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FE-01

Optimization of Fibrolytic Enzymes Production by *Aspergillus niger GS1* Using Solid-State Fermentation and Corn Stover as Substrate

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Corn stover is a by-product resulting from the agroindustrial activity, it can be used for ruminants feed due to its large amount of fiber content. However, the fiber is not used to its maximum potential, because the microorganisms present in the rumen are inhibited by lignin. In addition because of the hemicellulose content of fiber, a poor digestibility has been observed. The aim of this work was to produce fibrolytic enzymes by *A. niger GS1* using solid-state fermentation (SSF) and corn stover as substrate. Corn stover was milled to different particle sizes and pretreated with 4% NaOH to partially remove lignin. The particle size, moisture and fungal incubation time were evaluated during SSF in a column reactor fill with supplemented corn stover, to induce fibrolytic enzymes. Xylanase, mannanase and cellulase activities were determined by measuring the release of reducing sugars during the enzymes-substrate reaction. One unit of enzyme activity (U) was defined as the amount of enzyme that releases 1 μmol of reducing sugar equivalent to the substrate per minute, at 50° C. A fractional factorial design was applied to determine the effect of nine supplementing nutrients. A central composite design was then used to optimize the production of fibrolytic enzymes, using the significant nutrients resulting from the previous design. The growth of *A. niger* was determined indirectly by released CO_2, using gas chromatography. The maximum production of mannanase (16 U L^{-1}), xylanase (74.2 U L^{-1}) and cellulase (92 U L^{-1}) was obtained with the smaller particle size of corn stover, pretreated with NaOH at 80% (w/v) moisture content, after 48 h of SSF. Glucose, yeast extract, ammonium sulfate and copper (II) sulfate significantly contributed to an optimum production of 102 U L^{-1}, 386 U L^{-1} and 505 U L^{-1}, of mannanase, xylanase and cellulase, respectively, using specific supplementation for each one. The maximum CO_2 production was observed after 12 h of SSF (0.67%, 1.07% and 1.14%, respectively), which may indicate a diauxic behavior, because after this time the fungus probably started to release fibrolytic enzymes to obtain energy from the corn stover. These hemicellulases are being used for *in vitro* digestibility of corn stover.

(key words: corn stover, fibrolytic enzymes, solid-state fermentation)

FE-02

Isolation and Properties of AMP Deaminase from Jumbo Squid (*Dosidicus gigas*) Mantle from the Gulf of California, Mexico

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AMP deaminase is an important enzyme involve in ATP degradation pathway, long time ago was though that AMP deaminase was absent in cephalopod, however nearly it has been detected in cephalopod. Therefore in this study AMP deaminase was purified from jumbo squid mantle. SDS-PAGE showed a single band with 87 kDa molecular mass, native PAGE proved a band of 178 kDa, whereas gel filtration detected a 180 kDa protein, suggesting the homodimeric nature of this enzyme, in which subunits are not linked by covalent forces. Isoelectric focusing of this enzyme showed a pI of 5.76, which agrees with pI values of AMP deaminase from other invertebrate organisms. AMP deaminase presented a kinetic sigmoidal plot with Vmax of 1.16 µM/min/mg, Km of 13 mM, Kcat of 3.48 µM .s-1 and a Kcat/Km of 267 (mol/L)-1.s-1. The apparent relative low catalytic activity of jumbo squid muscle AMP deaminase in the absence of positive effectors is similar to that reported for homologous enzymes in other invertebrate organisms.

(keywords: AMP deaminase, characterization, jumbo squid mantle, purification)

FE-03

Saccharification: Sweet Liquor Obtained From Sugar Cane Bagasse

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There are different approaches to achieve the saccharification of lignocellulosic biomass; with physical and chemical methods a great amount of energy is consumed and inhibitory compounds are generated. The biological treatment by enzymes is a better alternative because of hydrolysis takes place at mild conditions. It has been reported as the best inducer of lignocellulolityc enzymes in *Cellulomonas flavigena.* Mutant PR-22 is a hyperproducer and deregulated cellulolytic bacteria. Its lignocellulolytic system has shown resistance to catabolic repression and inhibition phenomena. The aim of this work was the production of a liquor rich in reducing sugars (RS) from sugar cane bagasse using the enzymatic system of *C. flavigena* PR-22. These RS can be used as substrate to produce fuel stocks. Reducing sugars were determined with dinitrosalicylic (DNS) method. The sugar composition of syrup was done by TLC in silica gel plates in order to identify the hydrolysis products. Different ratios of sugar cane bagasse and enzyme were assayed. When 2 % sugar cane bagasse and 350 U of xylanase activity were incubated, a total of 17 $g \cdot l^{-1}$ of RS were accumulated. The system achieved 85 % of saccharification of substrate at 12 h of incubation.

(key words: Saccharification, sugar cane bagasse, cellulase, mutant, Cellulomonas)

FE-05

Production of Cellulases and Xylanases from *Trichoderma reesei*

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The filamentous fungus *Trichoderma reesei* produces and secretes profuse quantities of glucanases that act synergistically to degrade vegetal biomass. Recently, a great deal of attention has focused on cellulases and hemicellulases produced by these microorganisms because of their potential to be produced industrially and used in degradation of biomass for a number of applications, most notably biofuel production. *T. reesei* MCG80 is a mutant strain that overproduces cellulases and xylanases. The aim of this work was to compare the production of cellulases and xylanases using *T. reseei* MCG80 growing on sugar cane bagasse (agricultural waste), lactose (byproduct of milk process) or Solka flock (pure cellulose) as carbon sources. Batch cultures were carried out in a bioreactor Sixfors, with 500 ml of medium operated at 100 rpm, 30 °C and air flow of 1 vvm. Growth and enzyme activities were followed during 5 days. The maximum enzyme activities obtained were: 48, 12 and 449 IU/mg of carboxymethylcellulase (CMCase), filter paper activity (FPasa) and xylanase respectively when cells grew in 1 % Solka flock; in 1% lactose these activities were 35, 8.5 and 30 IU/mg of CMCase, FPase, and xylanase respectively. Sugar cane bagasse has been reported as a good inducer of cellulolytic complex in some bacteria and fungi, however for *T. reesei,* the cellulase and xylanase activities were lower than in the other substrates assayed. These results point out that cheap sources of lactose like milk serum could be used as good inducers of xylanase and cellulase activities instead of agricultural wastes.

(keywords: *Trichoderma reesei,* cellulases, xylanases, *FPasa*)

FE-06

Thermodynamic functions relative to the transfer of mycolytic enzymes of *Trichoderma spp.* from water to liposomes

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This work was undertaken to study the mycolytic enzymes encapsulation in liposomes. Antifungal activity of these enzymes can be effectively utilized in biologic pest control strategies. Cellulolytic complex, chitinase, laminarinase are enzymes that serve critical roles in fungal growth and development, in resistance of plants to fungal pathogens, and in parasitism of insects by entomopathogenic fungi. However, the enzyme applications *in situ* need to increase the enzyme stability. The thermodynamics of molal partitioning of hydrolytic enzymes of *Trichoderma spp.* (cellulolytic complex, chitinase, laminarinase) was studied in soya lecithin liposome system. In all cases the partition coefficients ($K^{m}_{o/w}$) subscript the m were greater than unity; therefore the standard free energies of transfer were negative indicating affinity of enzymes for microencapsulation in liposomes. Soya lecithin liposomes were stable during 70 days. The microencapsulation also led to increase of enzyme stability. It is important for enzymes application on bio-control of phytopathogenic fungi to decrease using of chemical fungicides and obtain the ecologically clean food.

(keywords: laminarinase; chitinase; cellulolytic enzymes; partition coefficient; soya lecithin liposome)

FE-07

Enzymatic Recovery of Maracuya Juice (Pasiflora edulis var, flavicarpa)

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Maracuya fruit (Passiflora edulis var Flavicarpa) is from tropical origin, specifically from the amazonic region of Brazil. Now, this fruit is cultivated in commercial form in Colombia, Venezuela, Ecuador, Australia, Indonesia and Mexico. For recovery the juice of this fruit, several mechanical equipment have been evaluated, the extractor of brushes that presents the biggest extraction efficiency (94.0%) has the disadvantage that during the process some seeds are broken and liberate oil, which generates the appearance of unpleasant flavors in the obtained juice. The use of enzymes in the recovery process is an alternative to avoid this problem. The aim of this work was to evaluate the enzymatic recovery of the maracuya juice. To carry out this work, we use the enzymatic preparations Pectinex USP-L and Pectinex AR provided by Novo Nordisk. Determination of the conditions for use of these enzymes was carried out with a factorial design 2^2, the experimental part was carried out using 1.0 kg of maracuya pulp; temperatures of 30 and 40°C; concentrations of enzyme of 3200 and 6400 U. Yields of juice obtained using anyone of the two enzymatic preparations fluctuated between the 60 and 75%. Statistical analysis of the data showed that there was not significant difference.

(keywords: maracuya, enzymatic recovery, passiflora edulis var. flavicarpa)

FE-08

Stability and Catalytic Properties of a New Tannase Produced by *Aspergillus niger* GH1

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Tannase or tannin acyl hydrolase (EC, 3.1.1.20), is an inducible enzyme produced by some special microorganisms. It catalyses the breakdown of hydrolysable tannins and gallic acid esters. Tannase transforms tannic acid into glucose and gallic acid. This enzyme is used in the industrial processing of fruit juices, beer and coffee-flavored soft drinks as a clarifying agent. The major commercial applications of tannase are in the manufacture of instant tea and it also plays an important role in the pharmaceutical industry. In this work, an extracellular tannase was produced by *Aspergillus niger* GH1 under solid state culture conditions. The fungal strain was previously isolated from Mexican semiarid region. For solid state culture, polyurethane foam (PUF) was used as support and tannic acid was employed was sole carbon source and inducer of tannase in an initial tannic acid concentration of 25 g/L. Tannase was concentrated using precipitation with ammonium sulfate in a percentage of saturation of 60% and precipitation with acetone in a relation 1:1 followed by dialysis with cellulose membranes reaching a specific activity of 23.5 U/mg. The assays were carried out based on the tannase activity detected by the HPLC method. Then, enzyme was purified though gel permeation chromatography. A physico-chemical characterization of purified enzyme was performed, evaluating the effect of pH and temperature obtaining results for optimal activities at 6 and 60 °C, respectively. The reaction time was defined as 20 minutes and the enzyme showed a K_M values of $1x10^{-3}$ M and Vmax 11.03 □ mol min^{-1} using methyl gallate as substrate. In conclusion, the new *A. niger* GH1 tannase is an attractive enzyme for industrial applications due its thermostability and catalytic capacity.

