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Annals of Biological Research, 2012, 3 (4):1712-1718
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

Production and Optimization of β-galactosidase by *Bacillus* Sp. MPTK 121, Isolated from Dairy Plant Soil

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ABSTRACT

Studies on the β-galactosidase production were carried out with a bacterial strain isolated from the soil sample near dairy processing plant. The production of extracellular β-galactosidase by Bacillus sp. was optimized in a submerged fermentation. The effect of incubation period, temperature and pH of the medium were optimized. The production of the enzyme was found to be high at the 48th hour after inoculation at 30°C and pH 7. Of the metal ions used, $MgCl_2$ was found to favor the enzyme production. Under optimal conditions, β-galactosidase was allowed to produce by the isolated Bacillus Sp. strain. The enzyme was purified using and the purified β-galactosidase was homogeneous with the molecular weight of 70 kDa as shown by SDS PAGE analysis.

Keywords: X-Gal, ONPG, Optimization, Ion exchange chromatography, *Bacillus* Sp.

INTRODUCTION

Beta-galactosidase s are the group of enzymes able to cleave β linked galactose residues from various compounds and is commonly used to cleave lactose into galactose and glucose **[1, 2]**. The enzymatic hydrolysis of lactose is one of the most important processes for food, dairy industry. Beta-galactosidase s preparations were used hydrolysis of lactose milk, milk products and whey **[3]**.

There are several advantages embodied in lactose hydrolysis: rapid fermentation of glucose, a higher degree of sweetness of the liquid in which lactose has been hydrolyzed which leads to the development of novel products and the production of new sweeteners **[4]**. On the other hand, the transglycosylation activity has been used to synthesize galacto-oligosaccharides (GOS), have been used in human nutrition in significant quantities as active components or as side products of processed milk or milk products **[5]**.

The activity and stability of enzymes is influenced by the type of strain, cultivation conditions (temperature, pH, aeration, agitation, incubation time) and the growth medium composition (particularly carbon and nitrogen sources). Hence the culture conditions and media components for the production of β-galactosidase using native bacteria from the soil sample collected near milk processing area were optimized.

MATERIALS AND METHODS

Strain isolation and screening
The organisms were isolated from soil sample collected near milk processing area (Aavin dairy plant, Chennai, TN, India) in a sterile polythene bag and precautions were taken to minimize the contamination. The strains showing blue colonies on Tryptic Soy Agar plates (g/l) (Tryptone-15, Soytone-5, NaCl-5, Agar-15) **[6]** with 0.5% lactose as the sole carbon source and 0.1% of 5-bromo-4-chloro-3-indolyl-β-d-galactopyranoside (X-gal) were collected and streaked separate onto NA medium. Once the isolates were purified, the strain showing high enzyme activity was selected and used for further investigation.

Characterization of organism
The morphological and taxonomic characteristics of the strain like grams reaction, spore formation and motility test were examined **[7, 8]**.

Identification of bacteria using 16S rDNA sequencing
The organism was identified by analysis of its 16S rRNA gene **[9]**. The DNA from the bacteria was isolated and the 16S rDNA was amplified using universal primers (8–27F 5' GTTTGATCCTGGCTCAG 3' and 1492 5'-GGTTACCTTGTTACGACTT 3'). The sequence similarity within the Genbank database was searched using the Basic Local Alignment Search Tool (BLASTN) **[10]**.

Enzyme production
In a 250ml Erlenmeyer flask containing 100 ml of the liquid production medium (g/L): lactose, 10; meat extract, 15; biopeptone, 5; yeast extract, 0.5; and sodium chloride, 1.5 **[11]**. 1ml of 18h broth culture of the organism was inoculated and incubated for 3days at 37°C under shaken condition. Crude enzyme preparation was obtained from the supernatant after centrifugation of the broth culture at 5000g for 20mins **[12]**.

Enzyme assay
Two hundred microlitre of crude enzyme was added with 0.1 M phosphate buffer and 500 µl of 6 mM ONPG (O-Nitrophenyl-β-D-Galactopyrinoside) and the reaction mixture was incubated for 30 minutes at 37°C. The reaction was ended by adding 0.5 ml of 1 M Na_2CO_3. The amount of ONP (O-Nitrophenol) released from ONPG was determined by taking the OD at 430 nm. One unit of β-galactosidase activity (U) was defined as the amount of enzyme that liberates 1 µm ONP per minute under assay conditions **[12]**.

Biomass yield
Bacteria biomass was determined by measuring the absorbance at 600nm **[13]**.

Effect of Incubation period, Temperature, and pH on enzyme production
The effect of incubation period, temperature and pH on β-galactosidase production was investigated by cultivating the organism at different incubation time (6–120 hours) and different temperatures (20-40) and various pH (6.0-8.0).The organism was incubated, the β-galactosidase activity was determined in supernatant.

Role of Carbon and Nitrogen sources:
Carbon sources such as glucose, xylose, maltose, starch, sucrose were tested at 1% (w/v) concentration. Nitrogen sources like peptone, beef extract, sodium carbonate, ammonium sulphate and ammonium chloride were also tested at 1% (w/v) concentration.

