**S5-P1**

**Isolation of Efficient Polyethylene Degrading Bacterial Species from Marine Ecosystem of Gulf of Mannar in India**

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Polyethylene (LDPE) is advantageous as they are strong, light-weighted, and durable. However, they possess disadvantageous such as they are resistant to biological degradation and harmful to the natural environment. These solid waste related problems pose threat to megacities. Biological degradation includes microbial degradation by microorganisms such as bacteria and fungi that consume the material. 15 (GM1- GM15) bacteria were isolated from the plastic wastes dumping places near sea shore in Gulf of Mannar, India. Among 15 bacterial isolates, GM5, GM7 were efficient to degrade the polyethylene. The efficiency test carried out by weight loss. GM5 and GM7 degraded the polyethylene by 13.73% and 12.15% of weight loss respectively and efficiency of degradation was confirmed by FTIR spectrometric analysis.

**Keywords:** Polyethylene; solid wastes; microorganisms; marine ecosystem.

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**S5-P2**

**Evaluation of Enzymatic Activity and Biodiversity in Soil with Methyl-Parathion Pesticide in Chinampas of Xochimilco**

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Denitrification is one of the main steps of global Nitrogen cycle and consists in the reduction of Nitrate to molecular Nitrogen via Nitrous Oxide. It is the most important biological mechanism by which Nitrogen returns to the atmosphere from soil and water. Denitrification has also received considerable interest recently because yields N$_2$O emissions, it is an important greenhouse gas and a natural catalyst of stratospheric ozone degradation. Denitrifying bacteria are widely distributed in the environment and exhibit a high taxonomic diversity, but the increasing use of pesticides and fertilizers during the last decades has negatively affected denitrifying populations in agricultural soils. Recently, the development of molecular techniques based on direct DNA extraction from the environment, which enable identifying functional genes, has helped understand the relation between microbial community composition and nitrogen dynamics in ecosystems. Now, in order to further understand denitrification processes in ecosystems, it is necessary to integrate physicochemical and molecular analysis. We performed microcosms experiments aimed at examining the persistence of nitrate, nitrite and nitrous oxide reductase, as well as the effect on the dynamics of NO$_3^-$, NO$_2^-$, N$_2$O y N$_2$ in soil with methyl-parathion pesticide in the so-called “Chinampas” of Xochimilco, in Mexico from Náhuatl or Aztec, chinamitl, bulrush or cattail stalks lattice for hydroponics cultivation. Studying the dynamics of the enzymes contributes to understanding the controls over N$_2$O production when anaerobiosis is rapidly induced in soils. We analyzed these enzymes’ activity after different periods of aerobic incubation by gas emission and analyzed one functional genes by TGGE. Our results indicate that there is variation of abundances at genetic level and of gas emission in incubations times, pointing at a correlation between gas emissions and molecular analysis.

**Keywords:** Methyl-Parathion pesticide; denitrification; enzymatic activity.
Determination of Changes in the Microbial Community in a Soil Amended with Liquid Cow Manure and Application of Atrazine through Molecular Techniques