(keywords: tannase, *Aspergillus niger* GH1, catalytic properties)

FE-10

Studies on the Regulation of *Aspergillus niger* GH1 Tannase Activity

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Tannin acyl hydrolase is an enzyme (EC. 3.1.1.20) that catalyses the hydrolysis of ester bonds presents in gallotannins, complex tannins and gallic acid esters. Tannase has been used in foods, beverages, pharmaceuticals and chemical industries. The use of tannase in large scale, however, is much limited due to a variety of factors including production cost and insufficient know ledge of the enzyme. The aim of this work was to study the effect of several additives on the activity of the tannase produced by *Aspergillus niger* GH1 in a solid state fermentation system using polyurethane foam as inert support. Fermentation conditions were 35 °C, 70 % of initial humidity, 25 g/L of tannic acid as sole carbon source and 40 h of incubation time. Enzymatic extract was obtained adding 2-4 volumes of acetate buffer and compressing. Tannase was partially purified by polyethyleneglycol concentration, gel filtration and ion exchange chromatography using a DEAE-FF column. It was studied the effect of the addition of several organic solvents, surfactants, chelating agents, metal ions, and other potential inhibitors. Ethanol, acetone, tetrahydrofuran and formaldehyde completely denaturalizated the enzyme, petroleum ether apparently improved activity. Surfactants (Tween 80, Tween 20, Triton X-100 and SDS) had no inhibitory effect at 0.1% (v/v). Strong inhibition by $FeCl_3$ and $CuSO_4$ was observed at 20 mM concentration after 60 min of pre-incubation; $ZnCl_2$, $MgSO_4$, $CaCl_2$ and $MnCl_2$ were less significant and an increment of TAH activity (16%) was observed during the first 5 min of pre-incubation. Strong inhibition (48%) by Phenyl Methyl Sulfonyl Fluoride was observed, indicating that TAH is a serin-hydrolase, while hydroquinone, mercaptoethanol and *o*-fenantroline had no modulator effect on tannase activity. Inhibition studies show that *Aspergillu niger* GH1 tannase is at least as stable as the other characterized tannases.

(keywords: tannase, *Aspergillus niger* GH1, inhibition)

FE-12

Potential of Mycolytic Enzymes Encapsulated on Soya Lecithin Liposomes as Biocontrol Agents against *Fusarium oxysporum*

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Antifungal activity of different hydrolases can be effectively utilized in biologic pest control strategies. In the present study antifungal effect of chitinase and laminarinase, encapsulated on lecithin liposomes, on *Fusarium oxysporum* was demonstrated. The microencapsulation was carried out to increase of enzyme stability. A synergic effect of both enzymes and a chemical fungicide was observed. The inhibition of mycelium growth, decreasing of spores number and their viability were detected on agar, while decreasing of CFU germination was demonstrated in soil. Enzymes application on biocontrol of phytopathogenic fungi led to decrease using of chemical fungicides. The gotten results support the theory that the hydrolytic enzymes reduce the growth of phytopathogenic fungi and demonstrate that their microencapsulation on liposomes don´t affect significantly their antifungal properties. The mechanism involves the degradation of fungal cell walls and probably hydrophobic affinity between liposomes and lipids of fungus cellular wall.

(keywords: laminarinase; chitinase; soya lecithin liposome; antifungal effect)

FE-13

Effect of Microwave Energy on Peroxidase, Polyphenoloxidase and Pectinesterase in Avocado (*Persea americana*) and Mamey Sapote (*Pouteria sapota*)

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Blanching focuses on heat resilient enzymes, like peroxidase, polyphenoloxidase and pectinesterase. Avocado and mamey sapote were placed in the microwave oven in intervals of 10s and enzymes extracted. Spectrometry evaluated POD and PPO. PE was quantified by titration. The avocado presented a decrease in POD and PPO after 20 s of treatment. The mamey had a decrease in POD and PPO at 30 s of blanching; but PPO was present. The pectinesterase in both fruits had no significant difference.

(keywords: avocado, mamey, blanching, polyphenoloxidase)

FE-14

Hydrolysis of Methylgallate for Gallic Acid Production using Catalytic Fermented Solids

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In this study, we report the production of tannase auto-immobilized during the growth of *Aspergillus niger* GH1 in solid-state culture, and the hydrolysis of methylgallate for gallic acid obtention through the use of the dried fermented solids containing tannase. The fermented solid material with tannase was treated with distilled water and then was dried by applying air current. Maximum tannase activity produced at 24 h of incubation was equivalent to 372 U/L with a specific activity of 27 U/mg. After 24 h of growth of *A. niger* GH1 on polyurethane foam (PUF) with 5% (v/w) of tannic acid as inducer, the fungus produced a tannase activity equivalent to 2.3 and 1.3 units per gram of dry solids (U/gDS) for fermented solids treated and untreated with water, respectively. The methyl gallate was hydrolyzed up to 41% after 30 min. Kinetic parameters of auto-immobilized tannase were calculated. Results demonstrated the possibility of using tannase auto-immobilized during the solid state culture process for antioxidant production, in this case, gallic acid. The results suggest that biocatalysis using fermented solids should be further explored.

(keywords: tannase, gallic acid, dry fermented solids, state-solid fermentation)

FE-15

Effect of Papain Against Phytopathogenic Fungus and Study of Stability of Pectin-Papain System under High Pressure

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In the present study antibiotic effect of papain on *Fusarium oxysporum* was demonstrated. Hosts of *Fusarium oxysporum* include: potato, sugarcane, garden bean, cowpea, etc. *In vitro* tests were carried out using potato dextrose agar (PDA) enriched with 0.025-0.15% of papain preparation, as well as using sterilized and no sterilized soil with the same enzyme concentrations. The inhibition of mycelium growth, decreasing of spores number and their viability were detected on agar, while decreasing of CFU germination was demonstrated in soil. The increase of enzyme concentration from 0.025 to 0.15% led to increase on antifungal activity. The papain activity was evaluated using Kunitz´s technique. The enzyme activity was detected in solution for 2 weeks and in soil for 4 weeks. To increase enzyme stability in solution and simplify the enzyme application on surfaces of some food products, a papain-pectin gel was prepared. The stability of the papain immobilized on pectin gel at 65°C under high pressure of air and nitrogen was studied. The concentrations of pectin and enzyme were selected by means of enzyme activity detection on systems with pectin at 1-10% and papain at 0.1-0.9% and by monitoring of film formation at short time. It was observed that increasing of pectin concentration led to significant decreasing of gel drying time. The papain immobilization on pectin gel increased enzyme stability at 65°C under atmospheric pressure and high pressure (up to 90 psi). Pressure increasing to 50 psi led to an increase of enzyme activity. The papain-pectin gel applied in spray may be one of the forms for enzyme application on fungi control.

(keywords: pectin-papain gel, *Fusarium oxysporum*, papain antifungal activity, papain stability under pressure in spray)

FE-16

Generation of a Heterologous System and Effect of Temperature on Expression of *nis*A gen in *Escherichia coli*

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Nisin is the most widely studied lantibiotic, produced by *Lactococcus lactis.* It is a 34 amino acid peptide which contains lanthionines. These unusual amino acids are produced by posttranslational modifications of the precursor peptide. In these reactions a ribosomally synthesized 57 amino acid pre-peptide (NisA) is converted to the final active form. Nisin has been worldwide used as a food preservative because of its antimicrobial activity against spoilage and pathogenic Gram positive bacteria, such as *Clostridium botulinun* and *Listeria monocytogenes,* and so far there are no reports on substantial development of bacterial resistance. The objective of this study was to generate a heterologous system, and study the effect of temperature on the expression of the structural gene *nis*A in *Escherichia coli*. Genomic DNA from *Lactococcus lactis* UQ2 isolated from Mexican style cheese was used as template to amplify the *nis*A gene by PCR. *E.coli* BL21(DE3) cells were grown and induced with 0.1 mM isopropyl-β-D thiogalactopyranoside (IPTG) at 14, 25 and 37 °C, and incubated for 60, 10.5 and 5 h respectively. The pET-28 system generated a fusion protein carrying a hexa-histidine sequence that was purified by Ni^{2+} affinity chromatography (IMAC). Plasmidic DNA was extracted from samples taken at different time intervals for each temperature, and the *nisA* gene was amplified by PCR. At the end of the fermentation, the cells were disrupted by sonication and the nisA-His_6 fusion protein was purified by IMAC. The recombinant protein was identified by Western blot using antibodies against the His_6 tag. Electrophoresis of PCR products showed that induction at 37°C resulted in a faster production of the recombinant peptide. However, induction was similar to that found at 25°C, while at 14°C the rate of recombinant peptide production was much lower.

(keywords: nisin, heterologous expression, lantibiotics)

FE-17

Polyphenol Oxidase, Catalase, and Peroxidase from Loquat Fruit (*Eriobotrya japonica* Lindl)

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Browning of damage tissues of fruits and vegetables occurs from the oxidation of phenolic compounds and contributes significantly to quality loss. The enzyme responsible for the browning reaction is polyphenol oxidase (PPO), Peroxidases are a ubiquitous class of enzymes present in various tissues and cell components in plants and they are involved in numerous processes, such as lignifications, wound healing, antipathogen defense and stiffening. Loquats (*E. japonica* Linds.) fruits were harvested at the ripe commercial stage (light orange) from trees grown on the Otumba, Edo. Mexico. A 100 g amount of loquat pericarp was homogenized with 100 ml of 5 % NaCl The PPO activity was assayed spectrophotometrically at 25°C using catechol as a substrate by monitoring at 420nm, Catalase the reaction medium contained catechol. One unit of enzyme was defined as the amount of enzyme that caused an increase in absorbance of 0.001/min at 25°C. Peroxidase activity was measured using o-dianisidine as a substrate and Catalase activity with 30 % H_2O_2. Specific activity of PPO in green fruit was 0.4 and 0.6 units, in mature fruit was decreased (0.92 to 0.35 U/mg) in relation to phenolic concentration (0.06-0.29 mg). The activity of Catalase decrease during ripening, 6.6 to 1.4 mM/min in green and mature loquat fruit, respectively.

