Editorial

Pela segunda vez a Revista *Holos Environment* disponibiliza gentilmente um suplemento dedicado às atividades do IV Simpósio de Microbiologia Aplicada – Prof. Dr. Choiti Kiyan.

Este simpósio optou por homenagear o Prof. Dr. Choiti Kiyan por considerar suas qualidades de cidadão, professor e pesquisador. Como descendente de japoneses, guarda cuidadosa memória das tradições e transmissão dos valores orientais. Foi um valioso líder estudantil, tendo lutado pela ética e democracia do país, desenvolvendo um profícuo trabalho na área política. Como pesquisador iniciou suas atividades sob a orientação do Prof. Dr. Alcides Serzedello com estudos sobre os microrganismos associados às formigas cortadeiras. Posteriormente, suas pesquisas direcionaram-se aos estudos sobre vinhaças (resíduos da destilação do etanol de cana de açúcar, madeira e mandioca), também na área de despoluição de substâncias químicas.

Recebeu em conjunto com outros autores pesquisadores, o prêmio FIPEC-Banco do Brasil sobre Despoluição Ambiental, em 1983.

O prof. Kiyan durante sua vida acadêmica participou de inúmeras atividades que beneficiaram o *Campus* da UNESP, dos quais destacamos: criação do curso de Pós Graduação do IB na área de Ciências Biológicas, tendo orientando nas áreas de Biologia Vegetal e Microbiologia Aplicada.

O IV Simpósio de Microbiologia Aplicada – Prof. Dr. Choiti Kiyan pode confirmar como os estudos sobre os microrganismos estão se direcionando também para as mais diversas interfaces da ciência. Os trabalhos apresentados na forma de resumos abrangendo o meio ambiente, fermentação, biologia molecular, bioquímica, genética, química, bioquímica e engenharia, permitiram demonstrar nesta conjugação quanto é abrangente o estudo dos microrganismos e seus relacionamentos.

O IV Simpósio teve a grata satisfação de acolher renomados conferencistas, como a Presidente da Sociedade Brasileira de Microbiologia Profª. Dra. Marina B. Martinez, a participação da Universidade de la Rioja (Espanha) - Prof.ª Dra. Fernanda Ruiz-Larrea, também com palestrantes das Universidades de Mato Grosso - Prof.ª Dra. Edna Lopes Hardoim (UFMT); Pernambuco - Prof.ª Dra. Galba Maria de Campos Takaki (Unicap); e de São Paulo: Prof. Dr. Márcio R. Lambais e Prof. Dr. Luiz Carlos Basso (ESALQ/USP); Prof.ª Dra. Cristina Paiva de Souza e Profa. Dra. Sandra Cecatto-

Antonini (UFSCar) ; Dra. Aurora M. G. F. Souza (CETESB); Prof. Dr. Roberto Berlinck (USP São Carlos); Prof. Dr. Humberto Milagre (UNESP - Rio Claro) e Dra. Marcia de Souza Carvalho Melhem (Instituto Adolfo Lutz).

Além disso, contamos com a participação de inscritos de todo país, em especial Pernambuco, Mato Grosso, Minas Gerais, Tocantins, Rio de Janeiro e Paraná.

Dentre os inúmeros palestrantes de reconhecida atuação na área de microbiologia foram ministradas palestras pelos alunos da Pós Graduação, cujas pesquisas estavam sendo concluídas.

Os resumos dos trabalhos apresentados na forma de painéis do IV Simpósio de Microbiologia Aplicada foram avaliados por uma comissão Técnica - Científica constituída por pesquisadores que premiaram o painel de maior destaque.

Os resumos dos trabalhos apresentados foram escritos em português/inglês e reunidos em CD pela comissão organizadora e entregues aos participantes.

Os resumos que se encontram na versão em inglês estão sendo publicadas pela Revista Holos Environment e objetiva conferir maior divulgação das pesquisas brasileiras no ramo da Microbiologia Aplicada. Os resumos em português encontram-se no site www.rc.unesp.br/ib/simposiomicro.

Profa. Dra. Dejanira de Franceschi de Angelis

Coordenadora Geral do Evento

A PROTEIN EXPRESSION SYSTEM FOR TANDEM AFFINITY PURIFICATION IN *XANTHOMONAS AXONOPODIS* PV. CITRI

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Keywords: Citrus canker, expression vectors, TAP-tag

Citrus canker, caused by the bacterium *Xanthomonas axonopodis* pv. citri (Xac), is a severe disease that affects all the varieties and species of citrus, and control is based solely on the elimination of infected plants in the field. Attempting to boost the understanding of the pathogenicity of Xac its genome was completely sequenced. Several biochemical traits were identified based on gene homology searches, but approximately 37% of the annotated ORFs did not display similarities with anything known (hypothetical). In order to start a systematic characterisation of novel factors codified by Xac we constructed two integrative vectors for protein expression in this bacterium. The expression vectors contain a xylose promoter for fine-tuned induction in Xac, an optimized RBS sequence based on consensus described for *B. subtilis* and *E. coli*, TAP-tag coding sequences for the expression of C- or N-terminal TAP-tagged protein fusions, and a fragment of the alpha-amylase gene of Xac for vector integration into its chromosome. Here we show that the expression vectors can be stably integrated into the *amy* locus of the bacterium, where Xac *amy*::pHF5Ca mutants did not exhibit any alteration in their pathogenicity phenotypes when inoculated in the host plant sweet orange. Furthermore, we show that the polypeptide TAP can be successfully expressed in Xac by detecting the production of functional Protein A, a constituent of the TAP-tag, in Western blotting assays. Finally, we overproduced the TAP-tag in Xac and purified it from the soluble phase of a Xac *amy*::pHF5Ca mutant cell extract. Our results corroborate the use of our TAP expression vectors in the production of TAP-tagged proteins in Xac, and constitute a novel tool for the characterisation of protein and protein complexes generated *in vivo* in this bacterium.

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ACTIVITY OF A *RICINUS COMMUNIS* L. (CASTOR OIL) DERIVATIVE AS BIOCIDAL AGENT AND VISCOSITY REDUCER ON MICROBIOTA OF SUGAR END ALCOHOL INDUSTRIES

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Keywords: Poliquilgerm®, *Leuconostoc mesenteroides*, castor oil, viscosity.

INTRODUCTION: Ethanolic fermentation is run in non-aseptic conditions, and bacterial contamination is frequent, which compete with *Saccharomyces cerevisiae* yeast for the available sugar, carrying out secondary fermentations that result in the production of other by-products than the alcohol. Many techniques have been used to control the bacterial contamination, such as addition of sulfuric acid to decrease pH, and biocides that support yeast. Due to its microbiostatic features (Messetti et al., 2005; Bertoletti, 2008), Poliquilgerm® was evaluated in relation to its capability to control contaminants in sugar and alcohol industries, among them *Leuconostoc mesenteroides,* which synthesizes dextran.

MATERIAL AND METHODS: A mixed culture of *S. cerevisiae* + *L. mesenteroides* was inoculated on CSN culture media in pH 6.0, which was maintained at 33ºC for 24 hours. 1.0 and 0.2% of Poliquilgerm® were added and the culture and controls incubated in a shaker at 33±1ºC and 100 rpm for 24 hours. Afterwards, the following analyses were carried out: measurement of viscosity (Brookfield viscometer), determination of *S. cerevisiae* viability (counting in a Neubauer chamber) and verification of *L. mesenteroides* growth (by Pour Plate technique). The experiment was designed in three replications in quintuplicate.

RESULTS AND DISCUSSION: A 6.80 and 6.12% decrease in culture viscosity was verified when 1.0 and 0.2% of the product were used, respectively. Poliquilgerm® did not affect the viability percentage, budding and viability budding of *S. cerevisiae* after 24 hours. The addition of 1.0 and 0.2% of the product resulted in 77.4 e 87.5% and 98 e 89% inhibition of *L. mesenteroides* UFC/mL, respectively at initial time and after 24 hours.

CONCLUSION: Poliquilgerm® showed efficiency when applied on mixed culture after 24 hours, because it acts inhibiting *L. mesenteroides* growth, as well as decreasing the viscosity produced, without causing damage to the yeast. Thus, this product can be considered a natural alternative to the methods used for controlling contaminants in sugar and alcohol industries.

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ADVANTAGE OF THE MILK SERUM AS ALTERNATIVE SUBSTRATE FOR PRODUCTION OF BACITRACIN FOR *Bacillus licheniformis* (UCP 1016)

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KEY WORDS: Milk serum, bacitracin and *Bacillus licheniformis*

INTRODUCTION: The *Bacillus* genus produces antibiotics polypeptides of low molecular weight, as the bacitracin, produced for *Bacillus licheniformis* and *B. subtilis*, which it directly intervenes with biosynthesis of bacterial cellular wall (DEMAIN, 2006). The milk serum is a product of the manufacture of cheeses, being considered a liquid and generating residue of great pollution of the hydrics resources (NITSCHKE et al., 2001). In the present work the recovery of the milk serum is aimed in the means of production of bacitracin through the substitution it glutamic acid for the same, for formularization of an alternative way of low cost.

MATERIALS AND METHODS: A sample of *Bacillus licheniformis* (UCP 1016) of ground was used for production of bacitracin. The media control : glutamic acid (10g/L), K_2HPO_4 (0.5 g/L), K_2PO_4 (0.5 g/L), $MgSO_4$. 7 H_2O (0.2 g/L), $MnSO_4$. $7H_2O$ (0.01 g/L), $FeSO_4$. $7H_2O$ (0.01 g/L). The alternative called way, presented the same previous composition, substituting the glutamic acid for the milk serum (5 and 10%). It was introduced in the composition of the ways, a supplemental carbon source, and the glucose (2 and 4%). The production occurred to 37°C, 96 hours, 150rpm, with daily accompaniment. They had been determined the kinetic one of growth, the variation of pH and the antibiotic activity using the *Micrococcus flavus* (UFPEDA 323) as tested microorganism.

RESULTS AND DISCUSSION: The initial studies that the milk serum demonstrated an inhibition of 40% of the growth of the *Bacillus licheniformis.* The studies involving the association of the glucose (2 and 4%) and of the milk serum (5 and 10%), had determined that the excellent condition of production of the antibiotic was gotten using the modified means of production contends 10% of milk serum and 4% of glucose, demonstrating the antibiotic activity front to the 43.61 mg/mL of *M. flavus* and a value of pH of 8.7, considered excellent for production of the bacitracin (AWAIS, et al., 2008).

CONCLUSIONS: One observes that carried through studies, the occurrence of 80% of production of the antibiotic in the alternative media, beyond one high biological activity, and mainly the recovery it milk serum, in view of that its discarding in the hydrics resources, raising considerably the ambient pollution.

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Agrobacterium tumefaciens-mediated transformation of *Guignardia citricarpa*

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Guignardia citricarpa, a plant pathogen which causes the disease called Citrus Black Spot (CBS), was successfully transformed via *Agrobacterium tumefaciens*-mediated transformation. The strain EHA105 of *A. tumefaciens* bearing the vector pPZP201BK was used for transformation. The *bar* gene, rendering ammonium glufosinate resistant transformants, and the *gfp* gene were employed as selection markers. The original protocols of *Agrobacterium tumefaciens*-mediated transformation of Guinardia citricarpa were adapted for the present work. Some of the changes were: i) Initially, spores of *G. citricarpa* were submitted to a temperature of 40ºC for 5 minutes. Such procedure increased the germination rate making agro transformation easier; ii) The culture medium pH was changed to 5,8, since this is the optimum pH for *G. citricarpa* to grow. iii) After co-cultivation between *G. citricarpa* and *A. tumefaciens*, selective medium was added to the membranes instead of transferring them to the selective medium. These changes significantly contributed for the success of agro transformation. Microscopic observations to confirm agro transformation were carried out using epifluorescence microscopy. Hyphaes and spores of *G. citricarpa*, which grew in the selective medium were observed and compared to control colonies and colonies bearing the *gfp* gene. The stability of T-DNA integration into the potential transformants was verified by growing them on minimum medium containing an amino acid solution but no ammonium glufosinate. The clones were transferred to selective medium after six consecutives periodic transfers on minimum medium. All transformants were still ammonium glufosinate resistant, therefore indicating mitotic stability. Molecular analysis of the potential transformants was done through PCR. The primers barR (TCAGATCTCGACGGG) and barF (ATGAGCGAACGACGC) were used to detect the *bar* gene. After amplification, a 600 pb PCR product from the transformants was obtained. No product was obtained after the amplification of control strains. *Agrobacterium tumefaciens*-mediated transformation was an efficient method to obtain ammonium glufosinate resistant transformants. Other methods of T-DNA insertion had already been tested, but none showed positive results. Having transformants is essential to study the interaction between plants and its pathogens, aiming the control of the disease.

Key-words: Agro transformation, *Agrobacterium tumefaciens, Guignardia citricarpa,* ammonium glufosinate, *gfp*.

AGROCHEMICALS INFLUENCE AT SOIL MICROBIAL POPULATION

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Keywords: agrochemicals, biodegradation, microbiota.

INTRODUCTION: The agrochemicals include fertilizers and pesticides, used to control plagues and increase productivity. Compounds behavior and their destiny in soil depend on several factors, such as chemical structure, quantity of product, application frequency, and soil physical, chemical and biological conditions. The number of microorganisms in soil and the proportion of its different species vary according to type and amount of food, humidity, temperature, and reaction and aeration type. This means that the different microorganisms present distinct capacities to use the compounds, performing distinct activities according to the environmental conditions. The purpose of this work is to verify the agrochemicals influence at soil microbial population in an African daisy plantation.

MATERIAL AND METHODS: Four soil samples were collected in private plantation, considering: control soil presenting a 5-year agrochemical free history, and soils presenting constant application of glyphosate herbicide, copper oxychloride fungicide and deltametrine insecticide history. Bacteria and fungi population was quantified at agrochemical application day, and 15 days and 30 days after it. Soil microbial activity at agrochemical application was assessed through Bartha's respirometric method for 28 days. Soil toxicity was verified at time point zero and after 28 days of respirometry, using the solubilized after 24 hours of agitation and 24 hours of rest, through the test organism *Daphnia similis.*

RESULTS AND DISCUSSION: The density of bacteria population and mainly fungi population was decreased, after what a balance similar to the original was established. CO_2 production was higher in soil presenting agrochemicals application, and was reduced after 3 weeks from application day. In agreement with observed values, samples showed no acute toxicity. Samples of soil containing insecticide showed evidences of toxicity to *Daphnia similis* at the beginning and at the end of biodegradation process, and an increased rate of immobility of organisms was observed.

CONCLUSION: The agrochemicals influenced temporally the soil microbial population. Probably in the soil presenting history of agrochemical application there are microorganisms capable to use agrochemical as a source of nutrients.

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AGROINDUSTRIALS PRODUCTS AS ALTERNATIVES SUBSTRATES FOR RADIAL GOWTH OF *Cunninghamella elegans* UCP 542

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Key Word: Agroindustrials substrates, radial growth, *Cunninghamella elegans*

INTRODUCTION: Industrial waste involve quantities of shells, flour, stones, and as economic sources such as substrate or carbon source in the fermentation process to obtain compounds with large value (Coelho, 2001). The *Cunninghamella elegans* under appropriate conditions of cultivation is able to produce substances of great commercial value. In this sense, it was investigated the effect of culture media on the peanut, sesame, flaxseed and brown to evaluate the radial growth of *C. elegans*.

MATERIAL AND METHODS: The research was accomplished with *Cunninghamella elegans* Lendner isolated of the sediment of swamps in the Municipal district of Rio Formoso-PE, deposited in the Bank of Cultures of the Catholic University of Pernambuco - UNICAP (Gomes et al., 2000), classified under the number 542 and maintained in medium BDA (BIOLIFE®). After growth in YMA (Yeast Malt Agar) were inoculated discs of 5 mm diameter in the center of Petri dishes containing solid media (2% peanut meal, sesame at 2%, and 2% for flaxseed and brown for 2%). The plates were incubated at a temperature of 28 º C. The evaluation of radial growth was achieved through daily measurements of the diameter of the colonies with a ruler. The readings were taken at intervals of 24 h to 120h, and also observed the morphology of the fungus and the sporulation process.

RESULTS AND DISCUSSION: In all media used was observed microbial growth. The final period of 120h was observed that the brown to medium containing 2%, the growth of *C. elegans* was rapid, reaching across the plate (9.0 cm), and high sporulation. However, with the means peanuts to 2% there was a greater inhibition of growth (5.0 cm). For the medium with 2% linseed reached 5.8 cm and half sesame 2%, 5.23 cm. Spier et al (2004) analyzed the radial growth of some strains of *Aspergillus* and *Rhizopus* using starch from cassava and sugar cane bagasse ground with the aim of increasing the production of α-amylases. Kumar et al (2003) using Aspergillus niger to produce citric acid by means of milled sugarcane bagasse after a preliminary review of its radial growth. The results above were positive for the growth of the mushroom and corroborate with the literature, suggesting the continuation of the researches in that line.

CONCLUSIONS: The alternatives used in the way of cultivation do not prevent the growth of microorganism, and not change the morphology of the fungus. *C. elegans* have shown a good potential for adaptation to agro-industrial products and can serve as substrates for low cost.

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ANALYSIS OF ANTIMICROBIALS SUBSTANCES PRODUCED BY *Aeromonas spp*. ISOLATED OF FISH

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Key-words: *Aeromonas spp*., drugs resistance, inhibitory substance

INTRODUTION: The antimicrobial substances have acknowledged application in food industry, like natural conservants, and also in cosmetic and medicine industry [1]. Concerning drugs with quimiotherapic action, there is urgency in producing new medicines for which the pathogenic are sensible, especially if the great amount of antibiotics' multiresistants bacteria that have been described in the last years are taken into consideration. Many studies reported the bacterial production of a variety of biologically active extracellular substances [1]. In this study, *Aeromonas* spp. strains were assessed for their antimicrobials activity.

MATERIAL AND METHODS: The *Aeromonas* strains previously isolated of fish were grown in LBFM medium for 18 hours at 37 C° and re-inoculated in the next day in LAFM medium (glucose 0,01%, peptone 0,8%, yeast extract 0,2%, NaCl 0,5%, bacteriological agar 1,4%, K_2HPO_4 0,01% , 20% of fages solution containing $CaCl_2$ 0,14%, $ZnCl_2$ 0,13%, $MgCl_2$ 0,2%, $MnCl_2$ 0,19%) and incubated for about 18 hours. At the end of this period, the cells were centrifuged 10.000 rpm /10min and ressuspended in 10ml of a buffer containing MgSO4 0.1mM, CaCl2 4mM, NaCl 0.6% and gelatone 0.1% e and induced by UV irradiation for 10 seconds (3ergs/s) and inoculated in 10ml LBFM medium. After a period of incubation of 1 hour at 37°C, the cells were again centrifuged to obtain the supernatant. The antimicrobial activity of the supernatant was tested against 11 strains of *Aeromonas* spp, seven of them being of the *A. hydrophila* species, three of *A. caviae* species and one *A. veronni bv sobria*. These strains were sown with swab in LAFM medium and then were dripped 100 µl of each supernatant on the plates. The reading was held after 18 hours of incubation at 37°C.

RESULTS AND DISCUSSION: The lack of growth in the region where the supernatant was applied was considered inhibition. Each experiment was held in triplicate and dilution was made to verify if the inhibition was due to induction for profages.
Three lysates obtained showed inhibitory activity against five strains tested. The strains which presented inhibitory activity belonged to different species being TC3 *A. caviae*; TC4 *A. veronni bv sobria* e TC8 *A. hydrophila* and inhibited differentiated species. They were isolated from fish of the same source.

CONCLUSION: This study shows inhibition phenomenon mediated by *Aeromonas* against strains with same genus. The dilution of lysates did not produce observable plates of lyses, suggesting that these lysates are not a result of profage induction. Studies about resistance to proteolitic enzymes and neutralization are being made to the characterization of inhibitory substance.

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ANALYSIS OF PHYSICAL AND BIOCHEMICALS FACTORS FOR SOLID WASTE BIODEGRADATION BY *Fusarium sp*

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Keywords: biodegration, *Fusarium sp*., solid waste

INTRODUCTION: Organisms such as fungi have great capability of adapting themselves to environmental changes by metabolizing as nutrient practically every kind of substance, even waste. Among the waste and pollutants produced by big cities we can mention automotive tires that bring several problems to the environment and the populations. Filamentous fungi as *Fusarium sp* have shown potential for the bioremediation of contaminated soils, biodegradation of phenolic compounds and development in culture medium. The influence of physical factors and biochemicals on the *in vitro* cultivation of the fungus *Fusarium sp* isolated from the soil under a fragment of tire and its biodegradation potential in this waste have been analyzed in this paper.

MATERIAL AND METHODS: The tests took place with 300mL of medium, added or not with different carbohydrates, 0,3g of rubber of tire sanded, ground and autoclaved and 96 x 104 macroconídeos/mL of Fusarium sp isolated from a piece of rubber found in soil. The cultures were subjected also to different pHs and temperatures and they were still observed as for agitation or not of the medium. The cultivation rehearsals happened for 50 days and the biodegradation rate was determined through the dry weight of this waste.

RESULTS AND DISCUSSION: Among the kinds of sugar used in these rehearsals, glucose presented the best result as an energetic resource utilized by the fungus in the degradation process. The rehearsals disturbance was one of the analyzed components of greatest significance representing an average of 24% more degradation than the rehearsals that were kept in rest. There was waste degradation both in the absence and in the presence of light, however, the greatest values for this process were found in rehearsals submitted to room light. Neutral pH levels and temperature at 27ºC were the most appropriate to the biodegration of this waste.

CONCLUSION: Many tests still must be conducted, but results show the interference of physical and biochemicals factors in the cultivation and waste biodegradation by Fusarium sp. in vitro.

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ANIMAL FAT AND CORN STEEP LIQUOR AS LOW-COST SUBSTRATES FOR BIOSURFACTANTS PRODUCTION

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Keywords: Biosurfactants, animal fat, corn step liquor, industrial residues.

INTRODUCTION: microbial surfactants or biosurfactants are surface active compounds produced mainly by bacteria and yeasts, although some fungi also produce them. These compounds, due to its biodegradability and compatibility with the environment, unlike the similar petrochemicals (synthetic), have been increasingly studied (RON & ROSENBERG, 2002). Despite the advantages of biosurfactants over the petrochemicals ones, they are still not widely used due to high production costs associated with inefficient methods for recovering the product and the use of expensive substrates, although this cost can be significantly reduced through the use of alternative sources of nutrients, readily available and of low cost (CORTIS & GHEZZEHEI, 2007). This work describes the production of biosurfactants from *Candida lipolytica* cultivated in low-cost substrates with future application in the environment.

MATERIAL AND METHODS: the yeast *Candida lipolytica* was used as the producer microorganism. Different cultivation conditions using animal fat, corn steep liquor, glucose, $NaNO_3$, yeast extract, K_2HPO_4, KH_2PO_4, urea, $MgSO_4.7H_2O$ and NaCl were tested in various concentrations. All fermentations were conducted during 144 hours at 28°C with shaking at 150 rpm. After fermentation, samples were centrifuged and withdrawn for the following analyses: biomass determination, surface tension measurement in the cell-free broth and biosurfactant isolation through solvent extraction, after selection of the best medium composition.

RESULTS AND DISCUSSION: considering the surface tension as the most important parameter for biosurfactant detection, its measurement was selected for comparing the medium formulations used. It was observed that the lower surface tension, of 27.7 mN/m, has been obtained for the medium composed by 5.0% animal fat and 2.5% corn steep liquor in distilled water. Regarding the microorganism growth, it could be observed that the corn steep liquor exerted a positive effect on biomass accumulation, while the medium formulated only with animal fat did not favor cell multiplication. Four different isolation methods were tested for biosurfactant isolation. The best yield has been obtained for the method described by Ilori et al., (2005).

CONCLUSION: the biosurfactant produced presents attractive active surface features and can be produced at a low cost, a factor of great importance to its future application in cases of environmental decontamination.

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ANTIBIOTIC RESISTANCE OF PROBIOTIC LACTIC ACID BACTERIA

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Key words: probiotic, lactic bacteria, antibiotics

INTRODUCTION: The selection criteria for probiotic strains for humans should include also absence of undesirable properties such virulence factors, harmful biochemical activities and transmissible antibiotic resistances. For that reason, the objective of this study was to evaluate eight strains for their possession of antibiotic resistance.

MATERIALS AND METHODS: The analyzed strains *Enterococcus avium* CTC 469, *Enterococcus* sp. CTC 483, *Enterococcus* sp. CTC 141, *Lactococcus lactis subsp. hordinae* CTC 484, *Lc. lactis* subsp. *cremoris* CTC 204 and *Lactobaciççus plantarum* CTC 368, were previously isolated from meats and meat products samples by Bromberg et al (2004). The vancomycin used were supplied by Sigma® (0,33mg.mL^{-1}) and all other antibiotic: phenoximetilpenicillin (240ui.mL^{-1}), amoxicillin (300ug.mL^{-1}), chloramphenicol (4mg.mL^{-1}), clindamycin (300ug/mL), erythromycin (0,5mg/mL)' tetracycline (500ug.mL^{-1}) and metronidazole (40mg.mL^{-1}), were acquired from retail drugstores. The antibiotics were dissolved using sterile water and the suspension were sterilized by filtration (Millipore, 0,22µm). These suspensions were serial diluted (1:2; v/v) in plates of microtitulation using sodium phosphate buffer (10mM, pH 7,0). Ten microliters of each dilution were applied in MRS agar containing strains (10^{6}UFC.mL^{-1}). The inhibitory zone was recorded after incubation during 48 hours at 37^{o}C. The commercial strains: *Bifidobacterium bifidum* BB12® (Chr Hansen, Denmark) and *Bifidobacterium longum* BL-07 (Danisco, USA) were incubated anaerobically.

RESULTS AND DISCUSSION: All analyzed bacteria were resistant to metronidazole at the highest concentration (40mgmL^{-1}). The strains CTC204, CTC368 and CTC483 were resistant to vancomycin (>333ug) and all others, including commercial probiotic strains, were resistant only to concentrations lower then 5µg of this antibiotic. The BL 07 was the most resistant to chloramphenicol (5,00ug) and vancomycin (3,33ug), all others were susceptible to concentrations lower then 0,75ug. All strains were susceptible to amoxicillin (0,075 to 0,600ug), clindamycin (0,023 to 1,500ug), erythromycin (0,313 to 1,250ug) and phenoximetilpenicillin (0,075 to 0,600ug). Chloramphenicol, erythromycin and tetracycline were pour inhibitory against analyzed bactéria. The highest concentration of chloramphenicol that causes inhibition was 5µg for the BB12 and to tetracycline was 5µg for the CTC469.

CONCLUSIONS: All analyzed bacteria were resistant to metronidazole and were susceptible to amoxicillin, clindamycin, erythromycin and phenoximetilpenicillin. *L. plantarum*, *Ent.avium* CTC 469 and *Ent.avium* CTC 483 were resistant to vancomycin and others one, including commercial probiotic strains, were resistant only to concentrations lower then 5µg.

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ANTIMICROBIAL ACTIVITY OF PEDIOCIN AGAINST WINE BACTERIA

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Keywords: microbiological control, wine, bacteriocin, pediocin, *Oenococcus oeni*, lactic acid bacteria, acetic acid bacteria.

INTRODUCTION: Pediocin is a bacteriocin used against food spoilage bacteria (1). Sulphur dioxide is as well a potent antimicrobial agent widely used in the wine industry. In this study we describe the effect of pediocin on the growth of a collection of lactic acid bacteria (LAB) strains of a variety of species isolated from wines and grapes, and a number of strains of yeast and acetic acid bacteria (AAB) of enological origin.

MATERIAL AND METHODS: LAB enological strains (n = 55) included the following species: *Lactobacillus plantarum, L. hilgardii, Leuconostoc mesenteroides, Pediococcus acidilactici, P. parvulus* and *P. pentosaceus* and *O. oeni*. Sixteen yeast strains and 14 AAB strains of enological origin were also included in the study. Antimicrobial activity was determined by the microtiter dilution method as previously described (2). Antimicrobial activity was defined as the amount of antimicrobial agent that inhibited the growth of the indicator strain by 50 % (50% of the turbidity of the control culture without antimicrobial). The minimal inhibitory concentration (MIC) 50 was defined as the smallest concentration of antimicrobial agent that inhibited 50% of the tested microorganisms. Both antimicrobial agents, pediocin and potassium metabisulphite, were tested alone and in combination.

RESULTS: Results demonstrated that *O. oeni* species was more susceptible than the other LAB species to pediocin, which showed a MIC_{50} value of 0.01 μg/ml against *O. oeni.* Similarly, AAB enological strains were susceptible to pediocin (MIC_{50} < 0.005 μg/ml), whereas yeast and LAB strains different from *O. oeni* were more resistant to pediocin ($MIC_{50} \geq 0.156$ μg/ml). Results confirm previous studies reporting that potassium metabisulphite is an efficient agent against LAB and AAB growth (2).

CONCLUSION: The results reported in this study indicate that the use of pediocin could effectively prevent *O. oeni* and AAB growth in wines, and therefore, it could constitute a new method for wine microbiological control and preservation.

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ANTIMICROBIAL POTENTIAL OF FILAMENTOUS FUNGI FROM *Laguncularia racemosa* (L.) GAERTN. RHIZOSPHERE

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Keywords: Fungi, Rhizosphere, Mangrove, Antimicrobial Activity

INTRODUCTION: Rhizosphere is recognized as the region of the soil adjacent to plant roots, with intense biological activity and microbial diversity, being influenced by plant species and environmental factors. As a consequence, the identification of these microorganisms in unexploited ecosystems could be an alternative to search new bioactive natural products and metabolites. The aim of this study was to evaluate the antimicrobial potential of filamentous fungi isolated from the rhizosphere of *Laguncularia racemosa* (L.) Gaertn. (White-Mangrove, Combretaceae).

MATERIAL AND METHODS: The antimicrobial potential was evaluated using sixty six (66) filamentous fungi strains (25 *Aspergillus* spp., 23 *Penicillium* spp., 2 *Trichoderma* sp., 1 *Fusarium* sp. and 15 unidentified fungi) isolated from *L. racemosa* rhizosphere localizated on Rio Paripe estuary, Vila Velha, Itamaracá, Pernambuco, Brazil. All fungal strains were maintained on potato dextrose agar (PDA) slants at ± 4°C until further use. The antimicrobial activity test was performed by agar plug diffusion assay (ICHIKAWA *et al*., 1971), allowing fast and qualitative selection of bioactive metabolite producers towards Gram-positive *Staphylococcus aureus* UFPEDA 02, *Bacillus subtilis* UFPEDA 16, *Micrococcus luteus* UFPEDA 100, *Enterococcus faecalis* UFPEDA 138; Gram-negative *Pseudomonas aeruginosa* UFPEDA 39; acid-fast bacterium *Mycobacterium smegmatis* UFPEDA 71 and yeast *Candida albicans* UFPEDA 1007, all provided by the Microorganism Culture Collection, Department of Antibiotics, Federal University of Pernambuco, Pernambuco, Brazil. Pure cultures of each filamentous fungus were growth in Petri dishes containing PDA culture medium at 30°C for 5 days. After time, agar plugs (6 mm) were withdrawn from the mature culture and placed on the surface of Petri dishes uniformly inoculated with microbial suspensions adjusted to 0.5 McFarland and containing GL medium for *M. smegmatis, E. faecalis* and *C. albicans* and Müeller-Hinton medium for the remaining microorganisms. The plates were then incubated at 35°C and after 24 and 48 hours the inhibition zones were measured and expressed in millimeters. Inhibition zones≥ 10mm were considered positive.

RESULTS AND DISCUSSION: Only 12 (18%) filamentous fungi strains were active for one or more microorganisms tested. However, *Aspergillus* spp. strains showed the strongest antimicrobial activity, confirming the importance of this genus in the production of bioactive secondary metabolites. Moreover, the strain number 41 of *Penicillium* sp. presented relevant inhibition zone values and wide spectrum activity: 13.33 mm for *B. subtilis*, 16.83 mm for *M. luteus* and 14.83 for *C. albicans*. Regarding the microorganisms tested, *M. luteus* and *B. subtilis* were the most sensitive, with inhibition zones ranging from 11.66 to 21.16 mm and from 10.33 to 17.66 mm, respectively.

CONCLUSION: Based in our results, the filamentous fungi strains from *L. racemosa* rhizosphere are promising antimicrobial metabolites producers. The isolation, characterization and elucidation of these substances must be pursued in order to obtain new compounds with pharmaceutical interest.

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APPLICABILITY OF EQUATIONS OF FREUNDLICH AND LANGMUIR IN THE STUDY OF DYE BIOSORPTION PROCION RED MX5B BY LYOPHILISATE *Saccharomyces cerevisiae* CELLS.

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Keywords : *Saccharomyces cerevisiae,* biosorption, azodyes.

INTRODUCTION: The control of water pollution has become of increasing importance in recent years. The release of dyes into the environment constitutes only a small proportion of water pollution, but dyes are visible in small quantities due to their absorptivity. Currently, removal of dyes from effluents is by physio-chemical means. Such methods are often very costly and the accumulation of concentrated sludge create a disposal problem. There is a need to find alternative treatments that are affective in removing dyes from large volumes of effluents and are low cost.

MATERIAL AND METHODS: It prepared a 10% solution containing *Saccharomyces cerevisiae* lyophilisate, separated in concentrations ranging from 0.2 to 2 mg / mL and placed in contact with Red dye Proción MX5B to a concentration of 100µg/mL over a period of 2 hours at 28 ^{o}C . Then centrifuged at 3500 rpm for 10 minutes and made the readings in Shimadzu spectrophotometer between 190 and 800 nm. The remaining dye was calculated and the relations of removing both the color determined by biosorption aspect of living cells in the values of pH 2.50, 4.50 and 6.50 as the equations of Langmuir and Freundlich of the pH of best result in removal of color.

RESULTS AND DISCUSSION: The data were very significant in terms of removing the dye depending on the pH, that is, there was a correlation between cell concentration and dye removed and given an estimate of the total removal of the dye in different pH values. For the values 2.50, 4.50 and 6.50 the amount of biomass needed to complete cell removal was 1.03, 29.70 and 35.23 mg/mL respectively. As the equations of isotherms we must of Langmuir describes an interaction in monolayers, ie, the yeast cell has a number of active sites and that according to the affinity of the dye molecule to the cell wall may reach a state of saturation depending on the physical-chemical conditions existing.When occurs the Freundlich equations, indicates the possibility of multiple layers with an initial interaction of cell wall-dye and a second interaction that would dye-dye. In this case the correlation coefficient was 0.9908 for the equation of Langmuir and 0.6190 to the Freundlich equation, indicating that this process is an interaction in monolayers.

CONCLUSION: For the data we can see that for the dye Proción Red MX5B the extent that it lowers the pH value improves biosorption, and that the phenomenon of the dye biosorption with . lyophilized *S.cerevisiae* occurs in monolayers

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APPLICATION OF CULTURE-INDEPENDENT MOLECULAR TECHNIQUES TO ACCESS BACTERIAL COMMUNITIES ASSOCIATED WITH TRANSGENIC SUGARCANE

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Keywords: PCR-DGGE, 16S DNAr, Microbial Ecology

INTRODUCTION: Molecular methods applied to microbial diversity studies have been largely contributing to understand these communities, supplying also information for the identification of new species and bioremediation agents discovery [1]. Current studies focus in the evaluation and quantification of possible impacts of transgenic plant in the soil microbial community. In this context, this work aimed to properly sampling and to measure the total bacterial community in sugarcane soil (variety SP80-1842) and your transgenic form (IMI-1) resistant to imazapyr herbicide, in three different cultivation conditions: *i)* conventional plants (SP80-1842) conduced with manual weeds control (CV); *ii)* transgenic plants conduced with manual weeds control (TC) and *iii)* transgenic plants conduced with herbicide imazapyr application (TH), using denaturing gradient gel eletrophoresis (DGGE) and 16S rDNA library construction and analysis.

MATERIAL AND METHODS: The total DNA present in 0.5 g of soil, collected in the middle of sugarcane field, was extracted using *MoBio Power soil DNA isolation* kit (MoBio Laboratories, EUA), following the fabricant protocol. The amplification of 16S fragment gene was conduced with specific primers where amplicons with 480pb were obtained. These amplicons were submitted to DGGE analysis in phorU2 system (Ingeny, Goes, Holland), with denaturing gradient vary of 45 to 65% [2]. To construct the 16S genes library, the amplicons were cloned into *pGEM-T Easy Vector System* (Promega, Madison, EUA), following the fabricant instructions. The obtained sequences were first submitted to similarity analysis comparison using the software RDPQuery. After, rarefaction analysis and estimative of Shannon diversity and ChaoI richness indexes were performed using the software DOTUR. In addition, multiple-comparison analysis among the three libraries was conduced by software S-LIBSHUFF.

RESULTS AND DISCUSSION: The results obtained by PCR-DGGE did not show any significant modification among the three treatments. However, the library analysis, despite the fact to show similar estimative of Shannon diversity and ChaoI richness indexes for all management conditions, showed a little shift in the TC library when compared to CV and TH. This result can be evidence for the presence of more bacterial Orders in this library and also by S-LIBSHUFF analysis.

CONCLUSION: These results show a little modification in the bacterial community in TC management condition when compared to TC and TH. However, this modification can be associated with the detection of less abundant bacterial groups in sugarcane soil. In this case, a new methodology to determinate bacterial soil diversity, based in soil total DNA hybridization with specific probes, has been developed with the objective to minimize this randomly factor and establish a standard methodology to study bacterial soil community.

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APPLICATION OF MOLECULAR MARKERS IN GENETIC IDENTIFICATION OF *Helicobacter pylori* STRAINS (ε-Proteobacteria; *Helicobacteraceae*)

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Keywords: *Helicobacter pylori* cagA; vacA; molecular marker

INTRODUCTION: *Helicobacter pylori* is a gram-negative rod-shaped bacillus included in the *Helicobacteraceae* family and frequently associated with gastric human diseases. The virulence level of *H pylori* changes according to the strain that infects the host. Combinations of alleles of the *vacA* locus and a fragment of 40kbp length called "pathogenic island" (*cagPAI*) are related to the most aggressive strains of the bacterium. The *cagA* marker allele indicative of the *cagPAI* and the $s1^+$ and $m1^+$ sequences of locus *vacA* are used to identify the most virulent strains of *H pylori*. Our aims were to estimate the prevalence of *H.pylori* and identify the genetic variations which indicate a possible correlation with different aspects of the host -parasite relationship **MATERIAL AND METHODS:** The genomic DNA was obtained from gastric biopsy samples (64) which were obtained in the Endoscopy Center of the Hospital das Clínicas Luzia de Pinho Melo (HCLPM) using phenol-chloroform system. The DNA was amplified by PCR using specific primers to the urease gene, vacA (s1a, s1b, m1 and m2) and cagA of *H.pylori*. The alleles were identified using the amplification pattern. This study was approved by the Institutional Ethics Committee (UMC/HCLPM) and registered in the CONEP on the number 0120.0.237.000-07. All the collected samples were obtained only after concordance of the patients. **RESULTS AND DISCUSSION:** Among the analyzed patients 78,1% were urease C gene-positive, indicative of the high prevalence of *H.pylori* in the population, of which 82% are adults (between 18 to 59 years of age) and 18% aged 59 or over. The $s1^+$ strains (locus *vacA*) were identified in 58% of the patients; 16% of them were infected by the variant $s1a^+$ and 42% $s1b^+$. To the $m1^+$ and $m2^+$ sequences, the obtained values were 46% and 48% respectively. The *cagA* marker was identified in 36% of the patients, 60% of them had peptic ulcer, which reaffirmed the correlation between the strain type and disease severity. A positive correlation was observed between the variants $s1^+/m1^+$ and patients who developed pangastritis ($P>0,05$). **CONCLUSION**: Through the use of specific primers it was possible to identify the genetic variants of *H.pylori* in human biopsies. From these biopsies it was possible to verify the existence of a positive correlation between disease and a genetic marker in *H pylori* strain.

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BACILLUS CEREUS DYNAMIC GROWTH IN THE PRESENCE OF ENDOSULFAN SULFATE

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Key-words: Pesticide, optic density (OD), nutrient medium

INTRODUTION: The endosulfan sulfate can be formed in natural environments from biological oxidation, being degraded slowly due microbial metabolism.

MATERIAL AND METHODS: *Bacillus cereus* (isolated from cropping ground) was cultivated in three culture medias to observe its behavior in accordance with the nutrients sources, being (1) Minimum Media contends the pesticide (5µg.mL^{-1}) as an only source of carbon, (2) Nutrient Broth + pesticide (5µg.mL^{-1}) and (3) Nutrient Broth endosulfan sulfate-free. The bacterial growth was followed by measuring in 600nm optic density [1], in different times: 0, 12, 24, 48, 72, 96, 120 and 144 hours.

RESULTS AND DISCUSSION: Evaluating the growth (OD 600nm) of *B. cereus* in three treatments (Fig. 1), perceives that the first one did not propitiate an abundant growth when compared to the others, what can be attributed to the lack of carbon sources, typical of this kind of medium. Comparing treatments 2 and 3, it is noticed that it did not have a considerable difference during the first 24h, having both presented a similar trend. However, in the treatment 2 at T24, it could be registered a more speed up growth until T72, when the culture started to decline. Differently, in treatment 3 a slower growth is observed, however, with a more delayed peak (T96), followed of accented decline.

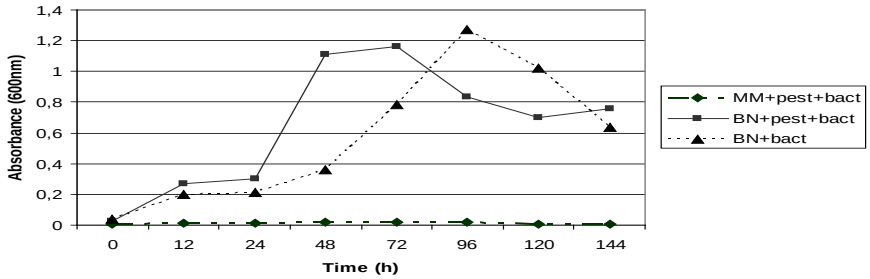

Figure 1 Growth of *Bacillus cereus* in three treatments (Fig. 1), being 1 Minimum Media + endosulfan sulfate (5µg.mL^{-1}) + bacterium, 2 - nutrient Broth + endosulfan sulfate (5µg.mL^{-1}) + bacterium and 3 - nutrient Broth + bacterium

Prado and Airoldi (2001) observed the effect of the herbicide Diuron in the microbial activity in soil, and one of the results was the delay of bacteria growth, in the Diuron presence. It is possible that the same has occurred in this experiment, therefore the supplemented production of biomass in the MM with endosulfan sulfate was fewer than that one observed in treatment 2 and 3 growth, showing that the generation time differed, for the same species, according to the culture media composition.

CONCLUSION: The growth of *B. cereus* in treatment 1 was revealed low, when compared with other studies [2]. However, the treatment 2 propitiated a great exponential growth in the first 72h, showing the importance of other nutrients sources in the biodegradação process.

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Biochemical characterization of a new strain of *Chromobacterium violaceum* UCP 1552 in Pernambuco, Brazil

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Key-words: *C. violaceum*, biochemical characterization, antibiogram.

INTRODUCTION: *Chromobacterium violaceum* is Gram-negative bacteria, optional aerobics that lives in tropical and subtropical regions, This bacterium has been found to be highly abundant in the water and borders of the Negro river, a major component of the Brazilian Amazon. Chosen for the sequence of your genoma in the Project Brazilian National Genoma. (BRAZILIAN NATIONAL GENOME PROJECT CONSORTIUM, 2003). This bacteria is characterized mainly by production of violet pigment denominated violacein, whose substance presents several biological applications as antibiotic, antitumoral, antichagasic and antioxidant, besides producing polyhydroxyalkanoates - PHAs (DURÁN, RETORI, MENCK, 2001).

MATERIAL AND METHODS: In the present work a new *Chromobacterium violaceum* strain found in waters in the city of Recife, Brazil was analyzed and stored in the Bank of Cultures of the Catholic University of Pernambuco as isolated UCP 1552. The bacterial identification was accomplished by automated methodology, being used the BD Phoenix System through a Panel for negative Gram where several conventional biochemical tests, cromogenics and fluorogenics are included, for identification and confirmation of the microorganism. The determination of the susceptibility profile to the antimicrobials was accomplished by conventional method of Kirby Bauer through disk diffusion, being tested the following drugs: amikacin, ampicillin, ampicillin-sulbactam, aztreonan, cephalothin, ceftazidim, ceftriaxon, cefepim, ciprofloxacin, ertapenem, gentamicin, imipenem, meropenem, piperacillin-tazobactam, polimixin B, sulfatrimetoprim and tigeciclin, once this species is not still included in the standardization extolled by CLSI.

RESULTS AND DISCUSSION: The new strain of *C. violaceum* presented positive results for the following biochemistries proofs: arginine, glutamic acid, leucin, fenilalanin, tryptofane, lysine, acetate, adonitol, citrate, colistin, D-manitol, alfa**-**cetoglutaric, malonate, polimixin B and range-glutamil and negative result for the following biochemistries proofs: prolina, L-prolina, phosphate, Beta-alose, dextrose, fructose, D-galactose, melibiose, sorbitol, sucrose, ac. galacturonic acid, L-arabinose, L-ramnose, maltose, ornitina, urea and esculin. In relation to sensibility tests was observed that the studied sample is quite sensitive to most of the tested antimicrobials. The reading for the method Kirby Bauer allows to interpret that it only happened resistance for ampicillin, ampicillin-sulbactam and cephalothin, in the isolated studied. For 2nd, 3rd and 4th cephalosporin class the sample was sensitive as well as for the other appraised antimicrobials. That can be explained by the little contact that those samples present in relation to antibiotics used in practice clinic, moving away a possible selective pressure exercised by the medicine.

CONCLUSIONS: The results obtained through the biochemical proofs, the new strain was identified as *Chromobacterium violaceum* with 99% of success for the equipment. With relationship to sensibility profile, the strain just presented resistance for ampicillin, ampicillin-sulbactam and cephalothin, presenting sensibility for the other antimicrobials tested.

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BIOCHEMICAL CHARACTERIZATION OF MICROORGANISMS OF RHIZOSPHERIC SOIL TREATED WITH DIURON

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Keywords: Diuron, microbial degradation, metabolites. sugar cane. rhizospheric soil.

