FT045B

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Keywords: *Leuconostoc mesenteroides*; structure characterization; dextran; dextransucrase

Introduction: Dextran is an extracellular bacterial homopolysaccharide of D-glucose composed predominantly (at least 50%) of α-1,6-glucopyranosidic linkages within the main chain. The different types of α-D-glucan also have side-chains, stemming mainly from α-(1,3)-branch linkages and occasionally from α-(1,4)- or α-(1,2)-branch linkages. The exact structure of each type of dextran and the degree of branching involving α-1,2, α-1,3 and α -1,4 linkages in dextrans depend on the specific microbial strain and, hence, on the specific type of dextransucrase(s) involved.

Material and Methods: In the present study, a water-soluble dextran was produced from dextransucrase by *L. mesenteroides* FT045B and purified through ethanol precipitation. The structure was determined by Fourier transform infrared (FTIR) spectroscopy and ^{1}H nuclear magnetic resonance (^{1}H NMR) spectroscopy. Structure characterization was performed using an acceptor reaction with maltose and endodextranase hydrolysis. Average molecular weight and degree of polymerization were determined from the measurement of the reducing value using the copper bicinchoninate method and the measurement of the total carbohydrates using the phenol-sulfuric acid method.

Results and Discussion: The polysaccharide produced by *Leuconostoc mesenteroides* FT045B was purified and analyzed using different structural techniques. The reference dextran B512B purchased from Sigma was also analyzed using these structural techniques. The average molecular weight was 91,535.56 Da and number-average degree of polymerization was 564.92, as determined from the measurement of the reducing value and total carbohydrates.

To ensure that the polysaccharide produced was a real dextran, with a main chain made up of predominantly α-1,6-glucopyranosidic linkages, the dextran was hydrolyzed with *Penicillium* sp dextranase. The major products were isomaltose and isomaltotriose, which represent the dextran hydrolysis products of α-(1,6) glucans chains. There was also less than 5% secondary linkages, such as α-(1,3), α-(1,4) or α-(1,2), as hydrolysis was greater than 20 to 30%. The acceptor reaction with maltose and dextransucrase FT045 yielded over 20% panose and 19% branched oligosaccharide (DP4), which generally appear in a dextransucrase maltose acceptor reaction.

The FTIR and ^{1}H NMR spectral analysis confirmed that the polysaccharide produced from *L. mesenteroides* FT045B is a highly linear dextran with α-(1,6) linkages and 2.1% α-(1,3) branching. The commercial dextran B512F from Sigma achieved very similar results in the spectral analysis as those of the novel dextran FT045, demonstrating that the structures are identical. It is very important to discover new microorganisms with the capability of producing highly linear dextran.

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STUDY OF ACID HYDROLYSIS OF SUGARCANE BAGASSE AND FERMENTATION OF D-XYLOSE MUST BY *PACHYSOLEN TANNOPHILUS*.

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Keywords: hydrolysis, organosolv, delignification, sugarcane bagasse, fermentation.

Introduction

The utilization of Organosolv solvents under acid and high temperature conditions in the delignification and hemicelulose and cellulose hydrolysis of wood and bagasse has already been reported in the literature for several experimental parameters. Organosolv treatment of sugarcane bagasse has also been reported and has shown good results with ethanol–water mixtures, normally at volume ratios close to unit. This study describes a method of delignification and hemicellulose hydrolysis of sugarcane bagasse combining the use of ethanol+acetone/water mixtures and diluted acid solutions.

Material and Methods

Ethanol+acetone/water mixture (1:1 v/v), acid concentration of 0,5 - 2,0% of H_2SO_4, reaction times from 30 to 120 min, and 5,0% of sugarcane bagasse (w/v) was carried out for bagasse pre-treatment. The effect of acid, solvent and temperature on the yield and extension of delignification and hemicelulose hydrolysis was studied.

Results

The obtained results indicate important differences from the Organosolv process, which may be due to the presence of acetone and acid concentration employed in this work. The best results were obtained at 1,0% of H_2SO_4 and 120 °C. Under these conditions the sugar yield obtained from hemicellulose hydrolysis of sugarcane bagasse was 7.53%. These results showed that the sugar yield obtained can be used as carbon source for *Pachysolen tannophilus* fermentation to produce ethanol.

Discussion

The conversion of D-xylose to ethanol by the yeast *Pachysolen tannophilus* is relatively inefficient in batch culture. The inefficiency has been attributed in part to concurrent utilization of ethanol, slow fermentation metabolism of the yeast and to the formation of xylitol and other by-products. The xylose concentration in the must was 2,0% during 120 hours of fermentation. The best ethanol yield was obtained in the first 24 hours, as the ethanol began to be consumed and the sugar concentration became low the yield became low. The yield of ethanol obtained with 24 hours of fermentation was 0,21% and the efficiency of the process was 0,234 with 12 hours of fermentation.

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Study of Immobilization of lipase produced by Bukholdeira cepacia in calcium alginate dry at room temperature.

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Keywords: Lipase; lipolytic activity, immobilization, *Bukholdeira cepacia*.

1. Introduction

The use of lipolytic enzymes is growing due its biotechnological potential to catalyze reaction of hydrolysis and esterification. These enzymes have preference by triglicerides and some of them are capable to synthesize esters beside to have many uses in industries. Several supports were assayed to immobilize lipase from *Bukholdeira cepacia* without good results, but when immobilized in calcium alginate dried at ambient temperature its activity was maintained, being the advantage of this method the mechanical resistance of the calcium alginate particles and their indissolubility.

2. Material and Methods

The enzyme was obtained from *Bukholdeira cepacia* cultured by 24 hours in orbital shaker at 15rpm and 30ºC. To determine the lipolítica activity of the supernatant from cells culture and in the particles of dried calcium alginate was used olive oil emulsified with solution of arabic gum 7% in the ratio 25:75. In 5 ml of this emulsion was added at 2 ml of buffer 10 mM and pH 8,0 and 1 ml of the supernatant culture or 10g of alginate spheres. These mixtures were maintained in bath at 37ºC. After the incubation period the samples were titled with NaOH 0,05N and the difference of NaOH volume between the sample and the blank title was used to determine the enzyme activity. To immobilize the enzyme, 36 ml of the supernatant were mixed with 48 ml of 2% sodium alginate solution. After this, the mixture was introduced in a burette which register was open to allow the mixture dropping on 2% calcium solution to form spheres with diameter of 0.5mm. The spheres were maintained in calcium solution during 30 minutes, being washed with distilled water and spread on Petri plate to dry at ambient temperature.

