ISSN: 0974-2115
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

B- Galactosidase Optimization Using Response Surface Methodology

M. R. K. Rao*, S. Ananda Narasimhan
Dept of In. Bio-Tech, Biher, 173, Agaram Road, Chennai-73
***Corresponding author: E-Mail:rao_mrk@gmail.com**

ABSTRACT

Lactatase or β-D-galactoside galactohydrolase is an enzyme which breaks down talyzes lactose to monosaccharides, glucose and galactose. They are naturally available and are produced by many microorganisms such as Bacteria, Fungi and Yeast. A loop of inoculum was used for inoculating the culture medium and was incubated at pH 4 and temperature 30°C at 125 rpm. The enzyme activity was checked for at 540 nm in DNS assay, and then the optimized enzyme was partial purified by Ammonium Suphate Precipitation. The concentrated enzyme was dialyzed and purified and the Molecular weight of the enzyme was measured using HPLC (High Performance Liquid Chromatography). Response Surface Methodology showed Good significance in the optimization of Culture medium parameters. In the present study it was found that the extracellular β-galactosidase production by the *Lactobacillus fermentum* in a submerged skim milk broth and the maximum amount of enzyme activity of 7.99U/mL/min was obtained by optimizing the culture medium of its Carbon and Nitrogen source.

KEY WORDS: B- Galactosidase, HPLC, DNS.

1. INTRODUCTION

Lactose intolerance, in other words inability to hydrolyze lactose is a problem prevalent in more than half the world population (Vasiljevic and Jelen, 2001). This intolerance is caused due the non availability of lactase enzyme. Thus Lactase enzyme is of great importance in milk related industry (Domingues, 2005, Montanari, 2000, Voget, 1994). Patients with this deficiency suffer with gastritis, bloating, indigestion and abdominal pain. The absence of β-galactosidase can be described as congenital, primary or secondary deficiency.

Lactic acid bacteria were known to synthesis β-galactosidase for the hydrolysis of lactose and to use them for their growth and these bacteria produces β-galactosidase inside their cell as it is an intracellular enzyme. It is also know to produce, in trace amounts, extracellular β-galactosidase. In the present work we have attempted to optimize the extracellular β-galactosidase production by *Lactobacilli fermentum* strain extracted from curd and cultured in Skim Milk broth.

In order to increase the production of this enzyme Siddique, (2010) experimented with five nitrogen substrates under slid state fermentation process. *A. niger* and its resistant mutant strain were grown in media with at pH 5.5, temperature 30°c for 144h and samples was collected at every 24h to find yhe parameters like substrate consumption, cell mass formation and enzyme production. Although sources such as ammonium sulphate, corn steep liquor, diammonium phosphate, fish meal and urea showed significant results enzyme activities of 168.0 and 371.15 µ/L/h were obtained in parent and mutant strains, respectively when 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).

In the present study the production of extracellular β-galactosidase from *Lactobacillus fermentum* strain extracted from curd. The study also included measuring the level of activity of enzyme by varying the optimal level using RM analysis and to measure the Molecular weight of the enzyme using HPLC technique.

2. MATERIALS AND METHODS

A strain of *Lactobacillus fermentum was* isolated from home made curd by an enrichment culture method in APT7302 agar on petri plates and maintained at 4°C. The inoculums are sub cultured in skim milk broth. 100µL of the suspension containing 10-30 CFU/ml was used as the seed inoculums. 2.8 gms of skim milk, 0.5gms of casein enzyme hydrolysate, 0.2gms yeast extract and dextrose in 250mL were added with 50mL distilled water in Erlenmeyer flasks and sterilized. After cooling, a loop containing *Lactobacillus fermentum* was streaked from the incubated inoculum on APT agar plate and mixed thoroughly. The incubation was done at 30°C for 48hrs in 125rpm in 50ml of seed medium. The activity of the enzyme is measured by using hydrolysis of lactose by using DNS method and one unit of glucose released was measured as equivalent to one unit of β-galctosidase that hydrolyze lactose.

To know the concentration of enzyme Folin-caocalteau raction was used. The blue colour was measured at 660 nm in a Shimadzu UV-VIS 2401 spectrometer and protein concentration is measured by Lowry's method.