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Application of animal manure amendments to agricultural soils is a common practice to improve soil fertility through the addition of essential plant nutrients. Applications of organic amendments have also been shown to increase the soil microbial biomass and stimulate microbial activity. This effect of biostimulation has been associated to modify the behaviour of pesticides in soil. Andisols in Chile are generally cropped to maize (Zea mays L.) and their management includes the application of liquid cow manure (LCM) at rates higher than 100,000 L ha\(^{-1}\). Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) is a herbicide widely used in corn production. However, the application of LCM has produced an increase in weed populations, which has caused farmers to increase rates of atrazine application through repeated application. The effects of herbicides on soil microbial communities are conventionally studied by techniques based on measuring their metabolic activities. However, in many cases detect significant effect. Recently, various molecular techniques, such as denaturing gradient gel electrophoresis (DGGE) have been developed to monitor temporal and spatial changes of the soil microbial community. In this study we investigated the response of microbial communities in unamended and LCM-amended soil treated with the herbicide atrazine. The LCM was applied at rates equivalent to 0, 100,000, 200,000, and 300,000 L ha\(^{-1}\), resulting in treatments S-0, S-100, S-200, and S-300, respectively. In the laboratory an aerobic incubation was conducted for 30 days. Then, was applied atrazine in concentrations of 0, 1, 2, and 3 mg kg\(^{-1}\). Bacterial community changes were assessed by 80 days after atrazine application using DGGE of polymerase chain reaction-amplified 16S rDNA fragments. The application of LCM at different doses modified the soil community, represented by appearance of new bands. The same tendency was observed with the application of atrazine at different concentrations in unamended and amended soils. Atrazine modified the soil community during the first 10 days after their application, and then returned at the previous condition. At day 1, 15 and 30 after LCM application, and at day 1 and 10 after atrazine application, major bands were excised from the gels and the DNA was cloned for sequence analysis. In amended soil the community was modified by the appearance of bacteria belonging of Phylum Bacteroidetes and Genus Acinetobacter mainly in amended soil S-300. While the dominant bacterium in unamended and amended soil with atrazine applications at different concentrations were belonging of Phylum Proteobacteria/Class Betaproteobacteria. The main changes were presented in unamended soil S-0. Our results indicate the existence of bacteria adapted to atrazine degradation in agricultural soil.

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Keywords: Microbial community; liquid cow manure; atrazine.
Perchlorate in Soils and Salary in the North of Chile, Iquique

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Perchlorate is regarded as a new emerging persistent inorganic contaminant because of its specific properties, such as highly soluble, mobile and stable in water, readily migrates to ground and surface waters that are used as drinking water and for irrigation. The Atacama Desert nitrate deposit in Chile used as fertilizer is also known to be a natural source of perchlorate. Recently it has also been reported that perchlorate is naturally formed in atmospheric processes. Perchlorate is known to interfere with iodine uptake by the thyroid gland. In 2005, the United States Environmental Protection Agency (US EPA) established an official referent dose (RfD) of 0.7 ug/kg/day of perchlorate and specified its drinking water equivalent level to be 24.5 ppb. Perchlorate can be uptake in plants, especially at environmentally relevant (low ppb) concentrations. In the present study we investigated the occurrence of perchlorate in the north of Chile, Iquique using IC-ESI-MS and illustrate the perchlorate pollution status in Chile. A total of 10 soils and 4 salary samples were collected from village around of Iquique. The soils in study is Canchones (6 samples), Tirana (4 samples) and Pintados Salary (4 samples). The quantification of perchlorate was performed by IC-ESI-MS, the methods detection limits (MDL) of perchlorate in soils were 0.03 ppb. The soils were characterized for pH, electric conductivity and bases of interchange. The result of the soils analyses, indicate the existence of perchlorate in 9 of the 14 soils in study: Canchones (496 ug/Kg, 430 ug/Kg, 140 ug/Kg and 280 ug/Kg), Tirana (257 ug/Kg, 600 ug/kg and 530 ug/Kg) and Pintados Salary (346 ug/kg and 1719 ug/Kg). The rest of soils is under the limit of detection of the method. The presence of perchlorate around of agricultural zones and habitet of the north of Chile in close concentrations to the established for international organism, generate a situation of precaution because considering the mobility and persistence of perchlorate the transference toward water sources used for the human consumption and for the irrigation of traditional cultivations and of accumulative vegetables like lettuces and spinach, can represent a real risk for the health of the consuming potentials.