3. Results and Discussion

The activity of the samples presents some variation. For one specific sample, the activity of the supernatant was 1,33 U/ml in 30 minutes of reaction and this same sample presents only 0.25 U/g when immobilized in spheres of dried calcium alginate also in 30 minutes. When these spheres were used again at the same conditions the activity reduce to 0.16 U/ml. The reduction of the activity of the supernatant after immobilized with calcium alginate was expected, since only the enzymes molecules on the spheres surface were able to react. On the other hand, the emulsified oil did not diffuse in the alginate spheres and enzyme molecules inside the spheres did not have contact with the substrate. Another supernatant was mixed with alginate at the same condition, being the activity of the wet spheres was 0.402 U/g and 0.162 U/g in dried spheres but in 18 hours of reaction.

4. Conclusion

The immobilization of lipase from *Bukholdeira cepacia* in dried calcium alginate appear to be interesting since it can be storage for long periods, therefore its stability must be better studied to avoid or minimize the activity lost and to allow its reuse by some times.

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STUDY OF THE REMOVAL OF CHEMICAL OXYGEN DEMAND OF THE CHEESE WHEY AND WASTEWATER FROM DAIRY INDUSTRY USING *Spirulina platensis*

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Keywords: *Spirulina platensis*, treatment of waste, cheese whey, dairy wastewater.

Spirulina platensis is a filamentous cyanobacterium, photosynthetic tissue that can live in soil, marshes, alkaline lakes and freshwater, brackish and marine. Microalgae cultivation is a beneficial process, not only due to the production of proteins of high biological value for human and animal nutrition, but also for obtaining other products like vitamins, pigments and lipids. The microalgae can be used for biodiesel production in the pharmaceutical industry and also in wastewater treatment because it has the ability to consume organic substances and inorganic nutrients from wastewater. The wastewater from a dairy industry refers to water for washing machines and production equipment. This carries significant quantities of dairy products. Whey is the residue from cheese-making which retains significant part of the nutrients of milk. Both the dairy effluent as cheese whey have high values of Chemical Oxygen Demand (COD) and therefore need appropriate treatment avoiding they become pollutants when reach water bodies. This study aimed to evaluate the potential of *Spirulina platensis* in COD reducing of two alternative cultivation medium: one containing wastewater from a dairy in three different dilutions (1000, 800 and 500 mg COD L^{-1}) and another containing whey, even in these three dilutions. Culture was performed in Erlenmeyer flasks of 500 mL containing 200 mL of culture medium, added 10 mL of the suspension of Spirulina. The bottles remained during the ten days of cultivation in a shaker at 100 rpm, room temperature and illuminance of 3000 lux provided by 6 fluorescent lamps Phillips, 20 watts, arranged at a distance of about 40 cm from the flasks and photoperiod of 12 h light / dark. Chemical oxygen demand (COD) were analyzed at the beginning, middle and end of treatment, in order to monitor the COD decrease. Analysis was carried out in spectrophotometer at 620 nm. The results showed that *Spirulina platensis* reduced the COD of the effluents studied. The largest percentage reductions obtained were 86% for the treatment with dairy wastewater and 83.5% for the treatment with whey. Therefore, the results were satisfactory and *Spirulina platensis* is efficient for COD removal of the dairy wastewater and the cheese whey.

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SURVEY OF YEASTS ASSOCIATED WITH THE EXOSKELETON OF THE LEAF-CUTTING ANT *ATTA SEXDENS* FROM PALMAS, TOCANTINS

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Keywords: yeasts, leaf-cutting ants, *Atta sexdens*, Tocantins.

During a survey of yeasts associated with exoskeleton of the ant species *Atta sexdens* collected from the state of Tocantins, a total of 63 yeasts including a novel yeast species (strain PBM 39^T) related to *Meira geulakonigii* were isolated. In order to isolate yeasts associated with the ants` exoskeleton, matured worker ants were allowed to walk on yeast-malt extract agar (YMA) plates (3 g yeast extract, 5 g peptone, 10 g D-glucose and 15 g agar per litre at pH 5.5) supplemented with 0.1 mg ml^{-1}) while additional sets were immersed in 10 ml YMA broth in test tubes. Using standard phenotypic (carbon and nitrogen assimilation tests) coupled with molecular taxonomic markers (microsatellites and DNA sequencing), the yeast isolates were identified and described. Distance phylogenetic analysis of concatenated sequences of the ITS region and the D1/D2 domains of the 26S rRNA carried out for strain PBM 39T were generated in MEGA v. 4 under the Kimura two-parameters substitution model. Among the ascomycetous yeasts (85.7 %), the majority were members of the genus *Candida* namely C. *zyelanoides* (1), *C. albicans*, (6), *C. metapsilosis* (19), *C. parapsilosis* (18) and *C. glaebosa* (1), 7.4 % belong to the genus *Pichia* being *P. caribbi*ca, (1), *P. burtonii* (2) and *P. guillermondii* (1) while the remaining belonged to the genus *Debaryomyces* (2) *Hanseniaspora* (1) and *Aureobasidium* (2). Among the basidiomycetous yeasts (14.3 %) the genus *Cryptococcus* was prevalent representing a total of 44 % of the basidiomycetous yeasts isolated. They include strains of *Cryptococcus flavus* (1), *C. laurentii* (2) and a *Crypococcus*. sp (1). Other basidiomycetous species isolated were distributed in the genera, *Pseudozyma* (2), *Sporobolomyces* (1), *Meira* (1) and *Rhodotorula* (1). Blast analysis of the sequence of D1/D2 domains of strain PBM 39^T showed that this putative novel species differs from *M*eira *geulakonigi* by 8 nucleotide substitutions and 3 gaps. Phylogenetic analysis assigned the novel yeast specie to Exobasidiomycetidae of the class Ustilaginomycetes and the taxonomic description is underway. This is the first record of finding *Meira* species in Brazil. The work revealed that ants may play major role in the dispersal and ecology of several yeasts.