Partial purification of the enzyme obtained was carried out by ammonium sulphate purification process. The crude enzyme collected after homogenization and to it ammonium sulphate (0-80%) saturation was added and equilibrated for 1hr at 4° and then the mixture was spinned at 10,000 rpm for 10 min and resultant precipitate containing the concentrated enzyme was suspended in 20mM acetate buffer at pH 4 and analyzed for β-galactosidase activity. The precipitate of the enzyme formed by ammonium sulfate precipitation was dialyzed extensively against 20mM acetate buffer, pH 4 using dialysis membrane (Himedia LA398) over night at 4°C and was repeatedly changed

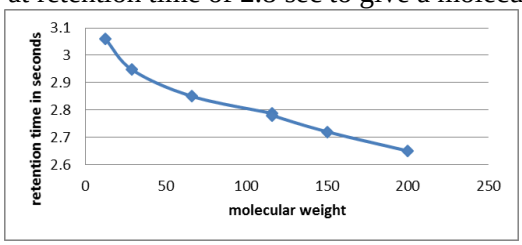
for new buffer to optimize the purification process. The resulting compound composes of pure enzyme concentration which was further assayed.

Response surface Method (RSM) was conducted to optimize the variables for increased lactase production. This method consists of three different sequences like, performing statistically designing, estimating the coefficients mathematically and finally understanding the response to check the validity of the model. Central composite design (CCD) under the response surface methodology (RSM) developed by the Design Expert software (Version 6.0.8, Stat-Ease Inc., Minneapolis, USA) was used to optimize the concentrations of the three significant factors namely Yeast extract, Casein and Fructose in the inoculums, to find a set of 20 experimental runs with six replicated center points. The independent variables were studied at three different levels, low (−1) medium (0) and high (+1). In the experimental design, Y is the dependent variable (Lactase production), X1, X2and X3 are independent variables (Casein, Fructose and Yeast extract concentrations), β0 is an intercept term, β1,β2, and β3 are linear coefficients; β12, β13,andβ14 are the interaction coefficients, and β11, β22 and β33 are the quadratic coefficients. ANOVA model was used for statistical calculations.

To purify β-galactosidase, to measure its MW, analytical Shimadzu C-18 column composed of an JASCO MD-2013Atvp pump and an PDA-10Avp detector was used. The mobile phase was 80% acetonitrile in water and the running time of 15min. Absorbance was measured at 220 nm. The measurement of the area of the new peak of the various molecular markers after hydrolysis of the enzyme allowed us to confirm the conversion yield. About 100µg of purified β-galactosidase was fed for HPLC analysis after the peaks of the molecular markers were analyzed. Run time was varied for each run based on the specifications. And for each marker their specific Molecular weight determines the Retention Time of the various peaks.

3. RESULT AND DISCUSSION

β-galactosidase enzyme was produced under Response Surface Methodology by varying three factors fructose which is the carbon source, casein and yeast extract which is the nitrogen source and the three factors were cumulatively optimized by using RSM. And the significance of the compounds were shown as 3D graph model. And the activity level of β-galactosidase has been significantly improved. And the molecular weight of the purified enzyme was measured using HPLC at retention time of 2.8 sec to give a molecular weight of 116 kDa.



4. CONCLUSION

The characterization studies on crude enzyme as well as immobilized enzyme showed that the maximum activity was at pH 4.0 and temperature at 30°C. The enzyme was stable in acidic pH range and a temperature range of 30-60°C. The enzyme was stable against various carbon sources and also showed increase in activity in the presence of Fructose. These features are desirable for this enzyme preparation to be used in various food processing and pharmaceutical application.

Partial purification of the enzyme by ammonium sulfate and dialysis was done which resulted in a yield of 43.5% at 0-80% concentration ammonium sulfate and also made to run under High Performance Liquid Chromatography and had resulted to a peak formation under a retention time of 2.76 seconds.

The application of enzyme was studied using hydrolysis of lactose. The hydrolysis of lactose finds major role in food processing industry (to improve the texture and sweetness of dairy production) and pharmaceutical industry (to treat lactose intolerant people). The optimum condition for the enzyme from *Lactobacilli fermentum* to hydrolyze lactose were: pH-4.0; temperature- 30°C; lactose concentration- 0.5%; reaction time- 60min.

In conclusion the enzyme β-galactosidase from *Lactobacilli fermentum* exhibits characteristic features that will enhance its scope of utility in wide industrial applications.

