H - 31

BIOCONVERSION OF D-XYLOSE TO XYLITOL BY YEAST

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Xylitol is used as an alternative sweetener as it has sweetening power as high as sucrose and promotes oral health and prevents caries. It is also an important sugar substitute for diabetics. Xylitol is currently manufactured by the chemical reduction of D-xylose. Bioconversion can be carried out by bacteria, filamentous fungi, yeast, or purified enzymes from these microorganisms. The best known xylitol producers are yeasts. In a preliminary screening of different yeast strains for xylitol production, one strain named 1.70 presented the best performance (Q_P=0.13 g L^{-1} h^{-1}, q_P=0.26 mmol g^{-1} h^{-1} and $Y_{P/S}$=0.58 g g^{-1}) and its optimal growth conditions were therefore determined. The best D-xylose concentration was 51.35 g L^{-1}, resulting in the production of 32.70 g L^{-1} xylitol (Q_P=0.49 g L^{-1} h^{-1}, q_P=0.49 mmol g^{-1} h^{-1} and $Y_{P/S}$=0.64 g g^{-1}). The best fermentation parameters were obtained using 1.45 g L^{-1} of cellular mass as initial inoculum (36.2 g L^{-1} xylitol from 56.80 g L^{-1} D-xylose, Q_P=0.60 g L^{-1} h^{-1}, q_P=0.58 mmol g^{-1} h^{-1} and $Y_{P/S}$=0.65 g g^{-1}). The best aeration rate was obtained at 200 rpm, which lead to the production of 27.20 g L^{-1} xylitol from 46.70 g L^{-1} D-xylose (Q_P=0.90 g L^{-1} h^{-1}, q_P=0.74 mmol g^{-1} h^{-1} and $Y_{P/S}$=0.57 g g^{-1}). The addition of 0.5% yeast extract from MERCK® lead to the production of 33.00 g L^{-1} xylitol from 49.40 g L^{-1} D-xilose (Q_P=0.64 g L^{-1} h^{-1}, q_P=0.67 mmol g^{-1} h^{-1} and $Y_{P/S}$=0.65 g g^{-1}) while the addition of 0.5% yeast extract from DIFCO® lead to the production of 33.65 g L^{-1} xylitol from 47.00 g L^{-1} D-xylose (Q_P=0.67 g L^{-1} h^{-1}, q_P=0.60 mmol g^{-1} h^{-1} and $Y_{P/S}$=0.72 g g^{-1}). The conversion ratios obtained ($Y_{P/S}$) indicate that the yeast strain 1.70 has a good potential for the industrial production of xylitol.

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H - 32

REDUCTION OF RAFFINOSE OLIGOSACCHARIDES IN SOYMILK BY α-GALACTOSIDASE FROM GERMINATING SOYBEAN SEEDS.

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Raffinose oligosaccharides (RO) are the principal factors responsible for flatulence associated to soybean ingestion and its products. Removal of those sugars from soybean seeds or soymilk would have a positive impact in soy-derived foods consumption. Several procedures for RO content reduction in soy products were described, however, enzymatic hydrolyze of RO by α-galactosidase seems to be the most efficient one. The objective of the present study was to verify the efficiency of α-galactosidase from germinating soybean seeds to hydrolyze RO present in soymilk. Soybean seeds (*Glycine max*, cv CAC) were allowed to germinate for 60 h, at 27 °C and the enzyme from germinated seeds was partially purified by separation in an Aqueous Two-Phase System and ionic exchange chromatography, CM-Sepharose column, pH 4.0. Purification factor was 12.4 fold with enzymatic activity recovery of 13.5 %. Maximum α-galactosidase activity was detected at pH 5-6 and 50 °C. The enzyme retained near 100 % of original activity after pre-incubation for 3 h at 35 and 40 °C, but only 50 % of original activity was retained after incubation at 45 °C. $K_{M\ app}$ values for hydrolyze of pNPGal, melibiose and raffinose were 0.30, 0.63 and 6.16 mM, respectively. The enzyme hydrolyzed raffinose, pNPGal, stachyose and melibiose. Galactose, raffinose, melibiose, $CuSO_4$, SDS and $CaCl_2$ inhibited the enzyme activity. Soybean milk was treated with the α-galactosidase and the HPLC profiles showed reduction of 72.3 and 89.2 % in the stachyose and raffinose contents, respectively, after incubation for 6 h at 40 °C. Therefore, α-galactosidase from germinating soybean seeds or the corresponding gene may be used for establishment of a process to improve the nutritional value of soymilk.