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Survival of *Lactobacillus delbrueckii* UFV H2b20 in ice cream elaborated with replace of fat and in conditions of acid stress and bile salts

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Keywords: Probiotic; Ice-cream; Survival

Lactobacillus delbrueckii UFV H2b20 is a probiotic microorganism that in according with many studies has performance as probiotic. However, their use as probiotic food still has not yet been evaluated. The effect of the conditions of food processing, the food composition and the effect of combination of these parameters in the tolerance to of acid stress and to bile salts are important factors for survival of microorganism in the food and also survival of microorganism mainly after contact with conditions of digestive tract. The objective of this work was produce a probiotic ice cream, and evaluate the effect of the replace of fat by inuline and to observe the *L. delbrueckii* UFV H2b20 viability in the process of ice cream production and storage time 150 days, and also on the tolerance to conditions acid stress and bile salts. Three ice cream formulations were developed, varying the fat percentage and replace of fat inuline (RFI): formulation①: ice cream with 12 % of fat; formulation②: ice cream with 6 % of fat + RFI; formulation③: 0,3 % of fat + RFI. The concentrate of cells of *L. delbrueckii* UFV H2b20 cultivated in whey of stabilized milk was added to the mixtures in the moment of the production of the ice cream. With 150 days of storage of ice cream at -20 ºC each sample was exposed to the conditions of acid stress (pH 3) for 60 minutes at 37 ºC, to stress with bile salts (0,5 %, p/p) for 30 minutes at 37 ºC, and also the succession to acid stress and bile salts, in other words, the samples of ice creams that were exposed to the acid stress for 60 minutes went soon afterwards exposed to the stress with bile salts 0,5% for 30 minutes. Probiotic bacteria counts were estimated by plating on MRS agar at 37 ºC for 48 h. The experiment was driven with three repetitions. The results were submitted to the variance analysis (ANOVA) and compared by the test Tukey to 5% of probability, used the program SAEG (version 9.1., 2007). The partial and total replace of the fat of the ice cream, didn't affect significantly ($p > 0.05$) the counting of viable cells of *L. delbrueckii* UFV H2b20 during the period of storage of 150 days. The replace of fat by inuline didn't also commit significantly ($p > 0.05$) the tolerance to the acid stress, however this replace affected significantly ($p < 0.05$) the viability of *L. delbrueckii* UFV H2b20, when exposed directly to bile salts, and also during the succession of acid stress and bile salts. Like this, the ice cream can be used as delivering probiotic in human diet, however studies that simulate conditions of digestive stress *in vitro* should be accomplished to confirm these results.

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TENSIO-ACTIVE PRODUCTION BY *Rhodotorula glutinis* UCP 1551 USING INDUSTRIAL WASTE

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Keywords: *Rhodotorula glutinis*, biosurfactant, industrial residue

Biosurfactants constitute an important class of chemical compounds used in various industrial sectors. The biosurfactant are compounds of microbial origin that exhibit properties of reduction of surface tension or emulsifier capacity. Due to their ability to concentrate at the air-water interface, they are commonly used to separate oily materials from a given medium. Surfactants increase the aqueous solubility of hydrophylic molecules by reducing their surface/interfacial tension at air-water and water-oil interfaces The aim of this work was the biosurfactant production by *Rhodotorula glutinis* using industrial residues cassava wastewater, corn steep liquor and ice cream effluent as substrates. *Rhodotorula glutinis* (UCP 1555) isolated from soil in semi-arid (Serra Talhada, PE, Brazil), identified using biochemical and molecular methods. The strain was supplied from the Culture Collection of "Universidade Católica de Pernambuco" (UCP), maintained in Sabouraud dextrose agar at 5 °C. The substrates used "manipueira" (obtained from cassava effluent), corn steep liquor (residue from the manufacture of corn), and effluent from ice cream. The medium composition using the agroindustrial residues for biosurfactant production was established according to factorial design, and variable response was surface tension and emulsifier index. The pre-inoculum was prepared using Sabouraud dextrose broth. The Erlenmeyer's flasks were inoculated with 5% of the pre-inoculums, and incubated under orbital shaker at 150 rpm for 72 hours at 28 º C. After this period the liquid metabolic free cells was used to determination of surface tension, emulsification index, and pH. The best results was obtained in the assay 17, containing 20% cassava, corn steep liquor 6% and effluent from ice cream to 20% indicated the reduction of surface tension of water 72 to 24.92 mN/m, and emulsifier index showed 86.36% using burned motor oil. The results obtained suggest that the cassava wastewater, ice cream effluent, and corn steep liquor as carbon and nitrogen sources, influencing the reduction of the surface tension during the biosurfactant production. In addition, the biosurfactant produced by *R. glutinis* showed high activity and emulsifying ability to stabilize oil/water emulsions, and the results showed 95% of confidence about the increased rates of emulsification and surface tension.

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THE EFFECT OF TEMPERATURE ON *Trichoderma sp* LIPASE PURIFIED BY REVERSE MICELLAR EXTRACTION

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Keywords: Lipase, reverse micellar extraction, *Trichoderma sp*.

Introduction: Lipases are triacylglycerol hydrolases enzymes (EC 3.1.1.3) that catalyze the hydrolysis of oils and fats, releasing free fatty acids and glycerol. They may also act in reverse reactions such as esterification and transesterification. Due to its high selectivity, the versatility of their properties and their easy availability, they can be widely used in food and biotechnological industries, such as biodiesel production and processing of dairy foods. The lipase can be produced by submerged fermentation by involving microorganisms. In this present study, the fungus *Trichoderma sp* was involved. The downstream process was composed by precipitation with ammonium sulfate and reverse micelles system, a purification technique for liquid-liquid extraction, which allows separating the cellular material and simultaneously concentrating the protein of interest. It is a technique with less difficulty and reduced cost compared to traditional chromatographic techniques. The present research work is focused on studying the effect of temperature on the lipase produced by *Trichoderma sp* and purified by reverse micelles.
Materials and methods: The filamentous fungus *Trichoderma sp* was cultivated in PDA (potato dextrose agar (39 g/L)) for 7 days at 28°C. A suspension of 10^8 spores/mL was used to prepare the pre -inoculum. Fermentation of 3600 mL containing 1% (v/v) of olive oil was performed in a shaker at 28°C, 180 rpm for 96 h. The fermented broth was filtered with gauze and cotton. Later, precipitation with ammonium sulfate to 80% saturation was performed. The supernatant with the salt was kept at 4°C for 12 h and centrifuged for 20 minutes at 3500 rpm and 4 ° C. The pellet was suspended in a minimum volume of phosphate buffer 0.05 mol/L pH 7.0. This suspension was dialyzed against this same buffer and the extract was stored at 4°C. In the purification by reverse micelles, pH was adjusted to pH 4. For forward extraction, we used 5 mL of crude extract and 5 mL of 0.1 mol/L of AOT in n-heptane. The solution was kept in strong agitation for 30 minutes and then was centrifuged. For the backward extraction, equal volumes of micellar solution and phosphate buffer 0.2 mol/L pH 7 containing KCl 1.2 mol/L were mixed and incubated under the same agitation, and then centrifuged. The aqueous phase containing the enzyme was used to study the effect of temperature on lipolytic activity, accompanied by hydrolysis of p-nitrophenyl palmitate (pNPP) at 410 nm between 20 and 70 º C.
Results and discussion: The crude extract obtained showed 1.36U/mL of activity and 1.06U/mg of specific activity. After precipitation, centrifugation and dialysis of the fermented broth, the activity recovery and the purification factor were found to be 25% and 3.77, respectively. After reverse micelles purification, the activity recovery was 0.17% and purification factor was 4.84. In literature are similar results regarding to purification factors after performing precipitation and reverse micelles extraction. In the temperature assay, it was found: 1.64U/mL at 20ºC;1.35U/mL at 30ºC;0.53U/mL at 37ºC;0.32U/mL at 40ºC; and no activity above 50ºC. Higher activity at 20ºC is unusual for lipases. In previous studies with crude enzyme showed higher activity at 50°C. It can be inferred that micellar extraction could have changed the properties of this lipase. Still, the characterization of the enzyme is necessary, as the purification method used is very efficient.
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THE EFFECTS OF DIFFERENT CARBON SOURCES IN LIPASES PRODUCTION BY *Fusarium sp.*