(keywords: *Eriobotrya japonica,* polyphenol oxidase, catalase, peroxidase)

FE-18

Evaluation of Orange and Apple Wastes as Fountainhead of Carbon to produce Pectinases from Mixobacterya

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The generation of industrial waste rich in cellulose and pectin from food industry grows up to 60 million tons per year, which leads to its accumulation in environment with fitting treatment. There is an increasing interest in the potential of this waste as raw material in biotechnological processes. There are over than 3000 known enzymes isolated from molds, and about the 25% are exploited in industry. This work intended to measure the pectilotlitic activity of myxobacteria over apple and orange skin and its potential use in biotechnology. The bacteria were isolated from different soil rich in decaying organic matter. The enzymatic activity was determined by culturing the bacteria in 1% orange and apple skin at 37°C and 200 rpm; the endo and exo pectinolytic activity was measured from this culture and the enzymes isolated; these were applied on a sample with 1% apple or orange skin and 1% pectin and incubated at 37ºC for ° hour, measuring the specific activity. The endopectinolitic activity was measured by the decrease of viscosity. There were many strains isolated which developed endo an exo pectinolytic, from this the 3 strains which developed the greater activity in apple skin were selected; likewise, the endopectinolytic activity was high in contrast to the substract alone (pectin), because the viscosity decreased more than 50%. This microorganisms and their enzymes have great potential for their application not only in the recycling of waste, but because they can make the extraction of natural colorants, essences, juices, food additives and syrups easier, and as an alternative source of enzymes isolated from other bacterial groups.

(keywords: pectinases, myxobacterias)

FE-19

Guava *(Psidium guajava l)* Fruit with a High Enzimatic Content

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The guava is a highly aromatic fruit and of great nutritional value like other tropical fruits, the guava is an original culture of Tropical America, but the main producers are India, Brazil, Mexico, the South Africa, Jamaica, Kenya, Cuba, Dominican Republic, Puerto Rico, Haiti, the United States (Hawaii and Florida), Taiwan, Egypt and the Philippines. (Gupta, 1995), (Quijano et at, 1999). One evaluated the activity of of enzymes invertase, catalase, polifenoloxidase, peroxidase, pectinmetilesterase PME, poligalactuonase PG of the rind extracts and pulp of green, mature guava in conditions of laboratory, first the extracts in NaCl stored 5% in freezing were obtained. The activity of each enzyme by the coloration method of valuation of glucose oxidasa was evaluated, the measurement of the speed of formation of the product of the reaction of the catechin of polifenoloxidase, the speed of decomposition of the peroxide of hydrogen by peroxidase with orto - dianisidina like hydrogen donor (460 nm) and catalase a (240 nm). Hydrolysis of the substrate saccharose for invertase The analyzed samples presented activity the extract of green guava and guava mature pulp. Where value with NaOH 0,1 N the change of the substrate to péctico acid by action of enzymes in the extracts (PME). In relation to the effect of the activity of the enzyme based on the time observed that activity in the times analyzed from (60 - 180 min). Where a 180 activity is to 120 min to min the extract of green guava and also realised the test to the 72 hours (190 activity of UK). The values of absorbance by factor 0,066 (0,2 ml extract/3ml) present activity the sample green guava where we can verify the presence of this enzyme catalase, glucosaoxidasa, not present activity the samples p1 EGV: extract green guava, p2 EGPM: extract guava mature pulp p3 GPCM: extract guava mature rind to the hour. The evaluate the activity of polifenoloxidasa in the guava extracts and presenting activity to 24 hours the mature pulp samples and green guava. for peroxidase the extract of p2 EGPM: extract guava mature pulp present values average of absorbance to 460 nm/min 0.107

(keywords: guava, catalase, polifenoloxidase, pectinmetilesterase, poligalactuonase)

FE-20

Expression and Partial Purification of an Alkalophilic α-L-rhamnosidase from *Acremonium murorum* in Submerged Cultures

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Rhamnosidase (Rhase), also known as α-L-rhamnopyranosidase (EC 3.2.1.40), catalyzes the hydrolysis of glycosidic bonds in both, natural or synthetic rhamnosides (heteropolysaccharides, flavonoids and aromatic compounds) which results in free rhamnose (6-deoxy-L-manose). *Acremomium murorum*, alkali tolerant filamentous fungi, produces □-L- rhamnosidase and a □-D-glucosidase (Gluse) active in alkaline conditions. The presence of the last enzyme could be inconvenient for some applications, such as the use of Rhase for the manufacture of hydrolyzed products from natural glycosides, which requires an enzyme free from Gluse. It is well established that certain carbon and energy sources (CES) induce the expression of hydrolytic enzymes. *A. murorum* was grown in complex media with five different carbon and energy sources in order to study its capability to produce Rhase and Gluse. CES used in this study were Pectin from citrus (Pec), Raffinose (Raf), Rhamnose (Rha), Soybean meal (SM) and V8 ® juice. Cultures were carried out in Erlenmeyers-flasks (200 rpm, 30°C). Samples were taken each 48 hs and enzymatic activity was determined at 37°C for 30 minutes using PNP-rhamnopyranoside and PNP-glucopyranoside as substrates. Enzyme was partially purified from culture supernatant by chromatographic techniques. All studied media could support fungal growth and enzyme production. Enzyme expression (Rhase and Gluse) exhibited different behavior in each media. Rhase expression shows a similar profile in each media, starting at day 14 with a maximum at day 24, while Gluse presents different profiles in each media. Among studied CES, rhamnose has demonstrated to be the most convenient for the production of Rhase with the lowest Gluse content. An activity of 140 mU/ml (Rhase) and 0.6 mU/ml (Gluse) were obtained in this condition. A culture extract was filtrated, concentrated under reduced pressure at 40ºC and then lyophilized. Concentrated enzyme extract was subjected to a Sephacryl ™ S100 column (pH 6.0) and then injected to a Sepharose ™ Q column (pH 6.0). Dialysis was then used to desalt the fraction containing Rhase activity. From this purification procedure, a fraction with Rhase free of Gluse was obtained. Biochemical characterization of the enzyme is under investigation.

(keywords: *acremonium murorum*, α-L rhamnosidase, β-D-glucosidase, rhamnose)

FE-21

Production of extracellular thermostable protease by *Yarrowia lipolytica* in solid state fermentation

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The dimorphic yeast *Yarrowia lipolytica* is one of the most extensively studied non-conventional GRAS strain and it has been frequently used to produce citric acid, lipases and esterases. However, there are scarce reports about protease production by *Y. lipolytica*. The aim of this work was to produce and characterize protease produced by *Y. lipolytica*. The extracellular protease was produced in solid state fermentation (tubular columns 25x2.5 cm) using polyurethane foam as inert support and fish flour as substrate. The enzyme was produced at 45°C at initial pH of 7. The effect of temperature (from 30 to 80 °C) and pH (from 6 to 10) on the proteolytic activity was evaluated; additionally, temperature stability was determined. Protease activity was assayed using the method described by Kembhavi *et al.* modificated by Jonhvesly and Naik (2001). One unit of protease activity was defined as the amount of enzyme required to release 1 microgram of tyrosine per min under assay conditions. The protease activity was reported per mL of enzymatic extract. The optimum temperature and pH for the proteolytic activity in the enzymatic extract were 50°C (364 U/mL) and 9 (395 U/mL), respectively. Studies on temperature stability (1 h) showed that the enzyme retained 40 % of activity at 60ºC. Hal life at 60°C was 27 min (R^2=0.984). The zymogram of the partially purified extract showed three major bands with molecular weights of 30, 20 and 14 kDa, approximately. There are few reports on the proteolytic activity from *Y. lypolitica* however most of them refer to the mayor intracellular proteolytic activity around 37ºC. The protease produced here was extracellular and exhibited maximal enzyme activity at 50ºC. The protease produced and characterized in this work is an extracellular enzyme with higher optimal temperature that those previously reported,which gives two important advantages over other proteases produced by *Y. lipolytica* in solid state fermentation. These findings can be used to study factors responsible for yeast protease thermostability.

(keywords: *Yarrowia lypolitica,* thermostable proteases, solid state fermentation)

FE-22

Production of Fibrolytic Enzymes by *Pleurotus ostreatus* Growth on Maize Straw

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Cellulose is the most abundant vegetable organic compound, being derived mainly from plant residues. The ability to degrade organic solid wastes by the fungus *Pleurotus ostreatus CP50* has been showed due to fibrolytic enzymes it can produce. These exogenous enzymes are being used to improve the ruminal digestion of fiber and starch in feeds used for feeding ruminants. In addition, may increase productivity and reduce the costs of feeding, reducing the use of grains in the diet and providing greater energy through the use of fibrous substrates. The decomposition of maize straw was studied during solid state fermentation with *Pleurotus ostreatus CP50*. Maize straw was milled to obtain a particle size of 5-7 cm and it was not supplemented with nutrients. The enzymatic activity ($UI\cdot g^{-1}\cdot$dry matter) of xylanases, celullases and laccases was evaluated at 5, 10, 15, 20 25 and 30 days. The activities were determined by measuring the release of reducing sugars during the reaction. Once the best conditions were established to produce fibrolytic enzymes, the fermented substrate was added to silage process. The highest activity of xylanases and lacasses ($P<0.01$) was expressed by *P. ostreatus*, with averages of 29.98 $IU\cdot g^{-1}$ DM at 25 days and 1086.28 $IU\cdot g^{-1}$ DM at 10 days respectively. The celulases activity was lower than the other fibrolytic enzymes, it was 2.4398 $IU\cdot g^{-1}$ DM after 5 days, but during the process the activity was decreasing. A fermented maize straw of 10 days was obtained, when the lacasses activity was highest, and it was added (10 % DM) like inoculum to maize straw in anaerobic fermentation (silage). Actually this experiment is being conducted. The activity of fibrolytic enzymes in *P. ostreatus* indicates a potential use for biotechnological application for example to elaborate ruminant feeds like an excellent option to improve animal production.

(keywords: fibrolytic enzymes, solid state fermentation, solid wastes degradation)

FE-23

Coupled application of aqueous two-phase systems and 2D-electrophoresis for three-dimensional characterization of soybean proteins

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To facilitate the general acceptance of plants as bioreactors, the establishment of efficient downstream operations is critical. General knowledge of the molecular properties of the potential contaminant proteins benefits the selection and design of suitable strategies for the recovery of recombinant proteins. Alternatively, such knowledge can be exploited for the selection of an adequate host by considering the molecular properties of the target protein. This research addresses the use of aqueous two-phase systems (ATPS) for the recovery and molecular characterization of proteins from soybean extracts. A recently reported experimental approach that resulted from the combination of quantitative 2D electrophoresis (2-DE) with hydrophobic partitioning in ATPS was applied for the three-dimensional characterization of soybean proteins. The three dimensional scatter plots of molecular weight (MW), isoelectric point (pI) and surface hydrophobicity (log K_p) were obtained using two different ATPS compositions, PEG 3350 (15.7%)-sodium sulfate (8.9%)-NaCl (3%) and PEG 3350 (14.8%)-potassium phosphates (10.3%)-NaCl (3%), to obtain hydrophobicity data. Six model proteins (lysozyme, bovine serum albumin, cytochrome C, □-lactalbumin, ribonuclease A and chymotrypsin A) with hydrophobicities characterized by other measures were used to validate the ATPS-based hydrophobicity scale. Molecular properties of soybean proteins were obtained (MW, pI and log K_p) simultaneously using two different ATPS combined with 2-DE. PEG/sulfate system resulted in a wider range of proteins characterized with this experimental approach. The presence of dominant proteins challenged the application of this new experimental approach to soybean. The majority of the proteins detected in 2D gels were derived from two main storage proteins and the limited number of spots detected in top phase gels restricted the number of 3D spots characterized.