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H - 33

Influence of heat stress combined with acidic, basic and bile salt incubations on trehalose level in natural yeasts strains (*Saccharomyces cerevisiae*) isolated from Atlantic Rain forest

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In the present work was investigated three diferents stresses (acidic condition, base condition and presence of bile salts) combined with heat shock. We considered the influence of theses stresses on the level of trehalose and on the viability of cells. Five natural strains of *Saccharomyces cerevisiae* isolated of Atlantic Rain forest (DM-UFMG 20, DM-UFMG 21, DM-UFMG 22, DM-UFMG 23, DM-UFMG 24) were studied. Simultaneous stresses is a experimental condition that is proximate to encountered during the passage of yeasts cells into the digestive tract. We try to simulated diferents injury that a yeast probiotic can be submitted on the gastrointestinal tract. A yeast probiotic is a live supplement which affects positively the human or animal digestive tract. The use of microorganisms for disease treatment and prevention as well as health restoration and maintenance of intestinal balance is not new, but studies of resistance on yeast probiotic strains are rare.
All the *S. cerevisiae* strains were resistant on the simulation of gastrointestinal tract. The level of trehalose after incubation of yeast cells at 37°C (heat shock) and pH 2 (acidic stress) is higher then cells submitted only to heat stress (classical stress, more studied in the literature). The trehalose level after incubation in basic condition (pH 8) and temperature elevated (37°C) depends on the strain utilized. Incubations of cells in presence of bile salts at 37°C maintain the same level of trehalose obtained in heat stress. Cell viability was determined microscopically by using a Neubauer counting chamber and vital staining with methylene blue and the correlation with the level of intracellular trehalose was done.

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H - 34

LABORATORY PRODUCTION AND FIRST CHARACTERIZATION OF PURIFIED RECOMBINANT HUMAN THYROTROPIN - IPEN

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Authentic recombinant human thyrotropin (rec-hTSH), an heterodimeric glycoprotein hormone containing two non-covalently linked subunits alpha(α) and beta (β),secreted by transformed Chinese Hamster Ovary (CHO) cells, was obtained in our laboratory at IPEN-CNEN/SP.

The production of rec-hTSH was carried out in hollow fiber bioreactor or in 162 cm^2 culture flasks, the concentration of the protein of interest in the hollow fiber bioreactor being ~ 20µg/mL in presence of dialysed fetal bovine serum (dFBS) or ~7µg/mL when dFBS is absent and ~ 5µg/mL in culture flasks, with or without dFBS. The medium total protein content in these two production systems, evaluated by micro BCA assay, was ~ 15 times higher in bioreactor-derived medium. Consequently, the starting material for purification, generated in this system, presented a 3 times lower specific activity.

A three-step purification procedure involving cation-exchange, dye affinity and size exclusion chromatography was utilized for obtaining purified, biologically active, heterodimeric rec-hTSH. A high purity level (~95%) and an overall yield of ~ 20% were obtained thanks to this purification process.

Identity and purity of rec-hTSH were evaluated through physico-chemical methods (size exclusion HPLC, reversed phase HPLC and SDS-PAGE), immunological methods (immunoradiometric assay, radioimmunoassay and Western blotting), N-terminal aminoacid sequencing and Maldi-Tof mass spectrometry. The physico-chemical and immunological methods were always carried out comparing our product with two well known reference preparations (rec-hTSH-Thyrogen® and pit-hTSH-NIDDK).

An "in vivo" bioassay confirmed that the relative biological activity of the purified hormone was 2.13 with relation to the international reference preparation of pituitary hTSH(NIDDK, USA).