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Keywords: Lipase, *Fusarium sp.*, carbon sources.

Introduction. Lipases are enzymes that hydrolyze ester linkages of trialcilglicerois (GAD) and also catalyze reactions of esterification and transesterification. These enzymes have great potential for application in various industrial sectors like food, biodiesel, detergent, among others. The use of microorganisms in the production of lipase has been targeted in the search for alternatives more economically attractive. To optimize the enzyme production various lipid substrates have been studied, because they act as inducers on lipase production in many species of fungi. This study aims to determine the effect of different carbon sources, which are olive oil, palm oil, soybeans, corn, chicken, crambe, linseed, and glycerol, the lipase production by *Fusarium sp.*
Material and Methods. The fungus was cultured in medium containing yeast extract 0.5% (w / v) Tween 80 surfactant 1.5% (w / v) and carbon source 1% (w / v; olive oil, palm oil, soybean , corn, chicken, linseed, crambe and glycerol) at 180 rpm and 28 ° C. The lipase activity was obtained after 24 h of culture by hydrolysis of p-nitrophenyl palm (pNPP) at 37 ° C and 410 nm. The protein determination was performed by Bradford method and biomass gravimetrically.
Results and Discussion. Olive oil has been commonly used as an inducer for lipase production due to its high content of triolein, standard substrate for these enzymes. As expected, there was lipase production by *Fusarium sp.* with the addition of olive oil (1.38 U / mL). Corn oil has also been reported as an inducer of lipase in *Fusarium*, as it was verified in this work to obtain 0.45 U / mL. However, due to the high cost of these oils, and its importance as food, other lipid sources were tested, at low cost or for not being a food source such as oil palm, linseed and crambe. Palm oil is produced from the fruits of oil palm (Elaeis guineensis) and, although it is also used for food, it has been used in the production of soaps and biodegradable detergents. Linseed oil is extracted from the seed of Linum usitatissimum and is used in cosmetics and inks. The crambe (Crambe abyssinica) provides 38% of oil in its seed, besides being a legume crop with low cost, high yield and early harvest. With the addition of palm oil, crambe and linseed to the culture medium was obtained activity of 1.71, 1.42 and 1.27 U / mL, respectively. Glycerol is a byproduct of biodiesel production, besides being a low-cost and excess demand. Enzymatic activity was detected (0.23 U/ML) with the addition of glycerol, although smaller than with vegetable oils. The chicken oil is a residue from the food industry and is rich in saturated fat and short chain, as opposed to plant sources. The addition of chicken oil provided the obtaining of 0.81 U / mL.
Conclusion. The analyzed data it is concluded that the lipase production by Fusarium sp is favored in culture media containing as carbon sources, palm oil, crambe and linseed, which are lower cost alternatives for enzyme production application in various industrial sectors.

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THE HYGIENIC AND SANITARY EVALUATION OF IRRIGATION WATER AND LETTUCE (*Lactuca sativa*) PRODUCED BY THE ORGANIC FARMING SYSTEM IN ARARAQUARA , SP

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Keywords: organic agriculture, thermotolerant coliforms, lettuce, irrigation water

The search for a healthier feeding has called the consumers attention to organic food. However, organic foods are more susceptible to microbiological contamination during the production process due to use of manure. The consumption of raw lettuce is common for the preparation of salads, sandwiches and other dishes decoration and these foods may potentially transport pathogenic microorganisms, mainly those of fecal origin. The main factors that determine the potherb contamination are the ecological conditions, the agriculture practices, the packing conditions, the transportation and commercialization. The present study aims to evaluate the microbial contamination of the irrigation water and the lettuce (*Lactuca sativa*) samples in the successive production stages of truck farm in Araraquara city, Sao Paulo state. Lettuce samples of the farm truck, after washing and commercial stages were collected during the months of February and March 2011. Samples of the water used to irrigate the farm truck and to wash the lettuce were also collected in different stages: the main and the intermediate tank, irrigation and the washing tank. The microbial groups analyzed in the lettuce were mesophilic aerobic bacteria, mould and yeast, thermotoletant and total coliforms and *Salmonella*. In the water samples thermotoletant and total coliforms were analyzed. The results were evaluated according to the limits set by the Brazilian legislation - RDC number 12 from January 2, 2001 for potherbs and Ordinance number 518 of 2004 of the Brazilian Ministry of Health, for the water. The results showed there was no *Salmonella* in the lettuce samples which were in accordance to the established patterns. Whereas the Legislation does not include a determination of mesophilic aerobic bacteria, mould, total and thermotolerant coliforms in lettuce, it has been verified that a hundred percent of the samples had amounts of those microbe groups. The higher levels of the microbes were verified in the truck farm and the commercial lettuce with levels above 10^6 UFC/g of mesophilic bacteria and mould, an average of 10^4 NMP/g in the truck farm and 10^3NMP/g in the commercial lettuce, for the last two microbial groups. The water samples also presented irregularities in all the stages analyzed through the production chain in which were verified high levels of total and thermotolerant coliforms. The results lead to the conclusion that either the lettuce and the water used to irrigate them presented unsatisfying hygienic and sanitary conditions because they may transmit enteropathogens to the consumers. Therefore it is necessary to intensify the quality control along the production chain.

