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BT-P13.
BIOEMULSIFIER-PRODUCING *Aspergillus niger* MYA 135: EFFECT OF CULTURE CONDITIONS

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Bioemulsifiers are surface-active molecules synthetized by microorganisms, which play an important physiological role in hydrocarbon degradation. Few reports have shown that the bioemulsifier production by filamentous fungi are amount, quality and nature dependent not only on the microorganism but also on the culture conditions. Bioemulsifiers have the advantages of biodegradability, low toxicity, effectiveness and these properties enable their wide application on bioremediation, food, cosmetic and pharmaceutical industries. The aim of this work was to study the bioemulsifier production by *Aspergillus niger* MYA 135 under different culture conditions. Methods: The bioemulsifier production was conducted in mineral medium during 96 hours, at different initial pH and with the addition of $CaCl_2$ or $FeCl_3$. The emulsification index was determinated after 24 h (E-24) in supernatants using kerosene as immiscible liquid. E-24 was estimated as the height of the emulsion layer divided by the total height and multiplied by 100. Results and conclusions: Bioemulsifier production reached maximum levels at 2 days of cultivation suggesting that its accumulation was growth-associated. Although all the emulsions were stable, the maximum values of E-24 were obtained at initial pH 2 (60.0 ± 0.71 %) and with the addition of$FeCl_3$ (50.5 ± 0.70%).
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BT-P14.
SELECTION AND CHARACTERIZATION OF HYDROLASES SECRETION MUTANTS IN *Tetrahymena thermophila*

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The non-pathogenic ciliate *T. thermophila* converts cholesterol from food stuffs into pro-vitamin D derivatives, increasing the nutritional value of milk and egg products. Unfortunately, the process is accompanied by the release of hydrolytic enzymes that impair food's sensorial properties, i.e., taste and odor. Hydrolytic enzymes are present in lysosomes, which are secreted after fusion with the cellular membrane. Our strategy was to block the secretion process by mutation with the mutagen N-methyl-N'-nitro-N'-nitrosoguanidine followed by conjugation, for the isolation of stable genotypes. Following this protocol, 20 sec- mutants were obtained, based on decreased (acid) phosphatase activity (70%). These clones were further characterized with respect to several other lysosomal enzymes, such as protease, ß-N-acetylglucosaminidase, galactosidase and ß-glucosidase. Only two of the mutants showed similar decrease in all tested enzymatic activities. In spite of these results, the mutants showed a high level of secretion upon stimulation with Dibucaine, approximately 60% more than the wild type and other sec^- mutants (MS-1). Assuming that this drug increases the intracellular level of free Ca^{2+}, the mutants must be affected in a pathway that has not been targeted in previously characterized sec- mutants.

BT-P15.
ISOLATION AND CHARACTERIZATION OF PLASMIDS FROM *Shewanella* CSQ2. A SHUTTLE VECTOR CONSTRUCTION

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Marine microorganisms present biotechnological and industrial interest becuase of its capability to develop cold-active biocatalizers, among others. Cold-active enzymes offer economic profits due to incremented reaction rates, high stereospecificity, meager undesirable chemical reactions, lack of heating costs and easy inactivation by its thermal lability. *Shewanella* is a marine bacterial genus cold-active enzyme producer that presents a big biotechnological potential and grows using a variety of substrates.
The aim of present work was to isolate and characterize by restriction profiles, the plasmids present in *Shewanella* C_8Q_2 strain, isolated in our laboratory from Beagle Channel samples, and to build a bridge vector capable of replication in *E. coli*. For that, plasmidic DNA extractions and conventional and pulsed field gel electrophoresis analyzes were performed. Restriction profile and cloning of selected plasmid were carried out in the pBluescript vector.
Results and conclusions: two plasmids of ~3,000 and ~6,000 respectively and a megaplasmid were isolated. Restriction profile of P1 plasmid (3,000 pb) was carried out and obtained fragments were cloned into pBluescript vector, subsequently used in *E.coli* XL1 blue transformation reactions. The shuttle vector obtained is a valuable tool that would allow to study and express genes of interest of *Shewanella*' strains into *E. Coli*.

BT-P16.
BEHAVIOUR OF TWO RECOMBINANT BACULOVIRUSES FOR PEROXIDASE EXPRESSION IN LEPIDOPTERA

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Baculovirus-insect cell system is a popular choice for heterologous gene expression when an eukaryotic environment is required. *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) is by far the most widely used baculovirus expression vector. In this work two recombinant viruses were constructed in order to compare the performance of HRPC expression in Lepidopteran hosts. The first one (AcMNPVHRPC occ-) was conventionally formed by introducing the HRPC gene in locus polyhedrin. The second construction (AcMNPVHRPC occ+) was identical except for polyhedrin gene presence under p10 promoter. *Spodoptera frugiperda* larvae (approximately 200 mg each) were infected by intrahaemocoelical injection with 50 µl of 6.10^6 ufp.ml^{-1} of both viral suspensions. At different days post-infection (dpi) HRPC expression in haemolymph was measured by its enzyme activity. HRPC levels achieved with both type of virions were not significantly different at any dpi. Thus, polyhedrin gene presence under p10 promotor does not affect the HRPC expression. AcMNPVHRPC occ+ showed to be as effective as AcMNPVHRPC occ- with the great advantage of being suitable for oral infection of permissive lepidopteran species.