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Keywords: Perchlorate, inorganic contaminants; Atacama Desert.
S5-P5
Characterization of Phytate-Utilizing-Bacteria during Aerobic Degradation of Dairy Dung

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Phytate-utilizing-bacteria (PUB) have potential biotechnological applications in agricultural fields, such as animal nutrition and soil fertility. Amendments of organic waste such as manure, compost or sludge contribute to increase phytate concentration in soil. Due to phytate is strongly linked to colloids of soil; it is stabilized in unavailable forms for plants nutrition. Improvement of P utilization could be achieved by using PUB to increase the availability of P in dung in order to produce an organic fertilizer rich in available P. In this study, culture-dependent methods, genetic and enzymatic analyses were used to characterize PUB populations during aerobic degradation of dairy dung. The culture-medium indicated the occurrence of PUB throughout degradation process. The phylogenetic analyses based on 16S rDNA gene sequences showed high diversity of PUB, which resulted similar to bacteria belonging to genus Bacillus, Escherichia, Enterobacter, Ochrobactrum, Rahnella, Shigella and Streptomyces. Two morphotypes with high phytate-utilizing capacity were genetically characterized as member of genus Enterobacter and Rahnella. Moreover, these morphotypes showed capacity to produce alkaline and acid phosphatases as revealed the enzymatic characterization by using Api Zym system. PUB could be useful tools to produce a fertilizer rich in available P to plants.

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Keywords: Phytate-utilizing-bacteria; aerobic degradation; dairy dung

S5-P6
Rhizospheric Bacteria Community Structure Perturbation of Acacia caven in the Presence of the Herbicide 2,4-Dichlorophenoxyacetic Acid and the Bioprotective Effect of C. necator JMP134 on the Plant

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Cupriavidus necator JMP134, is a b-proteobacterium well studied for its great catabolic versatility and whose genome sequence is now available. This bacterium can degrade and use more than 50 aromatic compounds as carbon source. Among them, xenobiotic compounds like the 2,4-dichlorophenoxyacetic acid (2,4-D), used for many years as an herbicide. Acacia caven is a leguminous shrub belonging to the Mimosaceae family. This plant has a wide distribution on Chile, associated with dry places, with a great resistance to the drought. A. caven is sensitive to 2,4-D, when used at standard field concentration. The aim of this study
was to test the effect of the 2,4-D on Acacia caven and in its community structure of rhizospheric and non-rhizospheric bacteria, in the presence of C. necator JMP134. Using plant-soil microcosms with or without exposure to 2,4-D and inoculated or not with a green fluorescent protein tagged C. necator, we detected that this bacterium has a preferential distribution towards the rhizosphere of A. caven. The presence of 2,4-D clearly diminished the ratio of C. necator cells found in the rhizospheric soil compared with non-plant soil. The presence of the wild type strain protected the plant from toxicity of 2,4-D, because plants died in soil microcosms inoculated with a tfdA mutant, unable to degrade 2,4-D. The analysis of the bacteria community structure by the T-RFLP technique in the rhizospheric and non-rhizospheric soil of A. caven in the absence of 2,4-D, revealed that the community structure of these two kinds of soil were different. The presence of the herbicide in these plant-soil microcosms produces a remarkably decrease in the differences between the bacterial community structure of the rhizospheric soil and the non-rhizospheric soil. The non-rhizospheric bacterial community structure remained unchanged, but the rhizospheric bacterial community structure turned to be closer to that of the bacterial community that is not influenced by the plant. This effect of 2,4-D was observed even if the plant had been protected of the lethal effect of this compound by C. necator JMP 134. This, can be explained by a toxic effect of the 2,4-D on the rhizospheric bacteria or because a change of the composition of the exudates of the plant in a stress situation.

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Keywords: Rhizospheric bacteria community; Acacia caven; 2,4-dichlorophenoxyacetic acid.

S5-P7

Effect of Metal Speciation on the Functional and Morphological Responses of Soil Fungi: Case of Cu and Zn in Trametes versicolor