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TOXICITY OF SANITARY SEWAGE CONTAINING FORMALDEHYDE TO ALGA *Pseudokirchneriella subcapitata* – METHODOLOGY ADAPTATION

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Keywords: ecotoxicity, microalgae, formaldehyde

INTRODUCTION: The unicellular alga *Pseudokirchneriella subcapitata (Chlorophyta: Chlorophycea)* has been widely used in ecotoxicity studies on water and wastewater due to its ease laboratory culture, rapid population growth and high sensitivity. Moreover, because algae are the producer base of aquatic ecosystems, changes in their community dynamics can affect the higher trophic levels.
MATERIALS AND METHODS: The test-organism was used to evaluate the toxicity of sanitary sewage containing formaldehyde (100 mg/L), through a treatment system combined of anaerobic followed by aerobic reactor, both upflow and fixed bed, and a secondary settler. The aim of the study was to deploy a method for algae bioassays and evaluate the chronic toxicity level of samples at 3 points of the biological treatment: P1 (raw sewage), P2 (anaerobic output) and P3 (settler output). 5 assays were performed, with adjustments according to NBR 12648 standards (ABNT, 2005) and the Concentration Inhibition (%) of algal biomass growth was observed after 96 h. The 1st assay employed automatic stirring and side lighting in closed chamber; the 2nd assay employed automatic and open stirring; 3rd and 4th assays employed semi-automatic and open stirring and 5th assay used manual stirring.
RESULTS AND DISCUSSION: In the 1st assay temperature reached up to 35ºC due to side lighting adapted to the closed shaker; in the 2nd assay P2 and P3 samples disclosed algae predators protozoa; in the last three assays there were algae film in the glass test-bottles. Despite the recorded incidents during deployment, it was possible to estimate the samples average toxicity due to employing replicas and several dilutions. For P1 samples, the more concentrated the test-solution, the greater the inhibition displayed. P2 samples displayed equivalence in algal growth to control in test-solutions ranging from 15 to 20% and increasing inhibition above 20% sample concentration, whilst in P3 growth inhibition occurred up to a 13% sample concentration, from 13 to 20% algal growth was higher than in control and above 20% there was increasing average growth inhibition.
CONCLUSION: For an accurate assessment of the toxicity level in algae bioassays, some precautions are suggested, otherwise they may interfere with data interpretation, such as: not using manual stirring of the vials, preventing algae adherence during the breaks, making strict temperature control (23 to 27 ºC) and lighting with proper bulbs distribution on the shaker, evaluating turbidity and presence of primary consumers in the sample microfauna. Further studies are recommended with higher formaldehyde concentrations and the appropriate referred cares. Thus, algae bioassays may be promising in order to confirm rapid and low cost detection of toxicity in wastewater.
REFERENCE: ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS. Ecotoxicologia aquática – Toxicidade crônica – Método de ensaio com algas (*Chlorophyceae*), 2005.

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TOXICITY OF TEXTILE AZO DYE CHRYSOIDINE BEFORE AND AFTER TREATMENT WITH *Saccharomyces cerevisiae* .

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Keywords: azo dye, toxicity, biodegradation, *Saccharomyces cerevisiae, Allium cepa*.

Today the production and disposal of toxic substances by industries is one of the most important subjects in pollution control, which has led researchers to look for tools to reduce or eliminate effluent toxicity. Among these toxic substances have synthetic dyes, which are used in large scale, mainly in the textile industry. It is estimated that approximately 15% of world production of dyes is lost to the environment during its synthesis, processing or application. Understanding this problem, the objective of this study was to evaluate the toxicity of the azo dye textile Chrysoidine before and after treatment with the yeast *Saccharomyces cerevisiae*. For the toxicity study were used bulbs of *Allium cepa*, as test organisms, and the biodegradation experiment was conducted with the yeast *Saccharomyces cerevisiae*. The toxicity test was conducted at a temperature of 21 ± 1 ° C, in quintuplicate for each concentration tested (5, 10, 15, 20, 25, 30, 35, 40 and 45 µg / mL), and exposure period 72 hours. At the end of the exposure period measures were taken from the roots of each bulb with the help of graph paper, and the average growth was calculated. From the data set was reached at concentrations inhibiting 50 and 100% of the growth of roots, which were equal to 6.17 and 76.15 µg / mL. The statistical analysis were performed in the statistical program Origin. The biodegradation test was performed using a dye solution with initial concentration of 7.0 µg / mL (approximate IC_{50} concentration), conducted at a temperature of 30 ± 1 ° C for 2 hours. The molecule proved to be quite unstable and easily degraded, so the exposure time was short. Every 30 minutes were performed spectrophotometric analysis of the solution. With the wavelengths in nanometers used for the absorbance ratio (Abs_{450}/Abs_{260}), it was possible to determine the rate of degradation of the dye. At the end of the exposure period the remaining concentration of dye was 6.6 µg/mL, and significant changes in the spectral scans of the sample. The absorbance ratio ranged from 3.55 (control solution) to 1.52 (sample tested), indicating the occurrence of degradation of dye molecules in the presence of yeast explaining the variation in the spectra of the solution. At the end of the exposure period were conducted toxicity tests with new bulbs of *Allium cepa* and the supernatant from the treated solution showed 82.18% inhibition of root growth. Indicating that there was an increase of approximately 31% degree of toxicity of the sample. These results led us to conclude that there was the formation of highly toxic byproducts after breakdown of the dye molecules. So from the toxicological point of view is not interesting incompletely degrade the molecules of this dye during the treatment process because this process can lead to increased toxicity.

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TOXICOLOGICAL TESTS WITH *Cucumis sativus* AND *Brassica oleracea* SEEDS IN SOIL CONTAMINATED WITH HYDROCARBON

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Keywords: plant, bioassays, soil

Abstract

During germination period occurs numerous physiological processes in seeds. The presence of a toxic substance can interfere in plant development and survival due to the high sensitivity of this period. Bioassays with seeds are based on the plant sensitivity in growth stages to analyze the contaminant toxicity. The study aimed to evaluate the toxicity of used lubricant oil in soil during biodegradation. Thus, seeds of *Cucumis sativus* (cucumber) and *Brassica oleracea* (kale) were used as test-organisms. Two assays were prepared for each specie: control without the oil and soil contaminated with the lubricant. Soil samples were buried into plastic bags with several holes to allow the microbial flow from the external environment to sample. One of the plastic bags was removed from soil each month for toxicity tests. Hence, the used lubricant oil toxicity was determinate according to biodegradation time by its effect on hypocotyl and root development. Each soil sample was divided into plastic cups seeded with 30 seeds of *Cucumis sativus* and *Brassica oleracea*. Later, they were incubated for 120 hours at 22 °C. Results showed different germination rates in the control test: cucumber (95%) and kale (78.52%). According to literature, only cucumber seeds are suitable for the methodology because their germination rate was above 90%. In relation to root and hypocotyls development, a toxic effect was demonstrated in the first months of contamination. Also, a gradual toxicity reduction was observed according to biodegradation time in soil. The used lubricant oil composition presented heavy metals and polycyclic aromatic hydrocarbons (PAHs) that are toxic to many organisms, for plants its presence caused less root and hypocotyl development as found in bioassays. After four months of biodegradation, the contaminated sample has enabled a greater root growth in *C. sativus* compared to the control test. Therefore, it was concluded that microbial metabolism in soil decreased the used lubricating oil toxicity in accordance with biodegradation time and *C. sativus* were able to be used as test-organism for germination test in soil.

