

Production and purification of β -galactosidase from *Aspergillus foetidus* MTCC 6322 using solid state fermentation.

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ABSTRACT

β -galactosidase (E.C.3.1.2.23) otherwise known as lactase plays a vital role in food processing and pharmaceutical industries in the present study this enzyme was produced from *Aspergillus foetidus* MTCC 6322, solid state fermentation. Maximum amount of enzyme activity of 83.40/mg was observed when gingilly oil cake (7.5g) and ground nut oil cake (2.5g) containing 60% moisture and 1.5ml of inoculums suspension were used at temperature 30°C and pH 4.0, respectively, after 96hrs of incubation. The enzyme was made to immobilize in calcium alginate and compared with its free form. Partial purification of the enzyme using ammonium sulphate precipitation resulted in a yield of 43.5% in 0-60% and 22.9 in 40-60% of salt concentration. The enzyme is made to dialyze and separated based on the molecular size by gel filtration chromatography under FPLC (fast performance liquid chromatography). Molecular weight of the enzyme was taken in to consideration based on graph.

KEY WORDS: MTCC 6322, *Aspergillus foetidus*.

1. INTRODUCTION

Lactose intolerance is a major and a common problem in among 70% of the world's population and it is mainly caused due to the insufficient or lack of production of β -galactosidase in the human intestine, and so this β -galactosidase is a viable and a major compound found in the hydrolysis of lactose process. And this β -galactosidase present in the lactose hydrolysis can be used in the treatment of Lactose intolerance. Siddique (2010) studied the production of β -galactosidase with five nitrogen sources by solid state fermentation using wheat bran as a substrate. *A. niger* and its DG resistant mutant were grown with initial pH of 5.5 and 30°C for 144h in culture media and samples were harvested after every 24h to analyse for substrate consumption, cell mass formation and enzyme production. All the five nitrogen sources namely, ammonium sulphate, corn steep liquor, diammonium phosphate, fish meal and urea showed considerably significant results. However, higher values of enzyme activity of 168.0 and 371.15 μ L/h in parent and mutant variety, respectively, was obtained from sample in which corn steep liquor was used as a nitrogen sources as compared to control (73.1 and 176.3u/l/h in parent and mutant respectively).

Microbial β -galactosidase enzyme has a great significance due to its use as biosensors, in the processing of milk in dairy industry and in ethanol production. Marrakchi (2008) have developed a biosensor using two distinct enzymatic activities of β -galactosidase and glucose oxidase, in order to apply it for the quantitative deduction of lactose in commercial milk samples. Panesar (2007) have reported the trails which were carried out to overcome the problem of enzyme extraction and poor permeability of cell membrane to lactose. Domingues (2005) have investigated the constant production extracellular heterologous β -galactosidase and ethanol by recombinant flocculating *S. cerevisiae*.