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Filamentous fungi are part of major biomass in soil and are key actors of soil functioning due to their ability to produce extracellular enzymes. It is known that metals can alter extracellular enzymatic activities as well as morphology in fungi. Thus fungal responses to metals were suggested to be used as biomarkers of metal exposure in soils. Such biomarkers are needed in the environmental risk assessment, to develop methods for measuring the impact of metal contamination on soil microorganisms. However it is not well known how the metal speciation, linked to the metal bioavailability, influences the soil fungi responses to the presence of metals thus limiting the predictive interest in such biomarkers. The present study aims at investigating both the morphological responses and the sensitivity and selectivity of extracellular oxidases produced by the filamentous fungus, Trametes versicolor, exposed to Cu or Zn, taking into account the speciation of these two metals. For that purpose, the activities of three peroxidases were used as biomarkers of exposure and of bioavailability: the laccase, manganese- and lignin-peroxidases. Activities were monitored from pure cultures performed in various liquid media exhibiting several levels of metal complexion in order to
vary metal bioavailability. Total metal contents from 0.1 µM to 1 mM and three liquid media, either rich or poor in organic ligands, were tested. Morphological responses were characterized in scanning electron microscopy. Theoretical speciation of Cu and Zn was calculated using the SOILCHEM thermodynamic program at the initial stage, and an Ion Selective Electrode was used to determine the free copper contents in the various media. Our results showed that Zn had no effect on the three oxidases tested. Cu however highly stimulated laccase and manganese-peroxidase activities. Lignin peroxidase was not measured in the control cultures without metals, but was found specifically produced after fungal exposure to Cu. Culture media exhibiting low complexing properties led to an increase in intensity and sensitivity of oxidase responses, suggesting a higher metal bioavailability. Moreover experimental speciation of Cu showed that Cu\(^{2+}\) contents were higher in the low complexing media. Theoretical speciation showed that only a few percentage of total Cu and Zn were in the free forms, the other forms were complexes of Zn and Cu with the organic or inorganic ligands. In our conditions, we were able to measure enzymatic responses to Cu at environmental levels of contamination (1 µM). Only Zn was found to impact the morphology of fungi during exposure. This result contrasted with the fact that no significant stimulation of oxidase activity could be detected in our experimental conditions. Our results show that the response of fungi is specific of a metal and depends on metal speciation: oxidases are stimulated in the presence of copper but no impact could be found on fungi morphology, while Zn had an impact on fungi morphology but had no effect on oxidase activities. This specificity and sensitivity confirm that fungal responses can be useful tools for metal ecotoxicity assessment that need to be tested in contaminated soils.

**Keywords:** Metal speciation, *Trametes versicolor*; Copper; Zinc.

**S5-P8**

**The Metabolic Response of Arabidopsis to a Combination of High Light and Sulfate Deficiency**

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One of the most crucial functions of plant cells is the ability to respond to environmental changes. Understanding the connections between a plant initial response and the downstream events is one of the open questions of plant biology. In the past effects of sulfate deficiency in plants has been intensively investigated to study the systems response to the applied stress. However, the majority of the performed experiments testing the response of plants to limiting sulfate levels were focused on single stress treatments applied to plants under controlled conditions. Responses of plants to sulfur nutrition and varied light intensities during growth have not been characterized so far. Chlorophyll fluorescence imaging and metabolite profiling were used to study the metabolic changes as read out for the plant response in leaf tissues of *A. thaliana* sulfate starved plants under different light intensities. Our study revealed a new pattern of response showing an initial event of inhibition in the photosynthetic...
performance associated with alterations at metabolic levels of different pathways. Furthermore, sulfate deprivation causes the disruption of metabolic pathways involved in the acclimatization process of plants to high light, such as the antioxidant defence system.

**Keywords:** Arabidopsis thaliana; sulfate deficiency; high light

**S5-P9**

*Rhizopus oryzae* Immobilization for Biodiesel Production Using a Soil Extract Media