INTRODUCTION: The diuron, N-(3,4-dichlorophenyl)-N,N-dimethylurea is a substituted urea herbicide widely used in the cultivation of sugar cane. Microbial vial is considered the main form of transformation of this herbicide and 3-(3,4-dichlorophenyl)-3-metilureia - (DCPMU), 3,4-diclorofenilureia - (DCPU), and 3,4-dichloroaniline - (DCA), are the main products of this degradation. This study aimed to isolate and characterize by biochemical evidence, the rhizosphere microorganisms of sugar cane, which has application of diuron.

MATERIAL AND METHODS: Rhizospheric soil samples of sugar cane were the growth in solution of WG® Hexaron (diuron + hexazinone) and incubated on shaker for 5 weeks. After plating in standard count agar (PCA), ten colonies were selected and characterized by Gram-staining and tests for catalase, citrate, sucrose and lactose fermentation, indol, motility, nitrate reduction, methyl red, Voges-Proskauer, spores formation and morphology.

RESULTS AND DISCUSSION:

Table 1 - Biochemical tests, formation of spores and morphology of selected microorganisms.

	Positive	Negative
Gram staining	4	6
Catalase	8	2
Simmons citrate	4	6
Sucrose and lactose fermentation	8	2
Indol	0	10
Motility	6	4
Nitrate reduction	10	0
Methyl red	0	10
Voges-Proskauer	10	0
Spores formation	7	3
Morphology	3 cocos	4 bacillus

Of the ten colonies were selected, two have the same biochemical and morphological characteristics. A systematic investigation of the characteristics and amplification of DNA from all microorganisms will select more precise information on the microorganisms involved in degradation of diuron.

CONCLUSION: This was important to investigate microorganisms not described in the literature on diuron bioremediation and its application in a later study of decreased concentration of the herbicide and characterization of the transformation process of the molecule in the ground.

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BIOCORROSION OF METALIC SURFACE IN CLAY SOIL

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Keywords: Biocorrosion, cathodic protection, clay soil.

The technological advance, stimulated by the use of oil, has brought many benefits to humanity. In contrast, the use of oil by the most varied industries has been the cause of major environmental accidents. Many of the injuries occur during the transport of oil and its derivatives, because of the leaks caused by corrosion of pipelines. When the corrosion of metal surfaces occurs through the participation of microorganisms it is called biocorrosion or microbiologically induced corrosion (MIC). Structures called biofilms caused by microorganisms alter the conditions of the interface metal / soil creating conditions favorable to the dissolution of metal. Among the methods of corrosion control one of the most employed is the cathodic protection (CP). The PC is one of the techniques used to establish the control of the process corrosion in metal structures, buried or submerged, especially when you can not perform periodic inspections. Thus, it is essential that the determination of the current necessary for the cathodic protection system is done in order to avoid mistakes, leading to a failure of protection or an unnecessary expense and even a weakening of the structure . The amount of current applied depends, among other factors, the electrochemical properties of the metal surface and the type of film that is formed on the surface. Both factors are influenced by microorganisms present. This study evaluated the influence of cathodic protection in the formation of biofilm on surfaces of carbon steel AISI 1020, buried on land belonging to the order of clay soils, classified as clay of low compressibility, representative of the municipality of Rio de Janeiro and adjacent municipalities. The tests were conducted at room temperature, in a glass recipient containing the soil, where the coupons were mantained in aerobic conditions. A comparative study was carried out with metal coupons with protection and with no cathodic protection and with different cell concentrations by the application of current supplied by a potentiostatic printed attached to a multimeter. The total time of each experiment was seven days when the cell measurements were made and the intensity of corrosion was obtained by determining the weight loss, and observing the morphology of the attack. It was possible to observe the colonization of metal surface by the microorganisms and the corrosion of the metal, where cell concentration influences the value of the potential to be applied, allowing then conclude that, under the conditions tested, the potential varies depending on some parameters and while the information dealing with the interaction of cathodic protection and microbial activity are very limited, a detailed study is essencial to assess the effectiveness of the potential used in the prevention of MIC.

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BIODEGRADAÇÃO DE ÁCIDO P-HIDRÓXIBENZÓICO POR *Chromohalobacter sp*.

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Palavras-Chave: Biodegradação, ácidos aromáticos, *Chromohalobacter sp.*

INTRODUÇÃO:
Todo ano, bilhões de galões de água de produção são gerados, e devido a essa grande quantidade, aliado a sua toxicidade, faz com que seu tratamento seja difícil. Entre os compostos tóxicos está o ácido p-hidróxibenzóico (PABA). Uma alternativa para a remoção do ácido p-hidróxibenzóico é a biodegradação desse composto, sendo este um método mais vantajoso que métodos físicos e químicos, por apresentar menores custos, alta eficiência no tratamento com pouco ou nenhum resíduo tóxico. Porém a alta salinidade da água de produção impede o crescimento de muitos organismos capazes de utilizarem hidrocarbonetos como fonte de carbono. As bactérias halofílicas constituem um grupo heterogêneo de microrganismos capazes de crescerem em uma ampla variação de salinidade (*Ventosa et al, 1998*), podendo ser utilizados para a biorremediaçao de locais com alta salinidade contaminados com hidrocarbonetos. Para isso, são necessários estudos, a fim de se identificar microrganismos aptos à biodegradação e as condições ambientais favoráveis a tal. O presente estudo teve como objetivo analisar a biodegradação de ácido p-hidroxibenzóico em alta salinidade pela bactéria *Chromohalobacter sp*. , a fim de utilizá-la para futuros processos de biorremediação.

MATERIAL E MÉTODOS:
A linhagem da bactéria halofílica *Chromohalobacter sp.* foi inoculada em 50 mL de meio HGM (*Fairley et al., 2002*) com 10% de salinidade e extrato de levedura. Posteriormente foi centrifugados e 0,01 de célula foi inoculada em 50 mL de meio líquido HGM suplementados com solução de vitaminas e 2,0 mM de PABA, mantido sob agitação por 132 horas. Após a visualização do crescimento a linhagem foi reinoculadas em 6 erlens contendo 30ml do meio HGM e PABA na qual foi observado o crescimento (mantendo-se todas as condições do estudo de crescimento). Os erlens foram retirados do shaker nos períodos de 0, 3 e 5. A análise quantitativa dos compostos aromáticos por CLAE foi realizada em cromatógrafo líquido Shimadzu, modelo LC-6A, com detector UV-Vis. Foi utilizada uma coluna de fase reversa Varian C18 (5 µm x 150 mm x 4,6 mm). A fase móvel utilizada foi: metanol: água : ácido acético.

RESULTADOS E DISCUSSÃO:
A análise por CLAE demostrou que a bactéria conseguiu degradar PABA. No tratamento de três dias foi observada uma degradação de 83,14% da fonte e no de cinco dias foi observada uma degradação de 98,9%.

CONCLUSÕES:
Frente ao exposto podemos concluir que a bactéria *Chromoalobacter sp.* apresenta potencial para a biodegradação dos ácidos aromáticos em alta salinidade e em um curto espaço de tempo.

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BIOEMULSIFIER PRODUCTION IN DIESEL-CONTAMINED SEA WATER SUPPLEMENTED WITH NITROGEN AND PHOSPHORUS SOURCES

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Palavras-Chave: Bioemulsifier, Candida lipolytica, Diesel, Sea water, Factorial Design.

INTRODUCTION: Hydrocarbon pollution of marine ecosystem due to refined and crude fuel is always a cause of concern (LEAHY & COHLWELL, 1990). Pollution due to light petroleum products like gasoline is treated efficiently by natural physico-chemical factors. However, heavier fuels like diesel take months for complete natural remediation due to their low volatility (PAVITRAN et al., 2006). Bioemulsifiers are directly involved in the process of oil and oil-related removal from the environmental. The aims of this work was to investigate the effects of nitrogen and phosphorus sources on bioemulsifier production by *Candida lipolytica* UCP 988 in natural sea water and contaminated in laboratory with diesel oil.

MATERIAL AND METHODS: Seawater was collected from a sampling point, at a beach near Abreu and Lima Refinery, at the Suape Port, Pernambuco. *Candida lipolytica* UCP 988 was provided by Bank of Cultures of the Nucleous of Researches in Environmental Sciences (NPCIAMB / UNICAP-PE). A 2^4 full factorial design was carried out to investigate the effects and interactions of the diesel oil, urea, ammonium sulfate and potassium dihydrogen orthophosphate concentrations on the emulsification activity of the emulsifier produced by *Candida lipolytica.* Sea water was supplemented with urea, ammonium sulfate and potassium dihydrogen orthophosphate and contaminated with diesel oil according to the factorial design specifications. The production of bioemulsifier was evaluated by determination of emulsification activity (CIRIGLIANO and CARMAN, 1984) using 168 h cell-free grow filtrates.

RESULTS AND DISSCUSSION: The results indicated that the interaction between diesel oil and potassium dihydrogen orthophosphate and the interaction between urea and potassium dihydrogen orthophospha, were statistically significant at 95% confidence level and favored the increase of emulsification activity. In the conditions studied, the others factors and theirs interactions were not statistically significant. The highest activity emulsification (6 UAE) was obtained with the medium containing 1% (v/v) of diesel; 0,10% (p/v) of urea; 0,10% (p/v) of ammonium sulfate and 0,68 % (p/v) of potassium dihydrogen orthophosphate.

CONCLUSION: The results obtained show the ability of *C.lipolytica* to degrade diesel oil in seawater and to produce emulsifiers with potential of application in environmental bioremediation.

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BIOFILM FORMATION AND MICROBIOLOGICAL QUALITY IN LETTUCE (*Lactuca sativa*) CROP IN HYDROPONIC SYSTEM

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Keywords: Coliform, lettuce, biofilm

INTRODUCTION: Foodborne diseases are responsible for thousand cases of diarrhea in the world. One food involved in these outbreaks is lettuce (*Lactuca sativa*) [1]. Currently, it has been observed an increase in production of these crops in hydroponic system[2]. Although this technique presents low risk of contamination by microorganisms, it has been reported presence of Salmonella and fecal coliform (FC) including *Escherichia coli* at levels not allowed by standard. Hydroponic irrigation system needs special attention. Certain pathogenic strains of *E. coli* are able to produce biofilms, which can adhere on surfaces of ducts and pipes that bring water to crops[3]. The presence of these pathogens in roots may indicate possible contamination of water and/or irrigation pipes. The aim of this study is microbial analysis of lettuce and the presence of genes involved in the formation of biofilms.

MATERIAL AND METHODS: We analyzed separately leaves and roots of 12 samples of lettuce for sale in Campinas/SP. The detection of *Salmonella* sp. was made by FDA's method[4]. Quantification of FC was conducted as described by Kornacki & Johnson[5]. The biofilm was analyzed by testing phenotypic polystyrene plate with crystal violet and the result confirmed by PCR, using genes *crl*, *fim* H and *Ag43.*

RESULTS AND DISCUSSION: According to ANVISA[6] all samples did not demonstrate the presence of *Salmonella* sp. and FC. Although within the specifications, 6 leaves had FC, and *E. coli* isolated in 2. Eight roots had FC and 6 *E.coli* were isolated. *E. coli* isolated from leaves showed gene *clr* and *fim* H and formed strong biofilm in plate and one had *Ag43. E. coli* isolated from root had presented genes *crl* and *fim* H and phenotypically strong formation of biofilm and one presented *Ag43*.

CONCLUSIONS: The hydroponic system presents low contamination when compared with other production systems. Our results demonstrate that while the lettuce is in satisfactory health conditions, isolation of *E. coli* with genes responsible for the formation of biofilms generates concern because they may represent a pathogenic potential. Further studies are necessary and greater surveillance on the health status of the systems involved in hydroponic production and finished products.

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BIOSORPTION OF TEXTILE DYE ACID BLUE 25 BY CELLS OF *Saccharomyces cerevisiae* IMMOBILIZED IN CALCIUM ALGINATE

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Keywords: Biosorption, *Saccharomyces cerevisiae,* Immobilization in alginate.

INTRODUCTION: Much of the contamination of water resources occurs due to improper treatment of industrial effluents. The presence of dyes that are not degraded in the conventional treatment systems, being the most common reactive of the type azo, are ones of the biggest problems of the effluent of this nature. Currently, the removal of dyes in economic and efficient way has become a stimulus for production of scientific research to propose practical solutions and of viable application by the industries. In this work was evaluated the rate of biosorption of acid blue 25 dye, present in industrial effluents, by yeast *Saccharomyces cerevisiae* immobilized in calcium alginate beads, the rate of dye biosorption in these beads whithout the presence of yeast and also was evaluated this dye in the presence of $CaCl_2$ 0,01M.
MATERIAL AND METHODS: In the pH 2,50 solutions were prepared with 9 mL of water and 1 mL of dye, which were placed in 5 tubes 2, 4, 6, 8 and 10 of calcium alginate beads with immobilized yeast, in another 5 tubes were placed 2, 4, 6, 8 and 10 calcium alginate beads without the presence of yeast and 5 more tubes were placed 0.1, 0.2, 0.3, 0.4 and 0.5 mL of $CaCl_2$ 0,01 M with 1 mL of dye obtained a final volume of 10 mL. The tubes were kept in furnace at 28 ° C for 10 days. The readings of the absorbance were made in spectrophotometer 190-800 nm every 24 hours from the time zero for a period of 1 to 10 days, thus getting the concentration of the remaining dyes.
RESULTS AND DISCUSSION: The rate of removal of dyes of the solutions was increasing gradually with elapsing of the time in the tubes which contained more of calcium alginate beads with immobilized yeast, when compared with the rate of removal of the other solutions that contained only calcium alginate beads and $CaCl_2$ 0,01M. In the tubes that contained 10 beads of calcium alginate with yeast, in 2, 5 and 10 days had a rate of decolorization of 11.66%, 67.5% and 91.66% respectively. In 5º day, the rates of discoloration in the tubes containing 2, 4, 6 and 8 beads of calcium alginate with S. cerevisiae immobilized, were 38.33%, 41.66%, 48.33% and 58.33% respectively. This technique showed that the encapsulation of microorganisms, in the case, the *S. cerevisiae*, offers some advantages in applications of bioremediation, guarantees the survival and promotes the physiological activity, increases the cell density and the cell growth preferably in several areas of aerobic and anaerobic gel encapsulated, can then be very useful in the stations of treatment of the textiles industries.
CONCLUSION: The immobilization of *Saccharomyces cerevisiae* in beads of calcium alginate was very effective in the removal of the color of solutions containing the textile dye Acid Blue 25, it was better observed in the solutions which contained the highest number of beads (10), while in the solutions that did not have the presence of the yeast and contained only beads of calcium alginate or $CaCl_2$ 0,01 M there was not discoloration rate, that is, it did not have adsorption.
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Biosurfactant production by *Bacillus subtilis* UCP 999 on adhesion polyethylene terephtalate-PET

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Keywords: Biosurfactants, PET, *Bacillus subtilis*

INTRODUCTION: The biosurfactants in function of the presence of the hydrophilic and hydrophobic groups, those compounds are distributed on the interfaces, with different polarity degrees (water-oil or air-water), and forming a molecular film, and reducing in this way the interfacial and superficial tension. (NITSCHKE; PASTORE, 2002). The biosurfactants showed environmental applications, as in the bioremediation processes, biosorption, in the dispersion of oily and in the recovery of petroleum, industries of cosmetics, of detergents and agricultural (TULEVA et. al., 2002). In the present was investigated the biosurfactant production by *Bacillus subtilis* during the phenomena's of adhesion of the surface of PET.

MATERIAL AND METHODS: Physicochemical treatments were carried out using UV irradiation (2.400 e 2.800nm) during 6 and 36h, respectively, and the treatment with temperatures of 35ºC and 50ºC during 72h. Plastics samples were submitted to aseptic conditions and were transferred to Erlenmeyer flasks of 250 ml capacity containing 100 ml of nutrient broth medium, added of 0.50% Tween 80. The flasks were inoculated with bacterial suspension of *Bacillus subtilis*, incubated at 150 rpm for 30 and 60 days, at the temperature of 28º C. After those periods of incubation, the cultures were submitted to filtration and metabolic liquid of free cells was used to: determination of the index and activity of emulsification, and surface tension. In the intervals was evaluated bacterial colonization on the plastic, following observation by scanning electron microscopy. The production of biosurfactant for adherence to PET was observed by reducing the surface tension.

RESULTS AND DISCUSSION: The colonization of *B. subtilis* was observed by scanning electron microscopy in control and all treatments. The production of biosurfactant was evidenced by the emulsification activity in all treatments, except to 35ºC temperature. The surface tension of the liquid metabolic free of cells was 39.89 mN/m, and emulsification index of 53,33% for the condition UV irradiation 6h, suggesting to be a good biosurfactant according Mulligan et al. (2005). Another important property of biosurfactants is the powerful and ability of reducing the surface tension in aqueous solutions (COOPER and ZAJIC, 1980).

CONCLUSIONS: The results suggest that the production of biosurfactant by *Bacillus subtilis* during adhesion phenomena's on PET is related to the increase of hydrophilicity, allowing the colonization of *B. subtilis* in the polymer. This phenomenon may be to contribute with the reduction of the polymer in the environment.

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BIOSURFACTANT PRODUCTION BY *Bacillus subtilis* USING RICE BRAN AS SUBSTRATE

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Palavras-Chave: Biossurfactant, *Bacillus subtilis,* Rice bran.

INTRODUCTION: The metabolic biossurfactants are composed of microorganisms capable of reducing the surface tension and interfacial, distributed on the interfaces between fluids with different degrees of polarity. This allows the dispersion or the mixture of liquids such as water and oil in the form of emulsions. The Surfactin is a heptapeptide, produced by strains of *Bacillus subtilis*, and is considered one of the most powerful surfactants. The Surfactin has several pharmaceutical applications due to its ability antiviral, antibacterial, antifungal and antitumor (NITSCHKE AND SHEPHERD, 2002). The production of large-scale biossurfactant difficulties due to the costs of the biotechnological process, and especially, the use of raw material, which in general is responsible for 30% of total production costs (CAMEOTRA and MAKKAR, 1998). In this context, this work was carried out to produce biossurfactant by *Bacillus subtilis* in a medium with a substrate of rice bran as carbon source, enriched with peptone as nitrogen source.
MATERIAL AND METHODS: The UCP999 *Bacillus subtilis* was isolated from the Port of Recife, in areas contaminated by oil. The sample is deposited in the bank of cultures of the Center for Research in Environmental Sciences at the Catholic University of Pernambuco, which is registered with the Federation Culture Collection-FCC. The *B. subtilis* was grown in medium with rice bran (100 g / L) and peptone (2g / L), pH 5.8, kept under agitation of 200rpm at 30 ° C. After 72h of fermentation were the surface tension (KUYUKINA et al. 2001), the rate of emulsification (COOPER and GOLDENBERG, 1987) and pH.
RESULTS AND DISCUSSION:
The nitrogen source is essential for the biosynthesis of surfactant compounds (DAVIS, et al. 1999) the way with rice bran supplemented with peptone as nitrogen source, showed production of biossurfactante, estimated by reducing the surface tension 37.29 mN / m. Emulsification of the rates of 90.0% in motor oil and kerosene at 22.3%, show that biossurfactante can be used in bioremediation. The surfactant showed a gross yield of 0.3 g / L and the final pH was 6.9. Similar results obtained by Morán et al. (2002) using mineral medium enriched with glucose to obtain 0.4 g / L Surfactin.
CONCLUSION: The biosurfactant produced shows characteristics similar to Surfactin.
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BIOTREATMENT OF OIL WASTE FROM PETROLEUM INDUSTRY BY SEQUENCING BATCH

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Key words: Periodic processes, sequencing batch, biodegradation.

INTRODUCTION:

Significant amounts of oil waste are generated by storage and distribution petroleum derivatives terminals. The present study was conduced by employing a stirred and aerated bioreactor, in order to treat this sort of residue. The bioreactor was operated by sequencing batch (SBBR – Sequencing Batch Bioreactor), with the purpose of to approximate the process of the conditions in the storage terminal.

MATERIAL AND METHODS:

The waste was characterized, and afterwards, factorial fractional design 3^{4-1} was elaborated with the purpose of to determine optimal of: C:N ratio; aeration condition; pH and initial waste concentration. Two sequencing batches were carried out at three cycles of 110h and four cycles of 72h, respectively (CASSIDY et al, 2001). The effectiveness of the process was assessed by the biodegradation rates at the end of each cycle of sequencing batches and by means of ecotoxicological tests employing representative species from the local biota (PAIXÃO *et al*, 2007). Studies on microbial molecular dynamics were conduced by means of DGGE techniques.

RESULTS AND DISCUSSION:

Conditions predicted in the 72h/cycle batch, demonstrate to be more effective in the reduction of hydrocarbons of waste. Biodegradation rates at the end of the cycles 1, 2, 3 and 4 were, respectively $0.74\%.h^{-1}$, $1.33\%.h^{-1}$, $1.06\%.h^{-1}$ and $1.04\%.h^{-1}$. Changes in the microbial community members, at the end of the cycle 1 and at the beginning of the cycle 2, are connected to the changes in biodegradation rates. Oxygen uptake rate of the acclimated community sampled at the end of each cycle corroborated with the biodegradation rates ($124.9mgO_2.L^{-1}.h^{-1}$, $252.9mgO_2.L^{-1}.h^{-1}$, $120.4mgO_2.L^{-1}.h^{-1}$, and $108.8mgO_2.L^{-1}.h^{-1}$). High values of percentage of ecotoxicity reduction were observed at the end of cycle 2 (70.1% when *Echinometra lucunter* was the tes-organism and 78.5% when *Crassostrea rhizophorae* was the test organism). The waste from cycle 4 did not promote toxic response to *E. lucunter* (reduction 100% of toxicity) and promoted lower toxic response to *C. rhizophorae* (reduction of 86.6% in the original toxicity).

CONCLUSIONS:

The studied waste is treatable, presenting low recalcitrance and high toxicity. The process showed primarily, efficient in the reduction ecotoxicity of the waste, from 70 to 78% of the original toxicity in the 2nd cycle. Studies of microbial molecular diversity when applied to biotreatment, processes, specially when operated by SBBR operation mode, show to be efficient when the aim is to understand changes in the community associated to the biodegradation rates.

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BLUE LIGHT INFLUENCING ASTAXANTHIN PRODUCTION POR *Mucor javanicus* USING AGROINDUSTRIALS SUBSTRATES

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

INTRODUCTION: Astaxanthin is considered an important and powerful antirust substance, many times bigger that other carotenoids, mainly due to its precursory activity of vitamin A. (PASHKOW et al., 2008) The astaxanthin can be make use of natural sources or synthetic, the preference exists in if to use astaxanthin of natural sources, extracted by saw microbiological (GONG and CHENG, 1998). A brief exposure to light, resulting in a substantial synthesis of carotenoids for microrganisms (TADA; SHIROISH, 1982). In this work, the wastes Corn Steep Liquor with "quirera" were investigated as alternative source for the production of astaxanthin for *Mucor javanicus*. And was evaluated too the influence of blue light in the production of astaxanthin.

MATERIAL AND METHODS: *Mucor javanicus* (UCP-69) belongs to the Culture Collection of Catholic University of Pernambuco, registered in Federation of Culture Collection-FCC. Conditions of culture and growth: A stock suspension of spores was prepared and adjusted to 107 spores/mL counter using a haemocytometer. For the production of astaxanthin 1 mL of preinoculum was transferred to Erlenmeyer flasks containing 100 mL of the medium Corn Steep Liquor with "quirera" and distilled water in the concentrations of 4%, 7%, and 10%, during 48h, 72h, and 96h, pH 6.5, using blue LED´s. The mycelia were harvested by filtration and the was lyophilized. Extraction and analysis of the carotenoids from the biomass was homogenized using ice-cold potter using methanol and hexane (1:1, v/v), as system of solvent. The astaxanthin was separated by centrifugation at 2000 rpm/10 minutes. The determination was carried out by spectrophotometer at the wavelength of 470 nm.

RESULTS AND DISCUSSION: The best condition verified was when it was used the concentration of 7% of Corn Steep Liquor with "quirera" in the time of 72 hours, with the income of 18,4μg/g, when utilized blue light and without blue light the production of astaxanthin was (13,4 μg/g). Fontana et al., (2000) described that the Corn Steep Liquor it's an excellent medium of culture for production of astaxanthin for microorganisms. Therefore this work contributed for the literature.

CONCLUSION: The utilization of blue light, the corn steep liquor and "quirera" shown a excellent substrates for the production of astaxanthin by *Mucor javanicus*. "Quirera" as substrate for astaxanthin production is described for the first time.

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CELLULOSE DEGRADING YEASTS ISOLATED FROM ATTINI ANTS

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Keywords: Cellulases, Yeasts, Attini ants

INTRODUCTION: In recent years, a lot of attention has been given to the renewable fuel production from plant biomass. Cellulose is a renewable and abundant source of carbon. Therefore, there is an increase interest in the search for microorganisms producing cellulolytic enzymes for the enzymatic hydrolysis of cellulose. In this study, yeasts and "yeasts like" organisms previously isolated from Attini ants nests, were screened for the production of endoglucanases/exoglucanases and cellobiases, with the aim of selecting strains with lignocellulose hydrolases.

MATERIAL AND METHODS: Two hundred five yeasts or "yeast like" used in this study were previously isolated from the following Attini ants: *Atta laevigata, Atta texana, Acromyrmex* spp., *Mycetarotes* sp., *Mycocepurus goeldii* and *Myrmicocrypta* sp. Microorganisms were inoculated on the surface of specific media for detection of enzymes, one containing 0,5% of carboxymethylcellulose (CMC) and other containing 0,5% of cellobiose, to detect endoglucanases/exoglucanases and cellobiases, respectively.

RESULTS AND DISCUSSION: In this study, 205 strains of yeasts and "yeast like" organisms belonging to 21 different genera were used in the assays. The most predominant genera include *Trichosporon* (n=69), *Cryptococcus* (n=50), *Pichia* (n=19), *Candida* (n=15) and the specie *Geotrichum candidum* (n=16). Ninety (43.90%) strains produced enzymes that can degrade CMC. Species of the genera *Trichosporon* accounts for more than 33% of strains that present cellulolytic activity**.** In related studies on the selection of microorganisms that produce enzymes of industrial importance**,** the results were quite different. In a study by Buzzini and Martini (2002), 397 yeasts were isolated (in tropical areas); none of these possess cellulolytic activity. In another similar work using yeasts isolated form the different stages of wine production, Strauss and collaborators (2001) found only 3.67% of cellulolytic yeasts. Synergy among the enzymes that degrade cellulose in oligosaccharides (endoglucanase/exoglucanases) and enzymes that convert cellobiose in glucose (cellobiases) is necessary for the conversion from cellulose in glucose. Cellobiase activity was found in 95.60% of the isolates. Only one out of the isolates (*Trichosporon gracile*) displayed endoglucanase/exoglucanase activity but didn't possess cellobiase activity. The other isolates are potentially degraders of cellulose in glucose, allowing the fermentation for the ethanol production from cellulose. The highest percentage of yeast able to degrade cellulose to glucose could be related to substrate present in the microhabitat of the isolation. In cut ant nests (genera *Atta* and *Acromyrmex*) the substrate is composed entirely of plant material; in the nests of the other genera, in addition to plant material, mainly lignocellulosics material, were found including others materials, like insects remains.

CONCLUSION: The results obtained in this study have shown that nests of ants could be promising sources for the isolation of yeasts and "yeasts like" cellulolytic enzymes producers. Further studies about quantification, characterization and the gene sequences of these enzymes are recommended for the selection of strains useful for biotechnology application.

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CHANGES IN POLYPHOSPHATE CONTENT OF *Cunninghamella elegans* IN RESPONSE TO CADMIUM

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Keywords: *Cunninghamella elegans,* Polyphosphate, Cadmium

INTRODUCTION: Polyphosphate (polyP) is a polyfunctional biopolymer of phosphate widespread in nature that has been implicated in metal tolerance and removal in many microorganisms (Keasling et al., 2000). In this study we investigate the influence of cadmium on phosphate uptake and polyP fractions content in *Cunninghamella elegans*.
MATERIAL AND METHODS: *C.elegans* IFM 46109 was growth during 15 days, at 28°C and 250 rpm, in medium without (control samples) and with cadmium at the following concentrations: 5.62mg/L; 11.24mg/L and 22.10mg/L. Samples of culture supernatant were used to evaluate the phosphate consumption by using Biosystems kit. The biomass was collected and submitted to sequential polyP extraction using the method described by Smirnov et al., (2002).
RESULTS AND DISCUSSION: The results showed that the rate of phosphate uptake by *C.elegans* was 19% higher during growth in medium with cadmium in all concentrations. Phosphate accumulation during growth in medium with cadmium can be an mecanism of detoxification this heavy metal (Aiking et al., 1984). Our results also demonstrated significant variations in the polyP content among the fractions studied according cadmium concentrations. Thus, the polyP content in the acid-soluble fraction was completely degradeted under cadmium treatment of 22.10mg/L whereas content of the alkali-soluble fraction decreased 80% when 11.24mg/L cadmium was used. On the other hand, under cadmium concentration of 5.62mg/L an increase of 50% of the polyP content in the acid-insoluble fraction was observed. These results point to a balance among syntesis and degradation in all polyP fractions in response to cadmium. This is corroborated by Keasling et al., (2000) study, in which the ability to synthesize and degrade polyP is more important for tolerance to heavy metals than the accumulation capacity of large polyP content.
CONCLUSION: The highest phosphate uptake in presence of cadmium, suggest that phosphate can be involved in the metal detoxification. It is possible that the distinct polyP fractions exhibit different roles in the tolerance to cadmium.
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CHARACTERIZATION AND EMULSIFYING ACTIVITY OF THE EXOPOLYSACCHARIDE PRODUCED BY *Gordonia polyisoprenivorans* CCT 7137, ISOLATED FROM CONTAMINATED GROUNDWATER

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Keywords: Exopolysaccharides, biosurfactants, *Gordonia polyisoprenivorans*

INTRODUCTION: Biosurfactants are produced by microorganisms and have important natural roles in the growth of the producing microorganisms, related to their different chemical structures and surface properties. These include increasing the surface area and bioavailability of hydrophobic water-insoluble substrates, quorum sensing and biofilms formation. Biosurfactants are biodegradable, presenting lower toxicity than the synthetic ones and have potential use in bioremediation, food and oil industries. The present study aimed at the characterization of the exopolysaccharide (EPS) structure produced by *Gordonia polyisoprenivorans* CCT 7137 as well as its emulsifying properties on different hydrocarbons.

MATERIAL AND METHODS: *G. polyisoprenivorans* CCT 7137, isolated from groundwater adjacent of an old landfill area (São Carlos, SP), was cultured in GYM (commercial medium) and sugarcane molasses (alternative medium) media during 72 hours at 30°C and 150 rpm (Fusconi et al. 2008). After extraction and purification the main functional groups of the EPS were assigned using FTIR. Emulsification assays were carried out according to Iqbal et al. (1995) using cell free GYM and 6% sugarcane molasses media with benzene, toluene and *o*-xylene. Triton X-100 was used as chemical surfactant and a control was prepared using the same method but replacing the sample by non-innoculated media. Results were tested by analysis of variance (Anova), followed by the Tukey test at a 0.05 level of significance.

RESULTS AND DISCUSSION: The EPS samples showed a carbohydrate structure composed mainly of α-anomers with presence of carboxylic and amide functional groups. The presence of carboxyl groups can be responsible for the low pH of EPS aqueous solutions (4.0 – 4.5), probably associated with the protein structure, acting as binding sites for cations. This characteristic is important in biopolymer applications as bioemulsifier. The emulsifying activity in GYM culture supernatant on benzene (61.67 %) was higher than those observed for SM medium (34.91 %) and Triton X-100 (47.16 %). On toluene, the emulsifying activity in GYM culture supernatant (60.61 %) was higher than the one obtained in SM medium (27.88 %) and similar to that found in Triton X-100 (51.27 %). On *o*-xylene, both emulsifying activity observed in GYM (62.88 %) and SM (35.61 %) media were similar to that obtained in Triton X-100 (51.90 %).

CONCLUSION: The EPS produced by *G. polyisoprenivorans* CCT 7137 showed biosurfactant characteristics, and generally had superior or similar emulsifying activity than commercial surfactant suggesting its potential application in bioremediation studies.

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CHEMICAL METAL PRECIPITATION IN RESIDUARY WATERS TREATED BY THE MICROBIOLOGY

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Keywords: **Toxics, Removal and Recovery, Processes and Research**

INTRODUCTION: The harmful effect of heavy metals and other toxic substances to the environment comes to stimulating innumerable research that approaches the possibility for removal and recovery residual waters of industries. About the chemical treatments employees processes the most known are the chemical precipitation in hydroxides form, carbonates or sulphides and the oxi-reduction. All the processes that involve the metallic ions precipitation operate under the same chemical principle that is an alkaline reagent - hydroxide, carbonate or sulphide is added to the effluent to be treated reducing the solubility of the metallic constituent and thus favoring its precipitation, that is promoting the chemical balance alteration of the species that is desired to remove.

MATERIAL AND METHODS: : The process using itself hydroxides widely is utilized industrially since appropriate residual concentrations of metallic ions for its discarding are reached after the effluent treatment. Into this process that occurs to the temperature and ambient pressure, metallic ions in solution are converted to insoluble hydroxides after the addition of precipitation agents as calcium oxide (CaO), hydroxide of calcium ($Ca(OH)_2$) or hydroxide of sodium (NaOH). Precipitation mechanism:

$$M^{+2} + OH^{-} = M(OH)_2$$

The process using itself carbonates can be carried out so much by the direct precipitation with carbonate of calcium ($CaCO_3$) or sodium carbonate (Na_2CO_3) or ($Na(HCO_3)_2$. The advantage is that lamas (muds) gotten hydroxides are more easily filtered than the respective ones, however the efficiency of metals removal is lower than the one gotten with other bases and some metals as zinc are not precipitated. Precipitation mechanism:

$$M^{+2} + CO_3^{2-} = MCO_3$$

Another ion capable to form little soluble composites with several metallic ions is sulphide. The precipitation of weighed metal in the form of sulphides by the sodium sulphide dosage (Na_2S), hydrogen sulphide (H_2S) or iron sulphide (FeS). Precipitation mechanism:

$$M^{+2} + S^{2-}(Na_2S) = MS$$

RESULTS AND DISCUSSION: After the precipitation process the formed solids should be separate of the liquid mass what is performed by a complementary process like coagulation, flocculation and sedimentation or still for the filtration process. After the solids separation process the effluent treated also can require the use of an additional process as, for example, the neutralization since in the majority of the cases during the precipitation process of the pH of the effluent should be elevated for values more than 9.

CONCLUSIONS: The chemical precipitation is a relatively simple and economical method very used, however it generates a great volume of silt and can present residual metal concentrations in some cases above the standards demanded for the legislation being necessary the application of a complementary process for final scouring of the effluent.

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COLORIMETRIC ASSAYS APPLIED TO VEGETABLE AND AUTOMOTIVE LUBRICANT OILS BIODEGRADATION ANALYSIS

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Keywords: biodegradation; colorimetry; wastewater.

INTRODUCTION: The destination of both lubricant automotive oils and vegetable oils, and the knowledge about those substances environmental impact and persistence represent important data about the safety of releasing them in aquatic environments (Shogren et al., 2004). The objective of this study is to observe the biodegradation of automotive lubricant oils and vegetable cooking oils by *Bacillus subtilis* (CCT 2576), evaluated in a simulated wastewater medium using the DCPIP colorimetric technique quantification by spectrophotometry. The DCPIP is a substance biodegradation indicator.
MATERIAL AND METHODS: Colorimetry assays were made using a *B. subtilis* inoculum in tubes containing a saline medium (Bushnell-Haas) along with the DCPIP indicator and the oil subject to analysis (and the respective control assays) following the methodology proposed by Hanson et al. (1993). The biodegradation of different types of automotive lubricant oils (synthetic, semi-synthetic, mineral and used) and vegetable oils (new and used) was tested. The discoloration process is a property of the DCPIP indicator, and was used to evaluate by periodical analysis with spectrophotometry techniques the biodegradation occurrence.
RESULTS AND DISCUSSION: The colorimetry indicated that biodegradation happened during DCPIP loss of color pattern in the 600 nm specter. The control assays tubes kept the blue color, whereas the oil containing assays passed through biodegradation process. A general pattern in DCPIP change of color was observed in all the other types of oil, however, the biodegradation time necessary to the blue color completely vanish was different between the different types of oils. Also, the assays containing *B. subtilis* inoculum demonstrated a faster biodegradation compared to the assays without the inoculums which indicates a positive interaction of this microorganism strain in oil biodegradation, since all the inoculum containing assays yielded better results in form of a faster biodegradation. It is also important to remember that, even the non inoculated assays were supposed to biodegrade, since they already contained native oil microrganisms originally. The biodegradation sequence in ascendent order of total biodegradation time observed in the different types of oil was: synthetic oil (slowest biodegradation), semi-synthetic oil, mineral oil, used oil, vegetable oil and used vegetable oil (fastest biodegradation).
CONCLUSION: The results obtained in this study leads to a better handling of contaminated water medium such as industrial wastewater and can be applied to the development of new techniques in water treatment. From the experimental data discussed in this experiment, the used automotive lubricant oil and the used vegetable oil biodegradation process makes the microorganism's enzymatic mechanics action easier in the catabolic processing of the pollutants.
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COMPARATIVE STUDY BETWEEN BASIDIOMYCETES AND ASCOMYCETES FUNGI CAPACITY IN DYE OF REMOVING ACID BLUE 29

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Keywords: Fungi, azo dye, bisorption

INTRODUCTION: The Acid Blue 29 dye is classified as a diazo dye by presenting 2 azo groups (-N=N-) chromophores linking aromatic rings in its structure. These dyes have complex structural features that hinder its removal by conventional techniques that generate sludge class I, and therefore, require specific treatment techniques, capable of minimizing potential environmental risks (DULLIUS, 2004). As some microorganisms have a high metabolic versatility and are capable of degrading complex compounds such as dyes, this study aimed to compare the capacity of removal of dyes by three different species of fungi, *Phanerochaete chrysosporium* (basidiomycete) and *Aspergillus niger* and *Aspergillus oryzae* (ascomycetes).

MATERIAL AND METHODS: It was prepared samples containing 1mL of solution with 100µg/ml of "Acid Blue 29" dye, 8ml of water at pH 2.5 and 1ml of biomass into pellets of *P. chrysosporium*, *A. oryzae* and *A. niger*. The control solution was prepared with 9 ml of water at pH 2.5 and without biomass. The samples were incubated in the oven at 30° C and sweeps in the UV-VIS spectrophotometer were carried out after 30 minutes, after 24h and after 120h.

RESULTS AND DISCUSSION: After scanning in the UV-VIS spectrophotometer, the wavelength maximum of the acid groups of the dye chromophores Blue 29 was found in 602nm. On 1st analyze, 30 minutes after preparation of samples, the solution absorbance of control presented in 2,716, and the samples with *P. chrysosporium*, *A. oryzae* and *A. niger* showed absorbance of 2,535, 2,494 and 2,333, respectively. This result shows that the 1st contact between biomass and the dye solution has been biosorption of the dye by the fungi examined. After 24 hours of contact, there was another scan showing absorbance values of the following: 1,791 for *P. chrysosporium*, 1,641 for *A. oryzae* and 1,942 for *A. niger*. After 120h of contact, the absorbance was observed in 0,153 for *P. chrysosporium*, 1,072 for *A. oryzae* and 1,551 for *A. niger*. These values showed that the basidiomycete has a growing capacity to remove the dye, is the greater the time of contact between fungus and dye, was the largest removal. But the ascomycetes removed more dye in the first hours of contact and then decreased their capacity.

CONCLUSIONS: The results obtained by UV-VIS spectrophotometry showed that the first hours of contact, ascomycetes presented the ease in biosortion of molecules of dye. However, its ability to biosorption decreased after 120h. The basidiomycete removed a larger amount of color of the solution. This was, probably, because the metabolism of the *P. chrysosporium* was activated quickly, before the ascomycetes, releasing enzymes that biodegraded the dye. Thus, we find that the use of fungi for the removal of dyes can be used as an alternative method for cleaning of contaminated effluents and rivers.

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COMPARATIVE STUDY BETWEEN COMPOSTING AND SYNERGISTIC ACTION OF INTESTINAL FLORA AND EARTHWORMS IN THE STABILIZATION OF ORGANIC WASTE

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Keywords: vermicomposting, organic residue, stabilization.

INTRODUCTION

The use of microorganisms as a technique for stabilization of organic matter (OM) (compost) is an ancient practice. However the collaboration of earthworms during the degradation of OM is purely mechanical which results in a food cake with great surface contact when compared to the initial substrate. The stabilization of OM is promoted in the digestive tract of earthworms by its flora. At the end of the process, a product of not digested and not assimilated remains is expelled. This product along with the particles of soil constitute a fertilizer rich in humus and nutrients. This work had as main objective the comparison of substrates stabilized only with the action of microorganisms and stabilized through the synergistic action of microorganisms and earthworms, in order to determine which of the two processes has ameliorate the efficiency in the OM transformation or stabilization.

MATERIAL AND METHODS

Physical-chemical characterization of sewage sludge and vermicompost was performed by the following parameters: pH in CaCl2, total nitrogen Kjedahl (NKT) and phosphorous, by Hach method 399 and method 480, respectively, total organic carbon (TOC) via Shimadzu TOC-VOHC, cation exchange capacity (CEC), through the occupation of active sites to exchange with hydrogen ions in solution 1 mol l^{-1} of glacial acetic acid, OM content and humidity (U), by gravimetry. The functionality characterization was performed by infrared spectroscopy. Spectra were obtained in tablets of KBr, with about 0.50 mg of sample to 200.00 mg of KBr. All tests were performed in five replicates and the arithmetic mean and the standard deviation were determined.

RESULTS AND DISCUSSION

Analyzing the initial matrix, the compounds produced and vermicomposting, it was found that the physico-chemical properties of matrices after passing by the processes of composting and vermicomposting have changed, increasing the degree of humification of all initial substrate, with an increase in CECef, in TKN and in P, a decrease in C/N ratio, an increase in the ratio CECef/C, an increase in rates of hydrophobicity and aromaticity (which indicate a greater or lesser resistance to microbial degradation). These results show that both processes may be used in the stabilization of OM.

CONCLUSION

The process of symbiosis between earthworms and microorganisms proved to be ideal for stabilization of organic residues, because in the end of the process, it is obtained an organic fertilizer with a high amount of nutrients (in a short period of time), and another product can be marketed, the earthworms. For further studies, the isolation and identification of microorganisms in the digestive flora of earthworms is proposed.

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COMPARATIVE STUDY BETWEEN THE CAPACITY OF BIOSORPTION *Saccharomyces cerevisiae* LYOPHILISATE AND FRESH WITH THE AZO DYE DIRECT RED 23

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Keywords: *Saccharomyces cerevisiae*, biosorption, liophilisate cells, azodye

INTRODUCTION: The production of toxic products by industries is one of the most important issues in pollution control. Among these products are used in textile processing, which are varied, large and colorful. It is estimated that about 15% of world production of synthetic dyes is lost to the environment during its synthesis, processing and application. And besides the environmental pollution that cause these colors, another concern is the effect that these substances released into the environment can bring to human health because they are often mutagenic or carcinogenic. There is therefore a need to find methods that will help remove these pollutants from the environment.

MATERIAL AND METHODS: Two solutions were prepared with a mark of *Saccharomyces cerevisiae* Fleischmann, fresh in tablet and in concentrations ranging from 0.5 to 3.0 mg/mL the other 10% containing the lyophilized yeast, and at concentrations of 2.0 to 10, 0 mg/mL and both solutions were autoclaved for 15 minutes at 120 ^{O}C and then placed in contact with dye Direct Red 23 to a concentration of 100µg/mL and pH of 2.50 during a period of 2 hours at 30 ^{O}C. Then centrifuged at 3500 rpm for 10 minutes and the remaining dye determined spectrophotometrically in equipment Shimadzu model UV 2.401 PC between 190 and 800 nm. was made a correlation between biosorptive biomass and removed dye and shall estimate of the dye total of removing the solution

RESULTS AND DISCUSSION: The aim of this study was to evaluate the interaction biosorptive of *S.cerevisiae* autoclaved in two versions that are made in trade, fresh in tablets and yeast lyophilized. By data obtained shows that the cells come from the fresh yeast and autoclaved, had a capacity for total removal of the dye when the biomass reached 2.60 mg/mL, while the lyophilized and autoclaved cells had a lower in come, that is there is a need to 14.13 mg/mL to achieve the same result. The pH of 2.50 was chosen because it was described as a pH of high-capacity biosorptive for direct dyes.

CONCLUSION: The direct dye Direct Red 23, was well adsorbed by autoclaved biomass of S. cerevisiae prepared from fresh yeast while the yeast cells lyophilized and autoclaved has performed well below, that is, there is a need for a quantity of biomass approximately five times higher.