REFERENCES

Caroline M.L, Vasudevan S, Growth and characterization of bis thiourea cadmium iodide, A semiorganic single crystal, Materials Chemistry and Physics, 113 (42038), 2009, 670-674.

Domingues L, Lima N, Teixeira J. A, *Aspergillus niger* β-galactosidase production by yeast in a continuous high density reactor,Process Biochem, 40, 1151-1154.

Kerana Hanirex D, Kaliyamurthie K.P, Multi-classification approach for detecting thyroid attacks, International Journal of Pharma and Bio Sciences, 4(3), 2013, B1246-B1251.

Kumaravel A, Meetei O.N, An application of non-uniform cellular automata for efficient cryptography, Indian Journal of Science and Technology, 6(5), 2013, 4560-4566.

Langeswaran K, Gowthamkumar S, Vijayaprakash S, Revathy R, Balasubramanian M.P, Influence of limonin on Wnt signalling molecule in HepG2 cell lines, Journal of Natural Science, Biology and Medicine, 4(1), 2013, 126-133.

Leena Sankari S, Masthan K.M.K, Aravindha Babu N, Bhattacharjee T, Elumalai M, Apoptosis in cancer - an update, Asian Pacific Journal of Cancer Prevention, 13 (10), 2012, 4873-4878.

Lydia Caroline M, Vasudevan S, Growth and characterization of l-phenylalanine nitric acid, a new organic nonlinear optical material, Materials Letters, 63 (1), 2009, 41-44.

Miller G, Jarvis J and McBean L.D, Lactose Intolerance. In, Handbook of Dairy Foods and Nutrution. CRC Press, Boca Raton FL, 2005, 187-220.

Nagarajan C, Madheswaran M, Experimental study and steady state stability analysis of CLL-T series parallel resonant converter with fuzzy controller using state space analysis, Iranian Journal of Electrical and Electronic Engineering, 8(3), 2012, 259-267.

Parthasarathy R, Ilavarasan R, Karrunakaran C.M, Antidiabetic activity of Thespesia Populnea bark and leaf extract against streptozotocin induced diabetic rats, International Journal of PharmTech Research, 1(4), 2009, 1069-1072.

Saravanan T, Saritha G, Udayakumar R, A Robust H-infinity two degree of freedom control for electromagnetic suspension system, Middle - East Journal of Scientific Research, 18 (12), 2013, 1827-1831.

Saravanan T, Srinivasan V, Sandiya V.P, A two stage DC-DC converter with isolation for renewable energy applications, Indian Journal of Science and Technology, 6 (6), 2013, 4824-4830.

Siddique Awan S, Shahzad Akbar Khan Z.U, Rehman, Azhar Saleem, Rana S.M and Rajoka M.I, Influence of nitrogen source on the production of β-galactosidase by *Asprergillus niger*, African Journal of Biotechnology, 9 (20), 2010, 2918-2922.

Srinivasan V, Saravanan T, Analysis of harmonic at educational division using C.A. 8332, Middle - East Journal of Scientific Research, 16 (12), 2013, 1768-1773.

Srinivasan V, Saravanan T, Udayakumar R, Specific absorption rate in the cell phone user's head, Middle - East Journal of Scientific Research, 16 (12), 2013, 1748-1750.

Udayakumar R, Khanaa V, Kaliyamurthie K.P, High data rate for coherent optical wired communication using DSP, Indian Journal of Science and Technology, 6(6), 2013, 4772-4776.

Udayakumar R, Khanaa V, Saravanan T, Analysis of polarization mode dispersion in fibers and its mitigation using an optical compensation technique, Indian Journal of Science and Technology, 6(6), 2013, 4767-4771.

Udayakumar R, Khanaa V, Saravanan T, Chromatic dispersion compensation in optical fiber communication system and its simulation, Indian Journal of Science and Technology, 6(6), 2013, 4762-4766.

Vasiljevic T, Jelen P, Lactose hydrolysis in milk as affected by neutralizers used for the preparation of crude β-galactosidase extracts from *Lactobacillus bulgaricus*11842, Innovative Food Science & Emerging Technologies, 3, 2002, 175-184.

Zadow J.G, Lactose hydrolyzed dairy products, *Food Technol. Aust*, 38, 1986, 460-462.