(keywords: 2D-electrophoresis; aqueous two-phase systems; soybean; protein characterization)

FE-24

Effect of Proteses from *Aloe vera* Over Miofibrils of Bovine Meat

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Meat is one of the most nutritious foods for humans due to its contribution in proteins of high biological value. Meat products can vary their properties, depending on the race, age and species of the bovine, varying the content of collagen and miofibrillas, the meat appear in some zones of its body with greater amount of muscle and miofibrils, originating meat harder than in other parts of the animal. One of the zones with greater hardness is "*Longisimus thoracis"* known like "cuete", the hydrolysis with proteolytic enzymes originate the softening, originating polypeptides of different molecular weights. For enzymatic hydrolysis of the meat, there are several enzymes from plants like papain, bromelain, ficin and hemisphaericin; finding that each one has an specificity activity on the muscle. The zabila (*Aloe vera*) is a plant that grows in México; is rich in many enzymes like the proteases. The determination of the proteolytic activity is informed in UT (tyrosin units/30 min). Meat softening with *A. vera* (acibar) at 10%, was 33% higher than using papin latex (Karisa 1%), with 40,180 UT. The tenderness of parenquime-cuticle 10%, is similar compared to Karisa 1%, 23,986 UT. The activity between of aloe 10% and parenquim- cuticle 10%, is a different a 40%, being more active of the aloe 10%. The hydrolysis of miofibrils with proteases of *A. vera* acting on parenquim-cuticle acted on actin and myosin. The tenderizing treatment with aloe 10%, originated polypeptides from 23,500 Da; with parenquime-cuticle 10% gave polypeptides up to 30,000 Da, and respect to the tenderness of Karisa 1%, it gave polypeptides of 15,500 Da. The sensory evaluation of different tenders for meat with aloe 10% and parenquim-cuticle 10%, demostrating an significant difference respect to the texture of the meat with tenderness in relation to the witness. There was not significant difference in color. We conclude that proteases from *A. vera* are an alternative source of meat tenderizers.

(keywords: proteases, *aloe vera*, tenderness, meat)

FE-25

Proteolytic Pattern of Asadero Cheese as an Effect of Plant Coagulant and Chymosin

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An important step in cheese production is the gelation of milk. The gelation is a result of two reactions; an enzymatic hydrolysis reaction and a physical aggregation of the coagulant-altered casein micelles. The hydrolysis is carried out by enzymes from different sources. The aim in this work was to establish differences between proteolytic products from cheese made with chymosin and trompillo (*Solanum elaeagnifolium*). Trompillo is a wild plant that possesses proteases in its fruit; those enzymes exhibit general proteolytic activities, which are useful in traditional asadero cheesemaking. Commercial chymosin and trompillo's crude extract was evaluated. Crude extract of such fruit was obtained by salting out with ammonium sulfate (40%). Cheeses were obtained by a standardized process, just changing the enzyme. The hydrolysis profile of the main proteins was characterized by polyacrylamide gel electrophoresis (SDS-PAGE). Similar to chymosin, the trompillo extract exhibited proteolytic activity toward κ-casein, besides s-casein and β-casein. The native proteins were converted to lower molecular weight peptides in both cases. In both treatments there was no effect on whey proteins since they were observed in the exuded lactoserum. SDS-PAGE analysis revealed several proteolytic bands in trompillo's gel compared with chymosin gel, where just one remarkable proteolytic band was identified. Although the trompillo's enzymes have unspecific cleavage sites on milk proteins, the physical properties of the asadero cheese produced were similar to those presented by the cheese made with chymosin. Therefore hydrolysis of milk proteins with trompillo may produce peptide mixtures with functional properties perfect for asadero cheese production.

(keywords: cheese, chymosin, plant coagulant, hydrolysis)

FE-26

Biochemical Characterization of Sucrose: Sucrose 1-Fructosyltransferase (1-SST) from *Festuca arundinaceae*

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Fructans are polymers of fructose that occur as storage compounds in a large number of plants from different families, including grasses (Poaceae) such as *Festuca arundinacea.* Fructans with a lower degree of polimerization called fructooligosaccharides (FOS) have prebiotic characteristics and are highly appreciated in the food industry. Enzymes involved in plant fructan synthesis (fructosyltransferases) are of interest since they could be useful for the production of fructooligosaccharides. In this context our interest has been to study fructosyltransferases from different species. In particular, in this work we describe the heterologous expresion of Sucrose:Sucrose 1-Fructosyltransferase (1-SST) from *F. arundinacea* in *Pichia pastoris* X-33 and its biochemical characterization. A single transformant was inoculated in BMGY medium and incubated at 29ºC and 180 rpm until an OD600 of 5.0 was reached. Cells were harvested by centrifugation and resuspended in the induction medium with methanol 1% (BMMY) and incubated during 24 h. Recombinant protein was recovered from culture medium by ammonium sulphate precipitation and the 1-SST activity determined by measuring the release of reducing sugars (DNS method) in reactions with 300 mM sucrose in 50 mM sodium acetate buffer pH 5.5. Total activity recovered from 1 L of culture after ammonium sulphate precipitation was 230 U. The effect of temperature on activity was evaluated at pH 5.5 in the range of 15 to 55ºC, while the effect of pH was determined at 35ºC in the 3.5 to 7.5 range. It was found that the enzyme has an optimum temperature and pH of 40°C and 5.5 respectively, similar to other plant fructosyltransferases. Conversion was determined following a reaction with 0.5U/mL of enzyme and 300 mM sucrose during 20 h. 50% sucrose was converted almost exclusively to 1-kestose and glucose after 3 h. Maximum conversion (85%) was observed after 20 h of reaction while maximum 1-kestose production was observed at the 8th hour. It is demonstrated that recombinant 1-SST are an interesting option for the synthesis of 1-kestose one of the most active prebiotics. Currently, this enzyme is tested under reactions conditions compatible with industrial production such as high sucrose concentrations.

(keywords: 1-SST, 1-kestose, fructosyltransferase, fructooligosaccharides)

FE-27

Generation of antibacterial oligosaccharides derived from chitin using heterologous endochitinase synthesized in *Escherichia coli*

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ChiA Nima and ChiA74 are bacterial endochitinases synthesized by respectively *Serratia marcescens Nima* and *Bacillus thuringiensis* whose structural genes where recently cloned in our laboratory. The aim of this work was to synthesize those two heterologous endochitinases, in *Escherichia coli* and demonstrate their potential for applied use in generating antibacterial chitin-derived oligosaccharides (OGS). Different constructions were obtained by cloning *chiA Nima* and *chiA74* genes under the regulation of their wild promoter in vectors with the ability to replicate in *E. coli*. Heterologous endochitinases were expressed and recognized by the *E. coli* export machinery. Chitinase activities in supernatants were detected by using fluorogenic derivates and by ~20 h maximal chitinolytic activity was observed. With all constructions a high chitinolytic activity was detected; however with *ChiA Nima* the highest increment was recorded. When colloidal chitin was digested with heterologous endochitinases, [$(GlcNAc)_6$], [$(GlcNAc)_5$] and [$(GlcNAc)_3$] derivative were detected by TLC. These OGS showed antibacterial activities against *Enterobacter cloacae, Escherichia coli, Staphylococcus aureus* and *S. xylosus*. Our study suggests that it is feasible to synthesize endochitinases *ChiA Nima* and *ChiA74* codified by *E. coli* and mass-produce these enzymes in culture supernatants. As signal peptides in native *ChiA Nima* and *ChiA74* were recognized by the protein export molecular apparatus in *E. coli,* these short peptides could be included as signal sequences for transport in *E. coli* of other proteins with applied value. This is the first report suggesting that ChiA Nima and ChiA74 can be used to produce OGS to control food-borne pathogenic bacteria.

(keywords**:** endochitinases, oligosaccharides, chia nima, chia74)

FE-28

Cloning and Sequencing of a *ldh* Gene from *B. infantis*

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Bifidobacterium spp. are immobile gram-positive anaerobic bacteria that were originally isolated in the feces of breast-fed infants in 1899. They are considered probiotic organisms improving the microbial balance in the human gut. The application of bifidobacteria to food products has become popular worldwide in recent years. The strain *B. infantis,* is one of the species frequently used in functional and dairy products. There is an increasing interest in exploiting the enzymatic characteristics and the molecular biology of this strain. In order to investigate the expression of the genes involved in carbohydrates catabolism and in the production of short chain fatty acids (SCFA), our first approach was to isolate the gene that codifies to a lactate dehydrogenase enzyme. The primers forward and reverse were designed from the coding sequence of *ldh* gene from *B. longum.* A fragment of approximately 1,000 bp was amplified by PCR, it was cloned into the pGEM-T vector. Subsequently, the cloned fragment was sequenced. The nucleotide sequence has a high similarity with the *ldh* sequence of *B. longum*. This fragment will be used as probe in further experiments to analyze the expression of the lactate deydrogenase gene, under several culture conditions.

(keywords: *Bifidobacterium*, *ldh*, cloning)

FE-29

Polymeric Protein Contents of Mexican Wheat Cultivars of Groups I, III and V

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Wheat is a grain of a great importance in the economy and food for the Mexican population. Gluten proteins are the major component of wheat flour and they are responsible for the viscoelastic properties of dough, and for the ability of flour to form a cohesive dough, and thus to make bread. The distribution of molecular weight of the proteins, mainly the insoluble polymeric protein has been related to dough strength and breadmaking quality. The aim of this study was to characterize the main cultivars of the Mexican wheat in relation to the content and distribution of the polymeric and monomeric proteins. Size Exclusion High Performance Liquid Chromatography (SE-HPLC) was used to determine the distribution and percentage of the soluble polymeric protein, gliadins, albumins and globulins. Cultivars of group I showed higher contents of insoluble polymeric protein and gliadins in the soluble fraction, On the contrary, cultivars of group III have lower protein content in the grain, lower insoluble polymeric protein and higher amounts of albumins and globulins in the soluble fractions. It was found that high grain protein content corresponds to higher amounts of gliadins, and lower protein contents to higher amounts of the albumin and globulin fraction.