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COMPARISON OF BOTRYOSPHAERAN PRODUCTION BY SPECIES OF *BOTRYOSPHAERIA* IN THE PRESENCE AND ABSENCE OF SOYBEAN OIL AND TWEEN 80

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Keywords: Botryosphaeran, Beta-1,3-D-glucan, *Botryosphaeria rhodina, Botryosphaeria ribis*

INTRODUCTION: Botryosphaeran is an exopolysaccharide (EPS) of the β-(1,3;1,6)-D-glucan type produced by fungi of the genus *Botryosphaeria*; imperfect form *Lasiodiplodia theobromae*. This EPS when produced on glucose as carbon source presented 22 % side branching of β(1→6) glucosidic linkages, while on fructose 31%, and on sucrose 21%. Silva et al. (2007) optimized production of EPS by *B. rhodina* MAMB-05 by adding soybean oil and Tween 80 to the nutrient medium with glucose. Highest production was obtained on glucose 40 g/L (w/v), with soybean oil 10 mL/L (v/v) and Tween 80 4.5 g/L (w/v) added to Vogel minimum salts medium. The aim of this work was to compare the production of botryosphaeran by four *Botryosphaeria* isolates using glucose, fructose or sucrose as carbon sources under conditions previously optimized for *B. rhodina* MAMB-05.
MATERIAL AND METHODS: EPS production was compared for *B. rhodina* MAMB-05, *B.ribis* EC-01 (isolated from eucalypt tree) and two *Lasiodiplodia theobromae* strains isolated from (pinha, *A. squamosa*) and (eggplant, *Solanum* sp.). The cultures were developed in Erlenmeyer flasks at 28°C in shaken liquid submerged cultivation (180 rpm) on Vogel minimum salts medium (Vogel, 1956) during 3 days. The carbohydrate sources evaluated were glucose, fructose, and sucrose (40 g/L), resp., under the following conditions: *A)* Without addition of soybean oil and surfactant; and *B)* With addition of soybean oil (10 mL/L v/v) and Tween 80 (4.5 g/L w/v). EPS was precipitated from the extracellular fluid with 3 volumes of ethanol, then dialysed for 3 days against distilled water with several changes of water, and lyophilized and weighed.
RESULTS AND DISCUSSION: All of the *Botryosphaeria* isolates produced EPS, but in different amounts. The combined addition of soybean oil and Tween 80 enhanced botryosphaeran production under all of the culture conditions studied. Under condition A, *B. rhodina* MAMB-05 was the highest producer of EPS (1.9 g/L) when grown on glucose, while under condition *B*, the same fungal isolate produced 9.3 g/L when grown on sucrose. *B. ribis* produced less EPS under both conditions on all carbon sources evaluated. *L. theobromae* isolated from eggplant produced more botryosphaeran on fructose under *condition A* (2.0 g/L), as well in *B* (5.4 g/L). *L. theobromae* isolated from pinha produced more EPS on sucrose under *condition A* (2.2 g/L), while under condition *B,* glucose was best (5.6 g/L).
CONCLUSIONS: All fungal isolates produced botryosphaeran independent of the carbon source with or without adding soybean oil and Tween 80. *B. ribis* was the isolate which produced less EPS under both conditions, and on all carbon sources examined. The best EPS producer was *L. theobromae* isolated from pinha grown on sucrose in the absence of soybean oil and Tween 80. The best botryosphaeran producer in the presence of soybean oil and Tween 80 was *B. rhodina* MAMB-05 when grown on sucrose.
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CORRELATION BETWEEN *BRETTANOMYCES* SPOILAGE AND VOLATILE PHENOLS IN RED WINE

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Keywords: microbiological control, wine, *Brettanomyces*, aroma compounds, volatile phenols.

INTRODUCTION: *Brettanomyces* is a yeast genus responsible for wine spoilage. The metabolic products of *Brettanomyces* growth in wine lead to sensorial defects that compromise wine quality. Detection and control of this spoilage yeast is currently a major need for wine makers, mainly during the period of red wine ageing in wooden barrels (1). The aim of this work was correlating *Brettanomyces* population of contaminated red wines in presence of different concentrations of potassium metabisulphite, and the volatile composition of the red wines after a period of 20 months of ageing.

MATERIAL AND METHODS: Red wine was elaborated from c.v. Tempranillo local grapes with the indigenous *Saccharomyces cerevisiae* yeast and lactic acid bacteria strains. It was aged for six months in wooden barrels and the turbid fraction of wines ranging from 45 to 444 NTUs was mixed. Potassium metabisulphite was added to reach the following concentrations: 28, 50 and 100 mg/l of total metabisulphite. Experiments were performed in replicates (n=15) and wines were stored in glass bottles for 20 months. Wine samples were taken after 4 and 20 months and were spread on YPD-agar plates for total yeast counting, on modified DBDM-agar, Manitol-agar, and MRS-agar plates for specific *Brettanomyces*, acetic acid, and lactic acid bacteria counting respectively. After 20 months' storage the concentrations of wine volatile phenols were determined by gas chromatography-mass spectrometry as previously reported (2).

RESULTS AND DISCUSSION: Population levels for total yeast were in all cases < 500 cfu/ml, for *Brettanomyces* were in the range 0- 138 cfu/ml, acetic acid bacteria counts were < 10 cfu/ml, and no lactic acid bacteria were detected in the studied wines. Volatile phenol concentrations (mean values) ranged from 59 to 115 μg/l for 4-ethylguaiacol, from 2.4 to 4.2 μg/l for 4-propyl guaiacol, from 793 to 1495 μg/l for 4-ethylphenol, from 11.5 to 58.3 μg/l for 4-vinyl phenol, and 4-vinyl guaiacol levels were in all cases under detection thresholds (< 10 μg/l). After 20 months of storage viable *Brettanomyces* counts were obtained only in wine samples with the lowest metabisulphite levels (28 mg/l). The highest *Brettanomyces* populations were shown in wine samples with the lowest levels of metabisulphite that had been stored for 4 months (mean value of 43 cfu/ml). These samples showed as well the highest concentrations of 4-vinyl phenol and the lowest concentrations of 4-ethylguaiacol and 4-ethylphenol, as well as a 'mousy off-flavour' component.

CONCLUSION: The results reported in this study indicate that 4-vinyl phenol levels in the studied red wines directly correlated with viable *Brettanomyces* populations, and these were detected after 20 months of storage in wines that contained a low metabisulphite concentration (28 mg/l).

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CULTIVATION OF *Ceriporiopsis subvermispora* IMMOBILIZED IN POLYURETHANE FOAM FOR THE PRODUCTION OF LIGNINOLYTIC ENZYMES

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Key words: C. subvermispora, Manganese Peroxidase and Laccase.

INTRODUCTION: C. subvermispora, a white-rot fungus, has been applied in biopulping processes due to its high selectivity in the degradation of lignin, which allows the preservation of cellulose in the production of pulp and paper. When immobilized, the fungus is able to produce extracellular enzymes like manganese peroxidases (MnPs) and laccases (Lacs), able to oxidize phenolic and non-phenolic compounds (Bourbonnais and Paice, 1990; Bao et al., 1994). In order to produce such enzymes in a "clean" medium, which would facilitate their further purification and characterization, we evaluated the production of MnP and Lac by the fungus during immobilized cultivation in a medium with defined composition.

MATERIAL AND METHODS: The defined medium was prepared with glucose and ammonium tartarate, in agreement with Ruttiman et al. (1992). C. subvermispora was initially grown in 2% w/v malt-extract agar plate at 27 °C for 7 days. After this period, 3 discs of mycelium (0.8 cm of diameter) were taken from the edges of the colony and used to inoculate 125 mL Erlenmeyers flasks containing 12 cubes of polyurethane foam (1.5 cm of side) and 30 mL of medium previously sterilized. These flasks were incubated at 27°C for 21 days, with samples being collected periodically (3 days). The samples were analyzed regarding the production of proteins (Bradford), the consumption of reducing sugars (DNS), the growth of mycelium (dry weigth), and the production of MnP (phenol red oxidation) and Lac (ABTS oxidation).

RESULTS AND DISCUSSION: The reducing sugars concentration was maintained constant up to 15 days. Then, a steady consumption which almost reached 100 % at the 21st cultivation day began. An increase in the concentration of extracellular proteins was observed after the 9th cultivation day, the maximum concentration (0,08 ± 0,004 g/L) being reached after 21 days. The maximum activity of MnP (0.19 ± 0.07 UI/mL) was reached after 18 cultivation days, while that of Lac (0.004 ± 0.001 UI/mL) after 6 days. Very high concentrations of mycelium, inconsistent with the other experimental points, were detected at the 9th day of cultivation. Probably, this behavior was due to the heterogeneity in the quantity of biomass inoculated in each one of the different flasks used for the kinetic study.

CONCLUSIONS: The immobilized cultivation in defined medium showed to be appropriate for the production of MnP, with small expression of Lac activity. It was not possible to establish a consistent profile for the mycelial growth along the cultivation, wich may be related to the form used to inoculate the different flasks "sacrificed" as samples during the fermentation.

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Curvularia sp ISOLATED FROM DIFFERENT VARIETIES OF CORN (*Zea mays* L.) IN STATE OF PARANÁ, BRAZIL

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Keywords: endophytic fungi, *Curvularia* sp, *Zea mays*

INTRODUCTION: Corn (*Zea mays*) stands out as a culture of great importance in Brazil, mainly in the state of Paraná. Its main use is for animal feed, but in regions of low income represents most of the caloric intake of the human population (BRUNELLI, 2004). Demacious fungi, such as *Curvularia* sp, are described as plants pathogens, and in some cases, humans. However, these fungi, extracted from plants of *Garcinia* showed antifungal, antiviral and antioxidant activities (PHONGPAICHIT *et al.*, 2007), and indicate its possible use in control of plants diseases. The goal of this study was to isolate endophytic fungi from varieties of corn and to characterize the isolates of genus *Curvularia* sp using macro and micromorphology characters.
MATERIAL AND METHODS: Samples of leaves of corn were obtained from 10 varieties in the state of Paraná. Isolation was performed according to Pimentel (2001), and identification by macromorphology and microcultive (BLEVINS and KERN, 1999).
RESULTS AND DISCUSSION: Twenty five distincts groups of fungi were isolated, separated according to the colonial morphology characteristics. In these groups, the genus found more frequently was *Curvularia* sp (26,2%), whose exemplars were characterized. In plants, there are many examples of diseases caused by demacious fungi. However, in some cases, fungal growth and plant development can be in harmony, as in *Aegle marmelos correae*, where there was growth of *Curvularia* sp in healthy plants (GOND *et al*., 2007). Future studies will aim genetic characterization of isolates and investigation of possible role of *Curvularia* sp as endophytic in corn.
CONCLUSION: A great variety of morphological groups of fungi were isolated from differents varieties of corn, and *Curvularia* sp was the most frequent genus. Though this genus is associated to plant diseases, its occurrence as corn endophytic can be related to some kind of plant resistance against field pests.
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DETECTION OF COLIFORMS AND STECS IN LETTUCE

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Keywords: lettuce, coliforms, STECs

INTRODUCTION: Lettuce is the most consumed vegetable in Brazil wich ensure its considerable economical importance of culture[1]. *Escherichia coli* that are carried by food, result from the faecal contamination (human and animal)[2] and cause several frailty. The detection of *E. coli* is the test used in public health for the assessment of faecal contamination of water and food[3]. Vegetables such as lettuce has been linked to outbreaks of Shiga-toxin (VT)- *E. coli* (STEC) food-related infections[3]. These positive STEC bacteria are able to live in water for four months[4]. Hemorrhagic colitis and its complications are the main clinical manifestation of STEC, and than can be found in the faeces of various health animals and domestic cattle is the most important reservoir[3].
MATERIAL AND METHODS: Each sample is a lettuce purchased in the food trade of Campinas city, São Paulo state, and were analysed by Kornack & Jhonson method[5]. Of the same positive tube for faecal coliforms (FC), was sown in Mac Conkey agar for the isolation of *E. coli* and 500µl of this sample was centrifugated at 10000RPM/5 minutes. The pellet was diluted in ultrapure water for the extraction of DNA, as well as one scraped of each plate of MC. For the polymerase chain reaction (PCR) we used primers for VT1, VT2 and *eae*, and separated for eletroforese in agarose 2% and visualized in transiluminator UV.
RESULTS AND DISCUSSION: 42 samples were analysed; 14 of conventional tillage, 14 of organic tillage and 14 of hydroponic tillage. In 7 samples of conventional tillage the more probable number (MPN) for FC was higher than 10^2/g so at odds with ANVISA[6]. Organic and hydroponic MPN had not levels greater than 10^2/g. However, the analysis of total coliforms (TC) of all conventional samples, 8 organics samples and 2 hydroponics samples had levels over than 10^3/g. PCR assay disclosed VT2-toxin gene in 1 conventional sample dispite the counting of TC (460MPN/g) and FC (23MPN/g) are in accordance with the ANVISA laws. The STECs are able to survive in acid environments making low the number of bacteria it takes to cause infection (100 a 200 CFU)[3]. This sample is in accordance with the ANVISA laws relating to FC, but not secure whereas 90% of the analyzed colonies showed gene VT2.
CONCLUSION: Although the microbiological quality of lettuce meets the law, we need more attention with FC. The detection of the gene for VT2 makes this case in a problem of public health. Thus, lettuce should be free of FC and MPN's tolerance below 10^2/g must be reviewed.

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DETECTION OF ENZYMATIC ACTIVITY OF FILAMENTOUS FUNGI ISOLATED FROM SEWAGE SLUDGE OF THE TREATMENT STATION MANGUEIRA, RECIFE-PE, BRAZIL

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Key-words: sewage sludge, filamentous fungi, hydrolytic enzymes

INTRODUCTION: The filamentous fungi are extremely versatile and are responsible for the production of several enzymes (FRISVAD et al., 2008). Sewers Treatment Stations (ETEs) had been created to minimize the waters pollution and public health issues. However, the treatment stations produce the sewage sludge, which is a rich organic substance and nutrients residue, and also present an appreciable amounts of heavy metals. The sewer silt is an ideal environment for growth and esporulation of different groups of filamentous fungi. The microbial enzymes are a biotechnological product of great interest, and have importance and applications in the industries of food, pharmaceutical and cosmetic products (DEMAIN, ADRIO, 2008).

MATERIAL AND METHODS: The filamentous fungi had been isolated by using the Serial Dilution Technique of silt of sewer collected in the Mangueira Station, Recife-Pernambuco, Brazil. The identifications had been performed according to ALEXOPOULOS et al (1996) and HUNDER-CEVERA (1998). The hydrolytic enzymes in solid media (cellulase, urease and lipase) were performed by using the HANKIN and ANAGNOSTAKIS (1975) at different temperatures: 28^{o}C and 35^{o}C had been evaluated. The assays had been carried out by using triplicate replicas.

RESULTS AND DISCUSSION: In this work 10 isolates of filamentous fungi in the sewage sludge: 4 *Monotospora sp;* 2 *Penicillium sp;* 2 *Scedosporium sp*; 1 *Chrysosporium sp*. and 1 *Aspergillus sp*. Among the tested enzymes, lipase was determined in higher percentual compared to tested enzymes (90%). *Chrysosporium sp* presented activity for cellulase (17 mm) 35^{o}C and for lipase (10mm) for the both the tested temperatures, respectively.

CONCLUSIONS: The isolated filamentous fungi of sewage sludge present a biotechnological potential for hydrolytic enzyme production. Lipase was the enzyme which exhibited the highest e percentual 9(0%. Isolated of *Chrysosporium sp* demonstrated ability in producing cellulase and lipase in both the tested temperatures.

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DETECTION OF MYCOBACTERIA AND FREE-LIVING AMOEBAS IN GROUNDWATER SUPPLY OF THE CITY OF SÃO CARLOS (SP).

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Keywords: mycobacteria, free-living amoebas, water.

INTRODUCTION: Environmental mycobacteria are often isolated from water distribution systems, and the chlorine present in treated water eliminates certain micro-organisms, which can promote the growth of other resistant to it, as bacteria of the genus *Mycobacterium* and free-living amoebae. The interest in these amoebae is related to exercise that role as reservoirs of many micro-organisms. The water, thus a natural habitat for them, is one important vehicle for its dissemination.

MATERIAL AND METHODS: We collected 22 samples of "in natura" water from artesian well and 22 from water tanks of the SAAE (Serviço Autônomo de Água e Esgoto) which supplies the city of São Carlos. For the count of heterotrophic bacteria was used the R2A medium (APHA, 1998) and for coliforms, Chromocult Coliform Agar® (USEPA, 2001). To isolate mycobacteria, 1L of sample was filtered in sterile membrane of 47mm in diameter and 0.45µm pore size of which was macerated in conical tubes with phosphate solution buffer. The suspension went through decontamination with cetylpyridinium chloride and, after centrifuged, was inoculated in Middlebrook 7H10 agar. Colonies suspected to be mycobacteria were submitted to the Zihel-Neelsen staining. For the isolation of free-living amoebae 1L of sample was filtered by membrane, which was inoculated on plates with non-nutrient Agar medium containing *E. coli* killed by ultraviolet radiation.

RESULTS AND DISCUSSION: Of the 22 samples "in natura" analyzed, all showed up positive for heterotrophic bacteria and 3 (13.6%) exceeded the permissible value established by Brazilian Health Ministry MS nº 518/04, 500 CFU/mL. For the treated samples, 13 (59.1%) showed to be positive. None of the samples showed positive for total coliform and *E. coli*. We obtained the isolation of mycobacteria in 17 (77.3%) of artesian wells samples and 15 (68.2%) in SAAE tanks. For free-living amoebae, there was growth in 16 (72.7%) samples of artesian wells and 4 (18.2%) of SAAE tanks.

CONCLUSION: Based on the results, this water is not contaminated with coliforms been proper for human consumption. But the presence of the mycobacterium and free-living amoebas may represent a risk factor to imunocompromissed individuals, once this micro-organisms are resistant to common water treatments. Therefore, if that water won't be adequately addressed can lead to the risk for population.

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DETECTION OF MYCOBACTERIA IN GROUND WATER THAT SUPLIES THE CITY OF SÃO CARLOS - SP

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Keywords: Ground water, water microbiology, atypical mycobacteria.

INTRODUCTION: Water is an important vehicle to spread microorganisms in the environment. Contaminated or inadequately treated water can expose a large portion of the population to these microorganisms, which may be pathogens. There is no severe monitoring in locations where there is potential for contamination of wells. Furthermore, the absence of a precise water analysis doesn´t allow a reliable determination of risk to public health.

MATERIAL AND METHODS: One liter samples were collected in 21 artesian wells of the Autonomous Service of Water and Sewage (SAAE), in the municipality of São Carlos. The samples were concentrated on nitrocellulose membrane. The membranes were macerated in conical tubes containing phosphate buffer solution (PBS). Heterotrophic bacteria were counted in R2A Agar, as specified by APHA[1], and coliforms in *Chromocult* Agar, according to USEPA[4]. To isolate mycobacteria, the samples were decontaminated with 0.05% cetylpyridinium chloride. The sediment was inoculated Löwestein-Jensen and Middlebrook 7H10 agar. The tubes were incubated at 30° C and 37° C.

RESULTS AND DISCUSSION: Total coliforms were observed in 28.6% of the samples, which were considered inappropriate for human consumption, according to Conama's resolution[2] No. 20/86. *Escherichia coli* was not found in any of the samples, indicating that water was not contaminated by fecal bacteria. Heterotrophic bacteria counting exceeded the permitted values in 4 cases (19%), which were considered inappropriate for human consumption, according to the Ordinance 518/04[3].
Environmental mycobacteria were recovered at 48% of wells tested. There was predominance of slow-growing (82.1%) and pigment (75%) strains. Twenty eight isolates of mycobacteria were characterized: 16 colonies (57.1%) obtained in LJ medium and 12 (42.9%) in 7H10. Five of these strains grew only on LJ. Mid 7H10 showed a greater number of types of isolates per sample of water. Regarding the temperature of incubation, the isolated mycobacteria showed increased growth at 37°C (53.6%) than at 30°C (46.4%).

CONCLUSION: It may be observed that the waters are not considered properly treated and they could represent risk to population. So, it should be implemented different types of qualitative and quantitative assessment and monitoring appropriate in artesian wells.

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IV Simpósio de Microbiologia Aplicada
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DIVERSITY OF THE ARCHAEAL GENES 16S rDNA AND *amoA* IN MANGROVES FROM SÃO PAULO STATE

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Keywords: PCR-DGGE, 16S DNAr, qPCR

INTRODUCTION:
Mangroves are areas located at flooded tidal regions which are important in the exploration of microbial diversity. Member from the domain Archaea represent a considerable portion of the prokaryotic community in marine and terrestrial ecosystems, indicating that this domain might have a great role in the global biogeochemical cycles (Schleper et al 2005). The ammonium oxidation, at nitrogen cycling, is promoted in archaeas by the protein codified by the gene *amoA*. In this context, this work aimed to evaluate the diversity of archaea by the analysis of the genes 16S DNAr and *amoA* in different mangroves from the São Paulo State.

MATERIAL AND METHODS:
Samples from superficial sediment (0-30 cm) were collected in three distinct mangroves and in distinct points, in a trans-section area, from the sea to the land: *i)* mangrove in Bertioga contaminated with oil, *ii)* mangrove in Bertioga free of contamination *iii)* mangrove in Ilha do Cardoso (preserved mangrove). After the DNA extraction, samples were analyzed by PCR-DGGE and qPCR, using specific primers for the archaeal genes *amoA* and 16S DNAr. Additionally, clone libraries were constructed for these genes. The phylogenetic affiliations of the obtained sequences were performed by comparison with the matches at the *Ribossomal Data Project* and BLASTn. Later, making use of the software DOTUR (Schloss et al., 2005), it was possible to determine the number of OTUs in each library, and also to perform the rarefaction analysis and the determination of indexes of richness and diversity of species in each sample.
RESULTS AND DISCUSSION: A number of 62 clones of archaeal 16S rDNA were obtained, revealing that 54% and 46% belong the phyla Crenarchaeota and Euryarchaeota, respectively. The principal components analysis, based on the PCR-DGGE patterns for this group, presented little differences in the structure of archaeal communities, evidencing a group adapted to the saline conditions at mangrove from Ilha do Cardoso. The PCR-DGGE pattern for the gene *amoA* revealed a low diversity and variations among samples, while the sequences of this gene (40) formed a group distinct from those found in already studied environments. The quantification of the gene *amoA* showed higher values for samples collected in area with higher availability of oxygen.
CONCLUSIONS: The density and the diversity of communities and genes evaluated are determined by the environmental conditions found in distinct mangroves. However, variations within a mangrove affect directly the archaeal communities present in this environmentl
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EFFECT OF ORGANIC ACIDS ON THE GROWTH OF *Acidithiobacillus ferrooxidans*

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Keywords: jarosite, *Acidithiobacillus ferrooxidans* – LR, organic acids

INTRODUCTION: The bioleaching is a process that uses microorganisms in the recovery of metals from mineral sulfides. The main microorganisms involved are chemolithotrophic and acidophilic species of genus *Acidithiobacillus*, mainly *A. ferrooxidans,* which obtains its energy by oxidation of Fe^{2+}, besides reduced sulfur including insoluble metallic sulfides. During the bioleaching process it has been detected precipitates of ferric iron known jarosite. This precipitated can restrict the process efficiency by the formation of a passivation layer on the mineral surface, blocking bacterial action (1). An alternative to avoid this problem is the use of organic acids which form soluble complexes with ferric ion. However, it must be considered before, a possible inhibition of microorganism activity by action of those acids.
MATERIAL AND METHODS: The experiments were performed in triplicate using 100 mL shake flasks containing 50 mL of T&K medium (2) plus 0; 0.1; 0.25; 0.5; 1 and 2% (w/v) of the oxalic and citric acids. The flasks were incubated at 150 rpm and 30ºC with 5% (v/v) of *A. ferrooxidans*– LR. The bacterial activity was monitored every 12 hours by determination of residual Fe^{2+}.
RESULTS AND DISCUSSION:

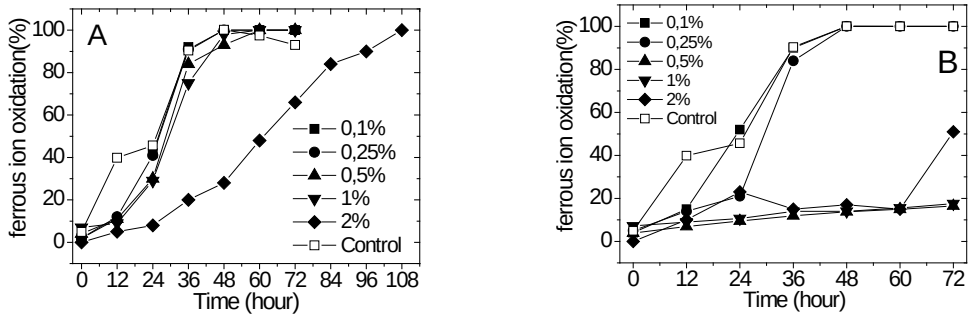

Figure 1: Effect of citric acid (A) and of oxalic acid (B) in Fe^{2+} oxidation by *A. ferrooxidans* - LR

It can be seen by Fig. 1A that citric acid didn`t inhibit cell growth when its concentration was 0.1 to 1%, but in presence of 2% there was extension of the “lag” phase of bacteria growth indicating partial inhibition. However, oxalic acid (Fig. 1B) in concentrations of 0.5 to 2% caused severe inhibition in the activity of *A. ferrooxidans* – LR. Even in 0.25% there was a delay in the “lag” phase during Fe^{2+} iron oxidation and only 0.1% did not cause inhibition.
CONCLUSION: The citric acid (0.1 to 1%) did not inhibit bacterial growth, whereas oxalic, even in low concentrations was inhibitory for *A. ferrooxidans* – LR. Therefore, in studies of bioleaching of metallic sulfides, citric acid can be tested to prevent jarosites precipitation.
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Financial support: CNPq, Companhia Vale.

EFFECT OF AQUEOUS EXTRACTS OF RUBIM, BASIL AND SPRING ON CONIDIA OF *BIPOLARIS SOROKINIANA*.

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Key-words: Rubim, Spring, Basil, *Bipolaris sorokiniana*.

INTRODUCTION: *Bipolaris sorokiniana* is the most serious diseases of barley (Agrios, 1988) affect the ears, darken the grains and impair the quality of malt and beer thus causing considerable losses in yield and quality. The aqueous extracts of rubim, basil and spring was been used as elicitor of resistance in barley plants against *Bipolaris sorokiniana*, that give protection at level ranged from 80 to 98% (Rodrigues et al, 2007). However, many of the elicitors can act as biological control that can be inhibiting the germination of conidia. In order to confirm the biological control, the objective of the present study was to evaluate the germination of conidia and the correspondent sporulation of fungi in culture medium with extracts.

MATERIAL AND METHODS: Plates of Petri of 5cm of diameter and 2 cm height received 5mL of medium potato-agar-dextrose (PDA) and 1mL of the extract solution at final dilution as: elicitor of rubim with 0,23mg of proteins, basil with 1,72mg of proteins and for spring 3,4 mg of proteins. Conidia from PDA with 10 days old were transferred for the same medium with extract, in total of six plates for each elicitor. The plates, thus prepared, had been placed in chamber type BOD with control temperature of 25^{o} C and at constant light. Diameter of the culture was average daily. After 10 days the conidia was removed from the substratum with 5mL of distilled water and 0.05% of Tween 20, filtered through gauze and counted in hematocitometer (Improved Newbauer 1/400 SQ. 1/10 mm deep ultraglides).

RESULTS AND DISCUSSION: The results demonstrated that the germination of the conidia occurred in all extracts as well as the growth tax varied from 0.86 to 0.95, agreeing to the control plate. In the tenth day, variation of 1.98 to 2.1 conidia/mL was carried through the counting of conidia but was equal the control plate without statistics difference.

CONCLUSIONS: The aqueous leaf extracts of rubim, spring and basil, don't presented the effect of biological control on the conidia of *Bipolaris sorokiniana* but has activity as elicitor of resistance in plants.

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EFFECT OF CAPTAN TS ON ENZIMATC HYDROLISIS AND ALCOHOLIC FERMENTION OF SORGHUM (*Sorghum bicolor* L. Moench).

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Keywords: Starch, Enzymes

INTRODUCTION: A seed infected by microorganisms usually has poor germination.This effect on germination may be beginning during the seeds development and maturation or during storage and post harvest, and even that environmental conditions are favorable. The fungi are the main responsible for the seeds deterioration.To avoid the seeds losses products are available for treatment of seeds. A product widely used in Brazil is the CAPTAN TS the trade name of N-[(trichloromethyl) thio]-4-cyclohexene-1,2-dicarboximide a not systemic fungicide belonging to the chemical group Phthalimide.Starchy raw material is widely and commercially used for good quality alcohol production. Many companies need to use fungicide treated seed for alcohol fermentation. There are doubts if the presence of this fungicide may affect the enzymes and yeast. The purpose of this study is to evaluate the effect of CAPTAN TS used as fungicide on sorghum (*Sorghum bicolor* L. Moench) seeds on enzymatic hydrolysis and alcoholic fermentation.
MATERIAL AND METHODS: The experiments were performed in the Center of Technology for Agribusiness, Mato Grosso do Sul, Brazil using a single batch 10 kg sorghum seeds, trademark Biomatrix treated with Captan 759 TS which gives a pink color to the grains. Seeds washing with water (5 times) take out the pink color. Washed and not washed seeds were prepared for hydrolysis by grinding. The granulometry was established by using sieves between 4.000 and 0.425mm with a bottom >0.045 mm. The grounded grains were used to prepare the worth 50% of sorghum grain in water by enzymatic process.The amount of Novozymes® enzymes was calculated on the sorghum starch content (30% by dry weight): SacchZyme (0.7 ml/kg) and AMG (2ml/kg). The worth was adjusted to pH 4.5 and 12° Brix with chemically untreated water and inoculated with 1% of fresh yeast. The fermentation was performed at room temperature (25 to 30^{o}C) and followed by Brix on hand refract meter, and reducing sugar (RS) and total reducing (TRS) were expressed as g.l^{-1}. At the end of fermentation alcohol content was determined by distillation and read with alcoholmeter. To evaluate the effect of fungicide on the growth of yeasts treated sorghum seeds were incubated in Petri dishes containing malt extract medium agar inoculated with 1% aqueous suspension of fresh yeast at 25^{o}C.
RESULTS AND DISCUSSION: The sorghum seeds were germinated in 3 days in the agar medium but molds not occurred proving the effect of the fungicide. There was no halo formation what will be considered as an indicative of inhibition of growth of yeast by fungicide effect of Captan 759 TS. The values of Brix, RS and TRS (g.l^{-1}) obtained with washed seeds were 17.5, 37.81 and 42.73 for AMG and 17.1, 28.33 and 29.59 for SacchZyme. For non washed seeds were 16.6, 36.91 and 40.82 for AMG and 17.3, 28.33 and 29.59 for SacchZyme. Residual RS and TRS as were similar for all but the alcoholic degree were higher (5 and 6 GL) for fermentation on non washed seeds than for the washed seeds (5 and 4 GL).
CONCLUSION: The results showed that under the tested conditions the fungicide Captan 759 TS did not affect the growth of yeast, the enzymatic hydrolysis or the alcoholic fermentation.
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EFFECT OF DIBENZOTIOFENO-DBT ON THE KINETICS OF GROWTH OF *Pseudomonas fluorescens*

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Palavras-Chave: DBT*, Pseudomonas fluorescens,* growth

INTRODUCTION: The automobile fuels have in its constitution heterocyclic aromatic compounds containing nitrogen atoms, mainly oxygen and sulfur, but consists basically of paraffinic hydrocarbons, olefins and aromatics. The thiophene compounds, such as DBT, are refractory to chemical desulphurization processes, and therefore a model compound for studies on the behavior of microorganisms in the biodessulfurização (WEBER and CORSEUL, 1994, and CORSEUL ALVAREZ, 1996, CARO et al., 2008). This study investigated the profile of growth of *P. fluorescens* against various concentrations of DBT, considering its potential for growth in a wide range of organic substrates.

MATERIAL AND METHODS: *Pseudomonas fluorescens* UPC1514 isolated from mangrove sediments of the Rio Formoso, Pernambuco, belongs to the collection of cultures of the Center for Research in Environmental Sciences, and maintained in nutrient agar and lyophilized in lactose at 5. C. The culture grown "overnight" in 50ml of nutrient broth, under orbital agitation of 150rpm, temperature 37 ° C, corresponded to 106 CFU / ml, and used as pre-inoculum. Then 1mL of pre-inoculum was transferred to the vials of with capacity of 250ml Erlenmeyer flasks containing 50ml of the same medium containing different concentrations of DBT (0.5, -1,0-2,0 mM) kept under agitation of 150rpm, the temperature of 37 ° C. Every 4 hours aliquots were removed for determination the number of viable colonies to complete 48 hours of growth. The growth was performed by the technique of "Pour plate, the pH, consumption of carbon source and activity of emulsification. The kinetic parameters of the maximum time of growth and generation were determined by Pirt (1975).

RESULTS AND DISCUSSION: In control and treated was observed the same behavior in exponential phase after 8h of incubation, except for 2.0 mM. However, the phase of decline occurred at 28 h. The maximum speed of growth was 0.27 h, with time of generation of 2.56 h. Treatment with 0.5 mM DBT showed a growth rate of 0.06 h and time to generate 11.55 h. The stationary phase occurred after 44h of growth, to decline from 48. Treatment with 1.0 mM DBT speed of growth was 0.38he time of generation from 1, 82h, to decline from 36h. Treatment with 2.0 mM DBT the exponential phase has been extended to 12pm until 24h. The maximum speed of growth was 0.05 h, with time of generation of 13.86 h. Stationary phase occurred from 28 to 32h, with decline from 36h of incubation. There was the formation of emulsification in concentration with 1.0 mM DBT. The data obtained are corroborated by Caro et al. (2008) informations.

CONCLUSIONS: The growth of P. fluorescens in DBT did not cause a significant inhibition, observing the formation of emulsifier in the use of DBT as a substrate, whereas no other source to enable its

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EFFECT OF METAL IONS, DETERGENTS, ORGANIC SOLVENTS AND DIFFERENT SUBSTRATES IN THE ACTIVITY AND STABILITY OF THE LIPASE OF *TRICHODERMA* SP.

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Keywords: lipases, *Trichoderma sp.,* characterization

INTRODUCTION: Lipases (EC 3.1.1.3) catalyse hydrolysis or synthesis of long-chains acylglycerols and due to their biocatalysts properties there is great interest in the industrial applications of these enzymes. The genus *Trichoderma* is constituted of saprophytic soil fungi that parasitize a range of other fungi, including phytopathogens ones**.** Although lipases could be wrapped in this infection mechanism, there are few references in the literature on lipases produced by this genus, so its study can be useful for industrial applications and also for understanding the fungi infection mechanism. The main purpose of this work was to determine some biochemical characteristics such as stability to the organic solvents, effect of ions, detergents and different substrates in the enzymatic activity of lipase of *Trichoderma sp.*

MATERIAL AND METHODS: The microorganism was cultivated during 48 hours in medium containing mineral salts, 0.5% (m/v) yeast extract and 1% (v/v) olive oil. In the filtered medium it was added ammonium sulphate to 80% saturation followed by dialysing it with 0.02mol/L pH 7 phosphate buffer. The enzymatic activity was determined by spectrophotometric method using different *p*-nitrophenyl esters and by for titulometric method using triacylglycerols. The effect of metal ions and detergents was determined by incubating it during one hour at 30^{O}C in the presence of salts and EDTA to 1mmol/L and detergents at 0.01; 0.1 and 0.5% (m/v) concentrations. The stability in organic solvents was determined by incubating it in hydrophylic solvents with its concentration ranging from 25 to 100% (v/v) for one hour at 30^{O}C each. In the case of butanol, toluene, hexane, isooctane and n-heptane, the crude extract was adsorved in Whatman filter paper no.4, in 1 cm^{2}, during one hour at 30^{O}C and then transferred to tubes with 1 mL of 0.02 mol/L pH 7,0 phosphate buffer. The activity was determined by the hydrolysis of *p*-nitrophenyl palmitate (pNPP).

RESULTS AND DISCUSSION: It was observed that the hydrolytic activity increased in the presence of Ni^{+2} and Na^{+}, none of the ions tested tested promoted decrease superior to 37% and even with Hg^{+2}, an denaturing agent, it was obtained 78% of residual activity. The addition of EDTA reduced 31% in the initial activity, while the addition of 0,1% (m/v) SDS lead to reduction of 92%, and the use of Triton X-100 and Tween 80 this reduction was not larger than 30%. The activity was stable for one hour in a presence of 25% of isopropanol, methanol, acetone and ethanol, when observating the residual activities at 97, 95, 86 and 85%, respectively. However, in hydrofobic solvents with n-heptane, the initial activity recovery was below 30%. When different *p*-nitrophenyl esters were used as substrate such as acetate (2:0), caproate (6:0), caprate (10:0) and palmitate (16:0), the activity was higher with the larger acyl chain substrate. Similar efect was observed for using the triacylglycerols such as triolein (18:1 (Δ^{9})), tricaprylin (8:0), tributyrin (4:0). This behavior was expected for true lipases.

CONCLUSION: The fungi *Trichoderma sp* produces a true lipase, with potential use in biocatalysis due to its enzyme stability in organic solvents, used for example in transesterification reactions for the production of biodiesel.

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EFFECT OF TEMPERATURE PRODUCTION ON XANTHAN GUM VISCOSITY

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Keywords: Xanthan gum, viscosity.

INTRODUCTION:

Bacteria of the species *Xanthomonas campestris* produce xanthan gum, an extracellular heteropolisacharide, water soluble, with high molecular weight and unique properties that differ from other gums. The xanthan gum is a biopolymer widely used as a thickener, jelling, emulsifier and stabilizer.

MATERIAL AND METHODS: *Xanthomonas campestris pv*. *campestris* FCLA 26 (from the collection of cultures of the Faculty of Sciences - UNESP Assis) produced xanthan gum at six different temperatures (23, 25, 27, 29, 30 and 31 ° C). The biopolymer produced were analyzed for the quality.

RESULTS AND DISCUSSION: The gum produced at 25 ° C obtained the gum produced higher viscosity at 30 ° C, temperature currently suggested by the literature for the strain NRRL-B-1459 (Viscosidade25 º C, 20rpm cP = 476.46; Viscosidade30 º C, 20rpm = 217.1cP). The concentration of gum was higher for the production temperature of 30 ° C (25 º C concentration = 9.53 g / L, 30 º C = concentration 12.81 g / L).

CONCLUSION: In order to produce a gum with better quality, it is concluded that the best temperature is 25 ° C.

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EFFECT OF THE ADDITION OF BUTANOL ON THE BIODEGRADABILITY OF GASOLINE

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Keywords: biofuel; biodegradation; biobutanol.

INTRODUCTION: Biobutanol, produced via fermentation of agricultural raw materials, can be utilized as a gasoline extender (Ezeji et al., 2007). With the commercialization of butanol or the butanol/gasoline blend, environmental damages due to spills can occur. The clean-up of these contaminated areas can be achieved with bioremediation, a technology based on the action of microorganisms, which has the advantage of turning hazardous contaminants into non toxic substances such as CO_2, water and biomass. Thus, bearing in mind the use of biobutanol in the near future as a gasoline extender and due to the lack of knowledge of the effects of butanol on the biodegradation of gasoline, this work aimed to evaluate the aerobic biodegradation of blends of gasoline with butanol and ethanol (20% v/v).

MATERIAL AND METHODS: Two experimental techniques were employed, namely the respirometric method (Bartha& Pramer, 1965) and the redox indicator DCPIP test (Hanson et al., 1993). In the former, an experiment simulating soil contamination (addition of 50 mL of fuel / Kg of soil from a non-contaminated site) was carried out in biometer flasks (250 mL), used to measure the microbial CO_2 production. The flasks were prepared in triplicates (50 g of soil) and incubated in the dark at 27^{o}C for 150 days. The DCPIP test assessed the capability of four inocula to biodegrade the blends considered in the respirometric experiment: *Pseudomonas aeruginosa* LBI; *Candida vismanathii*; consortium 1 (obtained from the soil); consortium 2 (obtained from Atibaia River). Inocula (0.2 mL, concentration not determined) were added to test tubes (duplicates) that contained sterile Bushnell-Hass (BH) medium (10 mL) and 1 % (v/v) of the blends. The concentration of DCPIP was 0.14 mg/mL.

RESULTS AND DISCUSSION: The CO_2 productions in the treatments with the butanol/gasoline blend and butanol were the same, while the CO_2 production for ethanol is 73% higher than that of the ethanol/gasoline blend. In the former the substitution of 80% of butanol by gasoline, which is less biodegradable, did not alter the biodegradation rate. It may suggest that butanol better enhanced the biodegradation of gasoline than ethanol. The results of the redox indicator experiment show that neat gasoline can not be biodegraded by the tested inocula. The biodegradation of ethanol for most of the cases started more promptly than butanol. Therefore, as verified in the respirometric experiment, the following order of biodegradability was found: ethanol>butanol>gasoline. In relation to the blends, with the exception of inoculum 2, the inocula achieved better results on the butanol/gasoline blend.

CONCLUSION: The addition of butanol to the gasoline resulted in positive synergic effect on the biodegradation of the fuel. Furthermore, results suggest that, butanol better enhanced the biodegradation of gasoline than ethanol in soil.

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Financial support: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (2007/07049-4) and ANP (PRH-05).

ENDOPHYTIC BACTERIAL COMMUNITY IN AXENIC PINEAPPLE MICROPLANTS

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Keywords: endophytes, DGGE, micropropagation

INTRODUCTION: The *in vitro* culture is based on axenic propagules, however the natural association between endophytic bacteria and microplants seems to be more common than it is reported, and it is not completely eliminated in the process of micropropagation. Aiming to demonstrate the omnipresence of endophytic microorganisms in plant cells, the objective of this work was to study the structure of the endophytic bacterial communities in micropropagated pineapple plants [*Ananas comosus* (L. Merrill) cv. IAC Gomo-de-mel].
MATERIAL AND METHODS: DNA was extracted from surface-disinfested roots, young and adult leaves collected from axenic pineapple microplants. Amplifications of 16S rRNA gene were made for the *Bacteria* domain and for *Actinobacteria*, α- and β-*Proteobacteria* groups. Products of amplifications were used as template in nested-PCR with the U984⊥GC and r1378 primers. The 16S rRNA gene fragments were separated using the INGENY phorU-2 system (Ingeny, Netherlands), using 6% polyacrylamide gels containing a linear denaturant gradient from 40-60%, at constant temperature of 60ºC for 4 h at 200V. The DGGE profiles were analyzed using the software program BioNumerics (Applied Maths).
RESULTS AND DISCUSSION: The hierarchical clustering procedures from the DDGE profiles were defined using Pearson correlation coefficient and revealed different structures of bacterial communities colonizing the whole body of the microplants. These results corroborate with studies that mention the omnipresence of covert endophytic bacteria from several *in vitro* cultures (Thomas et al., 2008). The cluster analysis of specific bacterial groups indicated differences between the structures of *Actinobacteria*, α- and β-*Proteobacteria* communities for the different examined organs and stages of development. These variations confirm that different bacterial groups perform different colonization in the body plant. Furthermore, the different colonization between young leaves and mature leaves indicates a population dynamics related to factors of plant metabolism, altered during the plant development (Baudoin et al., 2003). These changes indicate the occurrence of bacterial species succession colonizing actively the body of the microplants.
CONCLUSION: This study brings together evidences that pineapple microplants, characterized as axenic have an endophytic bacterial community composed of members of *Actinobacteria*, α- and β-*Proteobacteria* groups.
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EVALUATION OF ANTIOXIDANT ACTIVITY OF EXTRACTS FROM *ASPERGILLUS TERREUS* OBTAINED IN DIFFERENT CULTURE CONDITIONS

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Keywords: Antioxidant, *Aspergillus terreus*, secondary metabolites.

INTRODUCTION: In recent years there has been a growing interest in the discovery of the natural antioxidants due to the possibility of large-scale production at a cost lower than the chemical synthesis. Antioxidants may prevent damage caused by free radicals, which in excess are associated with various diseases such as Alzheimer and Parkinson. Fungi have been reported to be producers of secondary metabolites with broad range of biological activities. In early studies, we show that the melanin extracted from *A. nidulans* presents antioxidant activity for biological oxidants, as HOCl and H_2O_2 (GONÇALVES & POMBEIRO-SPONCHIADO, 2004). Studies in our laboratory also showed that the growth conditions affected the chemical profile of crude extracts obtained from 57 marine-derived fungal strains (VITA-MARQUES et al., 2008). In this work, we evaluated the influence of the culture medium and phase of fungal growth in the production of antioxidant metabolites by *A. terreus.*

MATERIALS AND METHODS: *A. terreus* (10^5conidia/mL) was cultivated in Sabouraud Dextrose Agar during 12 and 20 days or in artificial sea complete medium for 5 days, on orbital shaker (250rpm) at room temperature. Each culture broth was separated from mycelium by filtration under vacuum and submitted to three times partition with ethyl acetate and butanol, in sequence, and afterwards they were recovered by evaporation. The mycelia dried at 40ºC overnight was triturated and extracted for 30min with methanol under ultrasonically agitation. The resulting material was kept in the dark, for 5 days, a process repeated 3 times in order to complete the extraction of intracellular metabolites. The organic solvent was evaporated under airflow. All dried crude extracts were assayed for their antioxidant activity using ABTS (2,2'–azinobis-(3-ethyl-benzothiazoline-6-sulfonic) assay.

RESULTS AND DISCUSSION: With regard to Sabouraud's medium, the intracellular (MeOH) and extracellular (EtOAc and BuOH) extracts, obtained after 12 days of growth, exhibited lower antioxidant activity than those cultivated for 20 days, when the fungus is in stationary phase. These results are in accordance with literature because they showed that production of active metabolites is associated to growth phase. To evaluate the effect of the composition of medium in the production of antioxidants, the fungus was cultivated in artificial sea complete medium by 5 days, which corresponds to the stationary phase. In this condition, all obtained extracts showed the lowest antioxidant activity, while the biomass produced was 2-fold greater compared with Sabouraud's culture. This result can be explained by the fact that in the rich medium, as Marine Agar, the fungus uses the nutrients to produce biomass instead of active compounds, i.e., there is a inverse correlation between growth rate and production of secondary metabolites.

CONCLUSION: The results of this work showed that the variation of culture conditions may alter the production of active metabolites and the most promising antioxidant activity was obtained when *A. terreus* was cultivated on Sabouraud Agar for 20 days.

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EVALUATION OF *Burkholderia* spp. CHARACTERISTICS ASSOCIATED TO PLANT GROWTH PROMOTION AND CONTROL OF DESEASE IN SUGARCANE

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Keywords: indole-acetic-acid, plant growth, siderophores.