Effect of metal ions
The effect of metal salts on β-galactosidase production is determined by adding different metal salts in the fermentation medium. The metal salts selected for present study were $FeSO_4$, $MnSO_4$, $CuSO_4$, ZnSO4 $MgCl_2$ and EDTA at 0.1% concentration **[12]**.

Purification of enzyme
The pH of the crude enzyme was adjusted to 5.0 and with the help of 1M $ZnCl_2$, the protein contents were precipitated and separated using centrifugation. The precipitate was dissolved in 0.5 M EDTA and dialysed against 0.1 M Phosphate buffer (pH 7.5). The dialysed sample was run on anion exchange chromatography (Q-Sepharose) and eluded with the linear gradient in 0.1 M phosphate buffer at pH 7.5. 5 ml fractions were collected at the flow

rate of 1 ml/min. The fractions with maximum enzyme activity was concentrated and run on Sephadox-200 column (1.5 X 45 cm). The elution rate was adjusted to 0.5 ml/min and 1 ml fractions were collected and stored at 4°C **[11]**.

Characterization of purified β-galactosidase

The protein profile and the presence of purified β-galactosidase were confirmed by SDS PAGE analysis. Denatured Sodium Dodecyl Sulphate−polyacrylamide gel electrophoresis was performed with marker as described in Nakkharat and Haltrich **[14]**. Coomassie brilliant blue staining was used for the visualization of the protein bands.

RESULTS AND DISCUSSION

Beta-galactosidase has tremendous potential in research and application in various fields like food, bioremediation, biosensor, diagnosis and treatment of disorders. The enzyme β-galactosidase has two main biotechnological applications in milk and dairy industries, e.g. the removal of lactose from milk for lactose-intolerant people and the production of galacto-oligosaccharides (GOS) for use in probiotic food **[15]**. The present study aimed at the isolation, production and characterization of β-galactosidase producing bacteria from soil near milk processing plant. In the screening β-galactosidase production with the help of X-Gal, out of 29 bacteria isolated 13 strains shows positive β-galactosidase. Of that best producing strain was chosen and analyzed further. A large number of bacteria can produce β-galactosidases and only few were reported as safer sources **[12]**. Hence the isolated strain has to be characterized and identified. The isolated strain was gram positive, rod shaped and motile. Alignment of the 16S rDNA gene sequence of the isolated strain with the sequences available in the GenBank database showed the organism to have 100% identity with various *Bacillus* Spp. (e.g. JQ248587, JN990427). Hence, the strain was confirmed as *Bacillus* Sp. and sequence has been submitted to the Genbank (JQ6693396). The species level identification of the strain was analysed further. *Bacillus* spp. are the chief bacterial population of the microflora that are utilized in food and milk industries **[16]**. 16S rDNA sequencing was found to be most helpful in identifying *Bacillus* sp. **[17].**

According to the results taken at regular intervals, the maximum enzyme activity was obtained at 48th hour of incubation. Beyond this, the enzyme productivity was remains constant and no further increase in production was observed (Fig. 1). It might be due to decrease in the nutrient availabitity in the medium, or catalobic repression of the enzyme **[18]**.

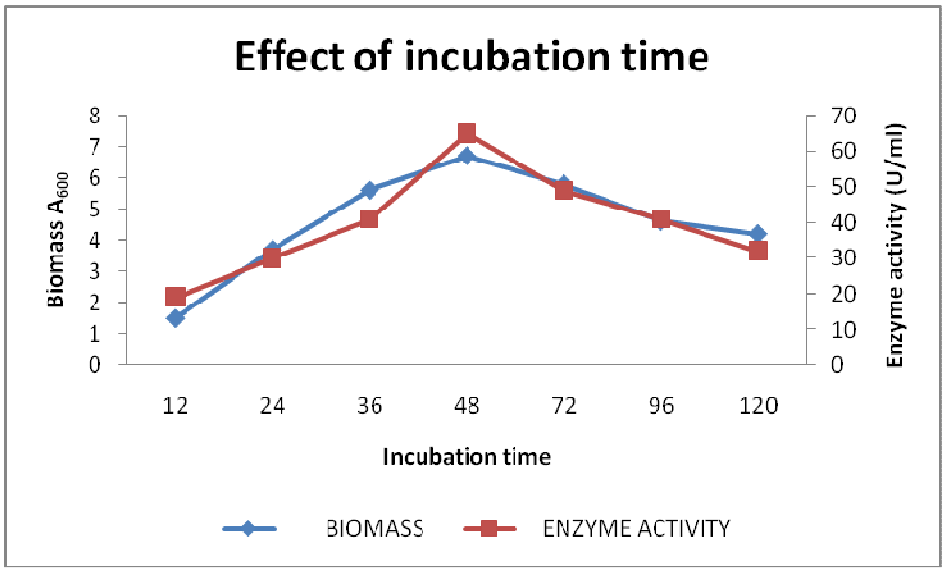

Fig. 1: Effect of incubation time on β-galactosidase production

The temperature was one of most important factor which influences the activity of metabolic enzyme. The increased enzyme activity of 62 U/ml was observed at the temperature range of 30°C and found as optimum temperature for β-

galactosidase production (Fig. 2). Similarly, the temperature ranges of 28-30°C were found as optimum for the β-galactosidase production **[19, 20]**.