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The use of chemical catalysts (acids and alkalines) in the transesterification reaction for biodiesel production have some negative aspects. Such as: difficult of glycerol recover, the need for wastewater treatment process and free fatty acids in the raw material can interfere with the reaction, generating saponified products. These problems can be minimized by using a catalytic enzyme (biocatalysts). The uses of extracellular enzymes are expensive (given its high purity level) and therefore are being used intracellular enzymes (whole cell) whose cost is closely related to the culture medium with which the organism grows. Soil is a known source of LPM (Lipase Producing Microorganism), such as *Rhizopus oryzae*. The main objective of this work was to immobilize *Rhizopus oryzae* using a growing low commercial value medium for produce biocatalyst particles for application in biodiesel production. *R. oryzae* was kept on potato dextrose agar plates (4%) and it was grown in a modified medium used for isolation of LPM from the soil and it consists of extract soil (10%), urea (0,02%) and rapeseed oil (3%) in 1 L of distilled water. The extract soil was prepared by stirring 5 min 100 g of sieved soil, 0.2 g CaCO₃ in 1 L of distilled water, filtered and sterilized. An airlift reactor with 550 mL of culture medium was inoculated with *R. oryzae* spores and zeolite was used as support material, the fungus was immobilized as result of natural growth. This process was carried out at 30°C and 1 lpm of airflow. After cultivation, the microorganism immobilized in zeolite was separated, washed with water for 1 minute and dried at room temperature for 48 h. To stabilize the lipase activity, the microorganism immobilized in zeolite was treated with a glutaraldehyde solution (0.1 - 1.0% v / v) at 25°C for 1 hour, washed with water and dried at room temperature for 48 h. For the transesterification reaction were used bottles of 30 ml with rapeseed oil and ethanol at 1:3 molar ratio and biocatalyst particles. The reaction was carried out in a shaker at 30°C and 150 rpm. The contents of alkyl esters were analyzed by gas chromatography. Conversion of rapeseed oil and alcohol into biodiesel evidenced the microorganism immobilization using soil extract medium and it increased with the glutaraldehyde concentration treatment. While this conversion was low it could be optimized and to compete with other culture mediums of higher commercial value that are normally used for growth of *R. oryzae*.

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**Keywords:** *Rhizopus oryzae*; microorganism immobilization; biodiesel.
Vegetable Meals as Substrate for Cellulase Production by *Aspergillus niger*

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Cellulose is insoluble and crystalline; hence, it is largely resistant to enzymatic hydrolysis. In many biomass utilization schemes, the raw material is first treated with dilute acid at moderate temperatures to remove hemicellulose and to speed-up subsequent cellulose hydrolysis by enzymes. Worldwide, screening of whole cellulase preparations is done predominantly using the International Union of pure and Applied Chemists (IUPAC) standard filter paper assay (FPA). In this assay the dinitrosalicylic acid (DNS) method is used to determine reducing sugars (RS) released from filter paper hydrolysis. Because cellulase activity in whole broths is nonlinear in regarding enzyme concentration, dilution is required to a point where 2 mg of RS equivalents are released in 1 h at 50ºC and pH 4.8. This amount of enzyme is defined as one filter paper unit (FPU). In this work cellulase production by two *Aspergillus niger* strains ATCC 20447 and ATCC 11414 was evaluated. Fermentations were carried out using rapeseed meal and a mixture of bran and wheat germ. For the submerged fermentations (SmF) the substrates (1% w/v) were inoculated with spores to a final concentration of $10^4$ cell mL$^{-1}$. These fermentations were carried out at 25ºC and enzyme production was followed during 7 days. In the solid state fermentation (SSF) the substrates were moisturized to 65% and inoculated with spores to a final concentration of $10^4$ cell g$^{-1}$ dry matter. Incubation was carried out at 25ºC in a water bath. Every 24 h two flask containing the fermented substrate were taken out for enzyme quantification. In the SmF, enzyme production presented a maximum (0.02 U) at 96 h with a significant reduction (0.007 U) after 120 h. Cellulase activity in the SSF of bran and wheat germ by *A. niger* ATCC 11414 presented a sustained increase and after 48 h, 0.56 U g$^{-1}$ dry matter was obtained. These results allowed us to conclude that best fermentation conditions for the production of cellulase by *A. niger* strains is SSF. The substrate also affected the enzyme production and best results were those obtained by using a mixture of bran and wheat germ. Probably the slow growth rate and water limiting conditions in SSF favor the enzyme production. Experiments for the optimization of fermentation conditions (moisture, fermentation time, size of the inoculum, substrate and substrate pre-treatment) are currently under study.