INTRODUCTION: Sugarcane (*Saccharum* spp.) occupies a prominent position among the crops with economic importance, especially in Brazil, the largest producer in the world. Recent studies, about the endophytic bacterial community associated with this culture, have been showing that several bacteria genus with plant grow promotion and control of disease potentials can be found. In this context, we must consider the *Burkholderia* genus with approximately 30 species, where some of then had been describe as plant hormones producer, inorganic phosphate solubilizing, nitrogen fixation and antagonists of pathogens (Coenye & Vandamme, 2003). This study aimed to evaluate the characteristics of 39 sugarcane *Burkholderia* spp. isolates, trying to establish parameters to be used in future studies associated to the biotechnology usage of this isolates.
MATERIAL AND METHODS: It was used 39 bacteria identified as *Burkholderia* spp., which ones 19 were isolated from rhizosphere and 20 from sugarcane root, as endophytic. It was determined, using specific methodologies, the potential of nitrogen fixation (NF) (Döbereiner et al. 1995), the production of plant growth hormone indole-acetic-acid (IAA) (Bric et al., 1991) and siderophores (SP) (Schwyn & Neilands, 1987), the inorganic phosphate solubilization activity (IFS) (Verma et al., 2001) and the antagonism potential against sugarcane pathogens: *Fusarium verticillioides* (Paired Culture Method) and *Xhantomonas albilineans* (Agar Underlayer Method) (Pugsley & Oudega, 1987, Gross & Vidaver, 1990).
RESULTS AND DISCUSSION: The obtained results show that, among the evaluated isolates, 87.2% were positive for NF, 100% were positive for production of IAA, 64.1% were positive for SP, 100% were positive for IFS, 97.4% of isolates were able to inhibit *F. verticillioides* and 74.4% to inhibit *X. albilineans*.
CONCLUSION: This study show the potential of *Burkholderia* bacteria genus in the production of compounds associated to plant growth promotion and pathogens inhibition, especially for sugarcane pathogens as *F. verticillioides* and *X. albilineans*. Future studies associated with specific methodologies for the practical usage of these isolates can be developed, aiming the production of bio-product, which ones show as a highly feasible in the current sustainable agriculture.

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EVALUATION OF HYDROLYSIS SUGAR CANE BAGASSE FOR FILAMENTOUS FUNGI

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Keywords: Sugar Cane Bagasse, Fungi, Hydrolysis

INTRODUCTION: Brazil, considered the greatest sugar cane producer in the world, presents a favorable potential to the biomass generation, as sugar cane bagasse (SCB), which could be used as industrial raw material for production of ethanol. This conversion includes two processes: hydrolysis of cellulose to soluble sugars and fermentation of these sugars to ethanol (PANDEY et al., 2000). One of the methods currently applied for the obtaining of the fermentable sugars is the acid hydrolysis, which uses acid diluted and high temperature. However, this treatment produces furfural that is toxic to many micro-organisms and the residual acid must be neutralized. In this sense, the enzymatic hydrolysis, carried out by celullolytic enzymes produced by microorganisms, reveals more advantageous because it is usually conducted at mild conditions, does not produce toxic compounds and does not have a corrosion problem. However, the commercial cellulase is an expensive product, which makes this process impractical in large scale (HAMELINCK et al., 2005). Thus, the objective of this work was to evaluate the production of cellulolytic enzymes in various filamentous fungi using Sugar Cane Bagasse (with and without acid pretreatment) as substrate.

MATERIAL AND METHODS: The fungi used were: *Aspergillus nidulans, Aspergillus niger and Trichophyton terrestre.* For fermentation in solid state, 10g of dry and triturated SCB was treated with 100 mL of distilled water or 100 mL of HCl 2.5% and autoclaved for 30 minutes at 122º C. After this, the SCB was inoculated with fungal mycelium or suspension of spores and incubated for 15 and 30 days at room temperature**.** After these periods, 100 mL of distilled water was added in each cultures and agitated (150rpm) during 60 minutes. In the crude extract, obtained after filtration, were analyzed Total Sugars (TS), Reducing Sugars (RS), Phenol (Phe) and Cellulase Enzyme (CE).

RESULTS AND DISCUSSION: The results with SCB without treatment showed that after 30 days the enzymatic activity was higher compared with 15 days of growth for all fungi tested, resulting in an increase in the amount of TS, RS e Phe. In this condition, the *Trichophyton terrestre* fungus showed the highest cellulase activity. Using acid pretreated SCB observed a 50% increase in enzymatic activity in all the fungi cultivated by 15 days as compared to the untreated SCB. However, the acid**-**hydrolyzed SCB did not showed advantage for the production of enzymes due to large amount of phenol released, which can act as inhibitor of alcoholic fermentation. The results also showed that the enzymatic activity did not differ significantly between the SCB inoculated with suspension of spores or fungal mycelium.

CONCLUSION: Therefore, the results of this work suggest that *Trichophyton terrestre* fungus seems promising for the production of cellulase using SCB without pretreatment, after 30 days of cultivation.

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EVALUATION OF THE PLASMID pYGFP3 EFFECT UPON YEAST STRAIN X2904-3C IN ALCOHOLIC FERMENTATION

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Key words: ethanol**,** GFP, ADH2

INTRODUCTION: The great deal about ethanol is the fact it is a low cost bio-fuel, less pollutant and from a renewable source. Besides that, contributes to the country economy, avoiding dependency on petroleum and international prices. New Technologies as metabolic engineering are being used to produce ethanol with higher yield and lower costs. This work aimed to evaluate the use of GFP3 protein, associated to the ADH2 as a fermentation ending marker and to evaluate its effects during fermentation process. Such mechanism is possible because ADH2 enzyme promoter is expressed when there are low sugar levels (lower than 2%).

MATERIAL AND METHODS: pYGFP3 PLASMID(GFP3, $TRP1^+$, AMP) (Gomes, 2000) constructed from pYADE4 plasmid, yeast strain X2904-3C (met^-,ura^- $trp1^-$) transformed according to Dohmen et al. (1991), and the transformed strains isolated in minimum culture media, without trp. Ethanol production was done in five fermentation assays and three replicates, using YEPS culture media. Sugar analysis were performed according to Somogyi & Nelson method and alcohol analyzed by distillation and densimetry (Anton Paar DMA-45 digital densimeter).

RESULTS AND DISCUSSION: Transformation was done and the GFP3 expression in X2904-3C yeast strain was modulated by the ADH2 promoter activity, which is expressed at low sugar amounts in the culture media. Fermentation assays demonstrated the transformed X2904-3C yeast strain with the pYGFP3 plasmid produces 15 % more alcohol than non-transformed X2904-3C strain. Such effect can occurs by the competition for ADR1 protein by the plasmid ADH2 gene promoter in the cytoplasm with the genomic ADH2 gene promoter from chromosome VIII, which is the transcriptional factor for ADH2 enzyme. Once the plasmid is in the cytoplasm, it has preferential conditions for the ADR1 enzyme capture, which explains the GFP3 enzyme expression by the end of alcoholic fermentation and the low alcohol intake by the transformed strain in comparison to the non-transformed strain.

CONCLUSIONS: The study of blocking metabolic chains linked to alcohol production and the association to GFP gene as a marker for promoter's activity, representing an improvement on yeast genetic breeding.

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Evaluation of the production of ligninolytic enzymes by *Ceriporiopsis subvermispora* in submerged culture

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Keywords: manganese peroxidase, laccase, *Ceriporiopsis subvermispora*

INTRODUCTION: In nature, the most efficient microorganisms in wood biodegradation are the white rot fungi. The basidiomycete *Ceriporiopsis subvermispora* is an example, which shows high selectivity in the degradation of lignin and, for this reason, is considered for application in the process known as biopulping (Akthar et al., 1998). Recent studies have shown that this fungus produces two major extracellular enzymes associated with the depolymerization of lignin, manganese peroxidase (MnP) and laccase (Lac) (Vicentim and Ferraz, 2007). This work intended to assess the production of such enzymes in defined and complex media, both in submerged cultivation.
MATERIAL AND METHODS: The complex medium was composed of 2.4% w / v potato dextrose extract supplemented with 0.7% w / v yeast extract, while the defined medium was prepared with glucose and ammonium tartrate, according to Ruttiman et al. (1992). The fungus was grown on malt extract agar (2% w / v) for 7 days at 27 ° C. After this period, 3 plugs of mycelium (0.8 cm diameter) were removed from the edges of the colony and inoculated in 125 mL-Erlenmeyer flasks containing 30ml of the specific, sterilized culture medium. The flasks were incubated at 27 ° C under agitation (180rpm) for 21 days, samples being taken every 3 days for analysis of the consumption of reducing sugars (DNS), cell growth (dry weight of mycelium) and production of MnP (oxidation of phenol red, ε610nm = 22,000 M-1.cm-1) and Lac (oxidation of ABTS, ε420nm = 36,000 M-1 cm-1).
RESULTS AND DISCUSSION: According to the data obtained, it was found that the highest production of MnP in the defined medium (0.23 ± 0.32 IU / mL) and in the complex medium (0.08 ± 0.11 IU / mL) occurred on the 12th day of cultivation, time when the consumption of reducing sugars in both media reached near 100% and the concentrations of biomass approached their maximum values (0.0014± 0001g/mL and 0.0057± 0.0 g / mL, respectively). The h activities of Lac were 0.07 ± 0.04 IU / mL and 0.08 ± 0.01 IU / mL for the defined and complex media, respectively, both at 21 days. Large standard deviations were observed for the enzymatic activities.
CONCLUSION: It is believed that the form of inoculation with plugs of mycelium grown on solid medium may have contributed to the excessive deviations observed. Nevertheless, it is inferred that the complex medium favors the production of Lac, while the defined one that of MnP.
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FORMATION OF BIOFILMS FOR *LISTERIA MONOCYTOGENES* IN DIFERENT TEMPERATURES AND TIME OF TACK

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Keywords: *Listeria monocytogenes,* Biofilms*,* Dairy

INTRODUCTION: *Listeria monocytogenes* is a food-borne pathogen capable of forming biofilms, whose control is important in food industry. This study evaluated the influence of time and temperature on the formation of biofilms by *L. monocytogenes* on the surface of stainless steel
MATERIAL E MÉTHODS: Was used an experimental model, where the stainless steel was cut into chips of 10 cm^2 (swab) and 5 cm^2 (microscopy). These chips were immersed in a beak with UHT milk and TSB-YE artificially contaminated with a suspension of 10^8 CFU/mL of *L. monocytogenes*. The stainless steel surface chips remained for a period of ten hours under constant agitation at a temperature of 35ºC, for adhesion of the strains on the stainless steel surface chips followed by incubation at different times (18h and 5 days) and temperatures (5 and 35ºC) for the formation of biofilms. The viable counts of cells were determined by plating on TSA-YE and analyzed by scanning electron microscopy (SEM).
RESULTS AND DISCUSSION: We observed that the survival of *L. monocytogenes* on stainless steel was higher at 5°C than 35°C both in milk and in TSB. Certainly the bacteria are taken to produce more extracellular polymers under conditions of stress such as low temperatures (COSTERTON E LEWANDOWISK, 1995). This is evident in pictures examined by SEM, where extracellular material can be observed mainly in stainless steel surface chips incubated at 5°C.
CONCLUSION: So the cold (5°C) prolongs the survival of *L. monocytogenes.* The study of biofilms is an important step in decision making preventive to remove biofilms and persistent strains in the environment of food processing.
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Financial Support: Fapesp

FREQUENCY AND SPREADING OF PATHOGENIC MICROORGANISMS AT WATER DISTRIBUTION SYSTEMS

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Key words: frequency; pathogens; water.

INTRODUCTION: The easiest way to spread a disease is by the public water distribution system**,** which can causes epidemic and it demands rigorous monitoring as well as new analytical procedures to brings fast results about chemical and microbiological contaminations, hazardous to health before the water is distributed to the population. This work aimed to evaluate new culture media and molecular methodologies to detect pathogenic microorganisms in public water systems.

MATERIAL AND METHODS: Water samples were collected from supply plant water at Piracicaba-SP, from two year seasons (raining and dry) and analyzed in order to identify bacteria artificial cultivated and non-cultivated, from the capitation plant (origin), the treatment tanks, distribution net and from home tanks, totalizing 118 samples, done according to ALVES (2007).

RESULTS AND DISCUSSION: Bacteria isolation methodologies aimed to identify different metabolism species to visualize the real micro biota presented in the water distributed for the city population. Results showed some species resistant to the chemical and purification treatments, as the genus *Sphingomonas sp.*, first identified at the origin point and again in other 20 (twenty) points of sampling. A good indicative for contamination is the enteric group of microorganisms, as *Klebisiella sp.* and *Enterobacter sp.,* usually used as water quality indicators. Such microorganisms were not identify at the origin point or during the treatment process but were identified at three home tanks; in two houses entrance and at one pumping station. The genera o *Shigella,* a pathogenic specie, was also detected, probably resistant to all treatment phases, which must leads to an important concern about microorganisms adaptation and resistance to anti-microbial substances all over the water system chain, mainly because Chloride was present at all phases, compromising public health.

CONCLUSION: Data presented shows, despite efficient water treatment at Piracicaba city, there are bacteria resistant to the purifying and chemical processes, which can be contamination starting in the public distribution water system.

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Fungal microbiota of wet and dry processed green coffee, from regions of Paraná States, Brazil.

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Keywords: quality of coffee, coffee fungi and *Coffea arabica*.

INTRODUCTION: Brazil has been losing international market share due toits lower coffee quality when compared to other countries. Themicroorganism proliferation in the coffee fruit alters and damages theoverall quality of the final product, reducing its market price. Factors like genetic origin, environment, handling pre and pos harvestdirectly affect the coffee quality. Nonetheless, Krug (1940, 1945 e 1947) and Bitancourt (1957) correlate changes in the chemical composition of coffee with the presence of microbiota found in the grain, i.e. especially toxin producers fungi. The objective of this work was to identify and quantify the fungal microbiota of coffee produced in several cities in the state of Parana, Brazil; and to correlate these results with the coffee beverage quality, production method (wet or dry) and the altitudes where the samples were collected.
MATERIAL AND METHODS: We analyzed 55 samples of ground coffee beans from various coffee-producing towns of *Coffea arabica* in the state of Paraná, Brazil (harvest 2005/2006). The isolation and identification were performed through the technique "pour-plate" in PDA medium and microculture respectively. Analysis of variance ANOVA was held, followed by Tukey test.
RESULTS AND DISCUSSION: We found 8 genera: *Aspergillus, Penicillium, Mucor, Trichoderma, Rhizopus, Absidia, Nigrospora* and *Cladosporium*. Being *Aspergillus* the most frequent (76%). Significant differences were observed between the cities examined and the processing of coffee, where grains subjected to wet and peeled processing, presented a low rate of fungi. According to Thompson (1997), filamentous fungi and yeasts involved in fermentation of the coffee fruit, originate from the surface of the fruit, which explain the small number of fungi found in wet and peeled processed coffee. No relation was found between the quality of coffee beverage and the altitude, with the number of fungi.
CONCLUSION: The amount of fungi differed between the analyzed cities and the genus *Aspergillus* was the most frequent. The results also suggest that the husk removal of the coffee fruits in wet process significantly reduced the number of fungi.
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GENETIC DIVERSITY AND TOLERANCE OF COWPEA RHIZOBIA SYMBIOTIC STRAINS TO SALINITY

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Key words: Nitrogen biologic fixation; Leguminous; environmental stress

INTRODUCTION: The cowpea (*Vigna unguiculata* (L.) Walp.) is a leguminous of high proteic value, cultivated mainly in the North and Northeast regions of Brazil for subsistence. The nitrogen (N) necessary for the development of this leguminous and for grain improvement can be supplied by symbiosis with rhizobia (Soares et al., 2006). However, the process of FBN is dependent of several environmental factors. Soils with high saline concentrations such as the existing in soils of Northeast can affect directly the survival of rhizobium, and, consequently reduce the supply of N to the plant (Anthraper & Dubois, 2003). This study aims to evaluate the genetic diversity and tolerance of cowpea rhizobia symbiotic strains in relation to salinity.

MATERIALS AND METHODS:

Ten strains of isolate rhizobia from the soils of the South of Minas Gerais (Florentino, 2007) and two strains recommended as inoculants for the cowpea, UFLA 03-84 and INPA 03-11b were used. These strains were analyzed in relation to genetic diversity using the technique of Rep-PCR, such as the primer BOX, and in relation to tolerance to different concentrations of NaCl: (0, 5, 10, 15, 20, 25, 30, e 40 g L^{-1}). The symbiotic efficiency and the resistance to antibiotics of these same strains are presented in other work (Sousa et al., 2009, in this Symposium). The tolerance to NaCl was observed by presence (+) or absence (-) of growth in the culture medium 79 (Vincent, 1970), after seven days incubation at 28^{o}C.

RESULTS AND DISCUSSION: The analysis of diversity observed by the profiles obtained with primer BOX, revealed high diversity, evidenciating that each strain presented only one profile. The high diversity observed among the strains can be related to the promiscuous behavior of cowpea, which able growth of rhizobia belonging to several genera (Moreira, 2008). The tests of rhizobia in relation to resistance to salinity, it was observed that the strains grew up in the culture medium containing 5 g L^{-1} of NaCl. However, according to the increase of salt concentration, there was a reduction in the number of strains able to grow up in these concentrations. The strains Vu4IV4 and Vu4IV3b, which produced higher quantity of gum, tolerated concentrations above 40 g L^{-1} of NaCl.

CONCLUSIONS: The strains presented a great genetic diversity. The fast growing strains tolerated higher amounts of salt concentration which can be related to the higher production of exopolysaccharides.

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GLYCERIN AS AN ALTERNATIVE SUBSTRATE FOR BIOSURFACTANTS PRODUCTION

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Keywords: Surfactants; *Pseudomonas*; glycerin

INTRODUCTION: world production of surfactants exceeds three million ton per year and the vast majority of available surfactants is synthesized from petroleum derivatives. Among the renewable low-cost sources, the glycerin, a byproduct obtained in the transesterification process of vegetable oils and animal fats during the production of biodiesel, is an alternative of great interest because the large quantity generated worldwide is increasing as a function of the growing demand for biodiesel and other derivatives (DINIZ, 2006; SHARMA et al., 2008). Accordingly, this study aimed to use glycerin as an alternative substrate for low-cost production of a biosurfactant by *Pseudomonas aeruginosa*.
MATERIAL AND METHODS: the *Pseudomonas aeruginosa* UCP0992 was grown in mineral medium with different concentrations of glycerin (2-7%), under orbital agitation of 150rpm during 120 hours, at 27 ºC. After fermentation, the cell-free broth, obtained after centrifugation, was used for the following determinations: surface tension measurement and isolation of the biosurfactant with organic solvents (KUYUKINA et al., 2001).
RESULTS AND DISCUSSION: reducing the surface tension is used as a primary criterion for selecting biosurfactants producer's microorganisms, although dispersing and emulsifying agents have not, necessarily, ability to reduce the medium surface tension (COIMBRA et al., 2009). In this sense, it was observed that the best results were obtained in the condition containing the mineral medium supplemented with 3% glycerin, revealing the ability of the surfactant to reduce the water surface tension from 70 mN/m to values around 28 mN/m, with a yield of 3.6 g/L.
CONCLUSION: the results show the ability of *Pseudomonas aeruginosa* to produce surfactant agents in low-cost media, thus encouraging future researches for application of these agents in processes of environmental decontamination.
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GROWING EVALUATION OF *BACILLUS CEREUS* IN NUTRIENT BROTH USING DIFFERENT NACL CONCENTRATIONS.

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Keywords: Bacteria growth; sodium chloride,

INTRODUCTION: The *Bacillus cereus* species is generally found in different environments, as well as in foods and spice, therefore it resists a high range of temperatures and concentrations of salt (MARTINEZ et al, 2007). The aim of this work was to study the growth kinetic of this species in a gradient of sodium chloride (NaCl) concentration.

MATERIAL AND METHODS: The *Bacillus cereus* species was isolated from a cropping soil contaminated with the pesticide endosulfan and was inoculated in nutrient broth, supplemented with NaCl, reaching the final concentrations of 0%, 1%, 5% and 10% NaCl. The bacterium growth was evaluated by spectrophotometer in the intervals of 0, 12, 24, 48, 72, 96, 120 and 144 hours using 600nm of wave length (SHIVARAMAIAH and KENNEDY, 2007).

RESULTS AND DISCUSSION: The control (0%) has showed the highest values and was also the one that more quickly reached the climax in the growth curve, albeit 1% of NaCl final concentration was the treatment that reached the greatest values of optics density (OD). The 5% treatment has showed a little expressive growth, being the apex of its curve the value of 0,4º OD. Martinez et al. (2007) found a good growth of *Bacillus cereus* in 0,5% of NaCl, and while the concentration increased it was observed the decreasing of biomass.

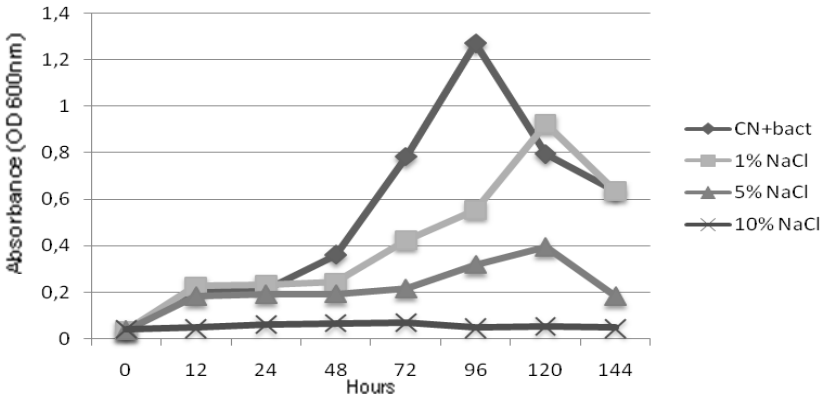

Figure 1 *B. cereus* growing in the presence of different concentrations of NaCl.

Mossel et al. (1967) concluded that the upper limit of NaCl concentration for *B. cereus* growth is 5%. Other studies (ex.: Claus and Berkeley, 1986) suggest that 11 to 89% of the B. cereus samples grows in 7%.

CONCLUSION: Considering these results we can conclude that despite *Bacillus cereus* tolerates 5% of NaCl, it had a more expressive growth in 1%, if compared with the other studies concentrations and NaCl at 10% didn't allow the species to grow.

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GROWTH OF AND BACTERIOCIN PRODUCTION BY *Lactococcus lactis subsp. cremoris* CTC 204 IN DIFFERENT CULTURE MEDIA

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KEYWORDS: Bacteriocin, Lactic Bacteria, Culture Media.

INTRODUCTION: The need to discover new natural and safe types of antimicrobials for use in the food industry boosts the interest in bacteriocins produced by lactic bacteria. For that reason, the objective of this study was to evaluate five culture media in order to ascertain which was most suited to cell growth and bacteriocin production by the *Lactococcus lactis subsp. cremoris* CTC 204 strain, previously isolated from fresh, natural chicken gizzard (Bromberg et al., 2004).
MATERIAL AND METHODS: The culture media evaluated: MRS Broth, Rogosa SL Broth, APT Broth and Soy-Tryptone Broth (Difco), were obtained from DIFCO. For the purpose of the experiments, these media were supplemented with 2% (w/v) sodium β-glycerophosphate (Sigma-Aldrich). 100 mL portions of each of the supplemented media were inoculated with 2% of an inoculum containing the bacteriocin-producing strain in MRS Broth (Difco) followed by aerobic incubation at 37^{o}C for 24 hours. Samples of the culture media were taken for analysis immediately after inoculation of the strain and at regular 2-hour incubation intervals. Bacterial cell growth was determined by the viable cell count technique (UFCmL^{-1}) in MRS Agar (Difco), the pH was measured with a pH-meter (Micronal), while bacteriocin activity was assessed by the critical dilution method (Mayr-Harting *et al,* 1972) and expressed in Arbitrary Units per ml (UAmL^{-1}). *Streptococcus salivarius* spp. TR 570, *Lactobacillus helveticus* ATCC 15009, *Staphylococcus aureus* CTC 033, *Bacillus cereus* CTC 01 and *Listeria innocua* LIN 11 were used as indicator strains for bacteriocin activity.
RESULTS AND DISCUSSION: The results show that the growth of the *Lc. lactis subsp. cremoris* CTC 204 strain varied with the culture medium evaluated: from 6,9 to 8,5 UFCmL^{-1} in Soy-Tryptone; from 7,1 to 8,9 UFCmL^{-1} in MRS Broth, from 7,9 to 9,1 UFCmL^{-1} in Rogosa SL Broth and from 7,0 to 9,5 UFCmL^{-1} in APT Broth. Maximum populations were reached after 8 hours growth of the bacteriocin-producing strain in the Rogosa SL and Soy-Tryptone broths and after 12 hours in the MRS and APT broths. With the exception of the Rogosa SL medium in which no inhibitory activity against any of the indicator cultures tested was found, bacteriocin production by the strain investigated in this study was detected in all the other culture media tested from 4 hours incubation onwards. In the Soy-Tryptone and APT broths, 0,4 UAmL^{-1} and 0,8 UAmL^{-1}, were respectively recorded after 6 hours incubation, whereas in MRS the maximum of 0,8 UAmL^{-1} was observed after 12 hours.
CONCLUSIONS: APT Broth favored cell growth and, consequently, the production of bacteriocin by *Lactococcus lactis* subsp. *cremoris* CTC 204 after a shorter incubation time as compared to the other culture media investigated.
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HEAVY METALS INFLUENCED THE PRODUCTION OF CHITOSAN BY *Absidia corymbifera* UCP 0134

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Keywords: Chitosan, Biosorption, *A. corymbifera*

INTRODUCTION: The heavy metals contamination is an environmental problem when the concentration of those elements reaches poisonous values for the organisms. The metals are normal constituents of the ecosystems. However, the human activities are contributing with the increase of circulation these metals in the atmosphere. Among the heavy metal copper is found in the nature on elementary state, as sulfide, arsenates, chlorides and carbonates. The presence of zinc in the atmosphere under rusted form. In the air the occurrence is related to industrial sources; mainly the matter transformation as the electroplating. The mechanisms of resistance have been identified in fungi, and some microbial system can be used in biotechnological processes, such as the environmental biosorption of heavy metals. Researches carried out with the filamentous fungi of the genus *Absidia, Cunninghamella, Mucor and Rhizopus,* demonstrated the ability of metallic ions removal (FRANCO et al., 2004; SUBUDHI & KAR, 2008). The aim of this work was to evaluate the potential of *Absidia corymbifera* adapted copper and zinc heavy metals on the chitosan production.

MATERIAL AND METHODS: Spores of *Absidia corymbifera*, maintained to 5°C in BDA medium were transferred to Petri dishes containing YMA (Yeast, Malt, Agar), for sporulation during 5 days. The spores were counted to 10^7cells/mL and 10% of suspension was transferred to Erlenmeyer flasks containing 400mL of the corn steep liquor 6% medium added of copper and zinc (2 and 4 mM) conditions, respectively. The flasks were maintained under agitation of 150 rpm, temperature of 28°C, for 96 hours. The pH was determined by potenciometry. The glucose consumption by DNSA method. The chitosan was extracted by Stamford et al. (2007) method, and the profile of Infrared Spectroscopy and Acetylation Degree.

RESULTS AND DISCUSSION: *A. corymbifera* showed biomass and chitosan of 6.97g/L and 67.3mg/g, respectively. The pH was 5.3 to 7.7 in the final of fermentation. The infrared ray spectra of the chitosan samples are according to the literature. The deacetylation degrees of the chitosan samples were 81 to 88%. Similar results were observed in commercial chitosan.

CONCLUSIONS: The obtained results evidence the potential of *A. corymbifera* in the conversion of industrial residue and chitosan production; as well the physiological adaptation for these used metallic ions, could be used, on the future, for metals biosorption.

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HETEROTROFIC AQUATIC BACTERIA OF AN AGRICULTURAL AREA, CAMPO VERDE, MATO GROSSO

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Keywords: agricultural area, pesticides, dynamics of growth

INTRODUCTION: One of the sources of the hydrics resources contamination is the pesticides and fertilizers used in agricultural activities that reaches waters by the runoff and/or leaching the in the drainage area. In these systems, significant and perceivable changes in microbiota are related to the ambient conditions, caused by the soil wrong use, resulting in the alteration of its dynamic balance, due modifications in the microbiological processes - physiological and biochemical.

MATERIAL AND METHODS: The Pirassununga farm is situated in Campo Verde, MT (Lat. S15º37'19,4" and Long. W55º10'29,6"), was the study area, where water samples of three points of an reservoir were collected: next the spring (P1), at the limnetic zone of the reservoir (P2) and in its exit (P3) in september of 2008 (dry period) and january of 2009 (rainy period) in order to compare the growth dynamics of its heterotrofic bacterial communities. For that, the samples had been diluted and plated by the "pour plate" method in Nutrient Agar for colonies general counting (APHA, 1998).

RESULTS AND DISCUSSION: In the graphs we plotted the bacterial growth average results of Colony Forming Units (CFUs). The S1 and the S2 had presented the same trend line in both seasonal periods. The samples from dry period, P3, presented an accented exponential growth in 48h. In the rainy period this result was observed in 72h. This small difference can be attributed to the dilution factor, common in this period, when the water level raises and the nutrients are less accessible comparing with the previous period.

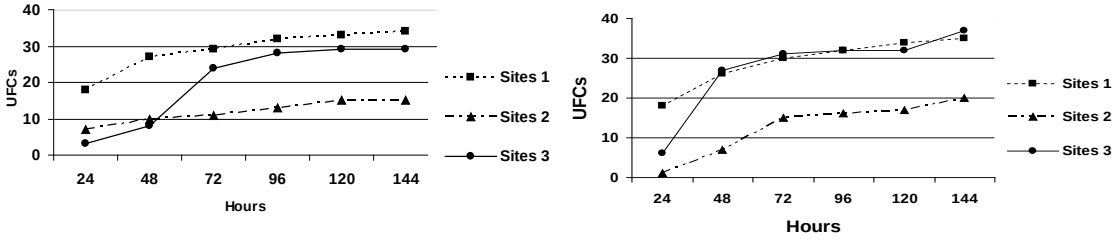

Figure 1 - Counting of heterotrofic bacteria collected samples september/2008 (a) and january/2009 (B)

Dahroug (2007) has also found colonies homogeneity sampled in the rainy period in the same study area. Such characteristics might be determinant for changing physical and chemical factors, beyond the degradation products accumulation. The microorganisms found in continental waters are determined by conditions that prevail in the environment. Such environmental conditions vary from an extremity of the aquatic environment to another. The bacterial growth also is influenced by these environmental factors, mainly by the nutrients, temperature, humidity, pH and the oxygen. All are important and can limit the growth and the bacterial development. The presence of some organisms also can cause the alterations of the microbial growth, either for food competition and space or for the presence of inhibitory substances for its growth.

CONCLUSION: The results indicate that there is lot to be done in relation to the studies of continental waters and its microbial diversity impacted by agricultural and industrial activities, aiming to quantify how much such communities are being affected.

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HIGH-THROUGHPUT ENZYMATIC EVALUATION OF METAGENOMIC LIBRARIES FROM OIL REFINERY EFFLUENTS

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Keywords: metagenomic and enzymatic high-throughput screening assays

INTRODUCTION: In the post-genomic age the interest in enzymes evolved from structural identification to functional analysis. The functional enzymatic screening detects the enzyme function without previously structural knowledge, revealing the relationship between the enzyme and the enzymatic function. These data are extremely relevant as insignificant structural modifications might produce drastic functional changes. To genetically improve an enzyme, the functional information obtained from enzymatic high-throughput screening of thousand mutants is frequently more useful than the detailed structural information of one enzyme. In this context, enzymatic high-throughput screening assays[1] were performed to evaluate the presence of lipases and epoxide hydrolases in metagenomic libraries built from brazilian soil microorganisms, sediment and mud from oil refinery effluents.

MATERIAL AND METHODS: The high molecular weight metagenomic libraries were built from mud samples collected in a pilot membrane bioreactor, located at COPE/UFRJ, which was fed during 30 days with effluents from REVAP-SP which had high fenolic content. Two additional libraries were created from mangrove sediment samples and soil of Guanabara Bay-RJ, both impacted with oil. The enzymatic assays in high-throughput screening format were performed in two steps; first in Petri dishes enriched with olive oil (lipase detection) and fosfomycin (epoxide hydrolase detection). The positive hits obtained from these preliminary evaluations were submitted to more refined assays, in 96 well polypropylene microplates using fluorogenic probes. The results obtained from both assays were validated through conventional biocatalysis and GC-MS and GC-FID analysis.

RESULTS AND DISCUSSION: Screening 16.000 clones of three metagenomic libraries detected 04 positive hits for lipases/esterases and 03 hits for epoxide hydrolases in the sediment library (1.000 clones). The metagenomic library of soil (5.000 clones) furnished 12 positive hits for epoxide hydrolases while the mud metagenomic library with 10.000 clones afforded 03 hits for lipase/esterase.

CONCLUSION: The application of enzymatic high-throughout screening techniques to monitor the enzymatic activity of whole cells allowed the evaluation of 16.000 clones in a straighforward manner. The selected clones are now under evaluation using synthetic substrates of industrial interest.

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Financial support: FAPESP, CNPq, CAPES and PETROBRÁS

HYDROCARBONS UPTAKE MECHANISMS IN *Candida* CELLS RELATED TO THE PRODUCTION OF BIOSURFACTANTS

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Keywords: cell hydrophobicity; surfactants; *Candida*.

INTRODUCTION: the distribution of the microbial cell plays an important role in the whole process of bioremediation (GAUTAM & TYAGI, 2006; SINGH et al., 2007). In this direction, the probable modes of hydrocarbons uptake in cells of *Candida* were studied based on emulsifying activity quantification, surface tension and interfacial tension measurement and cell hydrophobicity determination, once the elucidation of the microorganism hydrophilic and/or hydrophobic properties related to the capacity of biosurfactant production will allow a more effective application of the microorganism and/or of the biosurfactant produced in the bioremediation of petroleum and derivates.

MATERIAL AND METHODS: *Candida* species *(C. tropicalis, C. lipolytica, C. sphaerica, C. guillermondi, C. buinensis e C. glabrata)* were grown on a medium containing 2.5% of insolubles and solubles substrates, as n-hexadecane, soybean oil, ground-nut oil refinery residue, corn steep liquor and glucose, with shaking at 150 rpm for 144 h, at 27ºC. After fermentation, samples were withdrawn for the following analyses: surface tension, interfacial tension and cell hydrophobicity (BOUCHEZ-NAITALI et al., 1999).

RESULTS AND DISCUSSION: the yeasts showed similar culture characteristics, which means high values for cell hydrophobicity and values between high and intermediate for surface and interfacial tensions when cultivated in the insoluble substrates (n-hexadecane, soybean oil, and refinery residue), in the soluble substrate (glucose), or in the presence of both soluble and insoluble substrates (soybean oil and glucose). These results suggest that the direct interfacial uptake of hydrophobic compounds is the most frequent mechanism for these yeasts, thus allowing cell adherence to the insoluble phase.

CONCLUSION: the various possibilities of transference observed for these yeasts and the discussion of the results obtained with the data obtained over the years in the literature show that the mode of action of biosurfactants, in promoting solubilization or emulsification of substrate, or in modifying cell hydrophobicity, is a subject of great complexity, and relevant scientific discussion and not yet fully elucidated, since the production of biosurfactants also occurs during the growth on soluble carbon sources, suggesting a broader role for biosurfactants than just hydrocarbon uptake.

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IDENTIFICATION AND ENZYMATIC CARACTERIZATION OF BACTERIAL ENDOPHYTIC COMMUNITY FROM MANGROVE FOREST

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Keywords: endophytic bacteria, enzymes, biodiversity

INTRODUCTION: Mangrove is an important coastal ecosystem of transition between the land and sea. This environment maintains during all year a great amount of organic matter that is a source of food for associated community. The organic matter is decomposited for microorganisms, which is intimately associated with the local vegetation as the endophytic microorganisms. The endophytes may also be applied at agriculture acting as control agent of pest, diseases and plant-growth promoting (Hallmann et al., 1997). The research aiming was the evaluation of genetic and physiologic diversity of the bacterial endophytic community from two different mangrove areas with oil spills and non-oil spills at São Paulo States and the biotechnological potential characterization of this community at the enzymatic production for agroindustry.

MATERIAL AND METHODS: Endophytic bacteria were isolated from *Rhizhopora mangle, Avicenia schaueriana* and *Laguncularia racemosa* branches placed at Mangroves of São Paulo state. The production of amylase, lipase, protease esterase, endoglucanase, and phosphate solubilization by the isolates were evaluated on specific solid media and the producers were identified by 16S of the rDNA sequencing.

RESULTS AND DISCUSSION: The highest bacterial density was isolated from *A. schaueriana* branches. One thousand of endophytic isolates were sampled randomly for posterior experiments. Approximately 70% of isolates showed activity for at least one of the tested enzymes. In the oil spills area was observed a higher percentage of endoglucanase producers than at non-oil area. Highlight that just 7% of evaluated isolates showed esteratic activity. The highest percentage of producers for at least one of the evaluated enzymes and the highest bacterial density was isolated from *A. schaueriana*. Among the isolates the percentage of protease, lipase, endoglucanase, phosphate solubilation, amylase and esterase producers were respectively 84%, 47%, 47%, 29 %, 23% and 7%. By sequencing of 16S rDNA the predominant genera identified were: *Methylobacterium, Bradyrhizobium, Novosphingobium, Sphingomonas, Pseudomonas, Flavimonas, Microbacterium, Xanthomonas, Stenotrophomonas, Pantoea, Klebsiella, Salmonella, Escherichia and Enterobacter*.

CONCLUSIONS: This is one of the first reports about isolation, identification and enzymatic characterization of endophytic bacteria community from Mangroves of the Sao Paulo state. The bacterial density and diversity are affected according to vegetable and place of isolation. The enzymatic production by isolates shows the wide biotechnological potential of these communities as a wide source of bioprospection.

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Identification of the yeast fermentation process of ethanol

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Keywords: Identification, yeast and alcoholic fermentation.

INTRODUCTION: The selection process of yeast has been carried out to facilitate the fermentation and production of alcohol fuel (BASSO et al., 1993). Brazilian distilleries using yeasts selected for the process and during the season they are recycled at the end of each fermentation. It is believed that by recycling, the selected yeast suffering contamination of native yeasts, undergo polymorphic changes resulting in a different line of original. This work was aimed at the isolation and biochemical and molecular identification of yeasts cultivated during the harvest of a distillery in the region of Ribeirão Preto.
MATERIAL AND METHODS: The strains of yeast were isolated from samples fermented wine in YEPD medium and biochemically identified by API 20C AUX kit of Biomeriux ® and molecularly characterized by polymerase chain reaction (PCR). The primers used were ITS1 and ITS2, which encode the 5.8S ribosomal RNA.
RESULTS AND DISCUSSION: We isolated 14 yeast. These were identified as its biochemical properties using the API 20 C AUX kit as Saccharomyces cerevisiae 1 and 2, and 10 identified as S. cerevisiae 1 and 4 as S. cerevisiae 2. These were submitted to molecular characterization and by the technique employed, all strains had bands of 850 bp when the number of bands of the ITS region and two of these strains showed bands at 500 and 300 bp indicating a possible polymorphism.
CONCLUSION: The sample was identified biochemically with the 99% security of all isolates. However, molecularly, is necessary the assessment of new polymorphic techniques to confirm the polymorphisms between strains.
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Financial support: Capes

INFLUENCE OF DOMESTICS EFFLUENTS ON THE TOTAL HETEROTROPHIC BACTERIA COMMUNITY ALONG THE FEB STREAM, VÁRZEA GRANDE, MT.

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Keywords: bacteria, organic pollution, urban stream, organic matter

INTRODUCTION: Large amount of organic matter from domestic waste water reach urban streams, enriching water resources, and being used by bacteria as a carbon source. This situation leads to an euthophic stage, influencing heterotrophic bacteria community[3].

MATERIAL AND METHODS: During the 2009 rainy season, water samples were collected in five sampling sites, along the FEB's Stream, which is located in Várzea Grande/MT. The first sampling site (E1) was the region near the headspring, (E2) in a dam, (E3) in a spillway, (E4) the entrance of a high eutrophicated pond caused by large inputs of domestics effluents, (E5) the pond exit. Aiming to compare the growth dynamic of the heterotrophic bacteria community along the stream, and to characterize bacterial cells morphologically and considering differential Gram´s stain reaction[4], samples were diluted and plated in triplicate on Nutrient Agar using "pour plate" method to general counting of bacterial colonies[1]. Then, the colonies of the isolated strains were characterized[2].

RESULTS AND DISCUSSION: Most colonies showed yellow pigmentation, small size, irregular shape, flat elevation, corrugated board, smooth structure, and opaque brightness. Considering these morphologic and staining characteristics, the results showed that there were a prevalence of Gram (-) rods, followed by Gram (+) rods, which were not recorded in E3. Sporulated rods were observed only at E1 and E3, whereas coccus (+) at E1 and E5 (Figure 1 (A)). In Figure 1 (B) was plotted the triplicates average results of the Colony Forming Units (CFUs) counting. The E4 showed the highest count. This may have occurred due to the abundance of organic substances found in domestics and industrials effluents such as carbon[3] which is essential for bacterial growth and metabolism[4]. The bacteria sampled at the sampling points E1 and E2 had a similar growth due to this region being more conserved, and that receives waste from few cattle as the main source of nutrients, with little influence of organic matter from effluents, if compared to the points E3 and E5.

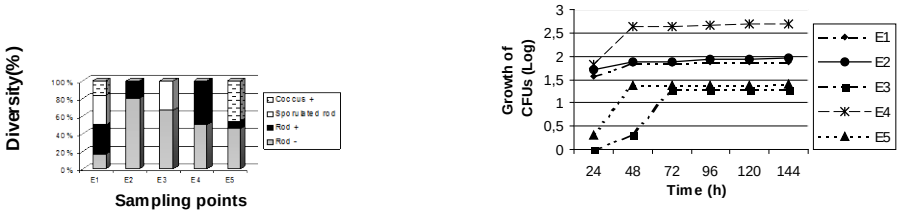

Figura 1 – (A) Diversity of bacterial morphologies (%) e (B) Microbial curve growth obtained in the samples from the FEB's Stream, Várzea Grande, Mato Grosso.

CONCLUSION: Considering this investigation results, one can observe the importance of studies about the effects of emissions of organic matter caused by domestics effluents of waste water in the dynamic growth of the total heterotrophic bacteria community.

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INTERFERENCE OF HEAVY METALS (Zn and Pb) OF THE CERAMIC INDUSTRY IN FUNGICAL POLISACARIDASES ACTIVITY

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Keywords: fungi, heavy metals, enzymes

INTRODUCTION: To quantify the microbial enzymatic activity is an important parameter to monitor and to optimize bioremediation processes, which can contribute to recovery of the degraded area (SINSABAUGH et al., 2000), since the fungi are the main ones responsible for the dynamics of the nutrients cycling and vegetable productivity. Santa Gertrudes's ceramic pole, SP, where locates the area of studies impactated, it is the largest in the country, where expressive amounts of toxic elements are used, like Pb and Zn in the composition of ceramics, enamels and dye, deposited inadequately in the soil (BONACIN SILVA, 2001).

MATERIAL AND METHODS: The activity of the polissacaridases, CMCase, AVICELASE, XYLANASE and POLYGALACTURONASE was evaluated by strains of *Aspergillus terreus* Thom, isolated of the soil by Warcup method (WARCUP, 1950). As control, were used strains CCAT 3320 and CCAT 4083 o*f Aspergillus terreus*, isolated of preserved areas. After cultivation in modified Czapek medium and obtaining of the crude extract, they were dosed the sugars reducers liberated by Somogyi-Nelson method (NELSON, 1944).

RESULTS AND DISCUSSION: it was detached a strain, isolated of concentrations of 1000 µg/mL of Zn, in the production of CMCase (0,45 U/mL), XYLANASE (1,06 U/mL) and POLYGALACTURONASE (3,06 U/mL). The activity of AVICELASE was not detected. The larger enzymatic activities were obtained by strains isolated of Czapek medium with addition concentrations of 100, 200, 500 and 1000 µg/mL of Zn. Pb inhibited the production of enzymes celulolytics and pectinolytics significantly (Tukey-Kramer p=0,01), in concentrations of 500 µg/mL and 1000 µg/mL, probably, interfering on the system of supply of CELLULASES and HEMICELLULASES energy.

CONCLUSION: The fungi suffer limitant influence by the heavy metals, adapting themselves to survive against adverse conditions. Such consequences may reflect on their growth as well as on their enzymatic production potentiality.

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ISOLATION AND SELECTION OF EXOPOLYSACCHARIDE-PRODUCING BACTERIA WITH EMULSIFYING ACTIVITY FROM *Sagittaria rombifolia* Cham OF A PALM SWAMP STREAM

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Keywords: Bacterial exopolysaccharides, emulsification, hydrocarbons

INTRODUCTION: Exopolysaccharides (EPS) are produced by several bacteria and have important structural role in biofilms, the natural habitat of many microbial communities, both in natural or artificial environments where a solid subtract remains exposed to moisture. Moreover, EPS are of great industrial interest. They possess a wide variety of properties that may not be found in traditional polymers of plant origin (Guezennec, 2002). The variety of chemical structures found in EPS lead to a broad spectrum of physical properties, allowing a great diversity of applications, such as emulsifying activity. The preset work aimed at the isolation, selection of EPS-producing bacteria from *Sagittaria rombifolia* Cham as well as the evaluation of its emulsifying properties on different hydrocarbons.

MATERIAL AND METHODS: EPS-producing bacteria were isolated from leaves of *Sagittaria rombifolia* Cham (of a palm swamp stream in Uberlândia, MG) on sugarcane 4% molasses agar and selected based on the mucoid mode of colonies and on Alcian Blue staining (Fusconi & Godinho, 2002). For emulsification assays (Iqbal et al., 1995), selected strains were cultured in sugarcane molasses medium for 48 hours at 30°C and 150 rpm. Emulsification assays were carried out using cell free 4% sugarcane molasses media with benzene, toluene and *o*-xylene. Triton X-100 was used as chemical surfactant and a control was prepared using the same method but replacing the sample by non-inoculated media. Results were tested by analysis of variance (Anova), followed by the Tukey test at a 0.05 level of significance.

RESULTS AND DISCUSSION: A total of 40 bacterial strains were isolated. F1CC and F1CA exhibited a high mucoid aspect and positive EPS production tested with Alcian Blue staining. Both strains were selected as EPS-producing bacteria for emulsification activity evaluation. The emulsifying index for Triton X-100 in benzene and toluene was higher than the one obtained in sugarcane molasses F1CC and F1CA culture supernatant ($F= 30.226$, $p=0,001$; $F=35.762$; $p<0,001$ respectively) suggesting a low activity of the bioemulsifiers on those hydrocarbons. However, in the culture supernatant of F1CC and F1CA with *o*-xylene, the emulsifying activity was similar than Triton X-100 ($F= 3.523$; $p=0,097$).