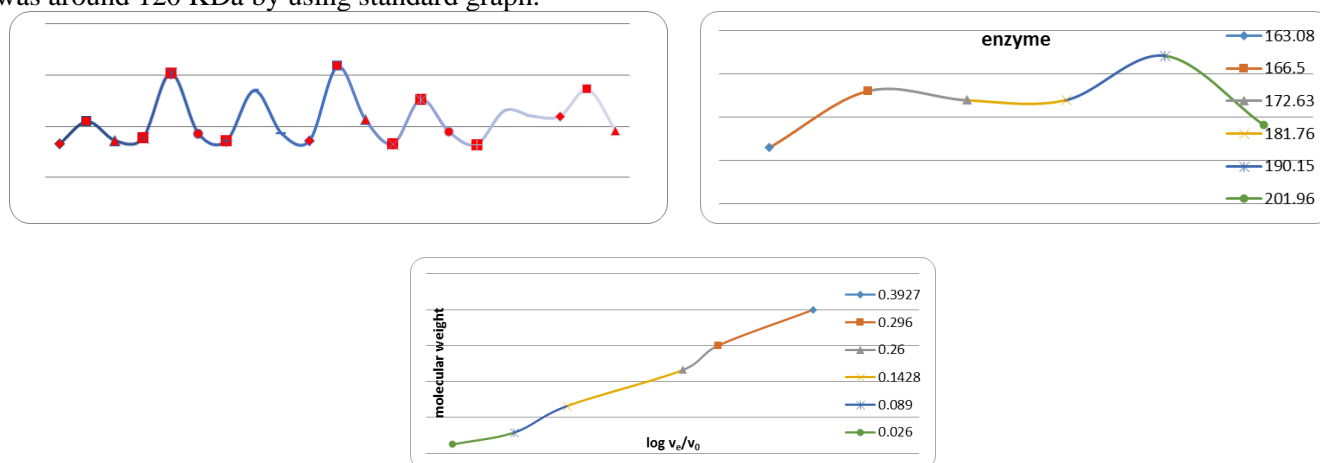
2. MATERIALS AND METHODS

A strain of *Aspergillus niger* MTCC6322 isolated from tannery sludge (common effluent treatment plant, Chrompet, Chennai) by an enrichment culture method and maintained at 4°C in Czapek-Dox agar slants. 100 μ L of the spore suspension containing 6.0×10^7 CFU/ml was used as the seed inoculums. Fermentation was carried out in 250mL Erlenmayer flasks. 10g of substrate in 250mL Erlenmayer flasks were moistened with 15.0 mL of distilled water and sterilized. After cooling, 1000 μ L of *Aspergillus foetidus* spore suspension was added to the substrate and mixed thoroughly. The flasks were incubated at 30°C for 96 hrs. Production of β -galactosidase was standardized by one factor at a time method (OFAT). The effects of different substrates viz., wheat bran, ground nut oil cake, gingilly oil cake, coconut oil cake, moisture content, inoculums concentration, pH, temperature for different periods of time were carried out for optimization of β -galactosidase production. 1g of moldy bran was mixed with 10ml of 20mM acetate buffer, pH 4.0 and centrifuged at 10,000 rpm for 10min and the mixture was filtered and enzyme extract was used for β -galactosidase assay. 3% of Sodium Alginate and 10ml of the crude enzyme solution were mixed well in a magnetic stirrer for a time period of 20-30min and the solution was added drop wise through syringe to 0.2M Calcium chloride of 10ml volume to form calcium alginate beads and were kept for hardening for 3h and the beads were washed and stored in 50mM of Calcium chloride. These β -galactosidase beads were used for enzyme assay. The β -galactosidase activity was determined by measuring the hydrolysis of ONPG. The incubation mixture comprised of 2mM ONPG, 0.2M sodium acetate buffer and optimally diluted enzyme in a total volume of 1mL. The reaction was carried out at 60°C for 10min and then stopped by adding 3mL of Na₂CO₃. The amount of ONP released was determined by measuring absorbance at 405 nm in a Shimadzu UV-VIS 2401 spectrometer. One unit of enzyme activity was defined as the amount of enzyme that hydrolyzed 1 μ mol of substrate per minute under assay conditions

(Norimassa, 1995). Partial purification of β -galactosidase was carried ammonium sulphate purification method. To the crude broth, ammonium sulphate (0-60%, 60-80%) was added. The enzyme which was precipitated obtained from the above step was dialyzed extensively against 20mM acetate buffer, pH 4 using dialysis membrane (Himedia LA398) over night at 4°C. It was further dialysed under FPLC using standard protein compounds like Cytochrome c from Horse heart, Carbonic anhydrase from bovine erythrocytes, Albumin bovine serum, Alcohol dehydrogenase from yeast, B-amylase from sweet potato, Blue dextran, acetate buffer (pH 4). Sephadex g- 100 gel and the recommended sample volume being less than 2% of the total gel bed volume was used. The sample was carefully added to the column (avoiding disturbing the gel bed surface) to determine V_0 and to check column packing. Immediately after applying the sample, fractions were collected of 0.5-1.5% of the total bed volume. The elution volume was spectrometrically determined under 280nm and the volume of effluent was measured after collecting from the point of sample application to the center of the effluent peak.

3. RESULT AND DISCUSSION

Enzyme β -galactosidase was produced in SSF in a 3:1 combination between gingelly and groundnut cakes. The enzyme activities were measured under free and immobilized states. It was partially purified using ammonium sulphate precipitation and the pellets were concentrated using membrane dialysis. The molecular size was measured by using FPLC and the peak was observed. The resultant molecular weight of β -galactosidase was measured which was around 120 KDa by using standard graph.



4. CONCLUSION

The optimization studies using one factor at a time (OFAT) for production of β -galactosidase revealed the optimization conditions as: Media- gingelly oil cake (7.5g) and ground nut oil cake (2.5g); humidity – 60%; inoculum- 1.0 ml; pH-4.0; incubation temperature -30°C; Culturing period-96hrs under optimized conditions the enzyme with specific activity 0.26U/mg was obtained. Partial purification of the enzyme by ammonium sulphate and dialysis was done which resulted in an yield of 43.5% at 0-60% concentration and 22.9% at 60-80% concentration of ammonium sulphate and also made to run under gel filtration chromatography and had resulted to a double peak formation under FPLC (Fast Performance Liquid Chromatography) and molecular weight of enzyme was measured to be 120kDa

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