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**Keywords:** Vegetable meals; cellulase; *Aspergillus niger.*
Biomass Production by a Native *Thraustochytrium* Strain

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Biomass production by a native *Thraustochytrium* strain isolated from the coastal area of Budi Lake was study. Budi lake is the only salt lake of América, located at La Araucania Region, Chile. The genus *Thraustochytrium* contains unicellular, zoospore-producing species of historically uncertain taxonomic affinity. These microorganisms have recently attracted the interest because of their capability to produce docosahexaenoic acid (C22:6, DHA), eicosapentaenoic acid (C20:5, EPA), and docosapentaenoic acid (C22:5, DPA). DHA is a major structural component of the gray matter of the brain and the eye retina and an important component of heart tissue. Regular DHA consumption has been shown to be important for proper development of the brain and eye in infants and supports good cardiovascular health. On the other hand, EPA acts as a precursor for eicosanoids, prostaglandin-3 (which inhibits platelet aggregation), thromboxane-3, and leukotriene-5 groups. In this work, the effect the carbon [glucose, rye, malt extract (ME) and malt syrup (MS)], and nitrogen (lupine and yeast extracts) sources on biomass production by our native strain was evaluated. The inoculum was grown for 48 h on media containing glucose 20 g/L, yeast extract 4 g L\(^{-1}\), and monosodium glutamate (MSG) 2 g L\(^{-1}\) in 50% artificial seawater. Flasks containing 100 mL of the following growth media: 1% (w/v) C-source, 0.4% (w/v) N-source and 0.2% (w/v) MSG, initial pH 7, were inoculated and incubated at 20\(^\circ\)C and 250 rpm. The results indicated that the best C-source was glucose with a final biomass concentration of 2.2 g L\(^{-1}\). Growth media based on ME and MS showed higher growth rates although final biomass concentrations were lower, 1.5 and 1.9 g L\(^{-1}\), respectively, suggesting a limitation by an unknown nutrient. Best N-source for biomass production in flask experiments was YE. The fast pH increase observed in the lupine based media was responsible of the low biomass production and thus this media should be evaluated under controlled pH conditions. Lipid content in the biomass of Th4 strain was dependent on growth conditions (10-40%). Saturated fatty acids in the lipids produced when biomass was grown in the Glu-YE-MSG media were 35.6%; monounsaturated and polyunsaturated fatty acids were 7.8 and 56.6%, respectively, and DHA represented 43.8% of the polyunsaturated fatty acids. Addition of vitamins (thiamin, biotin, cianocobalamin and Ca-pantothenate) and trace metals significantly increased biomass production under controlled pH conditions and highest biomass productivity was 0.033 g L\(^{-1}\)h\(^{-1}\). Under this growth conditions specific growth rate was 0.04 h\(^{-1}\).

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**Keywords:** *Thraustochytrium* strain; fatty acids; biomass production.
S5-P12
Consumption of *Ugni molinae* Turcz. Extracts and Its Effect on the Human Gut Microflora