Apoio: Capes, Fapesp e CNPq

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Use of ARDRA markers for analysis of genetic polymorphisms in *Candida* species isolated from HIV-positive patients

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Keywords:
ARDRA, *Candida*, HIV positive patients

Introduction

In recent decades, fungal infections are important causes of nosocomial infections, especially in immunocompromised patients and are now a serious social-medical problem. The species of the genus *Candida* have been the agents most frequently isolated, accounting for about 80% of fungal infections affecting patients with HIV. Despite the alarming numbers of current and growing fungal infections and the difficulty of treatment by synthetic antifungal, most clinical laboratories do not present an accurate diagnosis of routine and simple identification of *Candida* species and are using presumptive methods. Another limiting factor of these techniques is the reproducibility of results. The use of rDNA for identification of microbial species is highly sensitive due to the fact of the gene encoding rDNA is present in multiple copies in the genome of *Candida* spp. when compared to other PCR assays that utilize genes present as single copy.

Materials and Methods

Isolates of *Candida* species from patients with AIDS (Approval CEP num. 23115007226/2008-05) were subcultured in RPMI medium for 24 hours, centrifuged and resuspended in lysis buffer containing SDS. The samples were macerated and incubated at 65 ° C for 10 minutes and then extracted once with chloroform-isoamyl alcohol. The supernatant was precipitated with cold absolute ethanol and the pellet of DNA resuspended in pure water. An aliquot of DNA was used in PCR amplification reactions. The sequences of oligonucleotides used as primers in amplification reactions yearn for the initial portion of the 5S rDNA gene (primer "forward ") and the initial portion of the 28S rDNA gene (primer reverse). PCR products for each *Candida* species were digested with restriction enzyme *Dde* I and visualized in agarose gels.

Results and Dicussion

We have adapted a method for extraction of genomic DNA of yeast which provide fast and stable DNA. This study investigated the genetic polymorphism by ARDRA technique using only one restriction enzyme.

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USE OF THE BRAZILIAN TROPICAL BASIDIOMYCETE DECOLORIZATION OF SYNTHETIC DYES.

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Keywords: Remazol Brilhante Blue R, Yellow FN-2R Cibacron, Red FN-2BL Cibacron, textiles effluents, *Peniophora cinerea* (CCB 204), *Trametes villosa* (CCB 291)

The textile effluents are characterized by being highly colored due to the presence of dyes that are not fixed in the fiber during the dyeing process. Their very nature causes it easy to detect, being visible even at low concentrations. The treatment of these effluents is difficult and inefficient with conventional methods because the dyes have complex structures, synthetic, and are stable to light and temperature. The biological degradation using basidiomycetes is being widely used in the decolorization of dyes, because it is costly and, moreover, these fungi have the ability not only to discolor, but also to degrade and mineralize different textile dyes. In this study, we investigated the feasibility of using two cultures of basidiomycetes isolated from tropical ecosystems - *Peniophora cinerea* (CCB 204) and *Trametes villosa* (CCB 291) - the removal of textile dyes in aqueous synthetic test in discoloration. Discs of mycelium growth of both cultures were inoculated in 250 ml bottles containing 50 ml of modified liquid medium containing (g / L): ammonium tartrate 0.45 g, 0.049 g CuSO4, 0.05 g MgSO4, 0.016 g MnSO4 KH2PO4 0.2 g. Incubation was done in a stationary room temperature for 7 days. Two concentrations of sucrose (0.3%, 0.5%, 0.7%, 1.0%) as carbon source and glucose (0.5%) as a control, in triplicate. The percentage of decolorization of artificial textile effluent (3% NaCl, 0.2% dye Remazol Brilliant Blue-RBBR-Sigma, Yellow FN-2R or Red FN-2BL Cibacron) was evaluated in vivo and in vitro for both cultures, without adjustment pH by determining the change in absorbance (592, 432, 526 nm, respectively), 24 hours after addition of 5 mL of each effluent to fungal growth. It was concluded that higher percentages of decoloriZation of the dyes were obtained by treatment in vivo and in vitro cultures using both CCB 204 and CCB 219, grown in basal medium at 0.5% and 0.7% sucrose. However, comparing both, could be seen that the lower percentage of discoloration was submitted to the red dye, suggesting that another mechanism was involved in the enzymatic bleaching both in vivo and in vitro.

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UTILIZATION OF COMMERCIAL GASOLINE BY NITROGEN-FIXING LEGUMINOSAE NODULATING BACTERIA OF GENUS *CUPRIAVIDUS*

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Key Words: Bioremediation; Soil microorganisms; aromatic compounds

Introduction: Aromatic hydrocarbons are present in oil derivatives such as BTEX (benzene, toluene, ethylbenzene and xylene). These compounds are found in commercial gasoline and have a great potential to contaminate soil and waters. Several bacteria species, which are present in soil, are able to degrade pollutant compounds from these residues (Atlas, 1995). These bacteria include strains belonging to *Cupriavidus* genus which also includes nitrogen fixing Leguminosae nodulating bacteria (NFLNB). This work aimed to evaluate the ability to use gasoline as sole carbon source by two strains NFLNB belonging to *Cupriavidus* spp.

Material and Methods: The strains evaluated were *Cupriavidus taiwanensis*, LMG19424^T (Chen et al., 2001) and *C. necator*, UFLA02-129 (Silva, 2009). Strains were cultivated in 10 mL of mineral liquid media BH (Bushnell-Haas, 1941) containing the following treatments as sole carbon source: mannitol (10 g.L-1)(control) and commercial unleaded gasoline in concentrations of 1 and 3%. Glass bottles were sealed with rubber lids and aluminum rings and incubated at 28^{o}C and under agitation. Colony forming unities (CFU) were counted by Miles & Misra (1938) method.

Results and Discussion: Both *Cupriavidus* spp. strains were able to growth in the two gasoline concentrations tested. However, after 5 days growth with 3% gasoline, strain LMG19424^T has shown greater bioremediation ability in relation to UFLA02-129. However, after seven days, there were no significant differences in densities (UFC.mL-1) among treatments and strains studied. This corroborates other findings showing that *C. necator* strains have the ability to degrade several aromatic compounds (Clement et al., 1995), suggesting the need of essays involving kinetics of biodegradation of these compounds for these strains.