(keywords: polymeric protein, SE-HPLC)

FE-30

Preparation of Maltodextrins from Purple Banana (*Red Banana)* Starch by Enzymatic Hydrolysis

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The starch is the biggest component in the diet in all the human populations, it is excellent to modify the texture and consistency of the foods. It is the main storage polysaccharide of the plants. The most remarkable chemical change in the banana happens after the crop and it is the hydrolysis of the starch and the accumulation of sugars, near 20-25% of the fresh pulp of the green fruit it is starch. The maltodextrins has a great variety of application and they are oligosaccharides mixture constituted by D-glucose united by bonds glycosides □-1,4 and that are obtained starting from the controlled hydrolysis of the starch, by acids or enzymes to conserve a dextrose equivalence (DE) <20, they are classified based on their content of direct reducers sugars. In this work we studied the hydrolysis of the starch of purple banana using □-amylase of barley malt in concentrations of 50 and 100 □ g/mL and concentrations of substrate of 1, 2, 3 and 4%, the times of reaction were of 3, 5, 10, 15, 30, 45, 60, 90, 120, 240 and 360 minutes and temperatures of 35 and 45º C to obtain maltodextrins with an dextrose equivalence from 5 to 20, likewise the enzymatic activity was analyzed in each one of the described conditions. In the shortest times of reaction was obtained maltodextrins with an dextrose equivalence of 5 and 10, the enzymatic activity was smaller at these times and 120, 240 and 360 minutes was found maltodextrins with a DE of 15 and 20 and the enzymatic activity was bigger; with regard to the temperature, an important change is not observed.

(keywords: maltodextrins, starch, enzymatic hydrolysis)

FE-31

Fungi Lipase Production by Means of Surface Adhesion Fermentation

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The lipase (acyl gliceryl hidrolase E.C. 3.1.1.3) is enzyme that hydrolyzes the ester links between glycerol and fatty acids. In this work the extracellular lipases produced by fungus *Mucor griseocyanus* in a system of surface adhesion fermentation were studied. The production of lipase was carried out in an Erlenmeyer flask of 250 mL that contained 0.2 grams of polystyrene and 40 mL of whey at 140 rpm and 30°C. The system was inoculated with $1x10^6$ spores/mL. The inducer (olive oil at 0.5 %) was added at 24 h. Every 24 hours samples of 1 ml were taken from reactor to determine lipase activity. After 72 hrs of fermentation the quantity of free biomass and of biomass adhered to the support was evaluated, as well as the enzymatic activity of supernatant. The enzymatic activity was determined by p-NPP (p-nitrophenyl-propionate) method which consisted in detection of absorbance increasing at 348 nm related with p-nitrophenol liberation as a result of hydrolysis of ester in a phosphate solution 25 mM, at pH 7.0 and 37° C. International Unit is defined as the amount of enzyme necessary to hydrolyze 1 µmol of p-NPP per minute under the conditions described before. The experiments were realized by triplicate having obtained a free biomass of 0.470 g/L and a biomass adhered of 0.853 g/L, giving like result a total biomass of 1.330 g/L. The maxima activity detected was of 11.94 U L^{-1}. The adhesion of the fungus *Mucor griseocyanus* on the polystyrene support was possible. It was also observed that the lipase production was higher with whey alone as well as with olive oil as an inducer. The results suggested the application of *Mucor griseocyanus* for lipase production by means of surface adhesion fermentation applied whey.

(keywords: lipase, surface adhesion fermentation, polystyrene)

FE-32

Evaluation of Technologies for the Elaboration of Orange Segment (*Citrus sinensis*) in Syrup

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Mexico is ranking fourth in orange worldwide production which is mainly destined to the internal consumption as fresh fruit or for obtaining juice. In our country compared with other producers countries, this fruit is not processed to greater added value products, such as segments in syrup, consequently the possibility of increasing commerce profit is lost. The development and evaluation of technology for the elaboration of orange segment in syrup represents the possibility to have a product that conserves the properties of the fresh fruit making possible its transport to geographic areas where its production is limited or nonexistent. In addition, it also represents an economically viable alternative that can contribute in rising orange and citric fruits commerce profit. For these reasons, the aims of this work were: to evaluate different chemical and enzymatic methods for the flavedo and albedo elimination and then to establish the best conservation technique to obtain orange segments with fresh fruit organoleptic properties and with a minimum shelf life of three months. For the flavedo elimination, the treatment with boiling 5% NaOH by 2 minutes, generated the best results, facilitating the peeling, without causing loss of sugars. For the albedo elimination, the incubation of segments by hour and a half with a 0.5% enzymes solution (pectinolitics and celulolitics), pH 3.0, 40ºC, allowed to obtain intact vesicles without albedo whose organoleptics properties were preserved. The orange segments were packaged in a sweetened solution at 12ºBrix, pH 3.0 and 0.1% of sodium benzoate and then pasteurized. The product generated under the previous conditions was stored at room and 4ºC temperature without showing alteration after three months.

(keywords: orange segments, enzymology, food process, biotechnology)

FE-33

Molecular Detection of Lipolytic Microorganisms during the Processing Stages of an Artisan Made Mexican Cheese

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Cotija cheese has been manufactured by traditional means for four hundred years and it is one of the few Mexican raped cheeses. It is made with raw milk, there is no heat treatment during the manufacturing process, and has high salt content. We analyzed and compared samples from two small towns in the mountains of Jalisco and Michoacán where the Cotija cheese "Origin Denomination" is produced. By means of traditional microbiology techniques microorganisms were isolated from milk, from curdle before and after salting, and from the salt used during cheese processing. It was found that after salting the curd the lipolytic microorganisms were mainly halophylic Gram-positive cocci. No yeast or fungi were found up to this stage of processing. The molecular approach included PCR-DGGE. From analysis of the products obtained by amplification of the V3 region of the ribosomal gene 16S from DNA extracted directly from samples of each stage, the incorporation of lipolytic microbiota was studied. Further sequence analysis was performed in order to identify microorganisms. The lipolytic microorganisms found in this cheese are of major importance, because they contribute to its characteristic smell and taste. Bacteria such as S*taphylococcus psifermentans, S. Saprophyticus, S. xylosus, Bacillus pumilus* were identified.

(keywords: cotija cheese, lipolytic microorganisms, PCR-DGGE).

FE-34

In Vitro *Recombinant Lactoferrin Expression Analysis*

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Lactoferrin (Lf) is an 80-kDa iron-binding glycoprotein of the transferrin family that is expressed in most biological fluids and is a major component of mammals' innate immune system. Its protective effect has a ranges from direct antimicrobial activities against a large panel of microorganisms, including bacteria, viruses, fungi, and parasites, to anti-inflammatory and anticancer activities. This work describes the establishment of a method to produce recombinant bovine lactoferrin (rbLf) using an *Escherichia coli* expression system. The cDNA fragment encoding the bLf was obtained by RT-PCR from bovine mammary gland mRNA, amplified and cloned into pCR2.1TOPO. After the transformation into *E. coli* Mach1T1 host, two different constructs, pCRbLf53 and pCRbLf35, were characterized in which the bLf gene was oriented in opposite position in relation with the T7 promoter of the vector. The cDNA of bLf (pCRbLf53) was sequenced and submitted to GenBank of the NCBI with the accession number EU812318. To express the bLf, the cDNA was cloned into expression vector pET-32 and then introduced into *E. coli* BL21/DE3 expression host. *E. coli* carrying the pET-32bLf was cultured at 37 °C at 200 rpm, and the expression of the rbLf was induced by adding 1 mM (final concentration) IPTG. The expression levels of rbLF were analyzed by northern blot. Recombinant bLf mRNA´s were present at early stages after gene induction. Cloning and expression of bLf cDNA should provide of rbLf for biotechnological application in the improvement of nutraceutical feed and other industrial applications.

(keywords: lactoferrin, gene expression, functional protein)

FE-35

Sequence and Characterization of a Clone Containing the *xyncfl*A Gene from *Cellulomonas flavigena.*

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The xylanolytic bacteria *Cellulomonas flavigena* has been wide studied for its potential to produce enzymes with several applications like in baker industry to improve the quality of bread. Studies in the molecular biology of this microorganism had let us to isolate clones containing xylanase-coding genes from a genomic library. The C1 clone is a plasmidic construction containing a 3.8 kb fragment of DNA from *C. flavigena*. The 3.8 kb fragment was obtained from a Southern blot analysis after the digestion of a phage □ clone, the hybridization was made with a probe of 781 bp with 84% of identity with the *xyn*D from *C. fimi* gene. The sequence obtained in this work had an open reading frame of 1,605 bp, it presented high similarity with several xylanases from different microorganisms reported in the genebank.

(keywords: *Cellulomonas flavigena*, xylanase, sequencing)

FE-36

*Bifidobacterium infantis: V*iability and Protein Detection in Acid Conditions

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Bifidobacterium are natural inhabitants of the gastrointestinal tract (GIT). In order to colonize this environment, it is necessary that the bacteria resist extreme environments, as the acidity of the stomach and the presence of bile in the duodenum; these conditions affect the viability of bifidobacteria. Our interest is to develop methodologies to isolate bacteria with high resistance to acid conditions. The aim of this work was to determine the survival of *B. infantis* in low pH values and monitoring the protein expression under these stress conditions. *B. infantis* growth and viability of was carried out inoculating 100 µL TPY medium, containing 10^8 cfu/mL adjusted to pH 7.0, 4.0, 3.0 and 2.0. All the cultures started with 10^4-10^5 cfu/mL and they were incubated at 37°C. The growth was measured in a spectrophotometer at 600 nm and the protein was detected by SDS-PAGE. Results showed that the growth of *B. infantis* at pH 7.0 reached 2 orders of magnitude in 24 h. In the cultures with pH 4.0 and 3.0 the biomass was kept between 10^4 and 10^5 cfu/mL. At pH 2.0, the biomass decreased 2 orders of magnitude after 24 h. *B. infantis* showed two bands of proteins 22 and 28 kDa approximately in the different conditions of acidity. These proteins are in processes of purification to be sequenced and to determine if they are stress proteins.