This hormone, produced for the first time in our country, thanks to an original process, is extremely important for thyroid cancer diagnosis and therapy and for hyperthyroidism treatment.

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H - 35

Glycogen, Hsp26 and Hsp104 Stress Proteins Levels in Entrapped Cells of *Saccharomyces cerevisiae* Under Batch Cycles of Fermentation

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In last years, by virtue of their intrinsic characteristic and very long and safe use in industry and in human nutrition, biotechnological applications using entrapped yeast cell systems have recorded fast development. During industrial fermentation, domesticated yeast cells are subjected to several hard stress conditions for a long time resulting in a decrease cell viability, then imposing the need of cellular proliferation to system despite the existence in this organism of molecular mechanism to respond quickly against temporary adverse conditions evolved to preserve its viability. Ca-alginate entrapment cells enables yeast cells to be used for long periods of times in continuous fermentation eliminating the requirement for simultaneous cell growth. Glycogen recycle and Hsp(s) synthesis are mechanisms involved in temporary stress response and have been evaluated in laboratory strains but not in commercial ones under prolonged stresses. A better understanding of the cellular responses could open the possibility to improve this bioprocess. In this work Ca- alginate entrapped and free yeast cells where submitted to 75 cycles of batch fermentation. After each cycle cells were exposed to heat and osmotic stress conditions. Glycogen recycle was used as indicator of cellular viability. Hsp104 and Hsp26 stress proteins expression were determined using western blot using specific polyclonal antibodies as probe. The results show that under all stress conditions, Ca-alginate entrapped yeast cells and free cells respond similarly. Particularly, under osmotic stress up to 75 batches more intensive glycogen recycle and higher Hsp 26 level were observed in the former. Its seems that Ca-alginate matrix attenuates the environmental stress, increasing cell tolerance by protecting cell into the matrix and consequently decreasing cells exposition.

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H - 36

INFLUENCE OF MIXED SUGARS ON D-XYLOSE TRANSPORT AND CONVERSION BY XYLITOL-PRODUCING YEAST *Candida guilliermondii*.

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A method for efficient bioconversion of the D-xylose and L-arabinose commonly found in the hemicellulose fraction of lignocellulosic feed-stocks is essential for the development of an economical process for biomass conversion to fuel ethanol or sweetener xylitol. The objective of this study is to investigate the effect of different sugars, present in the hydrolyzed of biomass, on D-xylose conversion by *Candida guilliermondii* NRC5578. When cells grown in 2% xylose-medium (0.67%YNB without amino acids and 1%YE) were incubated during 15 s at 30°C in 50mM phosphate buffer pH 7 with D[1-^{14}C]xylose (55mCi/mmol), two xylose transport systems was induced: a facilitated diffusion system (Km 100mM) and an active system (Km 1.3 mM). The last one was inhibited competitively by D-glucose (4 and 40mM) and L-arabinose (4mM) added separately in the incubation system containing xylose. Cell extracts of the yeast in resting cells (cells pre-grown on 2% glycerol for 37h, washed and suspended in 100mM phosphate buffer pH 6.5 with 4% inducer sugar) contain NADPH-dependent aldose reductase (AR) and NAD^+-xylitol dehydrogenase (XDH) to utilize D-xylose by conversion to xylitol and D-xylulose. D-Xylose and L-arabinose were the best substrates of the enzymatic reaction for AR activity. 4% D-xylose or 4% L-arabinose were required for induction of both enzymes and 4% D-glucose repressed completely the activities of these enzymes. The addition of D-glucose with inducers repressed partially the induction of both enzymes, being dependent on glucose concentration. When D-xylose and L-arabinose were present in a mixture, no repression of AR and XDH activities was observed. The results suggest that the rate of xylose conversion is under control of catabolite repression and enzymatic inhibition. Glucose may prevent the entry of xylose or arabinose into the cells and therefore prevent enzymes induction. Intracellular glucose may reduce the rate of synthesis of both enzymes induced by xylose or arabinose.

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