Conclusions: NFLNB strains of *Cupriavidus* present great biotechnological potential to be used in bioremediation processes in gasoline contaminated soil.

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UTILIZATION OF COMMERCIAL GASOLINE BY SOIL BACTERIA

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Key Words: Bioremediation; Soil microorganisms; aromatic compounds

Introduction: Soil has a high diversity of organisms that play important roles for the maintenance of this ecosystem, including the degradation of xenobiotics by bacteria (Moreira & Siqueira, 2006). These organisms can be used in bioremediation processes for recovering polluted areas with petroleum and oil derivatives. The aim of this work was to evaluate the capacity of two bacteria strains, isolated from diverse soil samples, to use gasoline as sole carbon source.

Material and Methods: Two bacteria strains: EA4 e PF4I2BK, belonging to the collection of the Laboratory of Soil Microbiology-Federal University of Lavras - MG, Brazil, were evaluated. The first strain was isolated from nodules of *Enterolobium contortisiliquum* (Assis, 2009) and the second strain, was isolated from soil sample collected from an area of native Cerrado (savanna) in Minas Gerais. Strains were cultivated in 10 mL of mineral liquid media BH (Bushnell-Haas, 1941) containing the following treatments as sole carbon source: mannitol (10 g.L-1) (control) and commercial unleaded gasoline in concentrations of 1 and 3%. Glass bottles were sealed with rubber lids and aluminum rings and incubated at 28^{o}C under agitation. Colony forming unities (CFU) were counted by the Miles & Misra (1938) method.

Results and discussion: Strain EA4he grew utilizing gasoline at concentrations of 1 and 3%, however less than the control with mannitol. Strain PF4I2BK showed a high capacity to use gasoline as a sole carbon source, since its growth in these treatments was similar to the control containing mannitol. The concentrations tolerated by this strain are similar to those tolerated by bacteria belonging to genera *Bacillus* and *Pseudomonas* (Das & Mukherjee, 2007).

Conclusion: The strain isolated from soil of the native vegetation had a higher capacity of gasoline bioremediation compared to the strain isolated from nodules of *E. contortisiliquum*.

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Water reuse potential of filter backwash from a Water Treatment Plant in Cuiabá, Mato Grosso, Brazil.

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Keywords: Quality of water, filters, reuse.

In Water Treatment Plants, the formation of waste coming mainly from the washing of filters and discharges from the decanters. Long ago, the fate of this waste has been a water course near the station, and for technical reasons, environmental and legal, should be properly treated so that it can be arranged properly at your final destination. In this study we carried out a microbiological, physical and chemical water from washing the filters, checking the quality of the effluent in accordance with the Standards for effluent discharge legislation recommended in CONAMA 357/2005 and the possibility of reuse in the system. The study was conducted at Station Water Treatment Plant (WTP) San Sebastian, located in the city of Cuiabá - Mato Grosso, and nine samples taken from November 2009 to November 2010. All samples were analyzed in triplicate following the methods recommended by Standard Methods for the Examination of Water and Wastewater. Microbiological tests were performed and *Escherichia coli* were determined by the chromogenic / fluorogenic and overall count of heterotrophic bacteria by Pour Plate technique. The physical and chemical analysis were apparent color, turbidity and pH. The results of total coliform bacteria ranged from 2.36 to 4.38 E +00 E +00 (Log MPN/100ml). The results of *E. coli* ranged from 3.01 E-01 to 3.98 E +00 (Log MPN/100ml), these values are within the limit set by CONAMA Resolution 357, which establishes the limit of 1,000 coliforms per 100 milliliters 80% of samples. The values of the density of heterotrophic bacteria ranged from 2.46 to 5.01 E +00 E +00 (log CFU / mL). High densities of heterotrophic bacteria can be explained by agglomeration of suspended particles, originated from the raw water and form biofilms that end up stranded on the side of the filters during filtration, leading to the growth of bacteria. The results in the physical and chemical variables, the pH values found were within the limit set in legislation for release of water bodies to class II, ranging from 5.14 to 7.83. The turbidity values found show that 45% of the analysis results presented above the allowed maximum of 100 NTU and the apparent color values ranging from 10 to 866.67. The color values presented can not be compared with the permissible limit of 75 uH CONAMA 357, because this value considers the true color and not apparent. Analyses of filter backwash water show that the effluent does not have characteristics of pollution and bacterial contamination that could jeopardize the water body receiver. But for the reuse of effluent in water treatment process at Station Water Treatment Plant, it is necessary to the proper treatment for removal of turbidity and conducting research of *Giardia spp*. and *Cryptosporidium sp*. to meet the Ordinance nº 518 from Ministry of Health.

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WINOGRADSKY COLUMNS AND THE ECOTOXICOLOGICAL IMPACT OF TEXTILE EFFLUENTS

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Keywords: ecotoxicity, textile dye, photoelectrolytic process

Introduction

Pollution can be defined as all the environmental changes caused by human action which cause damages to the ecosystem and to the society. Textile industry is responsible for large amounts of wastewater which is unfit for consumption and survival of species found in rivers, causing harm to photosynthesis processes due to water turbidity. Once reactive dyes are hardly degraded by biological processes physical-chemical processes are more appropriate in this situation, as the photoelectrolytic process. After treated it is necessary to measure the impact of this effluent in the environment. One way is through Winogradsky columns. The Winogradsky column metodology is knowingly used in ecotoxicological studies of polluted environments.

Materials and Methods

The experiments were performed in a photoelectrolytic system containing: electrolytic reactor, stainless-steel chamber containing an ultraviolet lamp, flowmeter, PVC tank, valves, hydraulic pump and tubing. Effluent containing the textile dye (remazol red brilliant) was put on reservoir, from where it was pumped, passing through electrolytic reactor and UVC lamp, in a cyclic system and in clockwise direction for up to 30 minutes.
The Winogradsky columns were made using soil enriched with nutrients, water and the treated effluent. After four weeks, when the photosynthetic population was stabilized, it was started the identification of algae. Observation was done using a microscope (400x), so identification was done by comparison and taxonomic key.

Results and discussion

Photoelectrolytic treatment proved itself extremely successful in color removal from solution. Chromophore groups' destruction can be shown by a concentration decrease from 200 to 0 mg L^{-1}, analyzed with spectrophotometer. This can be explained by the fact that chloride is oxidized to chlorine/hypochlorite, which is responsible to increased efficiency of electrolytic process. Results of ecotoxicological tests using Winogradsky columns indicated that dye solution without any treatment affected the algae population less than the solution treated for 30 minutes. Also, in column containing solution treated for five minutes the population was closer to the control column (with no treated effluent), even though presenting a 97% color removal. This meant that 5 minutes treatment offers lower toxicity if compared with the other samples, and removes color satisfactorily.

