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Saccharomyces cerevisiae production on whey hydrolized by β-galactosidase: Fed batch process

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INTRODUCTION: Lactose from cheese whey is not a proper substrate for the production of baker's yeast. Its hydrolysis by β-galactosidase produces glucose, which can be used as a proper substrate in the case that glucose concentration is not higher than the inhibition value. The realization of a concomitant process in which lactose is hydrolyzed in a batch fashion and *Saccharomyces cerevisiae* is grown using this product in a form of continuos fed-batch fermentation enables the use of this effluent and eliminates an important environental contamination.

AIM: The aim of this work is to study the influence of different parameters (lactose and enzyme concentrations, aeration and hydrolysis time before inoculation) in the amount and quality of the obtained baker's yeast.

METHODS: An experimental design was used at a flask level and for laboratory continuos mixed tank reactors.

RESULTS: For the studied conditions the best results where obtain for a lactose concentration in whey of 80g/L supply with $(NH_4)_2SO_4$ 30 g/L, an enzyme concentration of 1000 LAU/L dosificated in three times (333 LAU/L each), klac* = 350 h^{-1} (900 rpm, 2300 mL air/min). In these conditions the hydrolysis is 62.5% in twelve hours with a yeast production of 31 g/L, productivity 2.58g/Lh and $Y_{X/lac}$= 0.371 in 18.5 h. The quality parameters of the obtained baker's yeast are better than those of the one obtained from the fermentation of molasses: moisture 73% , protein 47.6%, phosphates 2.23%, baking test 72 min.

CONCLUSION: The production of baker's yeast by a continuos fed-batch fermentation fed by the *in situ* hydrolysis of lactose from cheese whey is possible. The process is easy to perform and regulate maintaining all throughout the process a sufficient rate of hydrolysis but not so much to inhibit the yeast development.

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L-ASPARAGINASE PRODUCTION BY *Saccharomyces cerevisiae*

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This study reports the production of the periplasmic enzyme L-asparaginase (E.C.3.5.1.1) by *Saccharomyces cerevisiae*. The enzyme catalyses the hydrolysis of L-asparagine to L-aspartate and ammonia. The derepressed yeast mutant P40-2A was selected due to its capacity to produce twenty times more enzyme as compared to the parental strain. Fermentations were carried out in a 3L working volume instrumented fermenter at 29°C, 160 rpm and 0,5 vvm air flow rate. The medium composition was as follows: 2% dextrose, 0,99% ammonium sulfate, 0,67% yeast nitrogen base (YNB), suplemented by leucine, uracyl and histidine. The L-asparaginase activity was measured by the ammonia liberated in the reaction mixture in initial rate conditions. *E. coli* L-asparaginase is presently used for leukemia treatment. The yeast enzyme could replace the bacterial enzyme thus avoiding its unwanted side effects.

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LARGE-SCALE PRODUCTION AND PURIFICATION OF RECOMBINANT PROTEIN FROM INSECT CELLS-BACULOVIRUS SYSTEM IN ERLENMEYER FLASKS: APPLICATION TO THE CHICKEN POLY(ADP-RIBOSE) POLYMERASE CATALYTIC DOMAIN

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Poly(ADP-ribose) polymerase (PARP, EC 2.4.2.30) is an abundant, highly conserved zinc-finger enzyme involved in the detection of DNA strand breaks in nuclei of most eukaryotes. It has recently emerged as a critical regulatory component of cellular response to DNA damage. Being a potential target in cancer chemo- and radiotherapy, there is a need to know the three-dimensional structure of PARP and particularly its catalytic domain to generate new specific inhibitors. In order to produce large quantities of this protein domain for crystallization, we developed a simple system for its large-scale production using the insect cells-baculovirus system that proved more efficient than the conventional spinner flask system. On the basis of maximum cell density, average population doubling time and overproduction of recombinant protein a better result was obtained in the simplest and cheapest bioreactor composed of regular Erlenmeyer flasks and ordinary shaker waterbath. The recombinant protein was purified to homogeniety as evaluated by SDS-PAGE using a procedure involving 3-aminobenzamide affinity chromatography. Routinely, 30 mg of pure PARP catalytic domain was obtained for a total of $3x10^9$ infected cells in 1 L of culture.