CONCLUSION: F1CC and F1CA were selected as EPS-producing bacteria. EPS produced by the strains showed significant emulsifying activity with *o*-xylene, suggesting their potential application in bioremediation studies in contaminated sites with this hydrocarbon.

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KINETICS OF GROWTH ASSOCIATED TO THE PRODUCTION OF TOXINS BY *Bacillus thuringiensis* subsp. *Israelensis*

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Keywords: Bacillus thuringiensis, biological control, *Aedes aegypti*, bioinsecticide.

INTRODUCTION: *Bacillus thuringiensis* is a Gram-positive bacteria spore, which produces protein crystals with insecticidal activity. Despite the widespread use of *B. thuringiensis* in biological control, there are few published studies on its production, since many details are trade secrets. Therefore, the objective of this work was to study the kinetics of growth of *B. thuringiensis israelensis* involving the toxicity of culture, in an alternative medium based on meal chrysalis of *Bombyx mori* and also determine the time of cultivation of fermentation for the production of toxins.

MATERIAL AND METHODS: The microorganism used in this study was *B. thuringiensis* subsp. *israelensis* HD537, which was grown in a medium of cultivation-based on meal chrysalis of *B. mori*, glucose, ammonium sulphate, glucose and salts, at 30 ° C, 120 rpm for a period of 96 hours. The growth was evaluated through tests of gravity, the cell morphology by light microscopy and toxicity by bioassay against *Aedes aegypti* according to the protocol of the World Health Organization (WHO, 2005).

RESULTS AND DISCUSSION: The phase lag of the growth curve had the lowest toxicity and corresponded to about 9,4% of the time of cultivation. The exponential phase, which presented the highest growth rate, was about 76% of the time of cultivation, in which at the end of the biomass was 15.13 mg / mL. The toxicity increased gradually during cultivation, being maximum during the stationary and declining phases, when it corresponded to a lethal concentration for 50% of the larvae (LC50) of 0.50 ppm (v / v). In general, sporulation occurs in the stationary phase, when the bacteria has consumed most of the sources of nutrients and it becomes a factor limiting to their growth. Furthermore, during the fermentation, the microorganism may produce metabolites, which with accumulation, ultimately inhibit its growth. Most of the toxins of *B. thuringiensis* synthesized during sporulation is therefore different from other species, the stationary phase is very important in the fermentation of this bacteria. (Bravo et al., 2007; SCHNEPF et al, 1998). The lower values of LC50, ie the higher toxicity, was observed after 96 hours of cultivation. However, when analyzing it slides, the occurrence of germination of some spores can be noticed at 96 hours. The major toxicity of the period of 96 hours, probably occurred because the vast majority of cells of the growth phase had already released their crystals, while in 72 hours some cells, although little, were seen at the end of the sporulation.

CONCLUSION: The major toxicities of culture of *B. thuringiensis israelensis* HD537 occured in the final stages of cultivation, being up to 96 hours, time considered ideal for fermentation under testing conditions. Alternatively, the culture can be kept for 72 hours and submitted to a rest period of 24 hours so that the cells can release their toxins.

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LACTIC ACID BACTERIA AND BACTERIOCINS IN WINE BIOTECHNOLOGY

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Keywords: microbiological control, wine, bacteriocin, *Oenococcus oeni*, lactic acid bacteria

Abstract

Lactic acid bacteria (LAB) are responsible for the secondary fermentation of wine, and this fermentation is a requirement for quality red wines that are going to be submitted to the process of ageing in oak barrels. This secondary fermentation is named malolactic fermentation (MLF) and essentially it involves the degradation of L-malic acid into L-lactic acid and carbon dioxide, the consequence of which is a reduction in total acidity (deacidification) of the wine [1]. This biological deacidification is always accompanied by the metabolism of other molecules, which takes place on a modest scale and has as a result the increase of aroma components such as acetaldehyde, acetic acid, acetoin, diacetyl, etc. [2, 3, 4]. As a whole, wine gains in mellowness, roundness and fullness, and becomes more pleasant to the palate. The capacity of LAB to grow in wine is largely determined by wine pH, alcohol concentration, temperature, and sulphur dioxide concentration. *Oenococcus oeni* is the LAB species considered preferable for achieving MLF in wine because of its tolerance to low pH and high ethanol levels [5], and thus, this LAB is currently used as starter culture for MLF in wine.

Bacteriocins are peptides with antimicrobial activity that are naturally produced by some bacteria to inhibit the growth of other competing microorganisms. Currently, bacteriocins produced by LAB arouse most interest because LAB possess the status of QPS (qualified presumption of safety), i.e. they are regarded as safe microorganisms for human consumption because they and their metabolites have been consumed in fermented foods for countless generations without adverse effects in the population. Bacteriocins produced by LAB have found important applications as natural preservatives in food industry because they offer the possibility of preventing the development of food spoilage bacteria [6]. In the future this application could be useful as well in preservation of wine and it offers the additional advantage of allowing a decrease of the sulphurous anhydride levels that are currently used in wine preservation. Two of the most widely used bacteriocins in food industry are nisin [7] and pediocin [8]. A number of results will be presented showing the effect of nisin and pediocin, alone and in combination with sulphurous anhydride, to preserve wine during the ageing and storage process. Currently a number of studies are being carried out searching for bacteriocin active fermentates and wine indigenous lactic acid bacteria showing bacteriocin activity.

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LACTIC ACID PRODUCTION BY *Lactobacillus* sp. LMI8 FROM WHEY LACTOSE AT DIFFERENT NITROGEN SOURCES

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Keywords: cheese whey, lactic acid, nitrogen sources

INTRODUCTION: Lactic acid (acid 2-hidroxipropiônico) became commercially important since 1881 (BARUFFALDI, 1975) tends application in the nutritious, pharmaceutical, cosmetic, textile industries, of leather and chemistry. Lactic acid can be manufactured either chemically or by microbial fermentation. However, the fermentative processes is more advantageous for be more economic (MANCILHA & SILVA, 1991). The aim of this study was to produce lactic acid in modified MRS medium (Man-Rogosa-Sharpe) containing whey lactose (35 g/L) at different nitrogen sources by *Lactobacillus* sp LMI8 isolated from cassava flour industry waste.

MATERIAL AND METHODS: The experiments were performed in 250 mL Erlenmeyers flasks containing 50 mL of the production medium during 48 hours of fermentation, maintained in rotating shaker at 200 rpm and temperature of 37±1 °C. The nitrogen concentrations was (g/L) 5, 15 and 25 of corn steep liquor, profloo, yeast extract, peptone and autolysate yeast. The initial medium pH was adjusted to 6.7 by addition of 5N NaOH (no controlled). Lactic acid and lactose were determined by HPLC (High Performance Liquid Chromatography) using Aminex HPX-87H column (300 mm X 7.8 mm) and a refractive index detector. The mobile phase was sulfuric acid 5mM at 0.6 mL/min and temperature of 60°C. Biomass concentration was estimated by cell dry weight.

RESULTS AND DISCUSSION: The best results to the lactic acid production (19.3 g/L) indicated peptone and yeast autolysate at 25g/L as better concentration and with a conversion efficiency of about 80% of the initial reducing sugars.

CONCLUSION: The biological production of lactic acid from whey lactose at different nitrogen sources by isolated *Lactobacillus* sp. LMI8 was investigated. Considering the low cost and availability, may be suggest that autolysate yeast is a potential nitrogen source for lactic acid production.

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LACTIC ACID PRODUCTION BY *Lactobacillus* sp. LMISM6 GROWING AT DIFFERENT MOLASSES CONCENTRATION

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Keywords: fermentation, molasses, carbon source, lactic acid

INTRODUCTION: Lactic acid can be used in food, textile, and pharmaceutical industries (WEE *et al*., 2004). The aim of this work was to optimize the lactic acid production by *Lactobacillus* sp. LMISM6 growing at different molasses concentration.

MATERIAL AND METHODS: The fermentation broth was the MRS medium, without glucose addition and supplemented with hydrolised molasses at different concentrations (3%, 5%, 7%, 9%; 15%; 20% and 25%). These experiments were developed in 125 mL Erlenmeyers flasks containing 45 mL the medium added with 3% of $CaCO_3$, temperature of 35°C, pH of 6.5 and 180 rpm. The inoculums concentration was 10%. The experiment was carried out in triplicate. Samples of 1 mL were withdrawn from fermentation broth at 0; 24; 48 and 72 hours. The samples collected were centrifuged at 7826 x g by 10 minutes. The supernatant was analyzed by HPLC to know the sugar and lactic acid concentration. The biomass concentrations were determined by dry weight (g L^{-1}). Lactic acid concentrations were determined by a high performance liquid chromatography system equipped with UV detector at 210 nm. An Rezex ROA (300 x 7,8 mm, phenomenex) column was eluted with 5mM H_2SO_4 as a mobile phase at a flow rate of 0.4 ml/min and the column temperature was maintained at 60 °C. Total sugar concentrations were analyzed by the same methodology utilized for lactic acid using a refractive index detector instead a UV detector.

RESULTS AND DISCUSSION: The lactic acid production increased with molasses concentration until 20%. One decrease in lactic acid production was observed with 25% of molasses, probably due to substrate inhibition, as a observed by WEE et al. (2004). The highest lactic acid production was 70,58 g L^{-1} after 48 hrs of fermentation, in a medium with 20% of molasses. Monteagudo *et al*. (1997) obtained 80 gL^{-1} of lactic acid with *L. delbrueckii* in fermentation medium containing 107 g L^{-1} molasses after 38 h of incubation.

CONCLUSION: The isolate LMISM6 shown to be promising for lactic acid production from molasses. Molasses have been one economic and abundant residue to industrial lactic acid production.

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LEGAL REQUIREMENTS FOR DRINKING WATER

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Keywords: Water, drinking, laws

INTRODUCTION: Pollution, population growth and inordinate use of water resources, intensify the lost of a larger portion of potable water, suitable for human consumption.
Thus, the number of works whose purpose is to evaluate the potability of water or make it drinkable increases.(Flores,2000).

A review of the literature related to the legal requirements of the drinking water emphasizing microbiology is essential, because it will help water researches, such as those that assess the pathogenic microorganisms in suspension, to establish appropriate parameters to specific legislation in force.

In Brazil, the patterns of drinking water are provided by bodies such as CONAMA and Ministério da Saúde. The classification of water and its distribution is defined by CONAMA, which provides classes in the resolution No 357 of 17 March 2005. The water quality parameters most recent, are provided by the Ministério da Saúde, the Order No 518 of 25 March 2004. This ordinance establishes procedures and responsibilities for the control and monitoring of water quality for human consumption and patterns of drinking.

MATERIAL AND METHODS: As a main tool for consulting the standards of drinking water, we used the system in legislation Health Surveillance (VISALEGIS) a database with full text search for and consolidation of standards, available on the homepage ANVISA (Agência Nacional de Vigilância Sanitária).

RESULTS AND DISCUSSION: In accordance with Resolution No. 357 of 17 March 2005 of CONAMA, the waters are classified into four classes. Only three of them can be used for human consumption, from first to third grade levels is upward of fecal coliform limits. Class 1 should be consumed only after processing simplified, class 2, after a conventional treatment, while the Class 3 requires a conventional or advanced treatment. The treatments aim to bring the water to drinking standards, defined by the ordinance MS No 518/2004 laying down the responsibilities of companies and water supply authorities vigilant, the microbiological standards are defined in Chapter IV of the Ordinance in Article 11.

CONCLUSIONS: From the above, it was possible to identify the main organs that define the uses and water quality, respectively called CONAMA and the Ministério da Saúde, may also be made an intensive review of the literature made possible through consultation with the resolution of CONAMA and Ministério da Saúde, as well as its attachments, which would help further scientific work to obtain parameters of water quality.

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Resoluções do Conselho Nacional do Meio Ambiente – Resolução nº 357 de 17 de março de 2005

Apoio Financeiro: PET/Secretaria da Educação Superior/ Ministério da Educação

LIPASE PRODUCTION BY MICROORGANISMS ISOLATED OF THE "MANIPUEIRA" IN THE PRESENCE OF THE SURFACTANT TWEEN-80

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Keywords: lipase production, microorganisms, manipueira

INTRODUCTION: "Manipueira" is the liquid that comes out from the pressing of the cassava during the cassava flour production, a basic food of Brazilian population. This residue contains carbohydrates, proteins, lipids and minerals. Although it has high levels of toxicity, due to the presence of cyanide. The development of technologies for reuse of waste in the production of enzymes by microorganisms promotes sustainable development. The lipases are one of the most important groups of biocatalysts in technological applications, reaching approximately 10% of the enzymes used in biotransformations in the worldwide.[1] This study investigated the lipase production by microorganisms isolated from "manipueira" in the presence of this residue and of Tween-80.

MATERIAL AND METHODS: Two samples of filamentous fungi isolated from the effluent of the cassava flour production were cultivated under agitation of 150 rpm at 28 °C during 8 days. "Manipueira" concentrations (2, 7 and 12%), ammonium sulfate concentrations (0.25, 1 and 1.75%) and pH (4.0, 5.5 and 7.0) were investigated in the presence of Tween-80 (1%). The samples were centrifuged and the supernatants were used to determine pH and lipases. The enzyme activity was determined in the presence of p-nitrophenylpalmitate as substrate, using gum arabic and Triton X-100 at pH 7.0 and 28 ºC.[2]

RESULTS AND DISCUSSION: The lipolitic activities produced by the samples of filamentous fungi were influenced by the pH of the enzyme production medium. During the cultivation at pH 4,0, this parameter decreased until pH 3.4. In all submerged growth of filamentous fungi at pH 7,0, the pH values increased. The results of the experiments at pH 5,5 depended on the nutritional conditions and the sample of the filamentous fungus. In the nutritional condition composed by: 7% "manipueira", 1% ammonium sulfate and 1% Tween-80 at pH 5,5, the lipase was produced throughout the cultivation by one of the microorganism tested, reaching a maximum value of 2,5 U/L at 96 h. The highest yield of lipase production, 7,6 U/L at 96 h, was determined in the presence of 2% manipueira, 1,75% ammonium sulfate and 1% Tween-80 at pH 4,0 after 72 h of cultivation.

CONCLUSIONS: The nutritional value of "manipueira" enables the microbial growth of filamentous fungi and the production of lipase in the presence of Tween-80. The next step is to investigate the optimization of cultivation conditions in the presence of an oil as the enzyme inducer.

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LIPASE PRODUCTION IN LABORATORY SCALE BY FERMENTATION

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Keywords: Lipases, fermentation, effluent treatment

INTRODUCTION Lipases (triacylglycerol acilhidrolase 3.1.1.3) can catalyze both the hydrolysis of esters of fatty acids to mono and di - acylglycerides, free fatty acid and glycerols in media containing water, how to reverse reactions of esterification, transesterification, interesterification on apolar organic solvents. (Joseph et al., 2008). The production of lipases has been achieved through processes of submerged fermentation (SF) that gives advantage over the control of the process and ease to extract the enzymes. In contrast, solid state fermentation (SSF) using agro-industrial residues as substrate, requires less energy and space, and higher yield than in SF. Therefore, studies that aim the use of different microorganisms, supplements and substrates can help to find optimal combinations in order to reduce the production cost for an application of lipase in an industrial scale. Then, the objective of this work is the selection of lipase-producing fungi for subsequent production of the enzyme by fermentation and its application in the treatment of industrial effluents.

MATERIAL AND METHODS: The lipase production of 77 fungi (from microorganisms collection of the Medical and Biotechnology Importance Fungi Laboratory and Environmental Mycology Laboratory of UFRGS)was qualitatively analyzed, inoculating its in the medium with rhodamine B 2 % and olive oil 6 % and the lipase activity was detected by visualization of fluorescence in UV light. From this test, fungi were selected for quantitative analysis on the production of the enzyme in medium containing soybean oil, bovine fat and residual oil, using as substrate the *p*-nitrophenylpalmitate(*p*-NPP). In this test, the fungi were pre-inoculated and incubated at 28 ºC, 200 rpm, overnight. Subsequently, 10 % of pre-inoculum was added to medium for lipase induction containing 2% of the triglyceride source and incubated at 28 ºC, 200 rpm for 48h. After incubation, the supernatant was removed and lipase activity analyzed adding 0.9 mL of substrate in 0.1 mL of sample. The mixture was kept at 37 °C for 30 min and the activity detected in the 410 nm spectrophotometer.

RESULTS AND DISCUSSION: Through the qualitative test with rhodamine B, 10 fungi were selected to be tested with different sources of triglycerides, because these oils are raw material commonly found in most waste with high load lipolytic. From this, the yeast *Pseudozyma hubeiensis* FI59, which showed the best lipase activity in soybean oil (40.5 ± 0.8 U/L), bovine fat (34.8 ± 0.8 U/L) and residual oil (40.6 ± 1.7 U/L) was selected for lipase production in laboratory scale using bovine residual fat (BRF) as substrate. Thus, by the method of response surfaces, 3 variables will be analyzed as to the conditions of culture: BRF concentration (2 to 60 %), agitation (100 to 340 rpm) and the incubation period (10 to 48 h).

CONCLUSIONS: Based on the results, a protocol will be developed for fungal cultivation on a pilot scale by fermentation. From the fermentation, there is potential application of lipase from *Pseudozyma hubeiensis* FI59 in effluent treatment, as presented activity for the 3 different sources of triglycerides.

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LUBRICANTS BIODEGRADATION AND TOXICITY IN SOIL

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Keywords: lubricant oil, biodegradation, toxicity.

Nowadays, many of the environmental damages resulting of oil pollution are due to the indiscriminate disposal of significant volumes of lubricant oils in the environment. Thus, the use of microbiological treatment in environments polluted by hydrocarbons from oil is an efficient, economical and versatile alternative to physical-chemical treatments. The objective of this work was to monitor the biodegradation of different types of lubricant oils using the respirometry. Furthermore, toxicological tests were performed to evaluate the lubricants toxicity before and after 90 days of microbial activity. The test organisms were *Eruca sativa* and *Eisenia foetida*. Initially, the inoculum was prepared with sandy soil, lubricant oild, surfactant and distilled water. This mixture was homogenized in a plastic bag and buried for 30 days which occurs a previous microbial selection. Subsequently, it was prepared the "base liquid" with this inoculum and distilled water. The respirometric method used was the Bartha respirometry following the Technical Standard L6.350 (CETESB, 1990). Four assays were prepared, each containing 100 g of inoculum, 5 mL of base liquid, 1 mL of nutrient solution and 1 mL of lubricant in analysis: S1 (control) - distilled water, S2 - mineral oil, S3 - semi-synthetic oil and S4 - used oil. The toxicological tests evaluated the toxicity of soils contaminated with different types of lubricants before and after 90 days of biodegradation. In the toxicological tests with *Eruca sativa*, the oil toxicity was defined by the inhibition percentage of seed germination. In *Eisenia foetida* tests, the toxicity before and after biological treatment was analized by Lethal Concentration 50 (LC50) determined by the oil volume per 100 g of soil. The respirometry results with CO_2 production (mg) after 90 days of biodegradation appears in the following order: S1 (78.260) <S2 (241.315) <S3 (316.195) <S4 (344.01). It was observed that the used oil (S4) is the most biodegradable followed by semi-synthetic (S3). The mineral lubricant (S2) was the oil with lower biodegradability. In toxicological test with *E. sativa*'s seeds, all oils were toxic because they presented an inhibition percentage higher than 40%. Even after 90 days of biodegradation, all oils also inhibited the seeds germination of more than 40%. However, the test containing used oil established with highest toxicity to the *E. sativa*'s seeds. With *Eisenia foetida* as test organisms, the results showed that the used oil was also the most toxic because its LC50 was between 0.50 and 0.25 mL per 100 g of soil. The mineral and semi-synthetic lubricants had their LC50 in 0.50 mL:100 g. After 90 days of biodegradation, the LC50 increased in mineral and semi-synthetic oil to the range of 0.75-0.50 ml. However, for used oil the LC50 to *E. foetida* remained the highest (between 0.50-0.25 mL). These results indicate that the lubricants already used in automobile engines are highly toxic to biota when they are discarded in nature. And, despite the used oil presented as the most biodegradable in respirometry tests, it also had a high toxic potential both for seeds and earthworms. Moreover, even after the lubricants undergo the biological treatment, all were considered toxic.

CETESB - Environmental Sanitation and Technology Company. Soils – Residuals Biodegradation Determination – Bartha Respirometric Method. **Technical Standard L6.350,** São Paulo, Brazil, 1990.

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MELANIZED FUNGI ASSOCIATED TO LEAFCUTTER ANTS.

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Key-words: melanized fungi, leafcutter ants, symbiosis.

INTRODUCTION: Studies revealed the importance and complexity of the symbiosis between fungi and leafcutter ants (Rodrigues *et al.*, 2008). Efforts have been made in order to improve knowledge on the real role played by fungi inside ant colonies. This work has the purpose of isolating and identifying melanized fungi in ant nests, which were recently reported by Little & Currie, 2007. The cuticle of soldiers and workers of *Atta sexdens rubropilosa* (saúva-Limão), created in the laboratory, and the litter resulted from their activities were sampled. Thus, our final goal was to bring information to help the comprehension of this complex niche.

MATHERIAL AND METHODS: Fungal cultures were isolated by spreading different parts of soldiers and workers on agar and inoculating the cuticle after scraping. The flotation method was used for the litter samples (Iwatsu *et. al*., 1981), aiming the isolation of hydrocarbon-degrading representatives. Mycosel agar was used for the isolation step.

RESULTS AND DISCUSSION: Fifty-eighty strains were isolated from the ant's cuticle and 25 from the litter sample, resulting in 83 isolates, among filamentous fungi and yeasts. Thirty percent of the melanized fungi already identified revealed the presence of the following genera: *Curvularia, Cladosporium, Cladophialophora, Coniosporium, Exophiala* and *Phialophora.* The latest was already reported by Currie & Little, 2007 in the leafcutting ant *Apterostigma*. Fungi in this group tend to be opportunistic, making this study even more interesting.

CONCLUSIONS: It is evident that the niche ants and fungi share still has a lot to be investigated. More studies must be done to elucidate the ecological interactions between fungi and ants. Some melanized fungal species may present virulence factors like *Cladophialophora, Coniosporium, Exophiala* and *Phialophora,* and it will be important to know what this can mean concerning the ant colony. All strains will be identified at species level to bring more detailed information about the group under study, and clarify their relevance to the symbiosis.

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METABOLIC CHARACTERIZATION OF MICROBIAL ISOLATES THAT USES OIL AS CARBON SOURCE

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Key-words: Bioremediation, biosurfactant, soil microbiology.

INTRODUCTION: In front of the lately ambient disasters occurred in the world, bioremediation is featured as a major model of decontamination and a strong way to reduce the effects of xenobiotic compounds into different ecosystems. This technique consolidates catabolic procedures by native microorganisms into the affected environment (ATLAS, et al.,1993). The morphophysiological and biochemical characterization of the microorganisms presented in this procedure is one of the most important steps. Through the study of microorganisms' biochemistry we can to discern which presents metabolic particularities, and then prospect primary and secondary metabolites with application in research and industry fields. This work aims to characterize fungi and bacteria obtained from soils contaminated with oil in respect of their biochemical and morphophysiological aspects.

MATERIALS AND METHODS: Three microorganisms isolated according to Marcelino et al. (2007) were used in this study. The biochemical tests were realized according to the Microbiology Manual. The lipase activity was based on the Rhodamine B reaction with fatty acids liberated during the enzymatic hydrolysis of soybean oil according to Kouker & Jaeger (1987). Biosurfactant's emulsification and stability tests were performed according to Chen et al. (2007).

RESULTS AND DISCUSSION: Three isolates, two bacteria and one fungus, presenting high potential as oil degraders, were partially biochemical and morphophysiologically characterized. Funguses were submitted to emulsification and stability tests only.

Table 1: Morphophysiological and Biochemical Tests

Microorganisms	Morphophysiological and Biochemical Tests						Stability and Emulsification Index (%)	
	Gram Test	Motility	Glucoses Fermentations (Aerobiosis)	Glucoses Fermentations (Anaerobiosis)	Catalasis	Lipasis	T_0	T_{48}
Bacteria 12 A	+	-	-	+	+	+	9,9348	0,7299
Bacteria 12 B	+	-	-	+	+	+	3,5461	2,8571
Fungus 4 B	Did Not Applied!					+	26,087	24,192

CONCLUSION: Through biochemical and morphophysiological tests it was possible to determine the fungi isolate 4B with an intense lipase activity and a high biosurfactant production with high emulsification stability, showing up as a major candidate for future biotechnological applications. The phylogenetic classification of the microbial isolates is under way using molecular techniques.

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METHODOLOGY ADEQUACY FOR THE QUANTIFICATION OF *Enterococcus spp*. IN SANITARY SEWAGE ORIGINATING FROM AN ALTERNATIVE TREATMENT SYSTEM SEEKING REUSE

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Key Words: *Enterococcus spp.*, sanitary sewage, methodology adequacy

INTRODUCTION: Enterococci are gram-positive cocci which do not develop or grow well in agar or simple broths, making necessary the addition of glucose and thermocoagulation proteins. These bacteria are part of the human normal intestinal flora and are found in feces (OPLUSTIL *et alli.,* 2000). Due to the fact that they do not reproduce in polluted water, their presence is indicative of recent fecal contamination. Their stronger resistance to the sewage treatment processes, in comparison to coliforms, enable a direct correlation with virus survival. Based on these statements more studies that involve these microorganisms in effluent reuse and environment are necessary. The goals of this project were: adapt and implement the detection methodology; quantify these microorganisms in the compartments of the treatment system and evaluate de disinfection by sodium hypochlorite.

MATERIALS AND METHODS: The system configuration consisted of a baffled anaerobic reactor (BAR), followed by "constructed wetlands", slow filtration and disinfection by cloration. The BAR was operated with hydraulic retention time (HRT) from 7.5 to 9.0 hours and ascending flow. The used methodology was adapted from APHA (American Public Health Association) official methods. The *Enterococcus* quantification test comprised the following steps: (1) presumption test in Azide Dextrose Broth, (2) differential selective plating in Bile Esculin Agar (replacing the Pfizer Selective *Enterococcus* Agar) and (3) confirmation test in BHI+NaCl broth at 6.5%. The result was expressed in NMP/100 mL.

RESULTS AND DISCUSSION: In 12 months 48 samples were analyzed, 12 raw sewage, 24 treated sewage and 12 treated sewage submitted to disinfection by cloration. The concentration of *Enterococcus spp.* varied from 10^3 to 10^{10} MPN/100 mL in the raw sewage (P1). The P1 reduction in relation to the effluent treated for reuse (P6) varied from 2 to 10 log. In all samples the decreasing in number of *Enterococcus spp.* in each of the sequential treatment steps was noticed, however the association with cloration was necessary to achieve greater efficiency.

CONCLUSIONS: The baffled anaerobic reactor itself was not efficient enough for the removal of the studied microorganisms. It was noticed that in the studied sewage treatment system a reduction of the *Enterococcus spp.* occurred in each one of the stage, contributing to an elevated global efficiency of the system. It were obtained inferior numbers to the method's detection limit in some samples. The use of Bile Esculin Agar instead of Pfizer Selective *Enterococcus* Agar in the microorganism quantification was efficient in the methodology execution, lowering analyzes costs.

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MICROBIAL ACTIVITY IN SOIL WITH THE ADDITION OF COMPOST FROM DOMESTIC ORGANIC WASTE

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Keywords: microbial biomass carbon, respiration, metabolic quotient.

INTRODUCTION: Microbial communities are stimulated when organic compost is added to soil. This compost is usually rich in macro and micronutrients, and its addition to the soil promotes changes to its structure and function, which stimulates microbial activity (Tejada et al., 2006). Thus, the aim of this study was to evaluate some of the indicators of the microbial processes in the soil, after the addition organic compost.

MATERIAL AND METHODS: The soil samples used in this study were collected at a depth of 10 cm, from a Cerrado soil, with very loamy texture. The compost used originated from aerobic composting of domestic organic waste. The treatments were: control and soil samples with two doses of organic compost (10 and 20 g of compost per kg of dry soil). The experimental design was completely randomized, with four repetitions (in factorial 3x4 for respiratory activity). The attributes analyzed were: carbon of the microbial biomass (CMB) (Vance et al., 1987); soil respiration (Stotzky, 1965) after 15 days of incubation in a ½ L hermetic pot, in the absence of light; and metabolic quotient (qCO_2) (Anderson & Domsh, 1993). The air temperature in the laboratory during the experimental period ranged from 21.5 to 24 ^oC, (average T= 23 ^oC). The results were submitted to ANOVA, at 0.05 of significance.

RESULTS AND DISCUSSION: The daily rates of qCO_2 released from the soil increased from 8.61 mg per kg per day in the control to 14.99 and 25.58 mg per kg per day, respectively, with the additions of 10 and 20 g of composed per kg of soil. These results indicate that the basal soil respiration was different between the control and treatments that received doses of organic compost. This can be explained by the increase in the amounts of carbon and essential nutrients available to the microorganisms in the soil, which could have stimulated the microbial activity. The highest (CBM) was obtained with the addition of the largest dose of organic compost to the soil. The treatments which received doses of organic compost differed significantly from the control for the microbial biomass, which was increased by 2.13 and 3.8 times, respectively, with the addition of the smallest and the largest doses of organic compost to the soil. The qCO_2 values were not significantly different between the two treatments witch received doses of organic compost, but it differed significantly between these treatments and the control, which showed the largest qCO_2 values. This indicates that the treatments which received doses of organic compost had a more diverse and efficient microbial community in relation to energy use compared to the control.

CONCLUSION: The addition of domestic organic compost to the soil of Cerrado, at the doses of 10 and 20 g of compost per kg of soil resulted in significant increases in microbial respiration activity and the carbon of microbial biomass, and in the decrease of the qCO_2.

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MICROBIAL MONITORING GASOLINE-BLENDING CLAY SOIL, *in vitro*

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Keywords: Microbial monitoring, gasoline and clay soil.

Petroleum and its derivatives spills are a frequent problem in aquatic and terrestrial environments. In urban areas, soils usually suffer with fuel leaks in posts, due to little or no maintenance of underground storage tanks. Gasoline is considered the most toxic petroleum derivative for soil microorganisms, because this fuel contains higher content of volatile hydrocarbons which promotes greater contact with the indigenous microorganisms. The aim of this study was determine the effect of gasoline spill on soil microorganisms and isolate microorganisms with hydrocarbon degradation potential from soils without contaminations historic.

The microbial monitoring was done using the “pour plate” and Most Probable Number (MPN) techniques for total heterotrophic bacteria and hydrocarbon degrader bacteria quantification, respectively. The toxicity of gasoline during the incubation period was evaluated by ecotoxicity test of earthworms mortality.

Before contamination soil showed no toxicity to the earthworms applied on ecotoxicity test and present 10^5 CFU/g of soil heterotrophic bacteria and approximately 10^2 MPN/g of soil hydrocarbon degrader bacteria. However, one week after contamination it observed a decrease of two magnitude order on total heterotrophic bacteria population and a slight increase on hydrocarbon degrader bacteria quantification, and it was also observed that 100% of mortality of earthworms applied on ecotoxicity test. Weekly, for 60 days, the total heterotrophic bacteria and hydrocarbon degrader bacteria populations were quantified and the ecotoxicity test was done. During this period, there was an increase in bacteria quantification, mainly hydrocarbon degrader population and a reduction of toxicity to earthworms used.

The results indicate that even in soils without contamination historic is possible the detection of potential hydrocarbon degrader gasoline. We could also observe that gasoline contamination initially reduces the bacteria population, however after an adaptation period there were bacteria population growth. It probably occurs by selection of potential hydrocarbon degraders species which will use the contaminant as carbon and energy sources.

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MICROBIOLOGICAL ANALYSIS OF WATER AND FINGERLINGS OF SURUBIM HÍBRIDO (*Pseudoplatystoma corruscans X P. fasciatum*)

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Keywords: *Salmonella,* pisciculture, coliforms

INTRODUCTION: Due to the great development of pisciculture in Brazil, as well as in Mato Grosso do Sul, it is so important to study the different bacteria which affects the fish production surubim híbrido. Water analysis deriving from the commercial pisciculture is necessary since it may be a way of bacteriological contamination for the population which may swallow this used and refused water without any necessary treatment. This work aimed to analyse the microbiological condition of water during the entrance and the exit from the creation reservoirs and also to carry out the microbiological analysis of the fish surubim híbrido.

MATERIAL AND METHODS: Water was collected in sterile flasks during the entrance and the exit from the reservoirs. The fish were collected with the assistance of a net and carried in sterile flasks. In the laboratory, the bowels were removed. The fish were crushed with the same water they were carried, or better, with the same one from the reservoir of pisciculture. Total and fecal coliforms counting was achieved by MPN (most probable number) method, in accordance with the recommended procedures by American Public Health Association (1995). The positive samples for thermotolerant coliforms were sowed in Teague and incubated for 24 hours. The confirmation of the presence of typical colonies from *Escherichia coli* was achieved through biochemical tests. For the detection, isolation and identification of *Salmonella* spp was used the methodology suggested by "Food and Drug Administration" (FDA) described in the 5th edition of Bacteriological Analytical Manual by Andrews *et al.* (1978).

RESULTS AND DISCUSSION: The values for total coliforms obtained in the entrance water had the maximum value of 15 MPN/ML and the minimum value of < 3MPN/ML and during the exit, the values were between 93 MPN/ML and < 3 MPN/ML. In relation to the fish, the values for total coliforms were between 150 MPN/ML and < 3 MPN/ML. For thermotolerant coliforms in the entrance water, the values varied from < 3 MPN/ML to 15MPN/ML and in the exit one, the values were from 3 MPN/ML to 43 MPN/ML. In the fish samples, the values for thermotolerant coliforms were < 3MPN/ML and 75 MPN/ML. *Salmonella* spp were found only in the exit water and in six samples of fish.

CONCLUSION: Although this commercial pisciculture presents no kind of treatment for used water, the found values are in accordance with the parameters of the Resolution from CONAMA N 274 which classifies the water and its bathing use. For the analysis of fish coliforms, in literature, there is no parameter to compare with this proceeding.

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MICROBIOLOGICAL AND PHYSICOCHEMICAL EVALUATION OF SAVANNA SOILS IN TOCANTINS

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Keywords: microorganisms, soil analysis, Cerrado

INTRODUCTION: Soil microorganisms are essential for the sustainable function of ecosystems, being fundamental in the process of decomposition of organic matter and availability of soil nutrients (MOREIRA and SIQUEIRA, 2002). However, little is known about the role of microorganisms and the main physical and chemical properties involved in maintenance of Cerrado soils (MENDES et al., 1999). The aim of this study was to evaluate and quantify the major groups of microorganisms: fungi, actinomycets and other bacteria, and to analyze the main physical and chemical properties of savanna soils under native vegetation or different agricultural systems in the Cerrado of Tocantins - Brazil.

MATERIAL AND METHODS: Samples were collected at the depth from 0 to 20 cm of soils without litter coverage, using a Dutch auger, from April/2008 to February /2009, at 5 sampling points in the municipality of Lagoa da Confusão-TO: 1) Area under agricultural rice tillage, 2) area under Cerrado "*Sensu stricto*" vegetation, 3) area under natural Savanna grassland, 4) Area under agricultural rice tillage with annual flooding, 5) Area of agricultural rice tillage seasonally flooded. Samples were examined for quantification of microorganisms by using pour plate counting of colony forming units (CFU)/g of soil in selected media, chemical and physical parameters by the methods recommended by EMBRAPA, especially soil carbon stocks.

RESULTS AND DISCUSSION: Microbial counts were higher in the rainy season. The maximum counts were: 935 CFU / g for fungi (Point 2), 3600 CFU / g of bacteria (Point 1) and 1435 CFU / g for actinomycetes (Point 3). During the dry period, the maximum values were 260 CFU / g for fungi (5), 3250 CFU / g for bacteria (Point 2) and 520 CFU / g for actinomycetes (Point 2). The pH of the sampling points is acidic, ranging from 4.9 (Point 2) to 6.2 (5), except for point 1, which had pH 6.8 in the dry season and pH 7.1 in the rainy season. Changes in pH, for each sampling point, were not significant between the dry and rainy periods. The moisture content did not differ between the sampling points, ranging from 6.5% to 8.5% in the dry season, and reaching 99% in the rainy season. The carbon of microbial biomass in soil samples ranged from 0.08 to 5.24 in the sampling period. In the dry season, samples from Point 2 had higher organic matter content (44.8 g/dm^3) and total phosphorus concentration (10.7 mg/dm^3).

CONCLUSION: The bacterial counts were higher than those of actinomycetes and fungi in the dry and rainy seasons. The highest average values in three groups of microorganisms occurred in areas under native vegetation, possibly indicating that agricultural practices may alter soil microbial community. Also, the great variability in the physico-chemical parameters indicates that these soils may form a mosaic of habitat, enabling a high microbial diversity. The molecular studies may prove this hypothesis.

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MICROBIOLOGICAL AND SENSORIAL EVALUATION OF *Pleurotus ostreatus* IN DIFFERENT PROCESSES OF STORAGE

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Key words: *Pleurotus*, storage, microbiology.

INTRODUCTION: The fungi are perishable products, with limited time of useful life, being able to suffer contamination for diverse microrganisms if well they will not be stored. Extrinsical conditions, such as temperature of storage, lack of cares in the process of conversation and manipulation, beyond the intrinsic conditions (water, pH and nutrients) that can be accelerated the metabolism of the microorganisms responsible of contamination (SILVA JUNIOR, 2005). The National Agency of Sanitary Vigilant (ANVISA) has been the agency that regulated the tolerable microbiological standards in foods, because when contaminated was considered improper for the human consumption or bringing risk to health or for presenting sensorial characteristics (color, odor, flavor and texture) that can be rejected from consumer. The objective of the present work was studied different process for the fungi for a long time and evaluated by microbiology and sensorial analysis.
MATERIAL AND METHODS: *Pleurotus ostreatus*, of small size, was harvested in Brasmicel firm (Suzano) and carried in refrigerator "isopor" until the laboratory and soon processed. The fungi in natura was weighed and separate in groups of 50g. Group 1: control only washed with water. Grupo 2: submitted to a treatment with water and citric acid 0.25% and boil at 4 minutes. Grupo 3: same treatment but conditioned in plastic bag with or not simple vacuum and sealing, and than placed under refrigeration for 3, 7, 14 and 28 days. After the intervals of time, the packings was opened and evaluated the sensorial aspects (color, odor, flavor and texture) and submitted to microbiological tests.
RESULTS AND DISCUSSION: Microbiological results, for all the treatments, demonstrated that in PDA (potato-dextrose-agar) medium only yeast was grown and than was counted. In medium with shining green broth, shining green agar, XLD, Rappaport, agar water, was not been detected presence of gram-negative bacteria or the Salmonella type, in none of the treatments. Comparing the sensorial characteristics with the microbiological results of the fungi, the counting of yeast were higher in the stored sample from 28 days without vacuum ($3{,}85x10^3$UFC/g fungi), that also already it presented flavor and odor, color and soft modified and not degusted. Therefore, fungi stored with vacuum for up to 14 days the sensorial characteristics preserved and a small number of colonies of yeast ($2{,}8x10^2$UFC/g fungi) were detected if compared with the sample stored without vacuum for the same period of time that presented yeast ($2{,}5x10^3$UFC/g fungi).
CONCLUSIONS: The fungi *Pleurotus ostreatus* could be submitted the treatment thus increasing the shelf life keeping the characteristics for up to 14 days.
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Microbiological evaluation during the stages of strawberry production in four properties under the integrated production system in São José dos Pinhais, Paraná, Brazil

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KeyWords: microbiological monitoring, strawberry, *Fragaria* sp

INTRODUTION: The production chain of small fruits has in the strawberry the most expressive species in cultivated area and in economic value. It involves many municipal districts in the South, South-East and Middle-East regions of Brazil. The fruit, very much appreciate by the consumers, presents in its production system, many obstacles that difficult the obtention of quality fruits, free of chemical and biological contaminants (EMBRAPA, 2005). Nowadays, it has been searched an alternative approach for production, in regards to obtain safer procedures which would improve the strawberry lucrativeness and quality. The project PIMO (Produção Integrada de Morango – Integrated Strawberry Production) in the Paraná State, congregates producers and researchers from several areas, that analyze many intrinsic factors in strawberry production, looking for solutions that could beneficiate producers and consumers. Environmental, genetics and biological factors affect the plant sanity, directly or through their interactions (UENO, 2004). Pathogenic microorganisms found while strawberry processing make the fruit inappropriate for human consumption. Thus, it is necessary to take sanitary preventive measures, during the production process, to minimize the risks of microbial contamination (TIBOLA; FACHINELLO, 2004). In this work, we aimed to identify the microorganisms present in several steps of strawberry production, and also to propose simple solutions for decreasing microbial agents that could interfere in the quality of final product.

MATERIAL AND METHODS: Four properties producers of strawberry were analysed (harvest 2008). One sample from irrigation water and one sample from each utensil (triage baskets, tables and packages) were collected in each property. After the collects, triage and product packing, the producers hands analysis was performed in 10 repetitions, before and after treatment with 70% alcohol. The water potability was evaluated by the multiple tubes technique, according to Resolution Nº 12 (02/01/2001) of ANVISA. The bacteria were isolated and identified by Gram stain and selective media. Fungi were isolated and identified by macro and micromorphology, in PDA medium. The results obtained will allow the development of a procedure guide to be applied for producers from PIMO project.

RESULTS: Among the four properties analysed, two showed inappropriated water for vegetal irrigation, according the resolution N° 20 (18/06/1986) of CONAMA. The bacteria isolated from hands and utensils were principally *Staphylococcus aureus* and *Escherichia coli*, showing no significant difference among properties. Troller (1971) mention that alimentary intoxication outbreaks are frequently reported and those related to *S. aureus* are the most common. The fungi were observed in lower frequency in the water and belong to genera *Drecheslera* sp and *Scopulariopsis* sp.

CONCLUSÃO: The results show the urgent necessity of sanitary control in the strawberry production steps. Knowing the factors that promote their emergence and the ways of fighting them is essential to minimize the risks of microbial contamination and consequently improve the quality of the final product.

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MICROBIOLOGICAL EVALUATION IN A REGION OF THE MIDDLE DOCE RIVER / MG

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Keywords: Water, sanitation, coliform

INTRODUCTION: The ponds Prata, Pau, Vermelha, Nova and Silvana and the Ipanema stream, universes of this research, are located in the Middle of Doce's River in Minas Gerais. In the view point of Doce River two ponds and a microwatershed are located to the west and three ponds to the east, covering respectively municipalities of Santana do Paraíso, Ipatinga and Caratinga. In order to assess the conditions of balneability of water, much used in recreation, an assessement for bacteria in the coliform group was made, and some physicochemical parameters were measured.

MATERIAL AND METHODS: In December of 2007 and 2008, 55 water samples were collected in ponds and in the Ipanema stream, following the standard NBR 9898/1987 - ABNT. The most probable number (MPN) of coliform group bacteria, including total coliforms and *Escherichia coli* was determined by the technique of dilution in multiple tubes. The physical-chemical characterization was done in-situ. The parameters dissolved oxygen, temperature, pH, conductivity and turbidity were measured for the lotic environment.

RESULTS AND DISCUSSION: The Ipanema stream and ponds Prata and Pau had higher rates of contamination with coliform. The Ipanema stream went through a program of reform implemented by COPASA – Companhia de Saneamento de Minas Gerais and water should be a Class 2 water, where the count of coliform should not exceed 1000 coliforms per 100 ml. The test results of December 2007 and 2008 show that the goal of the program has not yet been reached, and confirmed the results of an evaluation carried out by Santos *et al*, 2002. Molluscs of the Biomphalaria genus, known by the capacity to disseminate schistosomiasis were found in an effluent of Ipanema stream (Taúbas creek) and the Prata pond. Although the results for conductivity have demonstrated an increase in the wastewater in the biggest demographic density region of the Stream Ipanema, the dissolved oxygen remained stable. The stabilization in the rate of dissolved oxygen in the lower region of the stream may be related to the increase in the rate of photosynthesis by algae in local place, which will receive the amount of phosphorus present in the environment (apud SANTOS BARBOSA, 1997).

CONCLUSION: According to the municipal law No 1535/97, the Ipanema stream is in the context of Ipanema APA (Protection Environmental Area). And by Art 4 paragraph I, CONAMA 357 of 2005, waters in preservation of aquatic environments in conservation units of integral protection should belong to the special class. As for balneability, according to the CONAMA Resolution 020/86, the lotic environments examined are classified as inappropriate.

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MICROBIOLOGICAL EVALUATION OF BROWN SUGAR

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Keywords: Brown sugar, quality, microbiology.

INTRODUCTION: With population behavior in changing for a healthy nutrition, the brown sugar has been widely utilized to replace the white conventional sugar because it is produced almost free of chemical agents. Due to the artisanal production, a little is known about the quality of brown sugar commercialized in our country. In this way, this paper aims to evaluate the microbiological quality of brown sugar available in the market.

MATERIAL AND METHODS: 31 brands of brown sugar were evaluated for total mesophilic bacteria (ICUMSA GS2/3-43), mold and yeasts (ICUMSA GS2/3-47), total coliforms and *Escherichia coli* and *Salmonella sp*, the last two analyses performed with kits, Petrifilm (AOAC 991.14 – 3M Microbiology) and 1-2 Test (AOAC 989.13 – BioControl), respectively. The analyses were carried out at Laboratório de Microbiologia Agrícola e Molecular (LAMAM) - DTAiSER/CCA/UFSCar.

RESULTS AND DISCUSSION: Ten samples did not follow the microbiological limit of "National Food Canners and Processors" (50 UFC/g) for mesophilic bacteria, probably associated with an unsatisfactory hygiene at filling and conservation during the production of brown sugar. For the other parameters, there were not unacceptable results. Brazilian legislation establishes coliforms as the the only microbiological parameter for brown sugar, and in this respect, all samples followed the microbiological especification.

CONCLUSION: It seemed that in brown sugar commercialization some hygiene precautions must be improved, mainly in filling and storage process, to assure the food microbiological quality, especially because it is a product of direct consumption. It is suggested that new standardization resolutions, besides coliforms, must be adopted to guarantee the product quality for consumers and to preserve the permanence of small manufacturers in the market.