(keywords: *Bifidobacterium infantis,* acid stress, protein detection)

FE-37

Development of a Multiplex PCR Technique for Simultaneous Detection of Different Annexed Sequences to Transgenic Events

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The aim of this work was the optimization of the Multiplex PCR technique for simultaneous detection of three annexed sequences to transgenic events. DNA of maize, soybean and cotton plants was extracted using the CTAB (Cetil Trimethyl Ammonium Bromide) technique. Later a PCR amplification of the annexed sequences was carried out. Each vegetal sample was individually amplified by PCR. Primer sequences previously reported by other authors were analyzed using the Fast PCR software in order to determine if matching among them took place. In the 16 sequences (8 primers pairs) analyzed, no matching was observed between primers. By this reason any primer combination could be used for PCR. After, the Multiple PCR was carried out combining first DNA with two annexed sequences and later DNA with three sequences. For the double PCR, the selectable marker *nptII* (271pb) and the reporter gene *luc* (450 pb) were amplified, and for the triple PCR, the sequences used were *nptII*, *luc* and the terminator *nos* (125pb). The double and triple PCR primer annealing temperature was 62°C. For optimization of the multiplex PCR, different concentrations of magnesium chloride were tested. In the double PCR was used concentrations of 0.41, 0.83, 1.25 and 1.6 mM and for triple PCR were tested the concentrations 0.37, 0.73, 1.10 and 1.74 mM $MgCl_2$; the best results with obtained with 1.6 and 1.47 mM respectively. The optimization of the multiplex PCR technique and the amplification of the annexed transgenic sequences to the event could become a useful tool for the detection of Genetically Modified Organisms.

(keywords**:** *ntp*II, *luc* and *nos* sequences)

FE-38

Biodiversity of Yeasts present in a Traditional Mexican Cheese Analyzed by a Molecular Approach: ARDRA and DGGE

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Cotija cheese has been manufactured by traditional means in the mountains of Jalisco and Michoacán for four hundred years. The process does not comprise any heat treatment. The purpose of this work was to compare the biodiversity of yeasts present in eight samples of Cotija cheese "Region of Origin" by molecular methods, dependent and independent of culture. Samples of cheese were homogenized in peptone water and serial 1:10 dilutions were made and inoculated in three different culture media: Nutrient Agar Wallerstein Laboratory (WL), Malt Extract Agar (MEA) and Potato Dextrose Agar (PDA) in order to isolate yeast colonies. A PCR amplification of DNA representative of the isolated colonies, and the subsequent restriction analysis of the domain D1/D2 of 26S rRNA gene amplicon (ARDRA), and of the internal region transcribed spacers ITS1 and ITS4 amplicon were performed. This methodology was carried out to group and further identify the main microorganisms present in the samples. For the culture independent method, DNA was extracted directly from the cheese samples and an amplification of partial 26S sequence was carried out using primers NL1 clamp y LS2. The PCR products were analyzed by Denaturant Gradient Gel Electrophoresis (DGGE) in order to get the biodiversity pattern. This pattern indicated a difference in the microbiota found in each sample, although some microorganisms were present independently of the region where the cheese was made. A complementation of the information obtained from both approaches, culture dependent and independent methods, was observed. The microorganisms identified so far are *Candida zeylanoides*, *Candida oleophila* and *Candida parapsilosis*. These yeasts have been reported as isolated from sea water and other food such as honey and some ripened fruits. Particularly *C. oloeophila* has been used as a biocontrol agent against some postharvest pathogens on a variety of harvested commodities, and *C. zeylanoides* is also know for its lipolytic activity which might be important for the cheese ripening process.

(keywords: cotija cheese, ARDRA, DGGE, yeast, PCR)

FE-39

Influence of Whey Protein Fractions on the Expression of Penicillin G Acylase

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The aim of this work was to study the influence of several protein fractions from whey on the penicillin G acylase (PGA) expression from *M. griseoacyanus*. The whey was fractionated using precipitation with ammonium sulfate at 20, 40, 60 and 80% saturations. Both supernatant and precipitate obtained from each saturation were dialyzed in cellulose membrane against water for 24 h. The influence of the different whey protein fractions over the expression of PGA from *M. griseocyanus* was evaluated kinetically. As controls, were used whey with and without inducer (penicillin G 0.5 g/L). The process was carried out in submerged fermentation (SmF) in 250 mL Erlenmeyer flasks (120 rpm, 30 ºC). The results showed that in all cases, the maximum expression of PGA was obtained after 36 h of incubation. The highest level of penicillin acylase was obtained in the fraction of 60 % saturation, two and three times more than the control with and without inducer, respectively. These results show that some protein components present in whey are essential for microbial growth, and PGA production when used as sole nutrient source. This study confirms that some molecules of protein nature may have a role as inducers of PGA expression.

(keywords: Penicillin G acylase, whey, *Mucor griseocyanus)*

FE-40

Biotin Increases Pancreatic Glucokinase Expression Throught cGMP, Insulin Secretion and its Autocrine Signaling Pathway

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Many studies in diabetic patients have shown a benefical improve in glucose tolerance with a treatment with biotin. Besides its role as carboxylase prosthetic group, this vitamin modifies the expression of critical genes in the regulation of glucose metabolism. In a previous work we demonstrated that biotin increases pancreatic glucokinase mRNA levels. In this study, we explore the mechanism responsible for biotin-induced pancreatic glucokinase expression. Pancreatic islets in culture increased 2.5 fold glucokinase mRNA after 2 h incubation with biotin. Inhibition of soluble guanylate cyclase or protein kinase G (PKG) signaling abolished biotin-induced glucokinase expression. Biotin increases 3-folds the cGMP content in islets. Since cGMP signaling pathway stimulates insulin secretion, we found that inhibition of insulin release with diazoxide or nifedipine prevented biotin-stimulated glucokinase mRNA increase. Biotin treatment also increased significantly the islet ATP content. Inhibition of PKG activity suppressed the effects of biotin on ATP content. Insulin-antibodies or inhibitors of phosphoinositol-3-kinase/Akt insulin signaling pathway prevented biotin-induced glucokinase expression. The nucleotide 8-Br-cGMP mimicked the biotin effects. Our data indicate that the mechanism responsible for the effect of biotin on glucokinase mRNA abundance involves soluble guanylate ciclase and PKG activation, insulin secretion, and insulin PI3K/Akt signaling, and offer new insights on the pathways that participate in biotin-mediated gene expression.

(keywords: biotin, glucokinase, insulin, cGMP)

FE-41

Mapping of Loci Affecting Protein Quality in Maize Endosperm

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The *opaque2* (*o2*) mutation improves the protein quality of maize (*Zea mays* L) endosperm by reducing the synthesis of zein storage proteins and increasing the accumulation of lysine-containing proteins. Elongation factor 1A (eEF1A) is one of these proteins and its concentration is highly correlated with the total lysine content. The *o2* mutation also increases the free amino acid (FAA) content of the endosperm. The aim of this study was to identify *loci* associated with these protein quality traits. The mapping population consisted of two inbred lines contrasting in FAA and eEF1A levels, Oh545*o2* (low eEF1A, high FAA) and Oh51A*o2* (high eEF1A, low FAA), as well as 76 recombinant inbred lines derived from their cross that showed continuous variation for both traits. Composite interval mapping using a linkage map of 77 microsatelites identified 7 *loci* for eEF1A and 10 for FAA, each of them explaining on average 13 and 9 % of the phenotypic variation, respectively. Two *loci* on chromosomes 4 and 7 were located near α-zein genes in both traits. Flour samples contrasting in the genotype of flanking markers were prepared for each *locus* and used for protein fractionation into zeins, which were separated by SDS-PAGE. A decrease in α-zein 19 kDa levels was observed for these *loci* in the samples with high FAA and low eEF1A content. One *locus* for FAA on chromosome 2 was coincident with an aspartate kinase *locus* (*ask2*), which is consistent with the fact that amino acids derived from the aspartate pathway are the most affected in Oh545*o2*. These results show that much of the increased accumulation of amino acids in maize *o2* endosperm can be explained by the reduction of α-zein content, as well as the pleiotropic effect of the mutation that results in alteration of important steps in carbon and amino acid metabolism.

(keywords: maize endosperm, *opaque2*, protein quality, genetic mapping)

FE-42

Identification of Proteins Affecting Free Amino acid Accumulation in Maize Endosperm

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The *opaque2* (*o2*) mutation increases the free amino acid (FAA) content of maize endosperm, including lysine, the most limiting essential amino acid. Most of the endosperm lysine is present in proteins, but in some genotypes such as Oh545*o2*, free lysine accounts for 33% of the total lysine. Genetic analyses of the cross between Oh545*o2* and Oh51A*o2* (low FAA) identified several *loci* associated with this protein quality related trait. The aim of this work was to identify proteins associated to these *loci*. Flour samples contrasting in the genotype of flanking markers were prepared for each *locus* and used for FAA composition analysis, total protein extraction and fractionation in zeins and non-zeins. The protein fractions were analyzed by SDS-PAGE and 2D-PAGE and some of the proteins differentially expressed between the samples were identified by mass spectrometry. Three *loci* on chromosomes 4, 7 and 10 were previously located near α-zein genes; the isoelectric points of the proteins encoded by these genes were coincident with those of the zeins found to be differentially expressed between the samples of these *loci*. With respect to the non-zeins, some *loci* showed an association with globulin storage proteins; a decrease in globulin levels was associated with high levels of arginine, the most abundant amino acid in these storage proteins. Other differentially expressed non-zeins were enzymes involved in carbohydrate metabolism, including glyceraldehide-3-phosphate dehydrogenase, ADP glucose pyrophosphorylase and starch synthase. The results of this study suggest that the high levels of FAA in maize *o2* endosperm can be explained by the reduction of storage proteins and the non incorporation of their amino acids into other proteins, as well as the alteration of important steps in carbon metabolism that may influence amino acid metabolism.

(keywords: maize endosperm, *opaque2*, free amino acid, protein quality)

FE-43

Molecular Genetic Diversity of Maize Landraces from the State of Sinaloa in Mexico

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Mexico is the center of origin of maize (*Zea mays* L), a cereal of great economic and social importance that was diversified in the highlands after its domestication. In the state of Sinaloa, small-scale farmers still cultivate maize landraces with a wide range of phenotypic characteristics that have been preserved from generation to generation. However, the gene reservoir maintained in these populations has been poorly studied and it is being lost, mainly because of the destruction of their habitat and the introduction of genetically uniform materials. The aim of this study was to evaluate the genetic diversity of some of these maize landraces as an initial effort to contribute in their preservation. Twelve populations (10 individuals/population) corresponding to six landraces were used for the analysis. DNA was extracted from the 120 individuals and probed with 20 microsatellites distributed across the maize genome. A total of 116 alleles were obtained (average of 5.9 alleles per locus) and a total genetic diversity of 0.7. The average genetic diversity within populations (0.52) was higher than that among populations (0.26). The average Rogers distance between populations was 0.35. Cluster analysis with the UPGMA method revealed a high similarity among populations of Concordia and San Ignacio counties, with the landraces Hibrido Blanco and Breve San Juan being the closest. The high number of alleles per locus and total genetic diversity found in this study show a wide genetic basis of the maize landraces from Sinaloa, representing a gene reservoir with great potential to be used in the solution of food and agriculture related problems.

