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EFFECT OF pH ON ACTIVITY AND STABILITY OF PROTEASE PRODUCED BY *Streptomyces clavuligerus*

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The effect of pH on activity and stability of protease produced by two strains of *Streptomyces clavuligerus* (NRRL 3585 and 644) have been studied. *S. clavuligerus*, producer of β-lactam antibiotic, excrete a metalloprotease (Bascaran,1990). The enzyme was obtained from crude extract in batch condition, carried out at 28°C with orbital shaking (200rpm) for 4 days, using the medium described by AHARONOWITZ and DEMAIN (1978), modified by PORTO *et al.*(Anais da XX Reunião Anual da SBBq, 12.23,1991). The supernatants after centrifugation were used for determination of optimum pH and study protease of stability. The pH study was carried out from 4 to 9, using the folowing buffers: citrate-phosphate (pH 4.0-6.0, 0.1M), sodium phosphate (pH 5.7-8.0, 0.1M) and TRIS-HCl (pH 7.2-9.0, 0.2M). The stability study at different pH values (pH 6.5-8.0), was carried out at 25°C during 1h in buffers mentioned above. Protease activity of the samples were measured a 25°C, using TRIS-HCl buffer (pH 8.0). The optimal pH for protease extracted from wild type 3585 and 644 were pH 7.5 (sodium phosphate, 64.2U/mg) and pH 8.0 (TRIS-HCl, 26.5U/mg),respectively. The protease was active at pH 6.5-8.0 during 90 min at 25°C. At pH 8.0, 50% of the residual activity remained whereas at pH 8.4 the enzyme was completely inactivated for both strains of *Streptomyces clavuligerus.*

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COMPARATIVE STUDY OF GLUTAMATE DEHYDROGENASE-NADP BETWEEN TWO STRAINS OF Saccharomyces cerevisiae CARRYING THE PLASMID pCYG4 RELATED WITH AMMONIA ASSIMILATION

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Ammonia can be assimilated into the cells following two different pathways. The first is catalalyzed by glutamine synthetase (GS) together glutamine 2-oxo-glutarate aminotransferase (GOGAT). The second is catalyzed by NADP-linked glutamate dehydrogenase. This work shows a comparative study of glutamate dehydrogenase activity in two strains of *Saccharomyces cerevisiae* AR5 and AR2 cells carrying the plasmid pCYG4, which encode the glutamate dehydrogenase gene conferring an 11 fold increase comparing with wild type. However, the AR5 does not have any GOGAT acitivity, but the AR2 still with this enzyme encode. in activity was investigated. Experiments were carried out in batch culture, under aerobic conditions in the following media: D(+) glucose 1,5%,ammonium sulfate 0.4%, difco yeast nitrogen base (YNB) w/o amino acids and ammonium sulfate 0.17% Yeast cultures were grown for 33h in the previous medium at 30 C under stirring (200 rpm). The results shown that maximum NADP-linked glutamate dehydrogenase activity in the AR2 cells was obtained after 16h of growth (205U/g) whereas with AR5 the acitivity was eached after 8h (395U/g). In both strains the NADP-linked glutamate dehydrogenase activity was bigger than with wild type cells (158U/g) reached with 6h of growth. These previous results suggest that absence of GOGAT in the cells can stimulate the NADP-linked glutamate dehydrogenase avtivity and can increase the amount of ammonia taken by the cells.

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PHYSICOCHEMICAL, IMMUNOLOGICAL AND BIOLOGICAL CHARACTERIZATION OF RECOMBINANT HUMAN GROWTH HORMONE

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Recombinant human growth hormone (rec-hGH) secreted in bacterial periplasmic space and purified through several chromatographic steps, has been characterized and tested for purity, potency, identity and safety, according to recent international guidelines provided by FDA, WHO and European Pharmacopeial Commisssion.

Purity was tested through SDS-PAGE, Western Blotting, Isoelectric Focusing, Size Exclusion and Reverse-phase HPLC. Potency was measured *in vivo* by two biological assays in hypophysectomized rats and little dwarf mice providing specific activity values always above 2.5 IU/mg. Identity was confirmed by tryptic mapping, DNA and protein sequencing. Sterility, bacterial endotoxins, contaminating *E. coli* proteins and DNA, vector stability and acute toxicity were also tested.

All these studies have shown that the recombinant product prepared at IPEN-CNEN/São Paulo is a suitable candidate for therapeutic use.

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ANTIMICROBIAL ACTIVITY OF ACTINOMYCETES ISOLATED FROM MATA ATLANTICA FOREST SOIL.

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The actinomycetes are Gram-positive bacteria with potential and practical relevance in biotechnology, since they are able to produce bioactive compounds, among them antibiotics.