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MICROBIOLOGICAL EVALUATION OF TEXTILE EFFLUENTS TREATMENT BY ACTIVATED SLUDGE *IN VITRO* AND REAL SCALE

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Keywords: Activated sludge, microfauna, textile effluents

INTRODUCTION: The activated sludge system is one of the most used technology for the purification of textile industries effluents. The occurrence, abundance and the biological succession of the microrganisms were compared with physiochemical parameters in different operational sceneries in an activated sludge of two Wastewater Treatment Plant (WTPs) in the south of the State of Minas Gerais, Brazil.
MATERIAL AND METHODS: The studies were conducted in two WTPs. One data set (WTP-1) operates in the extended aeration mode, treating wastewater from a cotton laundry and finishing. The second, (WTP-2), conventional aeration treating polyester dyeing unit wastewater. Weekly samples were collected for monitoring physical and chemical parameters of operational control and performance. The biomass microscopic analysis of the aeration tank and return sludge were carried out by the comparative method with morphologies of a particular database and literature, particularly Jenkins et al. For the characterization of filamentous bacteria, tests were used for Gram and Neisser staining. The quantitative analysis of the morphologies of interest were taken from the dilution of samples in chamber of *Sedgewick-Rafter* and interpreted as the relative frequency. Were performed also treatability tests with lyophilized commercial biomass (Biolen) and sludge already acclimatized to textile effluents.
RESULTS AND DISCUSSION: Over the period of study, the microbiological characteristics of the two WTPs showed significant variations, with the ETE-1, due to the higher biodegradability of the substrate, the analysis indicated the presence of a superior number of species. Correlations were found between the dominance of microrganisms in the aeration tank of the two WTPs and operational condition, such as the presence of testate amoebas (*Arcella* sp, *Difflugia* sp, *Euglypha* sp) and rotifers (*Philodina* sp e *Epiphanes* sp) was indicative, in WTP-1, advanced sludge age and low relative food / microorganism. Through tests of accomplished tractability, it was possible to confirm the microbial community's temporary variation with the modification of the affluent to be treated.
CONCLUSION: In qualitative analysis it is important to the overall assessment of the sludge, with emphasis on the diversity of species. Microbiological condition observed which correlates to a greater functioning of the stations is the one in which the diversity of groups is high. There are microbiologicals differences in the two systems for textile wastewater activated sludge, however it is considered that a consortium of organisms acclimatized can efficiently perform the biodegradation of sewage from textile industries.

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MICROBIOLOGICAL MONITORING FROM THE EFFLUENTS IN PIG SLAUGHTERHOUSE

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Keywords: *Salmonella,* effluents, coliforms

INTRODUCTION: The pig slaughterhouses produce residues which are thrown in the environment and the rivers are the receivers of these effluents that must undergo by a treatment process before the launching, to prevent an environmental impact. At the studied slaughterhouse, there are five treatment lakes: two are anaerobic; two are optional and one is for polishing. CONAMA (National Council of the Environment) is a national agency responsible for overseeing the parameters from the residues launching. The physicochemical parameters are usually evaluated, but there is no microbiological appraisal which may be extreme important, mainly when it is a matter of pathogenic microorganisms. This work intended to achieve the identification of total coliforms, thermotolerant ones and *Salmonella* spp from the effluents of the treatment lakes.

MATERIAL AND METHODS: Effluent samples were collected in sterile flasks from the anaerobic, the optional and the polishing lakes from the pig slaughterhouse. Total and fecal coliforms counting was achieved by MPN (most probable number) method, in accordance with the recommended procedures by American Public Health Association (1995). The positive samples for thermotolerant coliforms were sowed in Teague and incubated for 24 hours. The confirmation of the presence of typical colonies from *Escherichia coli* was achieved through biochemical tests. For the detection, isolation and identification of *Salmonella* spp was used the methodology suggested by "Food and Drug Administration" (FDA) described in the 5th edition of Bacteriological Analytical Manual by Andrews *et al.* (1978).

RESULTS AND DISCUSSION: The values for total coliforms obtained in the first anaerobic lake had values higher than 1100 MPN/ML and in the polishing lake, the indexes were below than 240 MPN/ML, except for one sample which presented 1100 MPN/ML. For thermotolerant coliforms, the found values in the anaerobic lakes were higher than 1100 MPN/ML and in the polishing lake, the indexes were below than 43 MPN/ML. These results suggest an excellent reduction of the found indexes from the first lakes and the polishing lake, which flows to the river. In relation to *Salmonella* spp research, it was noted its presence in all collections of the anaerobic and optional lakes, but it was not found in the polishing lake.

CONCLUSION: From bacteriological point of view, it is concluded that the treatment system of the effluents from the slaughterhouse present good results when compared to the Resolution of CONAMA N 274, the water with 1.000 faecal coliforms/ML is classifies the satisfactory water and its bathing use.

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MICROBIOLOGICAL QUALITY OF ICE FOR CONSUMPTION IN RESTAURANTS LOCATED AT SOROCABA-SP.

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Keywords: Consumed ice; total coliforms; thermotolerant coliforms.

INTRODUCTION: Ice, resulting from freezing drinking water is consumed by a great amount of the population. Many beverages are made cold by the addiction of ice for consumption and several commercial bars and restaurants have machines used for ice production. The Health's Ministry Decree sets that the standard of drinking water for the human consumption shall be the lack of total coliforms and thermotolerant coliforms in 100mL. The presence of a great number of total coliforms indicates lack of good sanitary practices. The over-bearable number of thermotolerant coliforms and the presence of *Escherichia coli* indicate human and/or animal fecal contamination and therefore can be used as contamination indicators. The objective of the present study was to verify the microbiological quality of ice in some restaurants in Sorocaba-SP with respect to the presence of total coliforms, thermotolerant coliforms and the bacteria *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* is considered because it possibly inhibits thermotolerant coliforms inferring on the colimetric counting and also represents risk for the food quality.
MATERIAL AND METHODS: Seventy-nine samples of ice were collected from eleven restaurants located in Sorocaba-SP. The analyses were realized by Multiple-Tube Technique to evaluate the presence of total coliforms. Sample that tested positive were further inoculated into EC Broth to verify the presence of thermotolerant coliforms. This was followed by the determination of the MPN/100mL with the aid of a standard appropriate table. The presence of *Pseudomonas* sp. was determined by the Membrane Filtration Technique. P. aeruginosa was identified using Mac Conckey Agar for the isolation of bacteria and after was realized biochemical tests to confirm (A.P.H.A., 1999).
RESULTS AND DISCUSSION: Out of the eleven restaurants that were sampled, ice collected from ten of the restaurants were found to be contaminated with total coliforms while ice obtained from eight of them were confirmed to contain thermotolerant coliforms, suggesting the lack of personal hygiene by the staff and the inadequate manipulation of the ice. The *P. aeruginosa* was observed in just one sample suggesting that the quality of water used before handling was good.
CONCLUSION: To guarantee a high microbial quality of ice, good hygienic practices such as keeping the ice in clean isothermal boxes and frequent inspection must be maintained until ice are needed for consumption. Handlers of ice should maintain a high standard of hygiene before and during the manipulation. Equipments used for ice production and all the utensils that come in contact with ice must be washed at regular intervals before and after use. Routine orientation and supervision of workers is also necessary. Maintenance of these practices will preserve the ice safe from microbial contamination and keep it within the standards established by the Health's Ministry Decree nº 518/2004, contributing for the ice ceases to be a potential risk to health.

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Moniliophthora perniciosa BIOTYPE CHARACTERIZATION USING DIFFERENT CULTURE MEDIA

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Key words: *Moniliophthora perniciosa*, biotypes, dye.

INTRODUCTION:

Moniliophthora perniciosa (Stahel) Aime & Phillips-Mora (2005) (Basidiomycota, Agaricales, Tricholomataceae) is a pathogenic fungi, hemitrophic, responsable by the Witches-broom-disease in cocoa plants (*Theobroma cacao).* This disease is fast and very devastating for cooca plantations and in some cases, the farm can be totally destroyed by the disease. Four fungi biotypes can be recognized according to it is hosts. Biotypes C, B, L and S, respectively, attack Sterculiaceae family (including cocoa), *Bixa orellana*, lianas and Solanaceae family. Studies about pathogen genetic diversity are being realized to understandfungi origin at Bahia State and to improve genetic breeding programs searching resistance sources, much more effective and ever lasting.

MATERIAL AND METHODS:

Different culture media were used in order to show physiological differences from biotypes of *M. perniciosa*. WDA (5% wheat, 2% dextrose, 2%agar), No-amoinoacids media (NAAM) , NAAM plus lysine, NAAM plus cisteine and NAAM plus methionine were tested. Thirteen isolates were used from biotype C, six from S and one form L, from different geographical origins. Colony diameter, using a scalimether was sused and clour and morphological characteristics were observed.

RESULTS AND DISCUSSION:

A great difference among growth speed was observed for most of the isolates, so it can not be used as a biotype distinguishable characteristic. Some isolates produced dyes in all culture media, except for WDA – only rich media. Only biotype C isolates showed strong red dye and the others biotypes presented yellow dye. This event can be explained by biotype C at sporulation phase, as observed in basiodicarps in the field. Three isolates form Manaus (AM) and one from Medicelândia (PA) had same dye in all culture media, showing low gentic variation among geographically nearby isolates, as observed by Andebrhan e Furtek (1994), using RAPD (Radom Amplified Polymorphism DNA).

CONCLUSIONS:

It was not possible to identify isolates by the growth speed but the dye process seams to be associated to slow growth.Dye production can be related to pathogenicity of biotype C and it can be used as a differential mark on minimum culture media (poor nutrient media).

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MUTANTS BANK EVALUATION FOR RHAMNOLIPIDS PRODUCTION

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Keywords: rhamnolipids, mutants, *Pseudomonas aeruginosa* LBI

INTRODUCTION: Biosurfactants are metabolites with surface-active properties and are synthesized by a large variety of microorganisms. (BANAT *et al.,* 2000), they present high structural diversity, low toxicity and biodegradable nature. Although biosurfactants have clear advantages, they have not been employed extensively in industry due to their high production costs**.** The development of highly producers strains by mutation or gene recombination have been adopted by different authors, aiming the reduction of biosurfactant production costs. This study evaluated the potentiality of mutants to produce rhamnolipids.

MATERIAL E METHODS: The mutants were obtained through Pseudomonas *aeruginosa* LBI randomic mutation, using the kit EZ-Tn5™ <KAN-2>Tnp Transposome (Epicentre). The clones (760) were singly inoculated in "Blue Agar" medium (SIEGMUND e FRITZ, 1991) to extracellular glycolipids detection. Initially the microorganisms were inoculated in microcultive plates, with 200 µL, in each well, of Luria Bertani (LB) medium and incubated for 36 h at 30 °C and 200 rpm in a rotary shaker. A volume of 30µL of each mutant suspension was added in the plates containing Blue Ágar, to verify the dark blue halos concerning glycolipids production.

RESULTS E DISCUSSION: Among clones evaluated there were not glycolipid production in 120 mutants**,** 36 did not grow and in 604 mutants showed the dark blue halos formation. According to Siegmund e Fritz (1991) the rhamnolipids form an insoluble ion pair with the cationic tenside cetyltrimethylammonium bromide and the basic dye methylene resulting in dark blue halos surrounded the productive colonies of *Pseudomonas aeruginosa*.

CONCLUSION: The mutants bank showed to be promising to rhamnolipids production, since there were dark blue halo formation in 79,5% of colonies.

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OILS BIODEGRADATION ANALYSIS BY RESPIROMETRY METHOD

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Keywords biodegradation; bioremediation; respirometry.

1. INTRODUCTION

Among the different strategies for removal of pollutant hydrocarbons derived from oil, biorremediation is considered sufficiently efficient, transforming organic composites that presents toxicity by the activity of natural microorganisms, reducing the toxicity or treating it, helping in the maintenance of the ecological balance. The research on the evolution of the biodegradation of oil and diesel in terrestrial environment, subject of this work, allows a bigger knowledge on the biorremediation techniques.

2. MATERIAL AND METHODS

The biodegradation was carried through respirometry based on Bartha & Pramer`method, measuring the evolution of resultant CO_2 of the aerobic metabolism of the microorganisms.

3. RESULTS AND DISCUSSION

The data collected for the method of Bartha & Pramer allowed to verify the toxicity and relative biodegradability of oils, and the diesel+biodiesel and vegetable oil new and used presented greater biodegradation level, when compared they to mineral and synthetic oils.

4. CONCLUSIONS

From the data obtained in the studies on the biodegradation of oils it was possible to classify which oils are more easily biodegraded that were the diesel+biodiesel and vegetable oil.

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Optimization of polygalacturonase production by *Trichoderma inhamatum* and characterization of its enzymatic activity

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Keywords: pectinase, polygalacturonase, *Trichoderma inhamatum*

INTRODUCTION: Pectinases constitute a group of enzymes that catalyzes pectic polysaccharides degradation. They have industrial and economic relevancy since they are a good alternative for many chemical treatments. Pectinolytic enzymes can be acid active or alkaline active. The acid one is largely used in juice industries for fruit juice extraction and clarification, maceration and solubilization of plant tissues, vine and purees production. The alkaline one are mainly used in the degumming and retting of jute, flax, hemp and ramie fibers, pretreatment of pectic wastewater from fruit juice industries, tea and coffee fermentation, oil extraction and paper making. Polygalacturonases (PGs) are pectinases that act preferably towards unmethylated galacturonic acid polimers by hydrolysis (KASHYAP *et al*., 2001).

MATERIAL AND METHODS: In sterile Erlenmeyer flasks, 25 mL of liquid Vogel medium (VOGEL, 1956) containing 1 % (m/v) carbon source was inoculated with 1.0 mL of 1.0 x 10^7 conidia/mL suspension. At chosen intervals, the mycelium was separated by vacuum filtration and the culture filtrate used as extracellular polygalacturonase. Enzyme activity was assayed by incubation of culture filtrate with polygalacturonic acid in sodium acetate buffer 0.05 M pH 5.0 for 10 and 20 min, at 50 °C. Reducing sugars were then measured (MILLER, 1959) using galacturonic acid as standard. Protein concentrations were measured by the method of Lowry.

RESULTS AND DISCUSSION: The peak of PG production was seen after 7.5 days of growth in media initially adjusted to pH 5.0, shaking at 100 rpm and 28°C. The best carbon source for PG production was galacturonic acid. Citrus and apple pectins also induced PG production in a smaller level. The natural substrates (wheat bran, oat bran, solid orange waste, dried apple pulp and sugarcane bagasse) were not able to induce polygalacturonase production. The optimal PG activity was assayed at pH 5.0 and 50°C. It is in agreement with optimal conditions usually observed for PGs produced by filamentous fungi, pH between 3.0 and 5.0 and temperatures from 50 to 60°C (Pedrolli *et al*., 2009).The enzyme was able to hydrolyse in some level all tested substrates: polygalacturonic acid, citrus pectin (methylation degree-MD 34, 72 and 90%), apple pectin (MD 73%), showing large specificity for the unmethylated substrate. The PG activity obtained after production optimization was 34.2 U/mL and 181.7 U/mg of protein.

CONCLUSION: The optimization process increased 7.6 times the PG production from *T. Inhamatum*. This pectinolytic enzyme has potential for industry application mainly in juice, food and wine industries since it is an acid active enzyme.

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OTIMIZATION OF LACTIC ACID PRODUCTION BY *Lactobacillus* sp. LMISM6. GROWING IN MOLASSES SUPLEMENTED WITH CORN STEEP LIQUOR IN DIFFERENT TEMPERATURES.

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Keywords: fermentation, molasses, nitrogen source, lactic acid

INTRODUCTION: Lactic acid can be used in food, textile and pharmaceutical industries (WEE *et al*., 2004). The medium to lactic acid bacteria growth isn't economically attractive, due to the elevated cost of nutrients, such as yeast extract and peptone. One alternative is corn steep liquor utilization to optimize the process. The aim of this work was to optimize the temperature and corn steep liquor concentration in fermentation broth with molasses to produce lactic acid by *Lactobacillus* sp. LMISM6.

MATERIAL AND METHODS: The composition of fermentation medium was 19% of hydrolized molasses, 5 g l^{-1} of sodium acetate, 0,1 g l^{-1} of magnesium sulphate, 0,05 g l^{-1} of manganese sulphate, 2 g l^{-1} of ammonium citrate, 2 g l^{-1} of potassium phosphate and 3% of calcium carbonate. Two nitrogen sources were evaluated: corn steep liquor (CSL) and yeast extract (YE) at 5, 15, 25 and 35 g l^{-1}. These experiments were carried out in 250 ml Erlenmeyers flasks with 45 ml of this medium, at 35°C, pH of 6.5 and 180 rpm. The inoculums concentration was 10%. The experiment was carried out in triplicate. Samples of 1 ml were withdrawn from fermentation broth after 24 hours and centrifuged at 7826 x g by 10 minutes. The supernatant was analyzed by HPLC to know the sugar and lactic acid concentration. The biomass concentrations were determined by dry weight. Lactic acid concentrations were determined by high performance liquid chromatography system equipped with UV detector at 210 nm. An Rezex ROA (300 x 7,8 mm, phenomenex) column was eluted with 5mM H_2SO_4 as a mobile phase at a flow rate of 0.4 ml/min and the column temperature was maintained at 60 °C. Total sugar concentrations were analyzed by the same methodology utilized for lactic acid using a refractive index detector instead a UV detector.

RESULTS AND DISCUSSION: In broth fermentation with 5 g l^{-1} of CSL and YL, lactic acid production was smaller. The best production of lactic acid (71,2 g l^{-1}) was obtained with 25 g l^{-1} of corn steep liquor. Otherwise, one inhibition in acid lactic production with 35 g l^{-1} of corn steep liquor was observed. The best lactic acid production was at 35°C.

CONCLUSION: The substitution of YL for CSL is viable. Considering the smaller cost and the high availability of the molasse and the CSL, this is a good medium for lactic acid production.

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POTENTIAL APPLICATION OF MARINE-DERIVED FUNGI FOR THE CONCENTRATION AND RECOVERY OF RARE EARTH METALS

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Keywords: Fungi, rare earth, biosorption.

INTRODUCTION: The rare earth metals (RRs) acquired a great importance due to their applications in high technology. However, these metals present a high value joined due to the difficulties separation and purification by the conventional methods. In this function has increased the interest for new technologies that are efficient for the obtaining of pure RRs with low cost, as for instance, the biosorption, that consists of the adsorption of metallic ions in certain sites presents in the cellular surface of microorganisms (ANDRÈS et al., 2003). This technology can also be used for the separation of RRs, because the sites of adsorption can possess different affinities for the metals, creating a heterogeneity in the intensity of the ligations metal-biomass, which permits the recovery of metals during the desorption. In this sense, this work had as objective to evaluate different fungi isolated from São Paulo state coastline in relation the maximum capacity of biosorption ($Q_{máx}$) and percentage of desorption, in order to select one that presents potential to be used in the concentration and recovery the neodymium and lanthanum metals.

MATERIAL AND METHODS: After the cultivation of the fungi in marine medium liquid at 30^{o}C, with and without agitation, the mycelial mass was dry to 70^{o}C for the obtaining of the inactive biomass. In the biosorption experiments, 0,4g of the inactive biomass of the fungi would *Alternaria alternata*, *Aspergillus terreus*, *Aspergillus versicolor*, *Chrysosporium* sp., *Cladosporium* sp. and *Rhizopus* sp. was incubated with 100mL of the neodymium (Nd) and lanthanum (La) solutions, in concentrations of 0,15, 0,175, 0,20, 0,25 and 0,30g.L^{-1}. After 90 minutes under agitation, a aliquot was collected, filtered and the metal concentration was determinated by EDTA complexometric titulation using xylenol orange as indicator (GUENTER, 1972). From the analysis of the linear regression of the isotherms it was possible to determine maximum biosorption capacity ($Q_{máx,}$) according to the Langmuir's model. The desorption was performed with the biomass from the biosorption experiment using HCl 0,1M as desorbent. After 24 hours, a aliquot was collected, filtered and the metal concentration determined as described above.

RESULTS AND DISCUSSION: Among the fungi studied, *Cladosporium* sp. presented the highest value of $Q_{máx}$ for Nd, whose values were, respectively 374μmol.g^{-1} and 1380μmol.g^{-1}, for the cultivation with and without agitation. In relation to La, the largest Q_{max} was obtained for the fungi *Chrysosporium* sp. (453μmol.g^{-1}) and *Rhizopus* sp. (1338μmol.g^{-1}) among those cultivated with and without agitation, respectively. Also was observed that the fungus *Cladosporium* sp., cultivated without agitation, presented a large difference in the percentage of desorption, with the values of 65% for La and 5% for Nd.

CONCLUSION: The results of this work showed that the fungi *Cladosporium* sp. and *Rhizopus* sp. (cultivated without agitation) present biotechnological potential to be utilized to concentrate and/or recover the rare earths metals, neodymium and lanthanum.

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Potential risk of biocorrosion and detection of Sulfate Reduction Bacteria in the Salto Pilão hydropower plant, Ibirama, SC, Brazil

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Keyword: biofilm, microorganisms influenced corrosion, metagenomics

INTRODUCTION: The Brazilian energy matrix comprises: hydroelectric, thermoelectric (gas, oil, biomass, nuclear and coal) and wind and solar power generation, however, 76% of Brazilian production is derived from hydroelectric power, with only 766 Central Generating of the total in 2022 already installed (ANEEL, 2009). One of the major problems faced by Central Hydroelectric Generating is the biofilm development on the surface of metals submerged and in contact with the waters from the reservatory, especially the manufactured parts from carbon steel. Several sectors are affected by microorganisms influenced corrosion (MIC): Industry in general, piping and seals of fuel tanks (BEECH and GAYLARDE, 1999). The MIC is the process of electrochemistry metal dissolution initialized or accelerated by microorganisms, mainly sulfate reduction bacteria (SRB). The isolation and identification of microorganisms involved in metals biocorrosion are important tools for understanding the process. The knowledge of the relationship among these microorganisms and their metabolism in the biofilm, can lead to strategies to reduce its effects, and, consequently, economic losses in industry and energy production of.

MATERIAL AND METHODS: Seven different alloys were analyzed, representing all metals exposed to the waters of the River Itajaí-Açú in Hydro-Power Plant Salto Pilão. Samples were obtained from corrosion tubers of Metal Coupons (MC's) after exposure for 1 to 2 months at stations where reservatory water flow. The total DNA of samples were purified and submitted to PCR with specific primers for RSB (DALY; SHARP; McCARTHY, 2000) and DNA sequencing to amplicon confirmation.

RESULTS AND DISCUSSION: In all metals tested, the presence of Desulfovibrio sp were detected, however the most affected metals were: the steel ASTM A53, ASTM A36 and AISI 410T. Cu / Ni 90/10 and steel AISI 304, ASTM A743 and SAR 50BN were less affected by biocorrosion. The genera *Desulfobulbus* sp, *Desulfonema* sp and *Desulcoccus* sp were also observed. In addiction, the use of specific primers for RSB detection has accelerated the identification process in comparison with conventional methods, which require the prior microorganisms isolation (APHA, AWWA, WPCF 1999).

CONCLUSION: The RSB genera found were: *Desulfobulbus* sp, *Desulfonema* sp, *Desulcoccus* sp and *Desulfovibrio* sp. The metagenomics approach has accelerated the microorganisms identification. Carbon steel was the most affected by biocorrosion. These data suggest that the protective painting of carbon steel pipes could be employed to minimize the corrosion damages in the hydropower plant.

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FINANCIAL SUPORT:

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PRELIMINARY RESULTS IN THE DETERMINATION OF THE ACUTE SENSITIVITY TO *CERIODAPHNIA SILVESTRII* CLADOCERA TO ARSENIC

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Keywords: *Ceriodaphnia silvestrii*, arsenic, toxicity tests.

INTRODUCTION: In Brazil, generally well-standardized internationally test organisms are used in Aquatic Ecotoxicology. Although extremely sensitive, they do not occur in Brazilian ecosystems, increasing the difficulty of assessing the impacts of chemicals on specific components of the local biota. Thus, ecotoxicological studies become relevant by using native species. In the present study were carried out preliminary tests of acute toxicity with arsenic metal in 3+ and 5+ inorganic forms with the native microcrustaceans, *Ceriodaphnia silvestrii*.

MATERIAL AND METHODS: The methodological procedures were performed according to standard protocols (ABNT, 2004). The preliminary tests consisted in the exposure of 5 neonates (less than 24 hours old) each replicate, to different As^{5+} and As^{3+} concentrations. For each concentration, five replicates and a control were established. The experiments were kept under temperature of 25± 2ºC, without light and food. After 48 hours of exposure, not moving organisms were counted, and the results were expressed as percentage, to calculate the median effective concentration (EC) of the toxic substance, causing effect to 50% of the population exposed (Hamilton et al., 1977). The data were compared with control using the Trimmed-Spearmann-Karber statistical program.

RESULTS AND DISCUSSION: *Ceriodaphnia silvestrii* showed acute sensitivity to arsenic 3+ and 5+ at the concentrations tested. Despite the importance of these chemical compounds in industry, their presence in water bodies can affect the survival and reproduction of aquatic organisms. Preliminary results of the sensitivity range obtained for *C. silvestrii* exposed to As^{3+} was 10^{-6} to 10^{-1} mg L^{-1}, while the value of $EC(I)_{50}$; 48h was 10^{-4} mg L^{-1} with 10^{-5} - 10^{-3} confidence interval. For As^{5+}, the range was 0,005 to 1 mg L^{-1}, while the $EC(I)_{50}$; 48h was 0,13 mg L^{-1} with 0,09 - 0,18 confidence interval.

CONCLUSION: Considering the values of EC_{50} we may conclude that the maximum value (0,5 mg L^{-1}) allowed of total arsenic, established by CONAMA Resolution n° 357/2005 for the Classes 1 and 2 for freshwaters could be down to improve security of the aquatic communities protection, as it is quite toxic to this test organism. It is worth emphasizing that the ecotoxicological studies are used, standardized and recommended by various institutions of environmental analysis, including at international level.

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Production by Bioemulsificant *Bacillus licheniformis* UCP1016 Using Peel of Pineapple (Ananas comosus L.), as Alternative Medium

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Keywords: Bioemulsificant, B. licheniformis, Biorremoção.

INTRODUCTION: The studies with microorganisms, especially with bacteria, have attracted great interest in the environmental area, being able to produce substances that have function to degrade, or promote the degradation of complex compounds such as hydrocarbons in nature. These tense active substances when produced by microorganisms are called biossurfactantes. The bacteria, yeasts and fungi when grown in different carbon sources biossurfactantes produce as a result of its metabolism (SARUBBO et al., 2006). The biossurfactantes have functional properties that include emulsification, separation, wetting, solubilization, desemulsificação, corrosion inhibition and reduction of surface tension. This study aims to evaluate the potential of *Bacillus licfheniformis* in the production of bioemulsificante using the pineapple peel as a culture medium.

MATERIAL AND METHODS: The *Bacillus licheniformis* UCP1016 maintained at 5°C belongs to the Culture Collection of the Center for Research in Environmental Sciences at the Catholic University of Pernambuco, which is registered in the Federation Culture Collection-FCC. The *B. licheniformis* was grown in nutrient broth for preparation of pre-inoculum. The inoculum was transferred to Erlenmeyer flasks of 1000mL of capacity containing 500ml of the peel of pineapple (100g / L, pH 6.5) medium, supplemented with 5% diesel. The tests were performed in duplicates at a temperature of 35 ° C, 150rpm for 72horas. This period was the index of emulsification and surface tension for the production of bioemulsificant evaluation and selection for the test to remove the oil.

RESULTS AND DISCUSSION: *B. licheniformis* UCP in 1016 showed growth equivalent to 4.43 x 10^7 cell / mL and 9.86 x 10^6 cell / mL, at 72 hours of cultivation. The surface tension showed a reduction of 57.73 ± 0.15 mN / m to 38.32 ± 0.16 mN / m in the production medium with 5% of diesel. The rate of emulsification showed 100% of emulsification of oil and engine oil burned, post-frying oil, motor oil, corn oil, oil Pequi. In the test of removing the biossurfactant to 1mg/ml was able to remove 85% oil and 80% of burnt motor oil. The results obtained with pineapple juice as the first information for biosurfactant producer medium.

CONCLUSIONS: The results indicate the formulation of a new medium for biosurfactant producer using the shell of pineapple, as well as, the new strain of *Bacillus licheniformis* as promising bacteria in bioremediation processes.

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PRODUCTION OF BIOEMULSIFIERS FROM *CANDIDA* SPECIES

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Keywords: emulsifier, *Candida*.

INTRODUCTION: an emulsion is formed when one phase is dispersed as microscopic droplets in another liquid continuous phase (SINGH et al., 2007). When a liquid is dispersed in another one, the microscopic droplets formed promote high interface and surface areas. In the presence of surfactants, the emulsions formed can be stable throughout the reduction of the interfacial tension and the coalescence rate. The stability of an emulsion is related to the behavior of the equilibrium of the oil/water/surfactant phase formed by the action of the surfactant (URUM & PEKDEMIR, 2004). Most microbial surfactants are substrate specific, solubilizing or emulsying different hydrocarbons at different rates (ILORI & AMUND, 2001). Poor emulsification of some of the hydrocarbons might be due to the inability of the biosurfactant to stabilize the microscopic droplets. The aim of this work was to evaluate the ability of *Candida* species to produce surfactants with emulsifying properties.

MATERIALS AND METHODS: *Candida* species *(C. tropicalis, C. lipolytica, C. sphaerica, C. guillermondi, C. buinensis e C. glabrata)* were cultivated in mineral medium supplemented with 2.5% of an insoluble or a soluble substrate, or the co-utilization of both of them, including n-hexadecane, soybean oil, ground-nut oil refinery residue, corn steep liquor, and glucose, with shaking at 150 rpm for 144 h at 27 °C. After cultivation, samples were centrifuged for cells removal and the cell-free broth used for emulsification activity determination with different hydrophobic substrates (COOPER & GOLDENBERG, 1987).

RESULTS AND DISCUSSION: the emulsification activities using kerosene, motor oil, and corn oil were quite different, which means that they did not follow a similar pattern, being sometimes high and others low. It can thus be assumed that in this case the formation of emulsions can be due to the presence of other materials with emulsifying properties excreted in the medium. Another possibility can be the production of amphiphilic molecules at concentrations under the CMC in the media studied.

CONCLUSION: the results obtained in this work show that depending on the strain and on the substrate used the emulsification ability of the yeast cells and the production of surfactants can take place simultaneously, thus increasing the efficiency of bioremediation treatment of petroleum and derivates.

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PRODUCTION OF ASPARAGINASE BY *Zymomonas mobilis* UTILISING SUGAR CANE JUICE AND YEAST EXTRACT.

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KEY WORDS: Asparaginase, *Zymomonas mobilis*, sugar cane juice.

INTRODUCTION:

The asparaginase, an anti-leukemia agent, is utilised to treat acute linfoblastic leukemia and other leukemic illnesses. The enzyme catalyses asparagin hydrolysis into aspartate and ammonium, leading to asparagin depletion in tumoral cells which are unable to produce asparagin. So the asparagin depletion caused by asparaginase endovenous administration causes death of the malignant cells. However the conventional use of *E. coli* asparaginase causes toxic side effects and it has a high cost of production, than new asparginase sources need to be found. This work aimed the production of asparaginase by *Z. mobilis* utilizing low cost raw materials from agroindustry: sugar cane juice and yeast extract.

MATERIAL AND METHODS:

Z. mobilis was mantained in agar plates. Sugar cane juice under white-egg and boiling treatment followed by filtration and dilution at 42,34g/L of Total Sugars was used as medium in which was added (g/L): KH_2P0_4 1,0; $MgS0_4$ 1,0; $(NH4)_2S0_4$ 1,0 and yeast extract 1,75. Batch fermentation used 125ml Erlenmeyer flasks with 25mL culture medium without agitation, at 28°C and run in triplicate. After 10 hours of growth enzyme activity, biomass and residual sugar (DUBOIS et al. 1956) were measured according to IMADA et al.(1973) one enzyme unit was the quantity of enzyme necessary to release one µMol of ammonium per minute at 37°C in pH 8,5.

RESULTS AND DISCUSSION:

The values of sugar consumption (g/L), biomass (g/L), activity (U/L of fermentation broth) and productivity (ativity/fermentation time) were: 34,7; 10,65; 8,84 e 0,88. CASOTTI et al.(2007) obtained the ativity value of 9,75U/L when sugar cane juice at 80 (g/L) of Total Sugars was used while CAMILIOS NETO et al.(2006) achieved the activity value of 16,55 (U/L) when sugar cane molasses at 100(g/L) of total sugars was used but the medium contained asparagin as inducer and fermention time was of 21 hours. This work obtained good sugar consumption and although activity value was lower than CASOTTI et al, (2007) the productivity was better . However the use of molasses and asparagin led better than values of activity.

CONCLUSIONS:

The use of sugar cane juice and yeast extract are promissing for asparaginase production by *Z. mobilis,* but other research are needed to established both ideal sugar concentration of cane juice and yeast extract quantity.

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PRODUCTION OF ASTAXANTHIN BY M*ucor circinelloides* FROM CORN STEEP LIQUOR

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Keywords: *Mucor circinelloides*, astaxanthin, corn steep liquor.

INTRODUCTION: The astaxanthin ((3, 3' - dihydroxy-β, β-carotene-4, 4' dione) is one of the most important carotenoid and can be synthesized by microorganisms (FONTANA et al., 2000). The chemical synthesis of astaxanthin is very complex and of high cost. That is why there is a great interest in the use of biological sources of this kind (REYNDERS et al., 1996).The Corn steep liquor (CSL) is a waste water from washing and imbibition of the grains for its fractionation in starch and germ (oil), containing 40% of solids (FONTANA et al., 2000). In this work, it was studied the use of industrial waste of CSL as an alternative substrate for the production of astaxanthin by *Mucor circinelloides*.

MATERIAL AND METHODS: It was used a strain of *Mucor circinelloides* obtained after 5 days of incubation at 28 ° C. It was tested two patterns of inoculums: 10^4 and 10^7 spores / ml. For the preparation of the pre-inoculum, the suspension of spores adjusted was inoculated in the middle of CSL for 24 hours at 25 °C and 150 rpm. It was added 1 ml of pre-inoculum in bottles containing 100 ml of the culture means of 4% of CSL(concentration indicated by Barbosa da Silveira (2007) as being favorable to the production of astaxanthin) supplemented with thiamine (0.0005 g). The pH was set at 6.5 and the cultures incubated in orbital shaker at 120 rpm, 25 ° C for 96 hours. At the end of the process the cultures were filtered, washed with distilled water and lyophilized (Freeze Dry, Freezone 4.5, LABCONCO). The extraction of the carotenoid was done with hexane / methanol (1:1, v/v). The sample containing the astaxanthin was separated by centrifugation at 2000 rpm for 10 minutes. The final fraction was analyzed by spectrophotometry (UV-visible), 470 nm.

RESULTS AND DISCUSSION: When used the inoculum pattern of 10^4 the production of astaxanthin was 5,1 µg/g of biomass. With the inoculum of 10^7 it was obtained a production of 1,1 µg/g of astaxanthin. The results show that *Mucor circinelloides* has a significant biological potential for producing astaxanthin, once it accumulates pigments during mycelial growth (ANDRADE, 2003).

CONCLUSIONS: The Corn steep liquor (CSL) has potential for producing astaxanthin, demonstrating the ability to be used as alternative substrate.

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PRODUCTION OF BIOSURFACTANT BY *Candida lipolytica* IN SUBMERGED CULTIVE OF MANIPUEIRA AND PINEAPPLE JUICE AS LOW COST SUBSTRATE

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Key words: Biosurfactant; *Candida lipolytica*; surface tension

INTRODUCTION: The surfactant is an important class of chemical compound widely used in various industrial sectors. Most commercially available surfactants are synthesized from oil products (NITSCHKE; PASTORE, 2002). Microbial origin compounds that exhibit surfactant properties such as decrease the surface tension and have high emulsifying capacity, are called biosurfactant and can be metabolized by bacteria, fungi or yeasts (MULLIGAN, 2005). Biosurfactant production by *Candida lipolytica* using manipueira, that is a wastewater from cassava roots pressing, is a low cost alternative. In this context, the objective of this study was to test a new biosurfactant production technique, consisting of pineapple juice as culture medium and using manipueira as substrate.

METHOD AND MATERIALS: *Candida lipolytica* (UCP0988) strain, from Cultures Collection of the Center of Research in Environmental Sciences – UNICAP, was maintained in YMA medium to obtain a new culture. The biosurfactant production was performed in Erlenmeyer flasks of 250 mL containing 100 mL of the culture medium (90 mL manipueira added to 10 mL of pineapple juice). The initial pH of the medium was adjusted to 6 and inoculated 5% of pre-inoculum (YMB). The cultivation was carried out for 72 hours at steady state temperature of 28^{o}.C. Subsequently, it was determined the surface tension and emulsification index of the cells free metabolic liquid. The biossurfactante was extracted according to the method of Cameron, Cooper and Neufeld (1987).

RESULTS AND DISCUSSION: After the cultivation with 72 hours the biosurfactant been manufactured with a surface tension of 32.51 mN / m. The extraction of biosurfactant from the cells free metabolic liquid was 6.0 g per liter. The best results for the emulsification index showed 48.57% using corn oil as substrate and 32.26% using cotton seed oil. According to Mulligan *et al.* (2005) a good surfactant should decrease the surface tension of water from 72 to 35 mN / m and its efficiency is related to a low critical micellar concentration (CMC).

CONCLUSIONS: The biosurfactant produced by *Candida lipolytica* in a medium containing an industrial waste - manipueira, will contribute greatly to the economy and environmental preservation.

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PRODUCTION OF BIOSURFACTANT BY *Serratia marcescens* USING AGROINDUSTRIALS SUBSTRATES (CORN STEEP USING RICINUS OIL)

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Palavras-Chave: Biosurfactant, *Serratia marcescens*, Agroindustrials substrates

INTRODUTION: The biosurfactant has many advantages over chemical surfactants as biodegradability, low toxicity and performance in extreme conditions of temperature and / or pH (BANAt et al., 2000). However, the biosurfactant production by fermentative processes and purification demanded costs about 3 to 10 times higher that the synthetic surfactants. Therefore, in recent years, has continually increased the number of investigations using different species of microorganisms, using alternative substrates, as well alternative substrates and variables fermentative processes. In this work was evaluated the biosurfactant production by *Serratia marcescens* using corn steep medium (residue from corn industry) and ricinus vegetal oil, as substrates of low cost.

MATERIAL AND METHODS: The bacterium *Serratia marcescens* was in Cultures of the Bank of Center of Research in Environmental Sciences, Catholic University of Pernambuco, registered on Federation Culture Collection-FCC. The strain was maintained on nutrient agar medium at 5^{o} C. In this experiment was used complete factorial design 2^{2}, with 4, and three replicates in the central point to evaluate the effects of corn steep and ricinus oil on the surface tension, emulsification index activities from metabolic liquid free of cells. In the culture media formulated according to the factorial design (using combinations of corn steep and ricinus oil to 5% v/v in the lower level, and 15% v / v in the higher level). The Erlenmeyer's flasks were incubated at 28 ^{o}C and 155 rpm, during 72 hours. The production of biosurfactants was evaluated each 24 hours for three days using determination of surface tension (CAMEOTRA SINGH, 2004), emulsification index (COOPER and GOLDENBERG, 1987) and emulsification activity (CIRIGLIANO and CARMAN, 1984).

RESULTS AND DISSCUSSION: After 72 hours the results were indicated biosurfactant production in all conditions studied. The best results was obtained was pH values 8.04 (±0,05), the surface tension was reduced to 70mN/m to 30.47 (±.0,29) mN/m, the emulsification activity of the 4.19 (± 0,18) UAE and emulsification index 72.71 % (± 1.25).

CONCLUSION: The results showed the ability of the bacterium *Serratia marcescens* to grow on corn steep and ricinus oil as substrate, and produced biosurfactant with tensoactive and emulsifying properties. In addition the low cost medium has higher potential for bioremediation process.

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PRODUCTION OF BIOSURFACTANTS BY C*ANDIDA LIPOLYTICA* USING KINETIC MODELS

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Keywords: Biosurfactants, Kinetic Models and C*andida lipolytica*

INTRODUCTION: The *Candida* genus sort has been described in literature as an excellent produced of biosurfactants, compound that possess high capacity of emulsification (RUFINO, et al., 2008). Many systems can be represented by models comprising more than a mathematical equation, such as the kinetic models associated with the production of biossurfactants. Thus, the choice of the best model must be made by means of proper techniques capable of identifying the one that could best represent the various components of the system, in accordance to a given level of reliability.

MATERIAL AND METHODS: In this study, tests have been made to identify two relevant kinetic models described in the literature, model 1 (GARCIA-OCHOA and HOUSES, 1999) and model 2 (RODRIGUES et al.,2005), using the experimental data described by Sarubbo *et al*. (2001), to anticipate kinetic parameters for the production of biosurfactants by *Candida lipolytica*.

RESULTS AND DISCUSSION: The model 2 perfectly fitted the experimental data, having received from the Statistica program a message of the kind “predictors are probably very redundant; estimates suspect”. Thus, the analysis of other statistical parameters, such as the normal behavior of residues can be discarded as a working tool for the comparison between the components of the models.

CONCLUSION: Due to the similarity between the mathematical structures of the models involved, it was possible to conclude that the statistical criteria adopted were an excellent working tool, as they could clearly point out the set of equations which best fitted the experimental data. Model 2 proved to be the best set of kinetic equations to describe the production of the biosurfactant from *Candida lipolytica* . The choice between two models of quite similar mathematical structures favored the one which best fitted the experimental data, presenting variances in the order of 0.999, 0.982 and 0.972, for correlations for the prediction on biomass growth, glucose consumption and biosurfactant production, respectively.

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PRODUCTION OF BIOSURFACTANTS FROM INDUSTRIAL RESIDUES

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Keywords: Biosurfactants, industrial residue

INTRODUCTION: 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 (SINGH et al.,2007). Many biosurfactants have been produced but few have been commercialized due to the high process costs, specially as a function of the use of expensive substrates and the purification processes (MULLIGAN, 2005). In this sense, six biosurfactants were produced from industrial residues as low-cost substrates and characterized regarding the efficiency (surface tension reduction capacity) and effectiveness (low critical micelle concentration).

MATERIAS AND METHODS: five biosurfactants were produced by *Candida* yeasts (*C. lipolytica, C. glabrata, C. sphaerica* and *C. tropicalis*) and one by the bacterium *Pseudomonas aeruginosa.* The substrates ground-nut oil refinery residue, vegetal fat residue, cooking oil and corn steep liquor were used, separately or together, were added to the production media. All fermentations were conducted at 150 rpm during specific periods of time and with specific inoculum concentrations, according to each strain. After fermentation, samples were withdrawn for surface tension measurements, isolation and determination of the critical micelle concentration (CMC). The isolated products were then weighed, characterized and the yields calculated**.**

RESULTS AND DISCUSSION: the biomolocules obtained were able to reduce the water surface tension from 72 mN/m to values between 27-31mN/m and the CMC were between 0.07% and 1.0%, showing yields from 3.0 to 8.0g/L. Considering the utilization of industrial residues as substrates, the values of surface tension are satisfactory, once surfactants are considered efficient when reducing the surface tension to values around 35 mN/m, according to the literature (RON & ROSENBERG, 2001). Regarding the yields obtained, they are also satisfactory when compared to values also described in the literature for other biomolecules produced in flasks (MULLIGAN,2005).

CONCLUSIONS: the biosurfactants produced show attractive properties as reduced surface tension and low critical micelle concentration. Once different microorganisms, media and cultivation conditions were used, probably different structures had been obtained.

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PRODUCTION OF ITACONIC ACID BY *ASPERGILLUS TERREUS* IN SUGAR CANE MEDIUM

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Keywords: *Aspergillus terreus*, organic acids, itaconic acid, biotechnology, fermentation, sugar cane.

INTRODUCTION: Biotechnology has been defined as the application of living organisms and their components in industrial processes to obtain products of interest to economic, scientific and social. Some of the most important microorganism today exploited are filamental fungi and yeast. Among the extraordinary capacity for bioconversion of fungi, the production of organic acids in high concentrations is observed. One of the acids excreted by fungi is itaconic acid, which is also known as methylene succinic acid, 3-carbobxy-3-butanoic acid and methylene butenedioic acid. This acid can be used as alternative raw material for various organic syntheses. The properties of the methylene group of itaconic acid, allows its use in the synthesis of polymers in radical polymerization reactions, or even in additions of Michael, thus enabling the achievement of various macromolecules. Through this and other possibilities of use, the itaconic acid has been produced in some countries using a chemical route, or by using microorganisms such as *Aspergillus terreus, Rhodotorula glutinis* and other species.
MATERIAL METHODS: The fungus *Aspergillus terreus* NRRL 1960 (TCC) was maintained in agar medium, malt extract, 5% at 5±2ºC. *A. terreus* conidia were suspended in Tween 80 (0,1%) in aseptic conditions, inoculated into clarified sugar cane medium, 5º and 10º Brix, pH 2.0, in 250 mL Erlenmeyer flasks. The flasks were incubated for 7 days on 170 rpm rotary shaker. The itaconic acid was analyzed by ion chromatography, using Ion Chromatograph Metrohm model IC 761 Compact, with column metrosep organic acids 250/7.8 and as eluent H_2SO_4 0,4 mM / acetone (88-12).
RESULTS AND DISCUSSION: The *A. terreus* in malt extract medium, had high formation of conidia. These in sugar cane medium, produced biomass and itaconic acid. The results in sugar cane medium (pH 2.0) after 24 h of cultivation in 5º Brix were 2.57 $g.L^{-1}$ and 19 $mg.L^{-1}$ for biomass and itaconic acid respectively. And for cultivation in 10º Brix were 1.66 $g.L^{-1}$ and 55 $mg.L^{-1}$ for biomass and itaconic acid respectively.
CONCLUSIONS: The results can predict the alternative use of sugar cane medium for cultivation of *A. terreus* in production of itaconic acid.
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PRODUCTION OF LIPASE BY *BOTRYOSPHAERIA RIBIS* EC-01 IN SOLID-STATE AND SUBMERGED FERMENTATION USING AGRO-INDUSTRIAL RESIDUES

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Keywords: lipase, agro-industrial residues, *Botryosphaeria ribis*

INTRODUCTION:
Lipases hydrolyse esterified triacylglycerols releasing long-chain fatty acids and glycerol as products, which are applicable in detergent, food and biodiesel industries.[1] *Botryosphaeria ribis* was previously selected as a producer of constitutive lipase. Enzymes are commonly produced by submerged fermentation (SmF), however, solid-state fermentation (SSF) is also promising, especially when agro-industrial wastes are the source of substrate. The objective of this paper was to compare the lipase production by SmF and SSF, using, separately, agro-industrial residues, such as soybean, castor, corn and olive cakes/meals, as carbon sources.