(keywords: maize, genetic diversity, microsatellites)

FE-44

Identification of Proteins Associated with Chilling Injury in Tomato Fruit

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Tomato (*Lycopersicon esculentum* Mill) is one of the most popular and economically important vegetables in the world. However, tomato fruit production and distribution are affected by its susceptibility to develop a physiological disorder known as chilling injury, when exposed to temperatures lower than the critical (12 ºC) but above the freezing point, developing several symptoms that decrease its quality. Little is known about the biochemical basis of this disorder. The aim of this study was to identify proteins related to chilling injury in tomato fruit. Fruits were stored at 5 or 12 ºC for 5, 15 and 25 days and then at 21 ºC for 0, 4, 8, 12 and 16 days for ripening. Chilling injury index was evaluated during the ripening of fruits stored at 5 ºC; the main symptoms observed were uneven ripening, surface pitting and rots. Protein expression changes were investigated between the pericarp of fruits stored at 12 ºC and 5 ºC, as well as between the pericarp with and without symptoms of fruits stored 25 days at 5 °C, using a combination of two dimensional electrophoresis and mass spectrometry. This proteome comparative analysis detected about 300 polypeptides, of which about 6% changed their expression in response to cold stress. One of the identified polypeptides was a RNA-binding protein with chaperone activity that showed a higher accumulation in pericarp with no symptoms than in damaged tissue, suggesting an important role for this enzyme in cold acclimatization through regulation of gene expression at low temperature. The other chilling injury related proteins are involved in carbon and amino acid metabolism, oxidative stress, photosynthesis, respiration and protein processing and degradation. These proteins might be working together to maintain the celular homeostasis under cold stress.

(keywords: tomato, cold stress, chilling injury, proteomics)

FE-45

Correlation between Changes in Aroma Profile of Tomato Saladette Stored at Low Temperature and its Alcohol Dehydrogenase Activity

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Refrigerated storage is the main postharvest technology to increase shelf life in horticultural products. Even though, optimal storage temperatures have been established for different commodities, they are based mainly on physical quality parameters, without considering the effect of low temperature in flavor. The characteristic flavor of fresh tomato is the result of complex interactions between organic acids, soluble sugars and over 400 volatile compounds. These volatiles are derived from different biochemical pathways such as the catabolism of lipids, amino acids, lignins and carotenoids. Alcohol dehydrogenase (ADH) is considered an important enzyme which contributes to flavor development by interconverting aldehyde and alcohol volatile flavors originated from lipids and amino acids. In this study, we analyze the effect of the recommended storage temperature (10º C) on the aroma profile determined by GC-MS of tomato '7705', an important cultivar grown in the state of Hidalgo, and its possible correlation with changes in ADH activity. The production of NADH was measured spectrophotometrically in an enzymatic assay containing ethanol as substrate. Refrigeration caused quantitative and qualitative modifications in the aroma volatiles profile. Among the main detected changes, was an increase of the ratio 3-methylbutanal/3-methylbutanol and hexanal/hexanol, as well as an increase in trans-3-hexenol levels. In addition, we observed a decrease on ADH activity that might explain these changes.

(keywords: volatiles, flavor , refrigeration, alcohol dehydrogenase, tomato)

FE-46

Preliminary Studies of "Nuez De Castilla" (*Junglans regia*) as a Source of Serinprotease and Aspartylprotease Inhibitors

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Studies on protease inhibitors have been focused to the utilization of leguminosae and certain cereals. Other sources of proteases, such as nuts, have not been explored. Proteases from nuts "Nuez de Castilla" (*Junglans regia*) among others, are GRAS substances that can be included in developing functional foods. The objective of this work was to study the inhibitory effect of a *J. regia* inhibitor extract on commercial proteases. Pepsin (aspartyl-protease) was analyzed at pH 2 to 4, and trypsin (serin-protease) at pH 4 to 6. Protease inhibitors were extracted from *J. regia* at non-denaturing conditions; its protein concentration was analyzed in a spectrophotometer at 280 nm; inhibitory capacity were analyzed by non-denaturing electrophoresis at the same pH ranges as before, reporting molecular weight distributions. Zymograms were also developed at the same pH ranges to study aspartyl- and serinprotease inhibitors. The extract obtained from *J. regia* inhibited both commercial proteases; however, trypsin was more efficiently inhibited than pepsin at all every studied pH. Electrophoresis did not show any change in proteases molecular weight profile due to action of the inhibitor extract. These results were confirmed with zymogram results where activity decreased in samples added with *J. regia* extract. Therefore, *J. regia* extracts can be used as aspartyl- and seri-proteases in foods.

(keywords: aspartyl-and seri-proteases, protease inhibition, *Junglans regia***,** nuez de castilla)

FE-47

Effect of Shrimp (Heads) Residues (*Litopeneaus vannamei)* on Endogenous Protease Activity, and its Inhibition with Rice (*Oryza sativa*) Extracts

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Utilization of marine residues, such as shrimp heads, is an alternative to recover bioactive compounds, proteases among others, for further use in biotechnology-related industries. Enzymatic hydrolysis has been applied to food as a means to improve flavor, color and texture. However, proteolysis must me controlled to avoid undesirable changes. Rice (*Oryza sativa*) extracts, can be an alternative to inhibiting proteases, in controlled proteolytic reactions in foods as these extracts are GRAS substances. The objective of this work was to study the inhibitory capacity of rice extracts on endogenous proteases extracted from shrimp wastes (heads) (*Litopeneus vannamei)*. Proteases were extracted from shrimp heads stored at -30°C for 10 months, and recently caught animals; maximum enzyme activity was analyzed at pH 2 to 11 by the Anson and Kunitz. On the other hand, a protease inhibitor extract was obtained from rice using phosphate buffer at 4°C, pH 7.0; protein concentration in shrimp endogenous protease extracts and inhibitor extracts was analyzed by the Biuret method. Changes in molecular weights were also studied in shrimp protease extracts. After treating shrimp extracts with rice extracts, protease inhibition ratio was reported. pH rage of maximum activity was pH 4 to 10. After 10-month storage maximum activity was observed at pH 4, 6 and 7; further inhibition studies were carried out at these pH values. Protein concentration in the rice extract was 3.91 mg/mL. During frozen storage, an increase in endogenous shrimp protease activity was observed. Inhibition percentage obtained by treatment with the rice extract was 30%, after 10-month frozen storage. Shrimp wastes are a potential source of proteases; however, controlled proteolysis can be achieved by the use of rice inhibitor extracts.

(keywords: rice extracts (*Oryza sativa*), shrimp wastes (*Litopeneus vannamei)*, protease inhibitors)

FE-48

A Xylanase Gene Codified by *Aspergillus niger* GS1

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Hemicellulose is the second source of renewable organic carbon on earth, with a high potential for degradation to useful end products. Xylan constitutes the major component of hemicellulose, while endo-1,4-β-D-xylanases (E.C. 3.2.1.8) catalyze its hydrolysis. Xylanases have applications in a wide range of industrial processes, such as the paper and pulp industry, and animal feed. *Aspergillus niger* GS1, isolated from copra paste, is a novel strain which has shown to produce xylanase activity. The aim of this work was to obtain a xylanase gene codified by *A. niger* GS1 induced with xylan. *A. niger* GS1 was growth in potato dextrose agar with gradual decrease of glucose (G) content and increasing proportion of oat spelt xylan (X), ranging 75-25 % G-X mixtures to 100 % X, supplemented with yeast extract, for xylanase induction. Oat spelt xylan was linked with remazol brilliant blue (RBB-Xylan) in order to prepare a colored culture media useful to detect xylanase activity (zymogram). The fungal induced xylanase gene was isolated from total RNA by RT-PCR, using a conserved consensus sequence for primers design shown by other fungal xylanases. *A. niger* GS1 grew in culture media with oat spelt xylan as the major carbon source. Endoxylanolitic activity, which means hydrolysis of RBB-Xylan, from *A. niger* GS1 was visualized by the presence of a colorless halo surrounded the growing apical hyphae. The sequence of the PCR product showed a homology up to 84% compared with other fungal xylanases reported.

(keywords: xylanase, solid state fermentation, recombinant protein)

FE-49

Enzymatic Extraction of Hydroxycinnamic Acids from Coffee Pulp

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Ferulic, caffeic, *p*-cumaric and sinapic acids are classified as hydroxycinnamic acids (HA). They are usually linked to polysaccharides of plant cell walls. HA exhibit anticarcinogenic, anti-inflammatory and antioxidant properties. These properties are of great interest for the food, pharmaceutical and cosmetology industries. The objective of this work was to determine the enzymatic extraction efficiency of the HA present in the coffee pulp. For that purpose, two enzymatic preparations were used, a commercial pectinase (Fluka) and an enzymatic extract produced by *Rhizomucor pusillus* (strain 23aIV) by solid culture on coffee pulp. Total HA quantification in coffee pulp was carried out after alkaline hydrolysis (2M NaOH), followed by HPLC analysis. Before enzyme treatment, free AH of coffee pulp was extracted with a methanol:water solution (80:20). After that, successive treatments of coffee pulp were performed with 10 international units of pectinase (50 mM citrate buffer pH 5, at 50 °C) for 30 min, and 10 international units of lipase (50 mM citrate buffer pH 6, 45 °C) for 12 h. The amount of HA in the coffee pulp before extraction was (mg/kg coffee pulp): 3 097 ± 3.5 of chlorogenic acid, 1 985 ± 3.6 of caffeic acid, 113 ± 4.7 of ferulic acid and 81 ± 6.0 of *p*-cumaric acid. After the enzymatic treatment, an extraction efficiency of 45%, 25.5% and 2.7% for chlorogenic, ferulic, and caffeic acids respectively, were obtained. These extraction efficiencies corresponded to the HA covalently bonded to the coffee pulp cell wall, whereas for *p*-cumaric acid only traces were detected. The affinity constant (Km) for commercial pectinase, and the fermented coffee pulp extract (FAE) produced by *R. pusillus* (23aIV) were 3.24 ± 0.06 mM and 8.39 ± 0.09 mM, respectively. FAE enzyme shows lower affinity for the methyl ferulate when used as substrate. These results demonstrate that the combined use of pectinase and esterase activities might allow an efficient extraction of hydroxycinnamic acids from coffee pulp.