Looking for new antibiotics for the isolation of actinomycetes, besides the conventional technique of the plate dilution, à new technique of dispersion and differential centrifugation has been used.This procedure separates microbial cells more intimately attached to the soil particules, and allows for the discovery of microorganisms that may produce more efficient and inhibitory substances.

The actinomycetes were isolated from "Mata Atlantica" forest soil using glucose-asparagine-tyrosine-agar and starch-casein-agar media, and malt extract for maintenance of the cultures.

For the tests of antimicrobial activity, the actinomycetes were grown in a glycerol-peptona liquid medium, and the extracts were tested against *Staphylococcus aureus, S. saprophyticus* group A *Streptococcus, Escherichia coli* and *Pseudomonas aeruginosa* using Mueller-Hinton-agar medium and the Kirby-Bauer technique. Two out of the 12 extracts tested, were active against the Gram-positive cocci. Whereas none was active against the Gram-negative strains.

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CLONING OF HIPERRESISTANCE GENES OF *Kluyveromices marxianus CBS 6556:* CONSTRUCTION OF EXPRESSION VECTORS.

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Kluyveromices marxianus is an industrially attractive yeast due to its short generation time and its ability to grow at higher temperatures than *Saccharomices cerevisiae* .
SFA1 gene contained in the multicopy vetor YFRp$_1$ (Yeast Formaldehyde Resistance plasmid) confers hyperresistance (HYR) to formaldehyde (FA). Neither stable auxotrophic markers in recipient cells nor defined synthetic media are needed when YFRp$_1$ is employed for yeast transformation (Wehner et al. 1993). *S.cerevisiae* YFRp$_1$ transformants acquired stability to the vector when propagated in complex media supplemented with 3 to 5 mM FA..
Our experiments demostrated that *K.marxianus CBS 6556* transformed with YFRp$_1$ show two fold higher hyperresistance to FA than *S.cerevisiae* transformants.
We isolated the SFA homologue gene of *K. marxianus* by ligating EcoRI digested genomic *K.marxianus* DNA into yeast vector YEP352 and screening the resulting *K.marxianus* gene bank for FA-HYR in *S.cerevisiae*,showing an effective expression.
We are now investigating the expression of SFA1 of *K.marxianus* and characterizing the gene sequence,as well as constructing suitable expression vectors for this yeast.

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GENETIC IMPROVEMENT OF THE YEAST *Saccharomyces cerevisiae* FOR ALCOHOLIC FERMENTATION OF STARCH.

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Eight constructions involving the *Bacillus subtilis* α-amylase gene (*amyE*), a mouse pancreatic α-amylase cDNA (*AMY2*) and an *Aspergillus awamori* glucoamylase cDNA (*glaA*) were made: Three fusion genes, involving one α-amylase and glucoamylase; two double cassette plasmids (expressing one α-amylase and glucoamylase) and three single cassette plasmids, expressing each one of the DNA. Each of these plasmids were transformed into *Saccharomyces cerevisiae*. A plate test for amylolytic activity revealed that the largest starch hydrolysis halo was produced by the strain bearing the *Bacillus subtilis* α-amylase/glucoamylase fusion (BsAAase/GAase), and the worst by the mouse pancreatic α-amylase/glucoamylase fusion (MAAase/GAase). Northern hybridization showed that all strains produced the mRNA molecules of the expected sizes, approximatelly 2.0 kb for the single genes and 3.5 kb for the fusion genes. When assayed for enzymatic activity in liquid medium, the strains bearing the fusion and the double cassette plasmids involving *Bacillus subtilis* α-amylase and glucoamylase exhibited both activities. Two fusion proteins were purified and partially characterized. MAAse/GAase fusion protein is a single polypeptide of 150 kDa that exhibits only α-amylase activity. BsAAase/GAase fusion protein yielded a 150 kDa and 75 kDa polypeptides, exhibited both α-amylase and glucoamylase activities and is able to adsorb and digest raw starch. Finally, the capacity to grow on soluble and corn starch were tested in liquid medium for the strains bearing plasmids coding for the fusions and the enzymes separately. The strain carrying the double cassette BsAAase + GAase, that produced one of the smallest hydrolysis haloes in the plate test, showed the best performance, not only in digesting soluble and corn starch but also in using all of its products to grow. The BsAAase/GAase fusion transformant was also able to grow on soluble starch but, in contrast to YA2, was not able nor to grow on corn starch or to utilise all of the substrate. MAAase + GAase double cassette and MAAase/GAase fusion coding strains, even though were able to digest starch, were not capable of using its product to grow.