MATERIAL AND METHODS:
B. ribis was grown by SmF in 1 % (w/v) using, alone as a carbon sources, agro-industrial residues (soybean, castor, corn or olive cakes) with or without the addition of minimal salts medium (VMSM)[2]. SSF cultures were developed at different concentrations of the cited residues. The cultures were kept agitated (SmF) or static (SSF) for 5 days at 28 ºC. Cell suspensions (200 - 260 µg of dry mycelium/mL) were used as inoculum. Fungal cultures grown by SmF were interrupted by centrifugation, and by SSF by addition of distilled water, homogenization, agitation in a shaker and then centrifuged. The supernatants were dialyzed and frozen for subsequent enzymatic determination. Lipase activity was assayed using *p*NPP (55 ºC, phosphate buffer 0.05 mol/L, pH 8, 2 min, 410 nm), and one unit was defined as the release of 1 µmol of *p*NP per min, per mL of enzyme solution, and was converted to U/g substrate.

RESULTS AND DISCUSSION:
Castor cake stood out as the best residue for the production of lipase by *B. ribis* in SSF at all concentrations evaluated, followed next by soybean and corn. The best activity was obtained using 40 % w/v of substrate (10.14 ± 0.65 U/g of substrate). Lipase production on olive cake wasn't detected. Highest lipase titres were obtained by SmF when soybean or castor cake were used as a substrate (1495 ± 138, and 1382 ± 21 U/g substrate, resp.), whereas lower activities resulted when *B. ribis* was grown in the presence of VMSM. There was lipase production on all residues examined by SmF. However, in cultures with corn and olive cake, higher enzyme titres were obtained in the presence of VMSM (350 ± 24 and 759 ± 8 U/g substrate, respect.).

CONCLUSION:
Lipase was produced by *B. ribis* under both types of fermentation. The SmF process turned out to be more appropriated for the production of this enzyme, since it provided the greatest titres of lipase by *B. ribis*.

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PRODUCTION OF ORGANIC ACIDS AND OTHER METABOLITES IN COCULTURE OF YEAST AND LACTIC ACID BACTERIA

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Key words: alcoholic fermentation, bacterial contamination, homo- and heterofermentative metabolism.

INTRODUCTION: Bacterial contamination is often considered a major drawback during industrial ethanol fermentation. Besides deviating feedstock sugars from ethanol formation, there are also detrimental effects of bacterial metabolites (such as lactic and acetic acid) upon yeast fermentative performance and viability (Bayrock & Ingledew 2004). In this context, the aim of the present study was to evaluate the effects of organic acids and other metabolites produced by two lactic acid bacteria strains presenting distinct metabolism types (homo- and heterofermentative) on yeast viability and fermentation parameters.

MATERIAL AND METHODS: Lactic acid bacterial strains used in this work were: FT025B (*Lactobacillus plantarum*), a homofermentative strain; and FT230B (*L. fermentum*), a heterofermentative strain. Both strains were isolated from ethanol plants located in Brazil. Four industrial strains of *Saccharomyces cerevisiae* (BG-1, CAT-1, PE-2 and backer's yeast) were employed in co-cultivation with the above bacterial strains. The experimental setup consisted of cultivations in which a bacterial strain and a single industrial yeast strain were inoculated in the same medium composed of (in g/L): 10 glucose; 10 fructose; 5 Bacto-peptone; 5 Yeast extract; 2 K_2HPO_4; 0.2 $MgSO_4$; 0.01 $MnSO_4$. The flasks, containing 10mL, were incubated at 32°C. After 24hs samples were taken for organic acids, ethanol, glycerol, mannitol and sugar analysis. Samples were also analysed for cell viability and cell count, for both bacteria and yeast cells. Organic acids, ethanol and glycerol were quantified by HPLC (HPX-87H column; 5mM H2SO4 mobile phase and IR detection), whereas sugars and mannitol were determined by HPAEC (Carbo-PAK1 column; 100mM NaOH mobile phase and amperometry detection).

RESULTS: The results have showed that homofermentative bacteria viability was lower when compared to heterofermentative bacteria. It was also observed for all yeast strains, that their cell viabilities were lower when they were cultivated with the homofermentative bacteria. Regarding organic acid and metabolite production, succinic acid concentration was similar at all treatments. On the other hand, acid lactic was higher in the presence of homofermentative bacteria, whereas acetic acid was higher in cultivations inoculated with heterofermentative bacteria. The same trend was observed for glycerol. Mannitol was only detected in medium containing the heterofermentative strain. Finally, ethanol concentration was lower in the presence of both bacterial types when compared to the treatment without bacterial contamination.

CONCLUSIONS: This study suggests that homofermentative bacteria appear to be more detrimental to yeasts when compared to heterofermentative ones. Even though viability of the former type was lower than in heterofermentative, homofermentative bacteria (or their metabolites, probably lactic acid) have caused a more pronounced reduction in yeast cell viability.

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Production of Prodigiosin by a New Strain of *Serratia marcescens*

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Palavras-Chave: *Serratia marcescens*, Prodigiosin, production

INTRODUCTION: Prodigiosin is a secondary metabolite, produced by *Serratia marcescens* belongs to the family Enterobacteriaceae. The prodigiosin has bactericidal activity, antifungal, antitumour, and recently, activity against Trypanosoma cruzi (GEBER., GAUTHIER, 1979). Considering its potential pharmaceutical, pigment that arouses the interest of pharmaceutical companies to develop new drugs, including antitumor is (NAKASHIMA et al., 2005). This work was performed to produce prodigiosin using a new strain of Serratia marcescens.

MATERIAL AND METHODS: *Serratia marcescens* was isolated from soil of banana plants in the state of Pernambuco and has been maintained in nutrient agar cultures in the bank of the Catholic University of Pernambuco. The production of prodigiosin was performed in Petri dishes containing the media: Peptone glycerol (10g peptone, 10% glycerol, 15g of Agar) Agar mannitol (5 g of peptone, 2.0G extract of yeast, 20 g mannitol, 17 of agar / L), rice bran (40g of rice bran 17g and 15g of agar / L) and sesame meal (17g sesame meal and 15g of agar / L), added to agar to 1.5% ¨, incubated at 28 C for 48h. The pigment was extracted with chloroform / methanol (2:1, 1:1, 1:2, 100% MeOH v / v), and purified by chromatography employing the exclusion of Sephadex LH-20, identified by spectrophotometer UV / Vis bands in 200 to 700nm, with maximum absorbance of 539 nm.

RESULTS AND DISCUSSION: The production of prodigiosin using the four culture media showed only intense red pigmentation in mannitol agar, corresponding to 1.1 g / g of biomass. The other media tested produced biomass, but without pigmentation. Based on the absorption spectrum of prodigiosin described in the literature, the results suggest that the pigment is purified prodigiosin (2-methyl-3-pentyl-6-metoxiprodigioseno) with absorbance maximum of 539.1nm and 533 nm produced by alpha - proteobacterium. The data is confirmed by Nakashima (2005) information.

CONCLUSIONS: The new strain of *Serratia marcescens* is a higher prodigiosin producer.

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PROFILE OF FILAMENTOUS FUNGI FROM THE SEMI-ARID SOILS OF THE PERNAMBUCO STATE

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Palavras-Chave: soil, filamentous fungi, semi-arid

INTRODUÇÃO: The fungi comprise a heterogeneous and heterotrophic, cosmopolitan and important microorganism component of ecosystems (GOMES, et. Al., 2008). The semi-arid presents rainfall concentrated in short time, so most of the year the soils are exposed to strong sunlight and high water deficit (ARAÚJO et. Al., 2008). Considering the necessity of information on the mycobiota of the semi-arid soil, in particular for filamentous fungi, in this work we studied the diversity of fungi in soils of Serra-Talhada, PE.

MATERIAL E MÉTODOS: The samples were collected to 10 cm of soil depth, on the campus of Serra Talhada Federal Rural University of Pernambuco were submitted to isolation of filamentous fungi using the method of serial dilution. From suspensions of soil pH studies were performed with the natural environment, and then adjusting the pH of the natural soil for pH 4 (acid) and pH 8 (basic). Then, the dilutions 10^{-3} and 10^{-6} for the soil, and selected pH were transferred to Petri dishes, added to Sabouraud dextrose agar medium, incubated at a temperature of 28º C for 7 days, with observations every 24 hours . After growth fungi were transferred to Petri dishes containing Sabouraud dextrose agar medium for confirmation of purity and maintained in test tubes with the same medium. The microorganisms isolated were identified as to gender, based on microscopic characteristics, selecting the samples with potential for production amylase and celulase.

RESULTS AND DISCUSSION: The soils collected from the semi-arid region showed higher number of isolates in the pH 5 (natural) with a predominance of the genus *Aspergillus* sp. The fungi isolated from the soil were *Aspergillus* sp (60.00%), *Penicillium* sp (6.67%) and *Trichoderma* sp (13.33%), and the most showed ability to produce amylase and celulase. According to Domsch et al. (1993) and Colla et al. (2008) the fungi identified in this study are considered common inhabitants of the soil, which occurred in forests soils, fields, sandy, and cultivated areas.

CONCLUSION: The genus *Aspergillus* is the main microorganism isolated from Serra Talhada land. The strains demonstrated higher resistance to acid and basic pH. The isolates are common microorganism that inhabits soils.

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PURIFICATION ON THE EXTRACT OF *Trichosporon asahaii* AND INHIBITORY ACTION IN *Pseudomonas aeruginosa.*

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Keywords: purification, extract, *Trichosporon asahaii*, Minimum Inhibitory Concentration (MIC), *Pseudomonas aeruginosa.*

INTRODUCTION

Pseudomonas aeruginosa is the most important specie of bacteria for public institutions of health. It is responsible for many cases of infection in hospital environments and it is resistant to many antibiotics (WHO, 2003; BRAGA et al., 2004).

MATERIAL AND METHODS

The inhibition of *Pseudomonas aeruginosa* was avaliated from the extract liquid of the culture of *Trichosporon asahaii* from laboratory of Biotechnology Industrial of UNESP – Assis and identified for Fernando Panhocca – UNESP – Rio Claro. This extract was submitted a separation liquid-liquid where some solvents were avaliated, among they: isobutyl alcohol, ethyl acetate, butyl acetate and chloroform. The fractions organic obtained were dried for evaporation and diluted in Tween 80®. The determination of the dried antibacterial yield and the activity against *P. aeruginosa* ATCC27853 and ATCC9027 were procedure following the Minimum Inhibitory Concentration (MIC) with growth determination of the turbidity and by plate count using Luria Bertani medium, according to JONES et al., (1981).

RESULTS AND DISCUSSION

The extract of *T. asahaii* submitted to chloroform extraction inhibited the growth of *P. aeruginosa* more than others solvents, reaching 100% inhibition. In opposite, the others solvents did not inhibit, or partially inhibit the bacteria growth. The MIC of the dried *T. asahaii* extract from the extraction of chloroform in *P. aeruginosa* it's been determined, and it is less than 21,611mg dried extract per litre of this extract and 16,2 times concentrated from the culture. The yield of dried extract from chloloroform extraction was 2,2g in 100mL of the culture. The use of the turbidity for quantify the *P. aeruginosa* growth was not valid because the chloroform showed turbidity in the culture medium and the bacteria showed pellets in the growth, causing problems in the turbidity quantification by spectrophotometer. For this reasons the plate counting showed more appropriated for this purpose.

CONCLUSION

The *T. asahaii* extract concentrated by separation liquid-liquid with chloroform is effective in the inhibition of *P. aeruginosa.*

The isobutyl alcohol, ethyl acetate and butyl acetate were not recommended for the separation of the inhibitory agent from *P .aeruginosa* culture.

For the quantification of the *P. aeruginosa* growth on MIC test the plate counting method is recommended and the turbidity is not recommended.

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QUANTITATIVE MICROBIOLOGICAL ANALYSIS OF THE AIR IN THE CENTRO UNIVERSITÁRIO SÃO CAMILO – CAMPUS POMPÉIA (SP) AND SURROUNDINGS

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Keywords: Atmospheric pollution. Environment. Environment/control.

Introduction: The air quality in some areas in the metropolitan region of São Paulo has become a threat to the health and welfare of the population, not only because of the chemical pollution, but also for the presence of pathogenic microorganisms that put the man, fauna and flora at risk. Within this context, the present work had as an objective to make a quantitative analysis of the microorganisms present in the air in different areas of the Centro Universitário São Camilo – Campus Pompéia (SP) and surroundings.
MATERIAL AND METHODS: The samples were collected in Petri dishes exposed for 5', 10', 20', 30', being used four culture media per period, naming them: TSA, PDA, BP, and BEM, with 4 repetitions each. The collecting places were: biology lab, men bathroom, women bathroom, classroom, canteen, library, research room (mastering area) and external area. The evaluation was made through the counting of the unit-forming colony (UFC).
RESULTS AND DISCUSSION: the amount of UFCs obtained in the collected samples in the 5', 10', 20', 30' periods were respectively: biology lab: 12, 8, 10, 34: men bathroom: 33,185, 60, 76; women bathroom: 26, 42 ,93, 130: classroom: 9, 7, 23, 15; canteen: 17, 18, 47, 51: library: 1, 5, 16, 33; research room (mastering area): 7, 8, 13, 19; external area: 15, 78, 146 ,226. The amount of UFC increased accordingly with the exposition period. The TSA and PB provided a bigger growing of colonies, due to the fact that the first doesn't show great microorganism selectiveness accordingly to its composition and the second provides *Escherichia coli* growing that are cosmopolite. Another fact seen was that in places where there is a bigger people movement and in open areas, the number of UFCs was considerably bigger.
CONCLUSION: the results obtained suggest that a constant air movement, mainly in the canteen and the external area, favors the distribution of the microorganisms in general. This way, it's important to make clear that in those places it is necessary a bigger attention to hygiene, having in mind minimizing the risks caused by pathogenic microorganisms.
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Financial support: Centro Universitário São Camilo – Campus Pompéia – SP

RECUPERAÇÃO E VISCOSIDADE DA B-GLUCANA DE *Botryosphaeria rhodina* EM DIFERENTES TEMPERATURAS, pH, SAIS MINERAIS E TAXAS DE CISALHAMENTO

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Palavras-chave: *Botryosphaeria rhodina*, viscosidade, glucana.

INTRODUÇÃO: Botriosferana é um exopolissacarídeo (EPS) do tipo β-(1→3;1→6)–D–glucana produzido pelo fungo ascomiceto *Botryosphaeria rhodina* (BARBOSA et al., 2003). O grau de ramificação deste EPS varia em função da fonte de carbono utilizada para o crescimento do microrganismo e o seu arranjo conformacional é em hélice tripla, o que parece estar vinculado à sua propriedade antimutagênica. Entretanto, existem poucos relatos sobre a recuperação deste EPS do meio de cultivo, bem como sobre suas propriedades físico-químicas. O presente trabalho teve por objetivo avaliar o efeito de diferentes parâmetros, tais como, taxa de cisalhamento, concentração de polissacarídeo, temperatura e pH, sobre a recuperação e a viscosidade do botriosferana.

MATERIAL E MÉTODOS: O *B. rhodina* foi cultivado em meio mínimo de Vogel com sacarose 50g/L (180 rpm, 28°C), por 72 h. Após centrifugação a 3100 rpm, 4°C, por 30 min, foram estudadas diferentes condições para precipitar e recuperar o botriosferana: a) ajuste do pH entre 4 e 10 com HCl ou NaOH 1 M seguido da adição de etanol (1:3); b) adição de KCl de 0,001% a 1% (m/v) e adição de etanol (1:3). O EPS precipitado foi seco a 70°C, triturado e solubilizado em água destilada. A determinação da viscosidade foi desenvolvida em viscosímetro Brookfield modelo LVDV I+, em diferentes taxas de cisalhamento (0,102 a 68 s^{-1}), temperaturas (10 e 100 °C), pH (2 a 12,5) e na presença de sais minerais 1% (m/v).

RESULTADOS E DISCUSSAO: O pH influenciou na precipitação e na viscosidade do EPS solubilizado em água. Quando o pH do sobrenadante de cultivo foi corrigido para 4 obteve-se o menor rendimento (1,9 g/L), enquanto em pH 6, 8 e 10 recuperou-se em média 2,6 g/L. O EPS precipitado em pH 4 ou 6, quando solubilizado em água a 1% (m/v), apresentou maior viscosidade (1076 e 736 cP) do que nos ensaios em pH alcalino (283 cP, pH 10). Houve alteração da viscosidade quando o EPS foi precipitado sem alteração do pH do sobrenadante de cultivo, porém o pH da solução de EPS a 1% (m/v) foi ajustado entre 2 e 12,5. No intervalo de pH entre 2 a 4 obteve-se maior viscosidade (640 cP e 520 cP, respectivamente), do que em pH 6 a 12,5 (média 330 cP). A adição de KCl ao sobrenadante de cultivo proporcionou maior recuperação do EPS (2,9 g/L) em relação ao controle (1,21 g/L), independente da concentração de sal utilizada. Quanto maior a concentração de KCl, menor a viscosidade do EPS quando solubilizado em água a 1% (864 cP com KCl 0,001% e 445 cP com KCl1%). A dissolução do EPS em solução de NaCl, KCl, $CaCl_2$, $MgCl_2$, $MnCl_2$ ou $FeCl_3$ reduziu de 60 a 85% a viscosidade. Analisando-se uma solução de EPS precipitado sem alteração de pH ou adição de KCl e solubilizado em água a 1% (m/v), verificou-se grande decréscimo da viscosidade com o aumento da taxa de cisalhamento, indicando comportamento pseudoplástico. Quando a medida de viscosidade foi realizada em diferentes temperaturas, verificou-se que embora nas temperaturas mais baixas tenha sido observada maior viscosidade (704 cP a 10 °C), essa propriedade não foi grandemente afetada pela temperatura, mantendo-se viscosidade média em torno de 500 cP, mesmo a 100 °C.

CONCLUSÕES: A β-glucana botriosferana produzida por *B. rhodina* apresentou comportamento reológico pseudoplástico, pouco influenciado pela temperatura e com aumento de viscosidade em pH ácido, propriedades desejáveis para o seu emprego em cosméticos e alimentos.

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RESIDUE DETERMINATION OF SODIUM MONENSIN IN DRY YEAST FROM ETHANOL FERMENTATION

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Keywords: Dry yeast, antibiotic, monensin, ethanol fermentation.

INTRODUCTION: The control of bacterial contamination in sugar cane fermentation by *Saccharomyces cerevisiae*, for ethanol production, is done mainly by antibiotics. One of the most effective products is sodium monensin, produced from *Streptomyces cinnamonensis*. Residuous of antibiotics could be found in dry yeast withdrawn from such process, destinated to animal feed additive. The aim of this work was to quantify the residual of sodium monensin in dry yeast from ethanol fermentation in order to give parameters to establish concentration limits in the end product.

MATERIAL and METHODS: Three batch fermentations (300.000 L each), fed with sugar juice and molasses (20°Bx) and inoculated with *S. cerevisiae* 10% (m/v), were treated each one with 900 g of Kamoran (sodium monensin cristaline 90%), previously diluted in water-ethanol soluction (50% v/v). At the end of fermentations, the following samples were collected in triplicate: fermentation broth; yeast cream; beer; yeast milk; and dry yeast. Samples of dry yeast, from sucessives fermentations, were collected after 24 e 48 hours from treatment. The samples were analysed by HPLC as well as mass spectometer according to method JJ62H (Eurofins Wiertz-Eggert-Jorissen) for monensin quantitative determination.

RESULTS and DISCUSSION: The samples collected after treatment showed the following average monensin concentration: Fermentation broth: 1,29 mg/L; beer: 1,14 mg/L; yeat cream (65% m/v): 1,45 mg/L; yeast milk (35% m/v): 0,25 mg/L; dry yeast: 1,51 mg/Kg. The amount of monesin found in dry yeast, collected in 24 and 48 hours, was 1,22 e 1,07 ppm respectively.

CONCLUSION: The results showed that some monensin get out in the beer and some remain in the yeast cream separated from the fermentation broth by centrifugation. The apparent amount of monensin in yeast milk decreases due to dilution of the cream, on the other hand, increases slightly in dry yeast, due to the concentration of the same cream. The period of 48 hours between the fermentation treatment with sodium monensin and withdraw of the yeast led to major decreasing of residual of such antibiotic in the end product.

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RESPIRATORY ACTIVITY OF FOREST, PASTURE AND ANNUAL CULTURE SOIL ECOSSYSTEMS

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Keywords: basal respiration, organic matter, microorganisms

INTRODUCTION: The effect of conversion of forests into pasture and agricultural areas is an opportunity to assess microbiological and chemical changes in different soil ecosystems. In soil, the microorganisms occurring in great number and variety and have a key role in the transformation of residual organic matter, thus contributing to soil fertility and plant nutrition. The changes that occur in soil, and the effect of the different ecosystems were evaluated through the respiratory activity of soil, considered among the most traditional of methods for assessing microbial activity. The study aimed to evaluate the respiratory activity in forest ecosystems, pasture and annual crop (maize).

MATERIAL AND METHODS: The soil of the areas of study were classified as Latosol purple, eutrophic, A Moderate, very clayey texture and belongs to the UNESP-Jaboticabal. The depths of sampling were: 0-10, 10-20 and 20-40 cm. The determination of basal respiration was performed through the production of CO_2 according to methodology proposed by Rezende et al. (2004), where it weighed 100g of moist soil in glass jars with lids (capacity 2.5 L), balance is the ability to retain water to 60%. Two beak of 50 mL, one containing 40 mL of deionized water and another with 20 mL of 1M NaOH were placed inside the vials, which were sealed with parafilm and then with the lid. We included three bottles without soil, those with beak, serving as a control. After that, were the camera incubator (BOD) at 30 ^{0}C in the dark. Every seven days, for a period of 105 days was to assess the soil respiration that was the removal of the beak with NaOH, and immediately replaced by another under the same conditions previously described. In beak removed, add 2 mL of barium chloride solution of 30% followed by 3 drops of Phenolphthalein solution 1%, and under agitation, titles are samples with 1M HCl until the turning point in the dark pink to colorless. Noted the amount of spent titrant and was made the proper calculations, taking into consideration the amount of spent HCl in samples without soil.

RESULTS AND DISCUSSION: The respiratory activity observed in the ecosystem of forest did not differ significantly ($p < 0.05$) to the pasture, respectively showing values of 143.73 and 128.58 mg CO_2 released in the month. The area of maize had lower basal respiration rate in the range of 117.15 mg CO_2, because the forests and grasslands can be considered permanent crops and are not subject to such management practices as observed in agricultural crops. The effect of depth of collection was statistically significant at 5% level of probability, where the largest amounts of CO_2 produced were detected in samples of 0-10 cm and 10-20 cm depths in the minors and 20-40 cm in three environments studied. The soil respiration in the 0-10 cm depth was 50-85% CO_2 production, a fact known that the amount of organic matter in the more concentrated the soil surface.

CONCLUSION: The ecosystems of forest and pasture had higher respiratory activity compared to the annual crop, reflecting the less human intervention in those environments. The respiratory activity was better observed in the 0-10 cm depth, the region of greatest concentration of biological activity in soil.

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RESPONSE OF SOIL BACTERIAL COMMUNITY TO DIFFERENT LAND USE IN A MOSAIC LANDSCAPE OF WESTERN AMAZON

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Keywords: Microbial Ecology, Soils, *Bacteria*, DNA, DGGE, ARISA

INTRODUCTION: The Brazilian Amazon has been the target of activities that are transforming extensive areas of humid tropical forest in areas destined to agriculture and pasture. The implications of land use change on soil microbial communities are still not well understood. Therefore, this work used the Denaturing Gradient Gel Electrophoresis (DGGE) and Automated Analysis Spacer Intergenic Ribossomal (ARISA) to evaluate changes in bacterial community structure due to the anthropogenic effect of clear cutting and land use change of traditional agricultural crops and pasture in the region of *Alto Solimões*, Western Amazon.

MATERIAL AND METHODS: Soil samples were collected at 0-20 cm depth under primary forest, agriculture, pasture and secondary forest from the sampled areas of the project "Conservation and Sustainable Management of Below-Ground Biodiversity". The total soil DNA was extracted using the kit *Power Soil DNA Extraction*™ (MoBio). Fragments of the gene 16S rRNA (430 bp) were amplified with the primers F984GC and R1378 (Heuer et al., 1999), and separated in the INGENY phorU-2 system (Ingeny, Netherlands) in gels containing polyacrylamide 6% and a linear denaturing gradient 40-65% at a constant temperature of 60^{o} C for 16 hours at 100 V. DGGE gels were analyzed using the BioNumerics platform software (Applied Maths). The intergenic space between the 16S and 23S rRNA subunits was amplified with the primers 1490-72F labeled with 5-carboxyfluorescein (FAM) and LSU21-38R (Ranjard et al., 2001). The amplified product was purified using the kit *QIAquick PCR Purification* (Qiagen). The heterogeneity intergenic length was analyzed in *Sequencer ABI PRIM 3100 Genetic Analyzer* (Applied Biosystems). Statistical analyses were calculated using the packages of CANOCO for Windows 4.5.

RESULTS AND DISCUSSION: The studied soils presented high contents of Silt, low pH levels and high levels of H+Al. The cluster analysis based on DGGE fingerprinting was defined with the Pearson coefficient and was also confirmed by Principal Components Analysis, showing distinct soil community structures under primary forest, agriculture, pasture and secondary forest. The ARISA profile patterns were analyzed by the same ordination method and revealed identical results as those obtained by the DGGE technique. Nevertheless, higher phylotype richness was detected with ARISA in all analyzed samples.

CONCLUSION: The anthropogenic effect of clear cutting and land use for agriculture and pasture in the region of *Alto Solimões* was characterized by changes in the bacterial community structure in relation to the organization of bacterial populations present in soils under tropical primary forest.

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RHAMNOLIPIDS PRODUCTION BY DIFFERENT STRAINS OF *P.aeruginosa* GROWING IN N-PARAFFIN.

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Key-words: *P. aeruginosa*, *n*-paraffin, ramnolipid

INTRODUCTION: Biosurfactants are amphipatic molecules produced by a wide variety of microorganisms and are mainly used in the petrochemical industry to enhance oil recovery and hydrocarbon remediation. *Pseudomonas spp*. are knows to produce rhamnolipids, which represents one of the most important classes of microbial surfactants (COSTA; NITSCHKE; CONTIERO, 2008). The aim of this work was to investigate the rhamnolipids production by different strains of *P. aeruginosa* in n-paraffin.
METHODS: Isolates of *P. aeruginosa* were cultivated in saline medium (Robert et al., 1989). N-paraffin was added as carbon source at concentration of 4% (w/v). Initially, a rhamnolipid solution in a concentration of 2% (v/v) was added in the medium. Cultures in 125 ml baffled flasks, containing 25 ml of medium were incubated in a reciprocal shaker at 200 rpm and 30 °C (COSTA; NITSCHKE; CONTIERO, 2008). The rhamnose concentration was measured in the cell free culture broth by the method described by Chandrasekaran & Bemiller (1980). The analyses were made in triplicate.
RESULTS AND DISCUSSION: The rhamnolipid production increased with the initial addition of rhamnolipids solution in the culture medium, reaching a final production of 100,5 ± 2,4 mg/l to *P. aeruginosa* 7a, 123,9 ± 1,9 mg/l to *P. aeruginosa* LBI and 138,9 ± 2,4 mg/l to *P. aeruginosa* 6c. The medium without rhamnolipids solution addition, the final rhamnolipids production was 94 ± 1,4 to *P. aeruginosa* 7a, 87,1 ± 24,7 to *P. aeruginosa* LBI and 97,6 ± 11,5 to *P. aeruginosa* 6c. The difference in the final rhamnolipids concentration suggest the rhamnolipids utilization as an initial carbon source, promoting cell growth and consequently rhamnolipids production or the rhamnolipid was able to solubilise n-paraffin, increasing the carbon source assimilation.
CONCLUSIONS: The assimilation of n-paraffin was facilitated with the addition of initial rhamnolipids solution in the culture medium, with consequently increased in the rhamnolipid production.

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SACCHAROMYCCES CEREVISIAE AS BIOINDICATOR IN PHOTOELECTROLYTIC TREATMENT

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Keywords: photoelectrolytic, textile dye, degradation

INTRODUCTION: The residues from industries can pollute thousands of gallons of water with substances that are hardly degraded by organisms in the rivers. Textile industries are responsible for a large among of this polluted water, that are mainly produced during dyeing process, releasing improperly water as for human use, as for the health of aquatic ecosystems (Dellamatrice, 2005).

MATERIAL AND METHODS: Degradation experiments were done using a photoelectrolytic system, where a solution of Remazol red brilliant, is exposed to a current of 5A e 7V and to a UVC lamp. The treatment lasts 30 minutes and the samples are obtained on the following times: 0 min, 3 min, 5 min, 15 min and 30 min (Carvalho, 2008). These samples are used for the toxicity tests, absorbance, COD, pH e conductivity. The toxicity test is made exposing the bioindicator (*S. cerevisiae*) to the samples for three days starting at the moment they are obtained from the reactor, 15 days later and 3 months later, in test tubes, three tubes are made per each sample and kept in constant temperature of 28°C, after this period, the counting of living and died cells is made, using Neubauer chamber using the dye Erythrosin, which show the dead cells are stained.

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RESULTS AND DISCUSSION: Although experiments appoint satisfactory color (100%) and organic removal (70%), solution became toxic after 15 minutes of photoelectrolytic treatment, killing all *S. cerevisiae* cells, the fact can be explained considering the utilization of sodium chloride, that forms a strong oxidant as chlorine during the photoelectrolytic treatment. Even in three months, the time necessary to evaporation of residual chlorine, the samples still presents a relative high toxicity. In experiments which ones sodium sulfate was used as electrolyte, this high toxicity doesn't appear, provided that the percentage of died cells in the latest part of the treatment doesn't show increasing. However this kind of experiment loses efficiency, because color removal is about only 30% and organic matter content do not decrease.

CONCLUSION: As biologic process has a low efficiency in degradation of reactive dyes (Kolonko, 2005) like Remazol red brilliant, the photoelectrolytic process is a good option in treatment of textile effluent, as seen it removes the color from the solution in short time. But its use is possible only if a point of equilibrium among removal of color and organic matter and low toxicity for the effluent to be discarded in the rivers is reached, such as a colorless and low toxicity effluent.

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Salmonella spp. DETECTION IN SANITARY SEWAGE ORIGINATING FROM AN ALTERNATIVE TREATMENT SYSTEM FOR SMALL COMUNITIES

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Key words: *Salmonella spp*., sanitary sewage, methodology adequacy

INTRODUCTION: *Salmonella spp.* comprise a genre of pathogenic bacteria, widely distributed in nature and responsible for an increased number of gastro-intestinal infections. The interest in re-using treated sanitary effluents for less noble means and the use of sewage sludge in agriculture still demands further study to define the minimum quality requirements inherent to each use. Seeking to offer the means for conducting research related to this microorganism a methodology to detect *Salmonella spp.* in sanitary sewage was implemented at the Sanitation Laboratory of the Environment and Sanitation Department at FEC/UNICAMP adapted from the official methods of CETESB and APHA.

MATERIALS AND METHODS: The configuration used in the study consisted of a baffled anaerobic reactor, followed by vegetated "constructed wetlands" (using crushed stone as medium), slow filtration and disinfection by cloration using hypochlorite. 28 analyses were conducted during a period of 12 months, obtaining 11 samples of raw sewage, 06 treated sewage and 11 samples of treated sewage submitted to cloration. The samples were concentrated in a filtering membrane, suspended in Tetrathionate broth with iodine and incubated in a 35 °C incubator. After 24 hours of incubation the sample was striped in plates with 04 different types of agar: MacConkey, Xylose Lysine Desoxycolate, Salmonella-Shigella and Brilliant Green. The plates were incubated at 35 °C. After 24 hours the suggestive colonies were inoculated in TSI (Triple Sugar Iron) and LIA (Lysine Iron Agar) inclined agar, and then incubated at 35 °C for 24 hours. With the suggestive tubes of these means the following biochemical tests were performed: polyvalent somatic serum test, urease test, dulcitol fermentation test, lactose fermentation test, sucrose fermentation test, indol test, methyl red test, Voges-Proskauer and Simmons citrate test (SILVA, *et. alii.,* 1997, APHA, 1998, OPLUSTIL *et alli.,* 2000). The result was reported in terms of presence and absence (P-A).

RESULTS AND DISCUSSION: *Salmonella spp.* was detected in all samples of the raw and treated sewage and in 06 samples of the treated sewage submitted to disinfection. It was concluded that the studied treatment system was not effective in removing *Salmonella spp.* and that association with cloration was only efficient in 45% of the analyzed samples, demonstrating the need for other more efficient disinfection methods.

CONCLUSIONS: The methodology adaptation, using clinical analyses, food and sludge techniques were efficient in the detection of the microorganism in sanitary sewage. The presence of *Salmonella spp.* was detected in raw sewage as well as in treated sewage of the studied system, but in 45% of the samples submitted to cloration the microorganism was absent.

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SCREENING OF MICROORGANISMS AS BETA-GLUCANASE PRODUCERS ISOLATED FROM BERRIES OF GRAPES CULTIVATED IN THE NORTH OF PARANÁ

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Keywords: Beta-glucanase, Beta-glicosidase, Beta-glucan

INTRODUCTION: Beta-glucanases (EC 3.2.1.6) are hydrolases which catalyse the hydrolisis of beta-(1→3)-D-glucans such as laminarin (*Laminaria digitata*) and botryosphaeran (produced by *Botryosphaeria rhodina*). These enzymes can be secreted in the culture medium by several microorganisms, including yeasts. Beta-glucanases as well as beta-glicosidases (EC 3.2.1.21) can be applied in wine processes once they hydrolise glycosidic linkings between the glucose and aglycon groups of phenolic or volatil aromatic compounds (monoterpens, aliphatic alcohols), contribuiting to improve their organoleptic characteristics. In the present work it was isolated filamentous fungi and yeasts form berries, fruit juice, and must of grapes collected in rural properties in the north of Paraná-Brazil, which were screened concomitantly with 15 yeasts strains from Spanish wineries as beta-glucanase and glicosidase producers.
MATERIAL AND METHODS: Eight cultivars of grapes (Vênus, Itália, Rubi, Isabel, Takassumi, Benitaka, Brasil e Moscato Bailey) were collected and used to isolate the microorganisms. The screening test for fungi and yeast enzyme producers were developed in Vogel's liquid culture medium with 0,2 % (w/v) of a no commercial beta-(1→3; 1→6)-D-glucan (botryosphaeran), as sole carbon source, which is produced as a rotin in the biochemistry laboratory of UEL. The beta-glucanase and beta-glicosidase activities were determined in the extracellular fluid (ECF) according to Giese *et al*.(2005), using laminarin and *p*-NPG, as substrates, respectively. A zymogram test was developed to detect qualitatively the beta-glucanase secreted by yeasts using agar 2% (w/v), minimum Vogel's salts and botryosphaeran 0.2% (w/v) in a small Petri dish, where it was possible to view the beta-glucanase halo.
RESULTS AND DISCUSSION: A total of 108 microorganisms was isolated from all collected samples. The zymogram test permitted to detect hydrolysis halos of yeast beta-glucanase activities on botryosphaeran, after flooding the agar plate with etanol PA. Nine filamentous fungi and twelve yeasts were selected as producers of beta-(1,3)-glucanase and beta-glicosidase. In the ECF of a fungus that produced highest beta-glucanase titre was used to determine some kinetic parameters of this enzyme. Ten minutes was the most adequated time to measure the beta-glucanase activity, and the optimum pH and temperature obtained were 4,5 and 50 °C, respectively.
CONCLUSIONS: One hundred and eight microorganisms were isolated, and only nine filamentous fungus and twelve yeasts secreted beta-glucanases in the liquid culture medium. Some of these selected microorganisms produced beta-glicosidase concomitantly. The zymogram test developed in this work was useful to detect qualitatively the exo-beta-glucanase activities for screening purpose. An epifitic fungus isolated from a Rubi cultivar collected in Marialva-Paraná, produced the highest titre of beta-glucanase between the fungi, and the optimum pH and temperture were 4,5 e 50 °C, respectively.
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SELECTION OF MICROORGANISMS ISOLATED FROM ATLANTIC FOREST PRODUCERS OF LIPASE TOLERANT OF SOLVENTS AND pH EXTREMES

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Keywords: Lipase, selection, biodiversity.

INTRODUCTION: Lipases (EC 3.1.1.3) catalyze hydrolyses or synthesis of acylglycerides of long chain and due to their biochemical properties arouse interest in various industrial segments. However, those enzymes may not support the necessary conditions for its application in biocatalysis (e.g.: high temperatures, solvents and pH extremes). The Atlantic forest, for its conditions of humidity and heat, has an enormous biodiversity with exceptional wealth of genetic patrimony and innumerable species to be yet studied, that enables the isolation of microorganisms which can produce lipases with special properties. This study aimed to select microorganisms of Atlantic forest microbiota producers of stable lipases in organic solvents and active ones in pH extremes.

MATERIAL AND METHODS: It was studied 50 strains of filamentous fungi isolated from soil and leaves of the Atlantic forest, still without taxonomic identification and kept in the Lab. of Industrial Biotechnology (UNESP-Assis/SP). Fungi were previously selected by the capacity of formation of fluorescent halo at 350 nm, when grown on PDA medium (39 g/L), Rodamina b 0,001% (w/v), olive oil 1% (v/v) and Tween 80 (2 drops), 5 days at 29 °C. The submerged fermentation was performed in medium containing olive oil 1% (w/v) Tween 80 1.5% (w/v) and yeast extract 0.5% (w/v) at 180 rpm and 29 °C. Every 24 hours the culture was filtered, the biomass measured by gravimetry and enzymatic activity accompanied by hydrolysis of p-nitrophenyl palmitate (pNPP), at 37 °C and 410 nm. The stability to organic solvents was determined by incubation of the supernatant at 20 to 90% (v/v) of ethanol, methanol and isopropanol, in 1 h at 30 °C. The effect of pH on activity was studied varying the pH of the reaction medium between 2.6 and 10 and following the release of p-nitrophenol for 7 min at 37 °C.

RESULTS AND DISCUSSION: The 50 strains initially grown in Petri dishes showed 14 of them with orange fluorescent halo and they were cultivated in submerged fermentation for quantification of enzyme activity. The strain identified as MA 41, showed maximum activity of 4.96 U/mL in 72 h of cultivation. After 1 h of incubation with different solvents, there was greater stability to the methanol than with isopropanol or ethanol. It was observed in until 60% of methanol that the loss of enzyme activity is the maximum 30%. The enzyme activity remained stable up to 20% of ethanol, but decays rapidly with 40% of this solvent, and found only 24% of initial activity. After incubation in 20% of isopropanol, there was 88% of initial activity and between 60 and 90% of this solvent was observed no more than 30% of residual activity. In acidic pH (2.6 and 3) the enzyme activity was not detected, on the other hand, in alkaline pH were obtained high values of activity. Although the highest activity was obtained at pH 8, at pH 9 and 10 was also obtained 75% and 65% of this activity, respectively.

CONCLUSION: The lipolytic activity detected in cultures with the strain MA 41 isolated from Atlantic forest shows stability in the presence of methanol and activity in alkaline pH. And they are important properties, for instance, to the application in the transesterification reactions for the biodiesel production or for the detergents industry.

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SENSITIVITY PROFILE OF BACILLUS sp. ISOLATED FROM CUIABÁ-MT SOIL TO ANTIBIOTICS

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Palavras-Chave:
Bacillus, antimicrobials, β-lactams.

BACKGROUND: Species of the genus Bacillus are commonly found in soil, air and water (STABB et al., 1994). They have a bacilli shape, are Gram-positive, aerobic or facultative anaerobic, producing endospores that are resistant to desiccation, heat, irradiation and organic solvents (RHODES, 1990). They secrete extracellular proteins, most of them with economic interest, as proteases, amylases, lipases, DNase and RNase (KULKARNI et al., 1999)

MATERIAL AND METHODS: Bacilli from soil samples of UFMT campus, Cuiabá-MT, were straked in soybean triptcasein agar and kept at 4 ° C degrees until the sensitivity test was done. The susceptibility of bacilli to drugs was performed with modifications according to Cavallo et al. (2002). In Petri dishes containing Mueller-Hinton agar, the inoculum with concentration of bacilli in the scale of 0.5 MacFarland was plated with sterile swab. Sterilized forceps were used to distribute the disks of ampicillin (10 mg), gentamicin (10 mg), neomycin (30 mg), kanamycin (30 mg), oxacillin (1 g), streptomycin (10 mg), novobiocin (30 mg) , cephalothin (30 mg), amoxicillin (10 mg), chloramphenicol (30 mg), nalidixic Ac (30 mg), vancomycin (30 mg), polymyxin (300 mg), rifampicin (30 mg), norfloxacin (10 mg), teicoplanin (30 mg), carbenicillin (100 mg), erythromycin (15 mg), clindamycin (2 g), amikacin (30 g), sulfonamide (300 mg), penicillin G (10 mg), tetracycline (30 g) and nitrofurantoin (300 g). Cultures were incubated for 24 hours at 37 ° C. Measurement of inhibition were performed with digital pachymeter (Digimess).

RESULTS AND DISCUSSION: There was a synergism between β-lactams (amoxicillin and carbencilina) and rifampicin. Selection of colonies resistant to penicillin G, oxacillin, amoxicillin and carbenicillin were found, indicating resistance of the microorganism to β-lactams. Clutterbuck et al (2007) considered penicillin G, among the antibiotics tested, less effective against *B. cereus*. The small difference between values of this author and the organism here studied, grant resistance to both microorganisms to penicillin G. Reva et al (1995) considered inhibitory a halo greater than 8mm, and resistant when there was no halo formation. Comparisons showed susceptibility to kanamycin, erythromycin, gentamicin, streptomycin, neomycin and low sensitivity to polymyxin (data not shown). Whong and Kwag (2007) point a high sensitivity of isolates of *B. cereus* to ciprofloxacin (100%), chloramphenicol (100%), gentamicin (99%), nalidixic acid (97%), tetracycline (93.3%) and resistance to penicillin G (82%).

CONCLUSIONS: Selective resistance may occur after the use of β-lactams. These data combined with the profile of sensitivity to antibiotics, indicate a possibility of the microorganism be *Bacillus subtilis*.

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SIZE OF WATER HYACINTH PARTICLES AND WHEAT BRAN EFFECT ON THE CULTIVATION OF *Pleurotus florida*

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Keywords: water hyacinth, biodegradation, edible mushroom

INTRODUCTION: The interest on growing mushrooms has increased over the last years in Brazil (Neves & Graciolli, 2008). However, growing it for commercial purposes is limited and focused on *Agaricus bisporus*. *Pleurotus* mushrooms are grown worldwide and are responsible for more than 800,000 tonnes of the mushrooms harvested annually. The most common substrate for growing *Pleurotus* commercially is wheat straw. Nevertheless, different substrates have been developed in many countries due to the availability and price of the material. This research work is one of a series of investigations on the use of water hyacinth (*Eichhomia crassipes*) on the growth of *Pleurotus florida*.

MATERIAL AND METHODS: The water hyacinth, discarded by the Hydroelectric Power Plant of Jupiá, SP, was processed and separated into particles of 1, 5, 10 and 20 mm. 500 mL glass pots with a density of 50 g of the moisturized substrate (70 to 75% moisture) per 100 mL volume, supplemented or not with wheat bran (20%) (WB) were used for growing. The pots were autoclaved at 121 °C for 1 h and after the inoculation, they were incubated in the darkness at 25 °C until complete substrate colonization. The fruiting occurred indoors and although there was no temperature control, the relative humidity was kept above 70%. The results refer to a yield flow.

RESULTS AND DISCUSSION: The total colonization of the substrate was faster when water hyacinth, without WB and with 10 and 20 mm particles, was used (15 days). The beginning of the pin head formation lasted 4 days and the period from inoculation to harvest varied between 23 and 34 days. All the treatments with WB provided better yields. The highest yield (173.2 $g.kg^{-1}$) and the biological efficiency (51.8%) were observed when 5 mm particles were used. These yields were higher than the ones obtained with other substrates commonly used for growing *Pleurotus* (Zanetti & Ranal, 1997; Dias et al., 1993). Contamination of the substrate was not observed during the experiment.

CONCLUSIONS: The size of the particles and the adding of WB affect the *P. florida* yields. According to the results and due to the great availability of water hyacinth throughout the year with no costs, this substrate is a viable alternative for the growing of *P. florida*.

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SOIL ENZYMATIC ACTIVITY AFTER APPLICATION OF ORGANIC COMPOUND

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Keywords: acid phosphatase, alkaline phosphatase, β-glucosidase.

INTRODUCTION: Soil enzymes is derived from metabolic activity of animals, plants and microorganisms. Particularly, the microbial population in the soil promotes elements biogeochemistry cycling in soil, like phosphatases enzymes (phosphorus mineralization) and β-glucosidase (carbon cycle) (Garcia et al., 2002). The microbial community is enhanced with the addition of organic compounds in soil.

MATERIAL AND METHODS: The study was conducted with samples of soil from cerrado, clay texture, collected at 10 cm depth. The compound used was obtained from the aerobic composting of organic household waste. The treatments were: control, soil and addition of two doses of organic compound: 10 and 20 g kg^{-1} dry soil. For the evaluations of the activities of β-glucosidase (EC 3.2.1.21), acid phosphatase (EC 3.1.3.2) and alkaline phosphatase (EC 3.1.3.1), the methods proposed by Eivazi & Tabatabai (1988) and Tabatabai & Bremner (1969) was used, respectively. The results were submitted to the ANAVA with 5% probability.