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*Murta* (*Ugni molinae* Turcz) is a wild shrub growing in the South of Chile. Studies on the chemical composition of the leaves indicate the presence of phenolic acids, flavonoids and tannins. Dietary flavonoids have recently attracted the interest because *in vitro* and *in vivo* studies suggest they have a variety of beneficial biological effects, which may play an important role in the maintenance of human health. The flavonoids are potent antioxidants, free radical scavengers and metal chelators; they inhibit lipid peroxidation and exhibit various physiological activities including anti-inflammatory, antiallergic, anticarcinogenic, antihypertensive, antiarthritic and antimicrobial activities. Many plant phenols are known to possess antimicrobial properties, so their consumption might change the composition of the human gut microbial flora. The aim of this study was to investigate the antimicrobial properties of murta leaves against human gut microorganisms isolated from faecal human samples after the regular consumption of infusions. The fecal samples were collected from 6 healthy human subjects of different ages (0 to 50 years) and sex (three women and three men). Subjects maintained their usual lifestyles and dietary intakes throughout the two-weeks period of study. The volunteers consumed 200 mL of a murta leaf infusion (5 g/L), twice a day. Samples were collected in sterile specimen jars and immediately taken to the laboratory. A control sample was requested at the beginning of the study. Samples were also requested at 5\(^{th}\) and 10\(^{th}\) day, during the infusion consumption. A last sample was requested at the 15\(^{th}\) day, time at which the infusion consumption was finished. The samples were homogenized in buffer sodium phosphate (0.1 M pH 6.5) to a final concentration of 10% w/v. A dilution series (10\(^{-1}\) to 10\(^{-4}\)) was made in the same buffer, and 100 L aliquot of each dilution was used to inoculate selective media by spread plating. Bifidobacteria, Bacteroides and Gram positive coccus were enumerated on blood agar; total anaerobic and aerobic bacteria on nutritive agar; clostridia on clostridial agar; coliforms on Wilkins Chalgren agar; *Lactobacillus* on MRS agar and *Staphilococcus* on Bair Parker agar. Bacterial stocks were isolated for the analysis of *in vitro* effect through the diffusion in agar method. The tested extracts were those obtained in water (100%) and water-ethanol mixture in volumetric ratio of 1: 1. The results showed that regular consumption of aqueous murta infusions do not affect the distribution of bacterial microflora population, at least in the concentration and frequency of consumption evaluated. The *in vitro* assays confirmed that aqueous murta extract do not have antimicrobial effects, even at higher concentrations. However, water-ethanol extracts displayed antimicrobial effect on 12 of the 70 isolate bacterial strains. It was also demonstrated that the *in vitro* antimicrobial effect was limited to aerobic strains.

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**Keywords:** *Ugni molinae*; flavonoids; human gut microflora.
Protease and Prolil-Endopeptidase Production by *Aspergillus niger* Under Different Culture Condition

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The celiac disease (CD) is a permanent gluten intolerance. Gluten present in wheat, barley, rye and oat, is partially degraded in the gastrointestinal tract. The resulting proline rich fragments are sub-sequentially modified by tissue transglutaminase (tTG) and following reactions induce epithelial intestinal damage. Several studies have demonstrated that proline peptides are efficiently hydrolyzed by prolil-endopeptidase (PEP) produced by some *A. niger* strains, suggesting a new therapy for CD treatment. Very few references describe PEP production by *A. niger* and no one has evaluated the synthesis in solid state fermentation (SSF). In this work proteases and PEP production by two *A. niger* strains ATCC 20447 and ATCC 11414, was evaluated in submerged fermentation (SmF) and SSF. Culture media used in SmF contained for 1 L: KH₂PO₄ 1 g, K₂HPO₄ 2 g, KCl 0.5 g, MgSO₄·7H₂O 0.5 g, FeSO₄·7H₂O 0.01 g and variable levels of glucose, yeast extract and gelatin. Data for the optimization of culture media composition was obtained from flask experiments. The effects of pH, aeration and temperature were evaluated in batch fermentations. In the SSF a mixture of bran and wheat germ moisturized to 65% was used. Incubations were carried out at different temperatures. Protease activity was quantified by incubating the protein solution with azocasein solution (2.5 g/L) at 37°C for 20 min; the reaction was stopped through the addition of TCA 1 M. One unit of protease activity was defined as the amount of enzyme that increased the absorbance at 400 nm by 0.1 per min. PEP activity was assayed using a synthetic peptide Z-Gly-Pro-pNA (Sigma, 100 µM in DMSO 20%). The reaction was carried at 45°C for 1 h and the released pNA was measured spectrophotometrically at 410 nm. One unit of PEP activity was defined as the amount of enzyme that release 1mM of pNA per min. Highest protease activity in SmF was 884.6 U obtained after 14 days cultivated by using glucose 5 g/L, yeast extract 20 g/L, gelatine 10 g/L at pH 4.5. Growth temperature significantly (p<0.05) affected protease production suggesting that slow growth positively influence protease production. Maximum specific PEP activity (0.004 U/g) was obtained after 100 h at 25°C. In SSF by *A. niger* ATCC 11414 maximum protease activity was 130.4 U/g wet matter after 4 days incubation at 25°C; specific PEP activity was 0.038 U/g. These results confirm the hypothesis that SSF can be used for the production of PEP. Experiments for the optimization of conditions for the PEP production in SSF are currently in progress. The possibility of PEP therapy against CD is currently a very important biotechnological application, because the unique treatment against CD is a gluten free diet.