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XYLANASE ACTIVITY FROM FUNGI ISOLATED OF ALKALINE AND SALTY PONDS IN PANTANAL WETLAND

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Keywords: Xylanase, enzyme production, alkaline and salty ponds, filamentous fungi, Pantanal Wetland.

Endo-β-1,4-xylanases (β-1,4-xylan xylanohydrolase, EC 3.2.1.8) are the main enzyme responsible for the degradation of xylan, the second main constituent of plant cell walls. They attack the xylan main chain liberating oligosaccharides. Xylanases have applications in conversion of lignocellulosic materials to chemicals and fuels, animal feed digestion, food and textile industries, and as bleaching agents in the pulp and paper processing. The aim of this study was to evaluate the xylanases production by eleven fungal strains, denominated: 30x02, 30x03, 30x04, 30x05, 30x06, 30x07, 30x10, 40x01, 40x02, 40x03 and 40x10, newly isolated from soil near alkaline and salty ponds in Pantanal Wetland. The activity of these enzymes in different pH was also investigated. The Cultures were carried out in Vogel's liquid medium with oat spelts xylan pH 10.0 at 28 °C. After seven days, the cultures were filtrate and the supernatant was used as enzyme source. The xylanase activity was assayed in a buffered medium reaction with birchwood xylan, at 50 °C, in a pH range from 6.0 to 10.0 with different buffers. Reducing sugars were quantified with DNS acid reagent. The xylanase production for the strains 30x02, 30x03, 30x04, 30x05, 30x06 e 40x03 was lower than 10 U/mL in all pH range. The other five strains (30x07, 30x10, 40x01, 40x02 and 40x10) presented higher activity, around 50 U/mL in Tris-HCl buffer pH 7.0. Therefore the activity presented a tendency to increase in pH around neutrality. New assays will be performed to verify the activity of the xylanases produced by these fungal strains in pH lower than 6.0, in order to determine the optimum pH for each crude enzyme.

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XYLANASE PRODUCTION BY A FUNGAL STRAIN ISOLATED FROM SOIL UNDER CAATINGA

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Keywords: Xylanolytic enzymes, enzyme production.

Xylanases (EC 3.2.18) act in the degradation of hemicelluloses, a plant cell wall component present in most of the raw materials of agro-industry, reducing polymers to xylooligosaccharides. The aim of this study was to evaluate the influence of different carbon sources and period of cultivation on the production of xylanases by a filamentous fungus isolated from Brazilian soil under caatinga, as well as on the growth of this strain. The fungus was grown in Vogel's liquid medium supplemented with different carbon sources at concentration of 1% in pH 5.0 and at 28 °C. Cultures with seven days of age were filtered, and the filtrate was used as enzyme source. The influence of cultivation period on enzyme production was evaluated daily in stationary cultures during 12 days. Fungal growth was indirectly assessed from the quantification of intracellular proteins. The xylanase activity was determined at 50 °C using the substrate birchwood xylan at 1.0% (w/v) in sodium acetate buffer 100 mM, pH 5.0. Reducing sugars were quantified with dinitrosalicylic acid. Among the carbon sources Avicel, cellobiose, oat xylan, xylose, wheat bran, sugar cane bagasse and brewer's spent grain presented higher enzyme production with values of up to 1.28 U/mL. The enzyme activity was not detected in medium containing glucose, carboxymethylcellulose (CMC) and oat bran. Maximal fungal growth was observed in medium containing the monosaccharides xylose and glucose (4.09 and 3.29 mg protein, respectively). Cultivation with brewer's spent grain occurred later in evaluating the best time for cultivation of this strain. Maximal fungal growth was observed on the 5th day of culture, with an average of 0.84 mg protein, whereas the highest xylanase activity was observed at the 7th day of culture with a maximum of 0.48 U/mL. New assays will be performed to verify the influence of other physical and chemical factors on xylanase production by this fungus and thereby optimize the process.

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α-L-ARABINOFURANOSIDASE PRODUCTION BY *Penicillium janczewskii*

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Keywords: α-L-arabinofuranosidase, enzyme production, brewer's spent grain, orange waste, *Penicillium janczewskii*.

The natural resources scarcity has increase environmental concern, inducing the search for new technologies that are more efficient, more competitive and less pollutant. Many industrial processes have been applied enzymes as an alternative to the conventional techniques. α-L-arabinofuranosidases are enzyme that assist vegetal material degradation by removing L-arabinofuranosil residues from the hemicellulosic polymers in the plant cell wall. This enzyme, individually or combined with others, represents a promising biotechnological tool when applied in industrial processes such as: biobleaching, oligosaccharides synthesis, second generation ethanol production and also in bread, juice or wine production. Enzyme production by microorganisms is influenced by many factors. The culture conditions, especially carbon source, temperature and pH are the most important ones. The use of industrial residues as components of culture medium for microorganisms is really interesting, due to the fact that they are available in large amounts and at low cost. *Penicillium janczewskii* was previously screened as a good producer of extracellular xylanolytic enzymes and some aspects of its xylanolytic system had already been studied. Brewer's spent grain (BSG) and orange waste (OW) were previously selected as the best substrates for an efficient synthesis and release of α-L-arabinofuranosidase by this fungal strain. In this work *P. janczewskii* was cultivated in Vogel's liquid medium with a 1:1 (w/w) mixture of BSG and OW for seven days. In the first step the pH of the medium was adjusted to 3.0, 4.0, 5.0, 6.0 and 8.0, and the cultures were carried out at 28 °C. In a second step, cultures were prepared in pH 5.0 and cultivation was performed at 20, 25, 30 and 35°C. The arabinfuranosidase activity was detected using ρ-nitrophenyl-α-L-arabinofuranoside as a substrate and proteins were determined by the Lowry method. The enzymatic activity was similar in the pH 4.0, 5.0 and 6.0, but the highest value was observed in pH 5.0 (0.18 U/mL and 0.13 U/mg prot); in pH 7.0 and 8.0 almost no activity was found. Similar activities were observed at 25 and 30°C, but the best production was achieved at 25°C (0.10 U/mL and 0.08 U/mg prot). At 35°C no enzymatic activity was detected. The period of cultivation should also be evaluated in order to obtain higher levels of enzymatic production, so the enzyme, obtained under optimized conditions can be, in the future, applied in biotechnological processes.

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