RESULTS AND DISCUSSION: The activity of acid phosphatase (pH 4.0) was higher than the alkaline (pH 9.0) at 2.25, 3.35 and 2.02 times in control and treatments with 10 and 20 g kg^{-1} dry soil, in that order. This result may be related to the low value of soil pH (Eivazi & Tabatabai, 1977). The activity of acid phosphatase increased 4.0 and 3.0 times, and alkaline phosphatase increased 4.8 and 9.6 times the lowest and highest dose of compound added in the control, respectively. It is important to emphasize that phosphatases activity in soil is very important, especially in natural ecosystems, since the availability of Pi to the plants is dependent on the cycling of nutrients in the soil. The β-glucosidase increased significantly with the addition of organic compound: 1.3 and 1.57 times in treatments with 10 and 20 g kg^{-1} dry soil, respectively. The detection of the enzyme β-glucosidase is related to the process of decomposition of cellulose, which is synthesized by fungi, bacteria and other organisms in the soil (Sylvia et al., 1999).

CONCLUSION: The enzymatic activities of β-glucosidase and phosphatases (acid and alkaline) had significant increments in the enzymatic activity of soil, and was higher in the highest dose of organic compost added to soil. The acid phosphatase activity was higher than alkaline phosphatase in all treatments.

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STABILITY OF EXTRACELLULAR POLYGALACTURONASE PRODUCED BY *Penicillium janczewskii*

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Keywords: polygalacturonase, enzymatic characterization, thermostability, pH stability, *Penicillium janczewskii.*

INTRODUCTION: Polygalactunases (PGs) are pectinases that catalyze the clivage of internal α-(1$\rightarrow$4)-glycosidic bonds between two residues of galacturonic acid non-methylate (endo-PGs) or are responsible for removal terminal residues (exo-PGs). PGs are mainly used in food, textile, cellulose and paper industries. The industrial application is influenced by enzyme stabilization in processing conditions (JAYANI et al., 2005; KASHYAP et al., 2001). The aim of this work was to determine the thermostability and pH stability of PG produced by *Penicillium janczewskii.*

MATERIAL AND METHODS: A strain of *P. janczewskii* was cultivated on solid Vogel medium with glucose 1 % during 7 days. A spore suspension from this culture was added in liquid Vogel medium pH 5.0 supplemented with 1 % apple pectin and the culture was maintained in stationary condition, at 30 °C. The enzymatic activity was assayed by the reducing sugars determination (MILLER, 1959) and one unity of activity was defined as the amount of enzyme required to release 1 µmol of reducing group per min. The crude enzyme was diluted (1:1) in different buffers in a range from pH 2.0 to 9.0 and it was maintained at 4 °C for 24 h, and the residual activities were determined. For thermostability determination, the crude extract was incubated at 40, 50 and 60 °C for different periods before determination the PG activity.

RESULTS AND DISCUSSION: PG from *Penicillium janczewskii* was more stable in acid than alcaline pH and showed highest residual activity in pH 5.0 and high in a range from 2.o to 6.5. At pH 7.0 the residual activity was near to 53.9 %. Above pH 7.5, the activity was lesser than 40 % or equal to this value. Stable pectinases in acid conditions are mainly used in extraction, clarification and to remove pectin on fruit juices, maceration of vegetables to produce pastes and purées and in wine-making (ALKORTA et al., 1998; KASHYAP et al., 2001). About the thermostability, PG was more stable at 40 ºC. After 240 min of incubation in this temperature the residual activity was higher than 50 %. At 50 °C, after 15 min the polygalacturonase activity was 37.1 % of the initial. At 60 °C, after 5 min of incubation, only 6.2 % was observed.

CONCLUSION: The polygalacturonase produced by *P. janczewskii* showed a high stability in acid pH, suggesting possible application in processing of juices, pastes and wine-making. PG also showed an high stability at 40 °C, but low stability at 60 °C

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STUDY OF EFFECT OF ENVIRONMENTAL PARAMETERS ON THE CULTIVATION OF THE CILIATE PROTOZOAN *PARAMECIUM CAUDATUM*

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Keywords: *Paramecium caudatum*, protozoan, monoxenic culture.

INTRODUCTION: The protozoans have been used as test organisms in toxicological studies and proposed as biological indicators of water pollution (Nicolau et al. 1999; Nalecz-Jawecki & Sawicki, 2002; Dias, Mortara & Lima, 2003; Nalecz-Jawecki, 2004). The *Paramecium caudatum*, used on this study, is one of the most well known protozoans and it is usually found in sediment and associated with roots of macrophytes.

MATERIAL AND METHODS:

Isolation of studied protozoan: The *P. caudatum* was isolated from water samples with sediment and roots of macrophytes from the Lagoa do Óleo, an oxbow lake of Luis Antonio town (SP-Brazil).

Standard culture medium for bacterivorous protozoans (monoxenic culture): It was used 10 mL of a bacterial suspension (*E. aerogenes*, at 10^6 organisms per mL^{-1}) in test tubes containing a 2% agar slant with a rice grain with chaff autoclaved.

Experiments for optimization of culture: It was tested four pHs (6, 7, 8 and 9) and four temperatures (20, 25, 27.5 and 30° C) in the cultures. At the end of 72h of culture, two aliquots of 1 mL of each culture tube were fixed with saturated solution of mercuric chloride. The fixed protozoans were counted and measured for biovolume estimations.

RESULTS AND DISCUSSION: Considering the mean population densities, the better conditions for cultivation were pH 6 at 27,5° C. Considering the mean biovolumes, the better conditions were pH 8 at 20° C, because higher biovolumes were found. At lower temperatures the population density is lower, however, the mean biovolume was higher. At 20° C the start of the logarithmic phase of growth (log) was delayed, and the higher the temperature was, more early was the log phase.

CONCLUSION: 1) We noted that the temperature of 30° C and also the pH 9 were not appropriate conditions considering density or biovolume. 2) Regarding the environmental parameters studied, it was noted that population growth behave in an opposite manner compared with cell volume. In other words, with increasing temperature, the metabolism was accelerated, increasing the frequency of cell divisions and promoting the early log phase. This resulted in less stored energy and lower cell biovolumes.

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STUDY OF THE MICROENCAPSULATION OF *Lactobacillus acidophilus* BY SPRAY DRYER

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Keywords: Microencapsulation, Probiotics, spray dryer.

I**NTRODUCTION**: The consumption of probiotic products in Brazil stepped up considerably in recent years. This fact can be confirmed by the development of new brands for various food industries (Oliveira et al., 2002). The microencapsulation of bacteria comprises a technology that can increase the resistance of probiotic microorganisms, ensuring cell viability in food and also during its passage throughout the gastric and enteric juice. Therefore, the study of techniques and materials that increase the efficiency of microencapsulation of probiotics is important. This study aimed to develop and evaluate microcapsules containing the probiotic Lactobacillus acidophilus culture using the method of microencapsulation by spray drying, and evaluate the stability of the microcapsules under different conditions of pH.

MATERIAL AND METHODS: The probiotic culture used was Lactobacillus acidophilus at a concentration of 1x1010 CFU / g. For the microencapsulation, the following materials were used: cellulose acetate phthalate, maltodextrin, glycerol, inulin, Tween 80 and reconstituted milk. Drying in spray drying occurred in the conditions of input temperature 110 ° C, air flow of 439 L / h and flow rate of 6 ml / min. Afterwards, tests of dissolution of the capsules were realized in buffers with pH 4.5, 6.0 and 7.5 and aliquots were withdrawn after 60, 120 and 180 minutes. The analysis of the capsules were obtained through captured images using OLY-200 camera attached to Olympus CX41 light microscope and image analysis software - Image-Pro ® Plus - version 4.0 for WindowsTM from Media Cybernetics. The microorganisms released were counted by plating on MRS Agar.

RESULTS AND DISCUSSION: The capsules were examined by optical microscopy corroborating a uniform view of the particles generated, with a good distribution and morphology. The results showed that the release of the probiotic culture at pH 4.5 did not exceed 2.3 x 10^5 CFU/g in the counting plate during the 180 minutes of dissolution. This demonstrated that the capsules were not completely dissolved by solution with acid pH. On the other hand for the tests at pH 6.0 and 7.5, a release of 2.01 x 10^8 CFU / g was observed, showing a good viability of the culture in post-processing. Studies by Fávaro-Trindade and Grosso (2004) showed results very similar.

CONCLUSION: Due to the high viability of the probiotic culture after the processing, the drying in the spray dryer proved to be a highly efficient technique for the formulation of CAP particles-based and added of probiotic bacteria.

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STUDY OF THE PRODUCTION OF LIGNINOLYTIC ENZYMES BY *Ceriporiopsis subvermispora* IN STATIC CULTURES

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Keywords: manganese peroxidase, laccase, *Ceriporiopsis subvermispora*

INTRODUCTION: The most suitable fungi for wood degradation are the white-rot fungi, which are used in biopulping and other industrial processes such as bioremediation and biobleaching (Akhtar et al. 1998). Among them, the basidiomycete *Ceriporiopsis subvermispora* stands out with its high ability to remove lignin due to the production of two extracellular oxidative enzymes, manganese peroxidase and laccase (Vincentim and Ferraz, 2007). The purpose of this study was to evaluate the production of such enzymes in static cultures, in complex and defined media.

MATERIAL AND METHODS: The defined medium was prepared with glucose and ammonium tartrate, according to Ruttiman et al (1992), while the complex medium consisted of 2,4% w/v potato dextrose extract supplemented with 0,7% yeast extract. *C. subvermispora* was initially grown on 2% w/v malt-extract agar plates at 27ºC for 7 days. After this period, 3 discs (0.8 cm of diameter) of mycelium was taken from the edges of the colonies and used to inoculate 125 mL-Erlenmeyer flasks containing 30mL of the sterile culture media. These flasks were incubated at 27ºC for 21 days, with samples collected periodically (3 days). The samples were analyzed regarding the consumption of reducing sugars (DNS), the mycelium growth (dry weight) and the production of MnP (phenol red oxidation) and Lac (ABTS oxidation).

RESULTS AND DISCUSSION: According to the results, a high MnP activity (0.01 ±0.01UI/mL) was found on the 18^{th} day of culture in the complex medium , time in which a great consumption of reducing sugars had been observed and the fungus was in the stationary phase of growth. The maximum production of Lac in this medium was of 0.04 ±0.06UI/mL, in 15 days. On the other hand, in the defined media, the highest production of Lac was of 0.012 ±0.01UI/mL (21 days), which also coincided with the maximum mycelial growth and consumption of reducing sugars. For this last medium, the highest activity of MnP occurred on the 18^{th} day, with a value of 0.09 ±0.01UI/mL. The large standard deviations observed in the results of enzymatic activity should be highlighted.

CONCLUSION: It is believed that the form of inoculation with plugs of mycelium grown on solid medium contributed to the high deviations observed. Moreover, the static culture has not led to a considerable production of enzymes under the conditions employed.

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SUBSTITUTION OF YEAST EXTRACT BY A LOW-COST NITROGEN SOURCE ON THE PRODUCTION OF A BIOSURFACTANT FROM *CANDIDA TROPICALIS* UCP 0996

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Key-words: biosurfactants, *Candida tropicalis*, residue.

INTRODUCTION: surfactants are amphipathic molecules derivate from petroleum with applications in different industries (MUTHUSAMY et al., 2008). The production of microbial surfactants or biosurfactants, as they are called, differently form their synthetic counterparts, has been intensified due their properties such as low toxicity, high biodegradability and obtaining by renewable resources (GAUTAM & TYAGI, 2006). Considering the importance of reducing costs associated to the production process, in special related to the medium composition, the present work describes the substitution of yeast extract by corn steep liquor, an industrial residue originated from corn products processing.

MATERIAL AND METHODS: the biosurfactant was produced by *Candida tropicalis* UCP 0996 cultivated in waste frying oil as the substrate. Fermentations were carried out in mineral medium formulated with 2% of the oil, 0.067% NH_4Cl, 0.025% $MgSO_4.7H_2O$, 0.067% KH_2PO_4, 0.0026% $FeCl_3.6H_2O$ and 0.01 and 0.005% of yeast extract or corn steep liquor, during 144 hours under orbital shaking of 150rpm at 28ºC. After cultivation, samples were centrifuged and analyzed for emulsification activity (COOPER & GOLDENBERG,1987) and surface tension measurement by the Du Nuoy ring method. The biomass was determined by dry weight and the biosurfactant was isolated according to Sarubbo et al. (2007).

RESULTS AND DISCUSSION: the results obtained showed that the substitution of the yeast extract by corn steep liquor did not favor the surface tension reduction neither the emulsification capacity. The high surface tension reduction (33 mN/m) was obtained when yeast extract was used in the production medium. High emulsification indexes were also observed (80-94% of motor oil) with yeast extract as the nitrogen source, showing the potential of this new biosurfactant for application in bioremediation processes.

CONCLUSION: the yeast extract showed to be better than the industrial residue for the production of the biosurfactant from *Candida tropicalis*. In spite of the high cost, the properties obtained will permit new combinations of residues and substrates, aiming the reduction of the costs.

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SYMBIOTIC EFFICENCY OF RHIZOBIA IN COWPEA AND ITS TOLERANCE TO ANTIBIOTICS

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Key words: nitrogen biological fixation; symbiosis, leguminous.

INTRODUCTION: The cowpea (*Vigna unguiculata* (L.) Walp.) is used in the North and northeast of Brazil and is able to adapt to different edaphic conditions. Although the production costs are low, it is produced with low technology and the grain revenue is very low. One of the limitations to productivity is the Nitrogen (N) deficiency in the soils, which can be reverted by the Nitrogen Biologic Fixation (NBF) by rhizobia in symbiosis with cowpea, supplying the demands of plant N. The caupi is a promiscuous species which can nodulate with strains of several genera of fast growing and slow growing (Moreira, 2008). Limiting factors, such as competition with other strains for infection sites and antagonist relations, such as antibiosis, may affect negatively the NBF by efficient strains selected introduced as inoculants. It was aimed to evaluate the symbiotic efficiency of rhizobia strains in cowpea and test its tolerance to 15 different antibiotics.

MATERIALS AND METHODS: The symbiotic efficiency, evaluated in a greenhouse, of 12 strains of rhizobia in cowpea: 10 isolates of caupi nodules from soils of the South of de Minas Gerais, using cowpea as trap species (Florentino, 2007) and 2 recommended as inoculants for caupi, UFLA 03-84 and INPA 03-11B, being 2 of fast growing and 10 of slow growing. Two treatments were used without inoculation: one containing nitrogen (N) mineral and other without N mineral. The statistical design was completely randomized (CRD) in three repetitions. The plants were grown for 50 days and were evaluated for Shoot Dry Mass (SDM) and the nodules (DMN), Number of nodules (NN), Relative Efficiency (RE) and shoot nitrogen accumulation (SNA). SDM, DMN, NN, RE and SNA. The variables were statistically analyzed with Scott-Knott test at 5% probability. Was used the disk diffusion method for evaluate the tolerance of 15 antibiotics in the following concentrations (µg L^{-1}): Azitromicine (15), Streptomycin (10), Eritromicine (15), Ampicilin (10), Sulfonamides (300), Chloranphenicol (30), Rifamicin (30), Kanamicyn (30), Nalidixic acid (30), Claritromicin (15), Tetracycline (30), Amoxicillin (10), Gentamicin (10) and Vancomycin (30). It was also tested Bacitracin (10 U.I). Aliquots of 100 µL of each bacterial culture (with 10^9 cells) were spread in Petri plates, containing culture medium 79 (YMA). The test was carried in three repetitions. Afterwards, it was inserted 3 discs, distant amongst themselves, containing 3 kinds of antibiotics per plate. Plates were incubated at 28^{o}C for 5 days. Inhibition zone was measured for each antibiotic, indicating sensibility.

RESULTS AND DISCUSSION: It was observed variability among strains. The strains UFLA 03-84 and INPA 03-11b promoted values of SDM higher than the treatment N mineral, proving the efficiency in fixing N2 in symbiosis with caupi (Soares et al., 2006). Two tested strains were highlighted in most of the variables, superating the control with N mineral and the UFLA 03-84 and INPA 03-11b, in several parameters. Opposite to realted by Xavier et al. (1998) it was found relation between growing and morphologic characteristics, once the slow growing strains tended to be more tolerant to antibiotics than the fast growing strains.

CONCLUSIONS: The symbiotic efficiency in cowpea was variable, highlighting two strains which presented potential for further tests of agronomic efficiency. The slow growing strains were more tolerant to antibiotics.

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THE DESINFESTATION OF CASING LAYER AFFECTS THE PRODUCTIVITY OF THE MUSHROOMS *Agaricus brasiliensis*

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Keywords: mushroom, casing layer, soil microbiota

INTRODUCTION: The productivity of the mushroom *Agaricus brasiliensis* is relatively low compared to other mushrooms to productivity and the production cycles are extremely long, particularly the rising cost of production. The fruiting induction is one of the key to the success of cultivation and for the *A. brasiliensis*, the use of a casing layer is essential to the process. There are reports that in *A. bisporus*, the presence of the bacterium *Pseudomonas putida* in the casing layer leads to early production and greater productivity. However, for *A. brasiliensis* there are no studies on the importance of the presence of microorganisms in the casing layer. Therefore, the aim of this study was to evaluate the effect of disinfestation of the casing layer and inoculation with a bacterial cocktail on the fruiting of the mushroom *A. brasiliensis*.

MATERIAL AND METHODS: For this work we used the compost colonized with the mushroom *A. brasiliensis* strain CS1. The colonized compost, after 20 days, was transferred into 14 L polyethylene pots (4 kg compost/pot) and pressed. A 5cm depth of damp casing material was used to cover the colonized compost. It was used soil Rhodic Hapludox from the horizon B mixed with 20% (v/v) of charcoal fragments from Eucalyptus. For the treatments with the casing layer disinfected, the mixture was subjected to autoclaving for two hours at 121 º C twice with an interval of 24 hours. The treatments consisted of: (i) casing layer not autoclaved, (ii) casing layer not autoclaved and inoculated, (iii) casing layer autoclaved and inoculated (iv) casing layer autoclaved and inoculated. The inoculation of the bacterial cocktail on casing layer was made at the time it was placed on the compost. The statistical design was randomized in blocks with four replicates (3 pots/ replicate). Treatment means were separated using Scott-Knott test at 5% level. Data were analyzed using the program SISVAR®.

RESULTS AND DISCUSSION: The mushroom productivity was higher in treatment with autoclaved casing layer (12.79%) in relation to casing layer not sterilized (7.32%). However, there was a significant delay in the onset of fruiting with autoclaved casing layer in relation to soil without treatment. The inoculation bacteria on the casing layer didn't increase mushroom production. Furthermore, when the casing layer was not autoclaved but inoculated with bacteria, time for fruiting induction was reduced. These results indicate that the presence of microorganisms in the casing layer is important, however, other studies must be conducted to identify which species are important for the process.

CONCLUSION: Reduction or elimination of microorganisms from the casing layer was important to increase mushroom production, but the fruiting induction was delayed too. Inoculation of the casing layer with bacteria resulted in a faster fruiting, but the productivity was not affected.

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THE ENZIMATIC ACTIVITY OF *Aspergillus nidulans* IN RESPONSE TO CADMIUM PRESENCE

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Key Words; phenoloxidases, cadmium, *Aspergillus nidulans*

INTRODUCTION: Heavy metal accumulation in agricultural soils is an aspect of serious concern related to environmental security, because those substances can express its pollution potential on soil organisms, on plants in phytotoxic levels, as well as by the transference to trophic chains through plants or by waters contamination (MARIA, L.S.S. et al., 2007). The correlation between enzymatic activity and the survival ability in presence of heavy metal in the environment its suggested (LLOYD, J.R., 2002). The aim of this work was evaluate the *Aspergillus nidulans* growth and the activity of tannase, phenoloxidase and amylase in medium containing cadmium in concentrations of 1mM, 2mM and 3mM.

MATERIAL E METHODS: The analysis of enzymatic activity was evaluated in culture medium containing different substrata. Galic acid, tanic acid and soluble starch were used for phenoloxidase, tannase and amylase, respectively. The fungus was growth during 5 days, at 28°C. The radial colony growth and the enzymes activities were observed each 24h.

RESULTS AND DISCUSSION: The results obtained for the fungus growth in the different media tested are presented in Table 1.

Table 1. Growth profile of *Aspergillus nidulans* in media tested

	TANASE					PHENOLOXIDASE					AMYLASE				
	24h	**48h**	**72h**	**96h**	**120h**	**24h**	**48h**	**72h**	**96h**	**120h**	**24h**	**48h**	**72h**	**96h**	**120h**
Control	1,4	2,8	5,1	7	9	2	5,8	7,9	9	9	1,5	3	4,45	5,05	5,6
1mM	0	0	0	0	0	1,1	3,3	3,9	4,5	5,3	0,6	1,2	1,75	1,9	2,45
2mM	0	0	0	0	0	0,7	2,2	2,6	3	3	0,5	1	1,45	1,75	2,05
3mM	0	0	0	0	0	0,6	1,8	1,9	1,9	1,9	0	0	0	0	0

It is possible to observe that the isolate exhibited a higher growth in control media, and the radial colony ramification was related to cadmium concentration used in culture media. For tanase medium the fungus grew only in control condition, but exhibited enzymatic activity in all media containing cadmium (figure 1-B), When tested in media for amylase the fungus exhibited a exuberant growth and the activity was detected in all conditions tested (figura 1-C). A similar response was noted for phenoloxidase experiments (figura 1-A). Those results are the first related to cadmium presence.

Figure 1. Colonies growrth of *Aspergillus nidulans* in media for enzymatic indicators. A. Phenoloxidase; B. Tanase; C. Amylase

CONCLUSIONS: The fungus exhibited a positive response for the enzymes tested. In cadmium presence the tanase activity was noted but the growth was inhibited; in medium for phenoloxidase activity, the cellular growth and enzyme activity were the highest; In medium for amylase activity the growth and the enzyme were inhibited only in presence of 3mM of cadmium.

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THE USE OF LOW-COST RAW MATERIAL TO BIOPOLYMER PRODUCTION

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Keywords: pullulan; biopolymer; industrial residual

INTRODUCTION: Microbial biopolymers are polysaccharides produced by microorganisms whose rheologic properties allow for additional applications in various industrial sectors. Among biopolymers, pullulan, a linear neutral homopolysaccharide mainly produced by the dimorphic fungus *Aureobasidium pullulans* under aerobic conditions, stands out. Pullulan's physicochemical properties enable its use not only in the food, cosmetic, and pharmaceutical industries, but also for more noble purposes, as for combined vaccine and interferon. This study aimed to evaluate the effect of five nitrogen sources in different concentrations and of two strains of *A. pullulans*, to reduce costs and maximize the process yield.

MATERIAL AND METHODS: The experiments were performed in batch reactors using basic mineral medium consisting of 30 g/L of crystal sugar and the nitrogen sources – ammonium sulfate, sodium nitrate, ammonium nitrate, urea, and residual brewery yeast – in order to establish a carbon/nitrogen (C/N) ratio of 5, and 150. After 48 hours of incubation in 28 ± 1°C, under agitation of 150 rpm, the following determinations were performed: cellular concentration, substrate consumption, polymer yield, pH, and viscosity.

RESULTS AND DISCUSSION: All nitrogen sources, in the proportions tested, were capable of fostering cell growth and biopolymer production by both strains. However, both the amount of gum produced and the fermented broth viscosity were dependent on microbial strain, the nitrogen source, and the C/N ratio. Overall, the best results for the different conditions were observed for the IOC 3011 strain. Among the nitrogen sources, the residual brewery yeast (LRC) fostered the greatest yield of biopolymer. The LRC was even better than the ammonium sulfate, which is the nitrogen source usually recommended to obtain this biopolymer. The brewery residue used also fostered the generation of fermented broth with high viscosity values – 0.06 Pa.s 0.008 Pa.s, respectively in the lowest (15.6 s^{-1}) and the highest (415 s^{-1}) shear rate.
CONCLUSION: The use of residual brewery yeast was the source of nitrogen that obtained higher values of viscosity and yield.

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TOLERANCE OF BASIDIOMYCETES TO DIFFERENT CONCENTRATIONS OF CADMIUM

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Keywords: tolerance, basidiomycetes, cadmium.

INTRODUCTION: The spread of cadmium in the environment occurs through various sources, the origin of the largest anthropogenic processes responsible for the contamination of soil and water. Considering that cadmium has long life and slow excretion, this may be accumulated in human tissues and organs causing various diseases. Fungi Basidiomycota group are known for the ability of bioaccumulation of metal ions and represent an alternative for decontamination of polluted environments. This work aimed to verify whether different species of basidiomycetes respond in a different way to the toxicity of cadmium.
MATERIAL AND METHODS: The species *Pleurotus ostreatus*, *Pleurotus sajor-caju*, *Pleurotus eryngii*, *Lentinula edodes* and *Agaricus brasiliensis* were grown in PDA medium with different concentrations of cadmium (10, 20, 40 and 80 ppm), and a control without the heavy metal. The parameter analyzed after eight days of incubation at 25 ° C was the mycelial growth in mm / day in a completely randomized design in a simple scheme bifactorial (5x5) with three replications. Data generated were subjected to analysis of variance and the Scott-Knott test at 5% probability, with the aid of statistical software SISVAR ®.
RESULTS AND DISCUSSION: The species *L. edodes* mycelial growth was completely inhibited at lower concentrations of cadmium tested (10 ppm), while *A. brasiliensis* was completely inhibited only at the highest concentration (80 ppm). The species of *Pleurotus* were the most tolerant, showing mycelial growth in all concentrations tested, especially *P. ostreatus*, which had the highest rate of growth in all treatments. The highest cadmium tolerance of this species may indicate a greater capacity for bioaccumulation of metals by the fungus, however, that need to be confirmed in future experiments to be suitable for studies of bioremediation of contaminated soil.
CONCLUSIONS: *Agaricus brasiliensis* and different species of *Pleurotus* show tolerance to cadmium in different concentrations.

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TOLERANCE TO ETHANOL AND MOLECULAR CHARACTERIZATION OF *Dekkera bruxellensis* STRAINS ISOLATED FROM ETHANOLIC FERMENTATION

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Keywords: *Dekkera bruxellensis,* contaminants, alcoholic fermentation

INTRODUCTION:

Dekkera bruxellensis yeasts are considered important contaminants in alcoholic fermentation in Brazil, whose control has been done by increasing the alcohol content due to their low resistance to the ethanol. However, contradictory results have been found, including proposing these yeasts as ethanol producers in consortium with the bacteria *Lactobacillus vini* (Passoth et al., 2007). So, this study aimed to evaluate the tolerance to ethanol presented by 3 strains of *D. bruxellensis* isolated from ethanolic fermentation, and their molecular characterization.

MATERIAL AND METHODS:

1 Growth of strains (CCA059, CCA077 and CCA155) in 100ml of liquid YEPD, 10% inoculum (vol/vol, 10^5cells/ml), added with 0% to 4% ethanol (vol/vol), with analysis of optical density (600nm) and pH for a period of about 48-60 hours;
2 Amplification and sequencing of ITS region from ribosomal DNA by PCR;
3 Fingerprinting using the primer $(GTG)_5$.

RESULTS AND DISCUSSION:

The ITS region showed PCR product of about 500bp and by multiple alignment of the sequences, a high similarity between the strains CCA059 and CCA155 was observed. The molecular typing by the primer $(GTG)_5$ also showed two patterns of amplification including CCA059 and CCA155 in a group and CCA077 in another one. These results indicate the existence of a pattern related to the geographical boundaries, as the first group of strains was isolated from the same distillery but from different samples and harvesting periods, and the second strain was isolated from another distillery. All the strains showed slow growth in YEPD medium, but CCA059 and CCA155 were more tolerant to ethanol than CCA077, which showed a much lower growth over the same period of time. For the first group, concentrations of 3-4% ethanol affected very much the yeast growth, although there were differences in growth rate between the strains. For CCA077, there was a drastic reduction in growth from 1% ethanol. The growth was always accompanied by a significant decrease in medium pH.

CONCLUSION:

The strains of *D. bruxellensis* belong to distinct groups related to geographic region of the isolation, which also exhibited differences in tolerance to ethanol. However, the three strains showed significant reduction in growth, from 3% ethanol to the first group, and from 1% ethanol for the second group, confirming the low tolerance of the yeast to this alcohol.

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TOTAL AND THERMO TOLERANT COLIFORM MONITORING IN WASTEWATER TREATMENT SYSTEM AIMING THE EFFLUENT REUSE IN AGRICULTURE

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Keywords: Reuse, Agriculture, Wastewater

INTRODUCTION: The water has become scarcer as the population is being expanded and the agriculture is the activity which consumes more this resource (PHILIPPI, 2005). Facing this problem, the agriculture reuse of treated wastewater would be a low in cost alternative which could minimize this lack. In small villages, simple wastewater treatment systems could be used which besides not damage receiving waters through the discharged of sewer, a suitable effluent could be produced to be used in agriculture. One of these systems would be the association of an anaerobic filter with a sand filter. Some parameters should be monitored in order to prove that the produced effluent won't bring risk for the public health; among them, the total and thermo tolerant coliform quantification become necessary.

MATERIAL AND METHODS: The wastewater was treated by anaerobic filters, filled up with bamboo pieces, associated with a sand filter. The loading rate used in the sand filter was 600 $Lm^{-2}dia^{-1}$. Collected samples for system monitoring were analyzed according to *Standard Methods for the Examination of Water and Wastewater* recommendations (APHA, 2005).

RESULTS AND DISCUSSION: Total and thermo tolerant coliform concentrations in the final effluent varied from 1x 10^4 a 6x 10^5 NMP.100 mL^{-1}. It was possible to verify that, with the loading rate of 600$Lm^{-2}dia^{-1}$ of anaerobic effluent on the sand filter, two logarithmic units were removed. Thus, if this effluent were reused in agriculture, according to Organização Mundial da Saúde – Health World Organization (OMS, 2000), the irrigation with this effluent should be through aspersion in cultivations of cereal crops and in fruit-bearing in which is allowed the use of effluent with a maximum thermo tolerant coliform concentration of 10^5 NMP.100 mL^{-1}.

CONCLUSION: The final effluent from a treatment system composed of anaerobic filter and sand filter may be reused in agriculture without bringing problems for the public health.

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TOXICITY ANALYSIS OF EFFLUENT FROM A RUBBER ANTIOXIDANT MANUFACTURER AFTER ELECTROLYTIC PROCESS

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Key words – effluent, electrolytic process, toxicity

INTRODUCTION – This piece of work aims at showing the relationship level between the toxicity testing results and the electrolytic analysis ones that have been performed.

MATERIAL AND METHODS – An electrolytic system with steel electrodes and continuous current of 60 A has been installed at the final effluent generated by the industry. The electrolyzed effluent streams, in different time frames (0 a 120 min), have been analyzed relatively to toxicity. An alternative toxicity test has been utilized considering the viability cellular (V) representative (%) which consists of the *Saccharomyces cerevisiae* exposure to dilutions of the electrolyzed effluent streams, in different time (RÉGIS, 2000). In order to avoid interferences, a synthetic effluent was adopted as the study matrix for the toxicity tests. The efficiency of the electrolytic process, in this effluent was studied based on the reduction of the concentration of *Escherichia coli* present in sewage samples in different times of electrolysis. The results are shown in disinfection rate, expressed as, -log (N/No), where N and No the final and initial concentration of the indicative microorganism, respectively, relatively to the retention time of the water sample on the plaque, in seconds (Toxtrak™).

RESULTS AND DISCUSSIONS – Several electrolysis effluents were tested and showed that the *S. cerevisiae* in effluents: *Raw* (no treatment) electrolyze allowed a lower *S. cerevisiae* toxicity from 60 min time on. *Final, (*after treatment*)* electrolyze allowed a cellular viability of 100% in 30 min time. That result matches the result which was obtained with the electrolyzed raw effluent, presuming that the electrolytic process allows for substances degradation, turning that effluent more biocompatible to the *S.cerevisiae*. The electrolysis showed that *Escherichia coli* in synthetic effluent: *Flexzone 3P and 7P*, the toxicity decreased in about 30% along with the electrolysis time, but has not reached a reasonable percentage for its survival. This is due to the fragility of the bacteria's cellular wall relatively to the *S. cerevisiae*. *Naugard Q*, the electrolytic process allowed toxicity for this microorganism to remain at around 50%. *Octamine and Aminox,* the electrolytic process caused a toxicity increase in the first 10 min, returning to the same toxicity percentage of the *in nature* substance at 20 min time and decreasing by 10% at 30 min time relatively to the beginning. That fact leads us to presume that the electrolysis has little influence on toxicity decrease of the *E. coli*, when applied to these substances. *Synthetic effluent* (blend of the five substances), All those substances together are 100% toxicity to the *E. coli* microorganism.

CONCLUSIONS – The substances that take part of the effluent generated by the Antioxidants manufacturing process in reference are extremely bio-resistant, inhibiting the reduction of the toxicity level at reasonable levels. On the other hand, after going through the biological and the electrolytic processes, that toxicity decreases significantly to an extent that a study on the re-utilization of the water could be performed, such as floor cleaning, as sanitary flushing or as process water itself.

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TOXICOLOGY STUDY DURING THE BIODEGRADATION OF AUTOMOTIVES AND VEGETABLE OILS

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Keywords: bioremediation, biodegradation, toxicology.

INTRODUCTION: activities connected with extraction of the oil, as well as the diverted activities wrap a large environmental risk. They can be in the straight form, like the spilling, or even indirect, like produced residues of his use, polluting the air, waters and soils. For so much studies that aim to put the situation right they are constantly fulfilled, as well as methods of control or even of evaluation of impact. One of the methodologies employed enough and studied is that of the bioremediation, which consists of the use of microorganisms to degrade a pollutant substance, turning it into other one like less toxicity or even none.
MATERIAL AND METHODS: soil of the sandy type (middle sand of construction) was used to prepare inoculum with six types of oils test when they are biodegradados (mineral, synthetic, used lubricant, vegetable, vegetable oil and biodiesel). The toxicologies tests were carried out to quantify if the inoculum produced a less toxicity due to the biodegradação of the oils. The tests of toxicity were carried out by the next organisms-tests: Eisenia andrei (worm), seeds of *Eruca sativa* (rucula) and of *Lactuca sativa* (lettuce). So, an pilot experiment was carried out by the next concentrations (0,5; 1,0; 5,0 and 10,0 ml of oil/100g of soil) and the toxicity by several organisms was valued.
RESULTS AND DISCUSSION: the following results were withdrawn of the pilot experiments. Only in two bigger concentrations (5,0 and 10,0 mL of oil/100g of soil) obtained significant difference for all the organisms test. The biggest toxicity was for the mineral, synthetic and used lubricant oils. However, for the vegetable and used vegetable oils and the control (without any type of oil) the toxicity was low or much goes down for all the organisms-tests. On the other side, for the biodiesel a more medium toxicity was quantified only for the Eruca sativa and *Lactuca sativa*, in other words, no toxicity for the organism test *Eisenia andrei*.
CONCLUSION: the real variation of toxicity could be observed for the concentrations of 5,0 and 10,0 ml/100g of soil in the inoculum, therefore for the subsequent experiments an intermediary concentration will have to be used to value better the by-products of the biodegradação of the oils.
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TRANSIENT FUNGI IN THE ATIBAIA RIVER, PAULÍNIA, SP.

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Keywords: fungi, Atibaia river, fungal biodiversity.

INTRODUCTION: Human activities promote changes that interfere in the environmental biodiversity, what may favour the growth of some species that are able to survive under adverse ecological conditions, while others decrease or disappear. The study of the water quality in the Atibaia river, in a region of a petrochemical influence, Paulínia, SP, Brazil, has been carried out since 1997, through chemical and physical-chemical analyses. This work aims to improve these analyses with a study of the water quality concerning its fungal biodiversity as well.

MATERIAL AND METHODS: The isolation method was based on Eaton et al. (1998). Thirty-six samplings were done between May 2007 and March 2009, when water samples from 6 different places along the river were collected. A seventh sample was taken from Jaguari River to be used as control. In total, 252 samples were analyzed. The isolates were purified and preserved both in slants at 10°C and in ultrafreezer at -80°C. Fungal strains were identified as classical methods, using slide culture, biochemical tests and DNA sequencing when necessary.

RESULTS AND DISCUSSION: A number of 450 isolates were reported. Filamentous fungi were found in higher number in comparison to yeasts. The most frequent genera were: *Penicillium, Aspergillus, Acremonium, Fusarium, Trichoderma, Cladosporium, Colletotrichum, Paecilomyces, Phialophora* and *Curvularia*. They all belong to soil ubiquitous representatives, which are commonly resistant to polluted environments. The number of colony forming units, UFC, of some samples was higher, although many strains were morphologically similar what probably means a low fungal biodiversity. The yeasts are still being identified.

CONCLUSION: The environmental impact found in the area through previous chemical and physical-chemical analyses was also observed in our results, showing that the fungal community was affected as well. It is assumed that the amount of effluent and other pollutants discharged in the river Atibaia were enough to disturb the diversity of fungal transient representatives. This may explain why only high-sporulating resistant fungi were reported. How impacted this area is at the moment will be better clarified after the identification step is finished.

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USE OF GLYCERIN AS CARBON SOURCE FOR THE PRODUCTION OF BIOEMULSIFIERS BY *Pseudomonas aeruginosa*

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Keywords: Bioemulsifiers, *Pseudomonas aeruginosa*, Glycerol

INTRODUCTION: surfactants are substances that adsorb and alter the prevailing conditions at interfaces. These features allow the reduction of surface and interfacial tensions and the formation of micro emulsions where hydrocarbons can be solubilized in water or where water can be solubilized in hydrocarbons (RON & ROSENBERG, 2001; 2002). Thus, the objective of this work was to evaluate the use of glycerin on the production of a biosurfactant with emulsifying properties by *Pseudomonas aeruginosa* in order to reduce the disposal of glycerin, from plants of oil refining and processing, in the environmental.

MATERIAL AND METHODS: the *Pseudomonas aeruginosa* UCP0992 was grown in mineral medium composed by yeast extract (0.5%), KH_2PO_4 (0.3%), K_2HPO_4 (0.02%), $MgSO_47H_20$ (0.02%) and glycerin as the carbon source (2%, 3%, and 4%), for 72, 96 and 120 hours under agitation of 150 rpm at 28 ºC. The production of biosurfactants was evaluated by the measurement of surface tension on automatic tensiometer (KSV Sigma 70-Finland) and quantification of the emulsification index (COOPER & GOLDENBERG, 1987) with different substrates tested (corn oil, soybean oil, sunflower oil , diesel, motor oil and n-hexadecane).

RESULTS AND DISCUSSION: the biopolymer produced showed significant reduction of the water surface tension and a small variation of the cell-free broth surface tension in the three concentrations of glycerol studied (2% glycerin - 28.80 mN/m, 3% glycerin - 29.23 mN/m and 4% glycerin - 29.72 mN/m). In the evaluation of the emulsification index of the cell-free broth during 72, 96 and 120 hours, it was only observed a small variation in the medium compositions tested. For the media containing 2 and 3% glycerin, the soybean oil more emulsified (81% and 84%), while corn oil (62.5%), diesel (54.5%) and n-hexadecane (56%) showed the formation of more stable emulsions. For the medium containing 4% glycerin, there was a small difference in the results, once emulsions the more stable had been observed with soybean oil (54 %) and with sunflower oil, (54.5%), while higher emulsification indexes had been obtained with corn oil (81%) and with motor oil (72.5%).

CONCLUSION: the results show that the in influence of the carbon source concentration in the production of the biosurfactant with emulsifying properties is important to evaluate the condition of greater efficiency and effectiveness, so the biosurfactant may be produced on a larger scale for application in the environmental area.

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USE OF INDUSTRIAL BY-PRODUCTS AS SUBSTRATE FOR MELANIN PRODUCTION BY *Aspergillus nidulans*

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Keywords: Industrial by-products, Melanin, *Aspergillus nidulans*

INTRODUCTION: Pigments produced by microorganisms have been widely employed in the pharmaceutical and food industries due to a serious safety problem with many artificial synthetic colourants and also to be economically advantageous. The melanin produced by *Aspergillus nidulans* fungi has considerable biotechnological interest as an antioxidant agent in cosmetic formulations to minimize UV light-induced damage (GONÇALVES, POMBEIRO-SPONCHIADO, 2005; PLONKA, GRABACKA, 2006). However, for a possible practical application of this substance, is necessary high productivity with low cost compared to synthetic melanin. In this context, the objective of this work was to evaluate the melanin production by *Aspergillus nidulans* using industrial by-products (sugar cane molasses and corn steep liquor) as nutritional sources.
MATERIAL AND METHODS: Approximately 10^5 conídios/mL from melanized strains (MEL 1 and MEL 2) of *Aspergillus nidulans* were inoculated in 500-mL flasks containing 200 mL of minimal medium (pH 6,8) supplemented with the nutritional requirements inositol (to MEL 1) and acid ρ-aminobenzóic (to MEL 2), nitrate (0.6%) or corn steep liquor (0.2%) as nitrogen source and sugar cane molasses (1 and 10%) as carbon source. After 5 days of incubation at room temperature on a rotary shaker at 220 rpm, the culture was submitted to vacuum filtration for determination of the biomass and extraction of the pigment.
RESULTS AND DISCUSSION: In the presence of nitrate, the growth of the MEL 1 and MEL 2 strains in medium containing 10% molasses was greater than those with 1% molasses. However, no pigment was produced in these conditions. When the nitrate was substituted by the corn steep liquor (0.2%), the fungal growth in 1% and 10% molasses was low compared with the condition above, but the melanin production was observed only in 1% molasses. To explain these results we can assume that substances present in corn steep liquor stimulated the melanin production whereas high glucose concentration had an inhibitory effect in the production of this pigment by strains studied. In addition, our results also showed that there is a inverse relation between growth rate and melanin production, as already mentioned for literature.
CONCLUSION: Therefore, the results obtained in this work suggest that 1% sugar cane molasses (as carbon source) in combination with 0.2 % corn steep liquor (as nitrogen source) can represent a low-cost fermentation medium for melanin production by strains MEL1 and MEL2 of *Aspergillus nidulans.*
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VIABILITY OF *ESCOVOPSIS WEBERI* CONIDIA AND IMPLICATIONS FOR BIOLOGICAL CONTROL OF LEAF-CUTTING ANTS (HYMENOPTERA: FORMICIDAE)

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Keywords: *Atta*, parasite, conidia germination

INTRODUCTION: Leaf-cutting ants are well-known agricultural pests due to the ability of cutting leaves and flowers to use as substrate for a symbiotic fungus that ants cultivate as food. Recently, fungi in the genus *Escovopsis* were pointed out to be specialized parasites of the cultivated fungus (Currie, 2001; Reynolds & Currie 2004), but little is known about the biology and distribution of this parasite. In this sense, the aim of this preliminary study was to evaluate the conidial germination of *Escovopsis weberi* and to discuss the possible applications of this fungus as a biological control agent of such insects.
MATERIAL AND METHODS: In order to test the conidial viability of *E. weberi*, seven strains (A – F) were isolated from laboratory colonies of *Atta sexdens rubropilosa* which were previously treated with the insecticide sulfluramide in several concentrations (0.1 – 0.3%). An additional strain (G) was isolated from a field colony of *Acromyrmex* sp. (control). Fungal strains were inoculated in potato-dextrose agar (PDA) medium and incubated for seven days at 25° C. After incubation, conidia were suspended in 0.05% Tween 80 supplemented with 0.2% peptone water and standardized to approximately 10^4 conidia/mL. Aliquots of 150 µL of this suspension were surface spread in PDA plates (n= 6 replicates) and after eight days of incubation, under the same conditions, the percentage of conidial germination was calculated.
RESULTS AND DISCUSSION: All *E. weberi* strains isolated from colonies treated with insecticide showed rates of conidial germination below 1% (0.10 – 0.17% for strains A, C and E; 0.05 – 0.06% for strains B, D and F). The *E. weberi* strain G, isolated from colony not treated with the insecticide, showed conidial germination rate of 4%. The results suggest there was no correlation between conidial germination and the different concentrations of sulfluramide used. Using data available in the literature for other fungi we observed that *E. weberi* has a comparatively low conidial germination rate. The possible causes of this apparent low viability of *E. weberi* conidia are still unknown but will further analyzed in future studies.
CONCLUSION: Methods that intent to use *E. weberi* as a biological agent for the control of leaf-cutting ants in field conditions should consider the low viability of its conidia in addition to other factors.
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XANTHAN PRODUCTION BY DIFFERENT FORMULATIONS OF GROWTH MEDIUM OF *Xanthomonas campestris pv. campestris*

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INTRODUCTION:
The bacteria of the genus *Xanthomonas* synthesize the biopolymer denominated xanthan gum, whose viscosity and resistance to the heat check it important properties to be used in the nutritious, pharmaceutical, among others area. In that work, it is aimed at to study the influence of the composition of the medium of cell growth on the quality of the gum produced by *Xanthomonas campestris pv. campestris*.

MATERIAL AND METHODS:
It were used for the growth of *X. campestris pv. campestris* the medium YM (Yeast Malt – Difco Lab.) that it was compared with other twelve media formulated in relation to the viscosity and yield of the xanthan gum and on the cell growth. The composition of the culture media varied the following nutrients: yeast extract (0.3-0.7% w/v), sucrose (1.0-2.5% w/v), $(NH_4)_2HPO_4$ (0-0.25% w/v), $MgSO_4.7H_2O$ (0.01% w/v) and K_2HPO_4 (0.15% w/v). Of the grown culture, it was used as inoculum for the gum production in the medium described by Souw and Demain (1979).The biopolymer was recovered with ethanol from the cell-free fermentation broth in a ratio 3:1 (v/v), respectively. Viscosity measurements of fermentation broth and xanthan gum (solution 1% w/v) were performed on a Brookfield LVDVI+ at 25ºC at a shear rate of 20rpm.

RESULTS AND DISCUSSION:
It was verified that happened the formation of a more prepared biomass for gum production with better viscosity when the medium of growth contained higher sucrose concentration (2.5% w/v). Maybe it was due to similar mechanism caused by osmotic pressure as it occurs in the medium 4% sucrose (Souw and Demain,1979) used for xanthan production. It was unfavorable for xanthan production the combination of higher concentrations of yeast extract (0.7% w/v) and ammonium (0.25% w/v). Probably, this fact is associated with the nitrogen excess to stimulate the cellular growth causing decrease in xanthan production (Lo et al.,1997). The YM medium was overcome by the most formulated media in production and viscosity gum .

CONCLUSION:
The present study showed that the growth medium for *X. campestris* pv *campestris* is a factor of great influence in the production and quality of the xanthan gum. A new growth medium superior to YM was proposed.

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