(keywords: FAE, lipases, pectinases, methyl ferulate, hydroxycinnamic acids, coffee pulp)

FE-50

Production, Immobilization and Enantioselective Properties of Lipases from a Thermotolerant Fungal Strain of *Rhizopus homothallicus*

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The objective of this study was to evaluate the growth and lipases production from a thermotolerant fungus (*Rhizopus homothallicus var. rhizopodiformis*) by solid-state fermentation (SSF). The obtained lipases were immobilized in order to modulate their enantioselectivity (*E*). Two different protocols of immobilization were used to: i) involve different areas of the protein, ii) promote different degrees of enzyme rigidity or iii) modify the microenvironment of lipases. SSF were carried out at 45°C, aeration rate, 40 ml/min; initial moisture content, 55%; initial pH 6.5 and an inoculum size of $3x10^7$ spores per gram of dry solid support (perlite). Olive oil was used as enzyme inducer. Two columns were sampled at 16, 20, 24 and 40 h of fermentation time. Native electrophoreses were performed using a modification of the Laemmli method. Gels were stained by incubation in a 0.1 M sodium phosphate buffer at pH 6.2 with 0.02% □-naphtyl acetate and 0.05% Fast Blue RR. The two different derivatives prepared were: 1) interfacially adsorbed lipase (using octyl-Sepharose 4BCL) and a 2) covalently immobilized lipase (using glutaraldehyde-agarose). Hydrolysis of (*R*,*S*)-Butyl mandelate was used as model reaction to evaluate the *E* value. Maximum lipase activity (49.7±1.4 UI g^{-1} dry matter over olive oil as substrate) was observed after 16 h of culture. Native electrophoresis of the crude extract showed two bands with esterase activity around 43 and 64 kDa. The *E* values of the different derivatives were found to be completely different. The interfacially adsorbed lipase presented a low enantioselectivity (*E* = 7, both enantiomers produced), whereas the E value of the glutaraldehyde derivative resulted in a single enantiomer (*E* = ∞). In both cases the enantioselectivity yielded the S isomer. These results demonstrate that lipases produced by *Rh. homothallicus* using SSF show high enantioselectivity after immobilization with glutaraldehyde-agarose. This approach allows the development of biocatalysts for the production of highly purified enantiomers.

(keywords: lipases, solid-state fermentation, immobilization, enantioselectivity modulation)

FE-51

Production of bacterial phytase in *Pichia pastoris*

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The improvement of phytate phosphorus utilization by a microbial phytases has been described, mainly in pigs and poultry. According to its biochemical properties, the phytase encoded by the *phyC* gene from *Bacillus subtilis* VTT E-68013 strain, is a potential candidate as a shrimp feed additive. The objective of this study was to construct recombinant *Pichia pastoris* strains that are able to produce and secrete the phytase C. Using standard Molecular Biology techniques, the *phyC* gene was cloned into the expression vector pPIC9. The constructed plasmid (pPIC9*phyC*) was used to transform the *P. pastoris* host strain GS115. The genotype and phenotype of the transformants were determined. Induction conditions of *phyC* gene in GS115*phyC* strains were established to enhance the production of recombinant phytase C with catalytic activity in liquid shake flask cultures, and the strain that produced the highest amount of the recombinant phytase C was selected. The constructed GS115*phyC* strains were confirmed as Mut^{+} and $phytase^{+}$ phenotypes. The cell-free culture medium of 14 recombinant strains showed phytase activity. Three of these strains were selected due to their high levels of recombinant phytase C activity in the cell-free culture media. The C6-2 strain showed the highest value of specific activity, IPC-PRBA and enzymatic activity (0.75 U/mL), eight times higher than that obtained for the native phytase C (0.096 U/mL) produced in a shake flask culture of *Bacillus subtilis* VTT E-68013, and 3 times higher than the reported activities for other phytase of *Bacillus subtilis* (0.24 U/mL). In this study, recombinant *Pichia pastoris* strains harboring the *phyC* gene of *Bacillus subtilis* were constructed for the first-time, and these strains produce and secrete to the culture medium the recombinant phytase C with enzymatic activity. The levels of enzymatic and especific activity of the recombinant phytase C obtained in this study, exceed the reported for the native phytase and its recombinant forms in other hosts, therefore the phytase C production in *Pichia pastoris* gives a competitive improvement to the existing processes. Furthermore, the fact that *Bacillus subtilis* is a GRAS microorganism makes possible to apply the phytase C in shrimp nutrition.

(keywords: microbial phytases, recombinant protein, *Pichia pastoris)*

FE-52

Blue Endosperm Maize: DNA Fingerprinting and Accelerated Ageing of Seeds

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The loss of vigor and the susceptibility to attack by warehouse plagues of maize seeds (*Zea mays* L.) with blue endosperm, constitutes serious inconvenience for its massive production, since seeds require more scrupulous handling. The deterioration of seeds quality begins at its physiological maturity, and occurs through a series of physical, physiological, biochemical and molecular events, including alterations in DNA metabolism. In this study the damages caused by artificial ageing in two modalities were examined: dry heat (60 ºC during 48 h) and humid heat (41 ºC, 100 % relative humidity, for 48 h), on seeds of four native blue maize genotypes (Oaxaca-711, Puebla-479, Cocotitlán and Cuijingo), produced in 2005. The seeds quality was evaluated using physical tests (fast green), physiological (normal germination and root protrusion), biochemical (viability, leaching of solutes and electrical conductivity). In addition, the profile of genomic bands after random amplification of polymorphic DNA (RAPD) was evaluated. The results showed the particular blue maize varieties genotypic response to each one of the conducted analyses. This effect was observed in diverse aspects of tolerance to ageing. The varietal characterization obtained was robust and integrated diverse events of the germinative process, mainly molecular analysis, leading to conclude that this methodology could be an option for plant breeding programs and production of maize seeds, to reliably identify their genetic materials.

(keywords: *Zea mays*, fingerprinting, RAPD markers)

FE-53

Genomic Repercussions of Maize Seeds Ageing

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One of the aspects of the viability loss in the maize seeds is the deficient storage, which generally lacks controls of temperature and humidity. In the present study the damages were observed at molecular level in aged artificially embryos of hybrid maize seeds with white endosperm, in contrast with others studies of our team. This technique of aging resembles the effects caused by the natural aging, being observed at various levels (morphologic, physiological, biochemical, and molecular). In the present study our objective was to correlate the physiological and the genomic impact imposed with the aging treatment. The artificial aging was carried out using two conditions: humid heat at 41 °C for 72 h with a 100% relative humidity, and dry heat at 60 °C for 48 h. After the aging treatment, it was made the test of normal germination; it was evaluated normal and abnormal seedlings, as well as the dead seeds. In addition, it was determinated the length and the dry matter of the normal seedlings. It was evident that the aged seeds by dry heat suffered greater damage, since they showed the smaller number of normal seedlings. The RAPD analysis showed differences between the genetic profiles of the control seeds and the aged seeds. Finally, we observed loss of polymorphisms in the DNA fingerprinting among the seeds, this effect is consistent with others works about this topic.

(keywords: *Zea mays*, maize genomic, accelerated ageing, RAPD markers)

FE-54

Molecular Comparison of Maize Seed Origins by RAPD Test

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The objective of this work was the molecular comparison of the origins 1994 (1996) and 2000 of 4 maize lines, generated by the Maize Program of INIFAP: L 1, L 2, L 3 (this was only with origin 2000) and L 4. DNA of the seeds embryos was extracted, and it was processed by the RAPD protocol. The amplifications developed were coded in a basic dates matrix, and their phylogenetic relationships were performed by the statistical program LCDMV (calculation software of molecular distances between varieties, CIMMYT). The following parameters were employed: distance of Seuil =0, value of confidence =95; Nei and Li distance, and the method of cluster average (UPGMA). The results indicated the effectiveness of the RAPDs to differentiate origins and genotypes genetically related. In the origins comparison the dendrograms grouped similar genotypes with their respective different origins, highlighting the coefficient of similarity (0.875) for the union of the origins (1994 and 2000) of L 2. A group was built for each genotype, very far away to each other, being the nearest so much for old seeds as new L2 and L4, with coefficients of similarity of 0.72 and 0.8, respectively. This indicated the sensitivity of the RAPD test to distinguish among these genotypes subjected to natural aging. It was evidenced a differential genotypic effect for the tolerance to longevity, it was bigger in L2 and L1, and smaller in L3 and L4.

(keywords: *Zea mays*, genetic identity, fingerprinting, RAPD markers)

FE-55

Isolation and Partial Characterization of Three Isoamylases of *Rhyzopertha dominica* F. (Coleoptera: *Bostrichidae*)

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Three isoamylases of *Rhyzopertha dominica* (termed RdA70, RdA79, and RdA90 according to their relative mobility in gel electrophoresis) were isolated by ammonium sulfate fractionation and hydrophobic interaction chromatography. RdA70 and RdA79 showed an optimal pH of 7.0 whereas for RdA90 the optimal pH was 6.5. The three isoamylases remained stable at 50 °C for 1h, but at 60 °C, all lost 50% of their activity in 20 min and were completely inactivated in 1 h. RdA70 and RdA79 were inhibited by albumin extracts from wheat samples varying widely in amylase inhibitory activity; however, RdA90 was highly resistant to inhibition. β-Mercaptoethanol up to 30 mM increased the activity of the three isoamylases by 2.5-fold. The action pattern of the three isoamylases was typical of endoamylases; however, differences were observed on the hydrolytic efficiency rates measured as $V\text{max}/K\text{m}$ ratio on starch, amylopectin and amylose. The hydrolyzing action of RdA90 on starch and amylopectin ($V\text{max}/K\text{m}=90.4 \pm 2.3$ and 78.9 ± 6.6, respectively) was less efficient than that on amylose ($V\text{max}/K\text{m}=214 \pm 23.2$). RdA79 efficiently hydrolyzed both amylopectin and amylose ($V\text{max}/K\text{m}=260.6 \pm 12.9$ and 326.5 ± 9.4 respectively). RdA70 hydrolyzed starch and amylose at similar rates ($V\text{max}/K\text{m}=202.9 \pm 5.5$ and 215.9 ± 6.2, respectively), but amylopectin was a poor substrate ($V\text{max}/K\text{m}=124.2 \pm 7.4$). The overall results suggest that RdA70 and RdA79 appear to belong to a group of saccharifying isoamylases that breaks down long fragments of oligosaccharide chains produced by the hydrolytic action of RdA90. The simultaneous action of the three isoamylases on starch, aside from the high resistance of RdA90 to wheat amylase inhibitors, might allow *R. dominica* to feed and reproduce successfully on the wheat kernel.

(keywords: *Rhyzopertha dominica,* isoamylases, inhibitors, kinetics)