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PRODUCTION AND CHARACTERIZATION OF BIOLOGICALLY ACTIVE 6HIS-ILE-GLU-GLY-ARG-HUMAN PROLACTIN, SECRETED IN THE PERIPLASMIC SPACE OF *ESCHERICHIA COLI*

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A near full-length human prolactin (hPRL) cDNA was obtained by screening of a pituitary cDNA library with a synthetic 21-mer oligonucleotide and with rat PRL cDNA

For its expression use was made of a vector, p3SN8, containing a bacterial cellulase leader sequence, joined to sequences coding for a 6 histidine affinity site and a factor Xa cleavage site, and under control of the tac promoter. The hPRL cDNA was inserted at the 3' end of the cleavage site sequences.

Expression in *E. coli* led to secretion in the periplasmic space of a fully bioactive hPRL variant constituting authentic hPRL with a peptide tag, i.e. 6His-Ile-Glu-Gly-Arg, at its N-terminal. This tag-hPRL could rapidly and efficiently be purified by metal chelate affinity chromatography, its correct processing and quality being monitored by SDS-PAGE, Western blot analysis, Immunoassay and Nb2 lymphoma cell bioassay wich provided a specific activity of 32.3 IU/mg. Only a partial digestion of tag-hPRL was obtained with factor Xa.

A periplasmic secretion of tag-hPRL of the order of 0.7μg/ml/A_{600} and its good, one-step purification yield indicate its usefulness for *in vitro* diagnostic and research applications. This is the first report describing periplasmic secretion of a biologically active form of hPRL.

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PRODUCTION OF POLY-β-HYDROXIALKANOATES BY *CHROMOBACTERIUM VIOLACEUM*

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Poly-β-hydroxyalkanoic acids (PHA) is a polyester of β-D(-) hydroxyalkanoic acids is a constituint of bacterial carbon reserve which is produced when carbon/nitrogen ratio in growth culture media is high. PHA have been finding applications as biodegradable materials characterized by a number of physical properties related to conventional plastics. The most commun PHA found in bacteria is poly-β-hydroxybutyric acid PHB therefore it is also the most studied PHA. However many bacteria are able to produce a copolymer of butyric and valeric acid (PHB-co-PHV). Only recently it was reported some *Chromobacterium violaceum* strains able to produce the homopolymer Poly-β-hydroxyvaleric acid (PHV). Until now little is known about the properties and degradability of PHV. Since a brazilian strain of *C. violaceum* was avaiable its ability to produce PHB and PHV was tested. The bacterium was cultivated in medium lack of nitrogen source containing 0.5 % (w/v) glicose or 1.0% (v/v) valeric acid as the only carbon source. After drying of the cellular mass the production of PHB and PHV was measured by a spectrophotometric method described by SLEPECK AND LAW, 1960 (An. Chem., 32: 1697). The results have shown that in presence of glicose *C. violaceum* produced 145 nmol/ mg of dried cell of PHA. Of these amount 55.8% were PHB while 44.2% were PHV. In presence of valeric acid it was produced 4,8 nmol/ mg dried cell of PHA (50% of PHB and 50% of PHV). It seemed that in both condictions it were produced the copolymer PHB-co-PHV and only the proportion of the monomeric units was changed according to the condictions.

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AMYLASE PRODUCTION BY *Aspergillus fumigatus* Fresenius.

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Amylases play a very significant role in starch-processing industries. Hydrolysis of starch is accomplished by amylolytic enzymes mainly α-amylases, β-amylases and glucoamilases. Previous work in our laboratory has shown that a strain of *Aspergillus fumigatus* Fresenius isolated from soil produces both α-amylase and glucoamylase induced by soluble starch and lignocellulosic materials (Domingues and Peralta, Can. J. Microbiol, 39:681, 1993). Then, the purpose of this study was to investigate the utilization of wheat bran and manioc meal as substrate for the production of amylase by this strain. The amylase activities obtained with 1% (w/v) substrate were 7.80 U/mL (manioc meal) and 6.90 U/mL (wheat bran) in 5 days cultures. The results obtained in solid culture systems were 18.40 U/mL (10% w/v manioc meal) and 13.8 U/mL (10% w/v wheat bran) in 6 days cultures.

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