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**Keywords:** Protease; prolil-endopeptidase; *Aspergillus niger*. 

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*ISMOM 2008 – POSTER ABSTRACTS*  
Session 5. Environmental Biotechnology: Biochemical and Molecular Mechanisms of Microbe-Plant-Root Interactions & Their Genomic & Proteomic Advances Pertaining to Restoration of Contaminated Soils
Microbial Community Structure in Pentachlorophenol Contaminated Soil under Fungal Bioremediation Treatment Amended with Wheat Straw

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We analyzed, by denaturing gradient gel electrophoreses (DGGE), the structure of bacterial and fungal community in a pentachlorophenol (PCP) contaminated soil under fungal bioremediation treatment amended with wheat straw. The effect of the bioremediation treatment on autochthonous microorganisms was evaluated in simple microcosms. Briefly, 75 g of soil (35% of humidity) were put into glass recipient and different treatment were established: T1 (soil), T2 (soil contaminated with PCP), T3 (soil mixed with wheat straw), T4 (soil contaminated with PCP and mixed with wheat straw), T5 (soil mixed with *Anthracophyllum discolor* grown in wheat), and T6 (soil contaminated with PCP mixed with *A. discolor* grown in wheat). The soil was artificially contaminated by spiking a stock solution of 10 g L\(^{-1}\) of pentachlorophenol (PCP) diluted in KOH 0.1 mol L\(^{-1}\) reaching a concentration of 250 mg of PCP per kg of soil. The samples were incubated at a controlled temperature (25±1ºC) for 28 days. Water losses by evaporation were compensated weekly to maintain soil water content. Treatments were set up in triplicates. At the end of experiment, the analysis of 16S rDNA gene revealed that the addition of PCP stimulated the presence of γ-Proteobacteria (Xanthomonadaceae) and β-Proteobacteria (Burkholderiaceae). The addition of PCP plus straw also stimulated the presence of Enterobacteriaeae. In the case of fungus, the analysis of 18S rDNA gene revealed that PCP stimulated the presence of Saccharomycetes, Tremellomycetes and Agaricomycetes. The addition of straw showed an increment of other basal fungal lineales, such as Ascomycetes. However, the addition of PCP plus straw revealed a different pattern of stimulation. Under the presence of *A. discolor*, the addition of PCP plus straw showed predominance of member of Agaricomycetes and Ascomycetes. This work emphasizes the importance of autochthonous microorganisms involved in PCP degradation.

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Keywords: Microbial community; pentachlorophenol; fungal bioremediation.
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Acquiring Tolerance to Solvents during a Vermicomposting Process

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In this work, a cultivable, Gram-positive, solvent-resistant bacterium was isolated from vermicomposted olive wastes. The highest 16S rDNA sequence similarity (99%) was found with *Brevibacillus brevis*. The genome of the isolate, selected for TCE-tolerance, contained a nucleotide sequence encoding a conserved protein domain (ACR_tran) ascribable to the HAE1-RND family. Members of this family are hydrophobic/amphiphilic efflux pumps largely restricted to Gram-negative bacteria. Since no cultivable solvent-tolerant bacterium was detected in the unvermicomposted olive waste, a transfer of solvent-resistance genes from Gram-negative bacteria during the vermicomposting process could explain the presence of HAE1 transporters in *B. brevis* isolated from the vermicompost. The RND gene expression induced by different levels of pollution was determined with real time PCR in the bacterium cDNA. Under TCE stress conditions, the acquired nucleotide sequence was translated into proteins, and tolerance to solvents was conferred to the bacterium.

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Keywords: Tolerance; vermicompost; *Brevibacillus brevis*. 