Research Article

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

M. R. K. Rao*, T. V. Rishikesh

ABSTRACT

Objective: β-galactosidase (E. C.3.1.2.23) otherwise known as lactase plays a vital role in food processing and pharmaceutical industries. The present study deals with the production of one enzyme β-galactosidase which could be useful for industrial implementation. This enzyme was produced from *Aspergillus foetidus* MTCC 6322 using solid-state fermentation. **Materials and Methods:** One strain of *A. foetidus* was isolated from tannery sludge and maintained at 4°C in Czapek-Dox agar slants. 100 µL of the spore suspension containing 6.0 × 10^7 CFU/ml was used as the seed inoculums. Fermentation was carried out in 250 mL Erlenmeyer flasks. 10 g of substrate in 250 mL Erlenmeyer flasks was moistened with 15.0 mL of distilled water and sterilized. After cooling, 1000 µL of *A. foetidus* spore suspension was added to the substrate and mixed thoroughly. They were incubated at 30°C for 96 h. Production of β-galactosidase was standardized by one-factor-at-a-time method. **Results:** The maximum amount of enzyme activity of 83.40/mg was observed when gingelly oil cake (7.5 g) and groundnut oil cake (2.5 g) containing 60% moisture and 1.5 ml of inoculums suspension were used at temperature 30°C and pH 4.0, respectively, after 96 h of incubation. **Conclusions:** Thus, it is clear that this process of obtaining the enzyme is feasible and it is suggested that this process can be implemented by the industry.

KEY WORDS: Agar, *Aspergillus foetidus*, Inoculation, One-factor-at-a-time, Solid-state fermentation, β-galactosidase

INTRODUCTION

Lactose intolerance is mainly caused due to the insufficient or lack of production of β-galactosidase in the human intestine which helps in lactose metabolism. Awan *et al.* reported the production of β-galactosidase using various sources of nitrogen by solid-state fermentation.[1] *Aspergillus* and its DG (2-Deoxy-d-Glucose) resistant mutant were grown in culture media with initial pH of 5.5 and 30°C for 144 h, and the samples were collected at every 24 h to find the substrate consumption, cell mass formation, and enzyme production. All the five nitrogen sources 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, were obtained from sample in which corn steep liquor was used as a nitrogen source as compared to control (73.1 and 176.3u/l/h in parent and mutant, respectively).

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Microbial β-galactosidase enzyme has a great significance due to its use as biosensors, in the processing of milk in dairy industry and ethanol production. Marrakchi *et al.* used novel biosensor using the enzymatic activities of β-galactosidase and glucose oxidase for the quantitative deduction of lactose in commercial milk samples.[2] Panesar *et al*. have reported the experiments to overcome the problem of enzyme extraction and poor permeability of cell membrane to lactose.[3] Domingues *et al.* have worked on the constant production of β-galactosidase and ethanol by recombinant flocculating *Saccharomyces cerevisiae*.[4]

MATERIALS AND METHODS

A strain of *Aspergillus foetidus* was isolated from tannery sludge by an enrichment culture method and maintained at 4°C in Czapek-Dox agar slants. 100 µL of the spore suspension containing 6.0 × 10^7 CFU/ml was used as the seed inoculums. Fermentation was carried out in 250 mL Erlenmeyer flasks. 10 g of substrate in 250 mL Erlenmeyer flasks was moistened with 15.0 mL of distilled water and sterilized. After cooling, 1000 µL of *A. foetidus* spore suspension was added to the substrate

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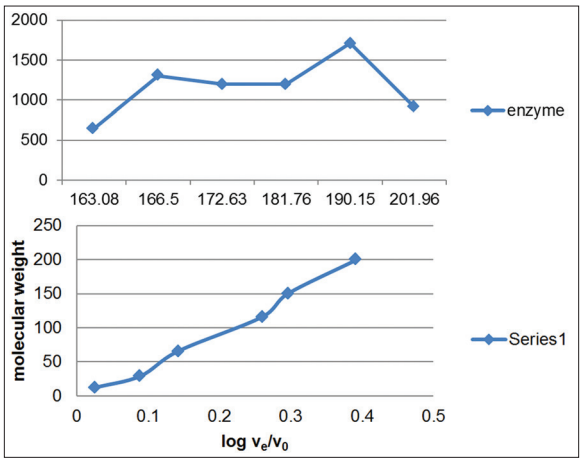

Figure 1: The enzyme activity curves

and mixed thoroughly. They were incubated at 30°C for 96 h. Production of β-galactosidase was standardized by one-factor-at-a-time (OFAT) method. The effects of different substrates, namely, wheat bran, groundnut oil cake, gingelly oil cake, and coconut oil cake were carried out for optimization of β-galactosidase production. 1 g of moldy bran was mixed with 10 ml of 20 mM acetate buffer, pH 4.0, and centrifuged at 10,000 rpm for 10 min, and the mixture was filtered and enzyme extract was used for β-galactosidase assay. 3% of sodium alginate and 10 ml of the crude enzyme solution were mixed well in a magnetic stirrer for a time period of 20–30 min, and the solution was added dropwise through syringe to 0.2 M calcium chloride of 10 ml volume to form calcium alginate beads and was kept for hardening for 3 h and the beads were washed and stored in 50 mM of calcium chloride. The β-galactosidase beads were used for further assay. The β-galactosidase activity was determined by hydrolysis of ortho-Nitrophenyl-β-galactoside (ONPG). The incubation mixture comprised 2 mM ONPG, 0.2 M sodium acetate buffer, and optimally diluted enzyme in a total volume of 1 mL. The reaction was allowed for 10 min at 60°C and then stopped by adding 3 mL of Na_2CO_3. The amount of ONPG released was measured at absorbance of 405 nm in a spectrophotometer. One unit of enzyme activity was defined as the amount of enzyme that hydrolyzed 1 µmol of substrate per minute under assay conditions.[5] Partial purification of β-galactosidase was carried by ammonium sulfate purification method. To the crude broth, ammonium sulfate (0–60% and 60–80%) was added. The enzyme collected by was dialyzed extensively against 20 mM acetate buffer at pH 4 using dialysis membrane (HiMedia LA398) over night at 4°C. It was further dialyzed under fast performance liquid chromatography (FPLC) using standard protein compounds such as Cytochrome c. The sample was carefully added to the column (avoiding disturbing the gel bed surface) to determine V_o. Immediately after applying the sample, fractions were collected 0.5–1.5% of the total bed volume. The elution volume was spectrophotometrically determined under 280 nm.

RESULTS 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 sulfate precipitation and the pellets were concentrated using membrane dialysis. The molecular size was measured using FPLC and the peak was observed. The resultant molecular weight of β-galactosidase was measured which was around 120 kDa using standard graph.

CONCLUSIONS

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

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