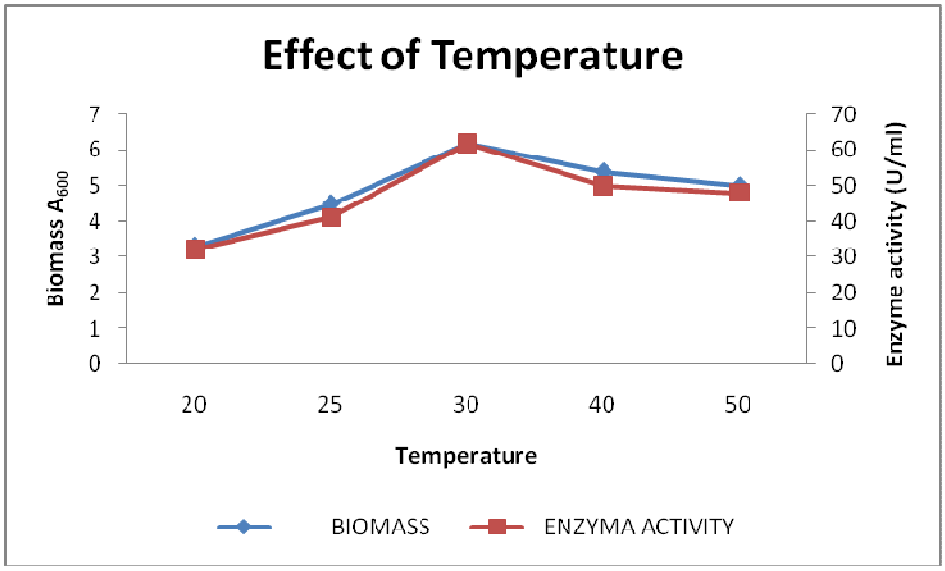

Fig. 2: Effect of temperature on β-galactosidase production

The hydrogen ion concentration of on environment has the maximum influence of the microbial growth and enzyme production. The effect of different initial pH in the β-galactosidase production was analyzed. β-galactosidase production increase concentration upto a range of 7.0 and decrease in enzyme production in respect to increase in pH was also observed (Fig. 3). pH 5.5 has been observed as optimum for the β-galactosidase production by Rajoka et al **[21]** and Hin **[22]**.

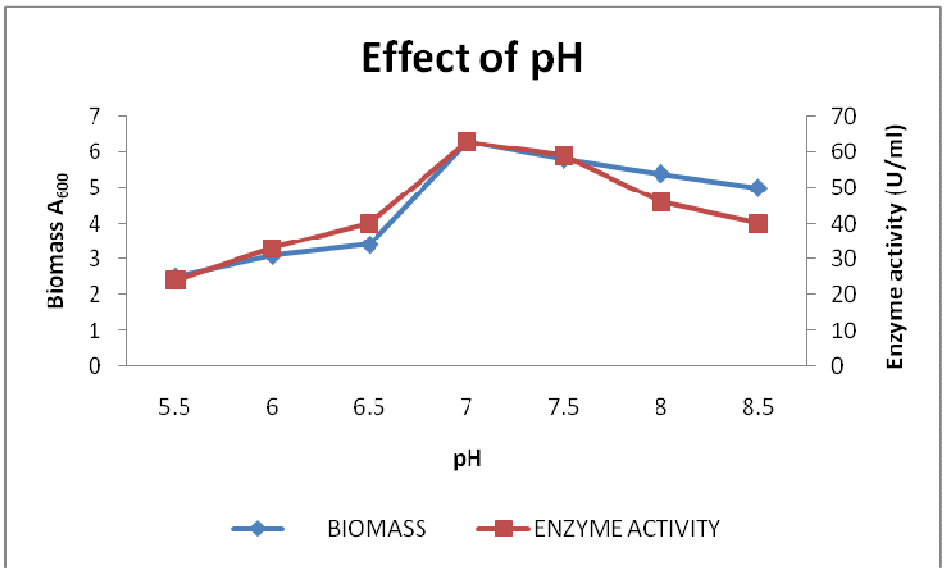

Fig. 3: Effect of pH on β-galactosidase production

Cheaper carbon and nitrogen sources are the key attraction for commercialization of the production processes and thus, ability of the microorganisms to grow and produce enzymes using these sources has been arguably a point of interest **[23]**. Selection of suitable carbon and nitrogen sources were the critical step during the enzyme optimization **[24]**.

While testing the β-galactosidase production in fermentation medium with different carbon sources and nitrogen sources, xylose (Fig. 4) and peptone (Fig. 5) were found to be the better carbon and nitrogen sources favoring maximum enzyme production. Next to them, maltose and beef extract also found to be increasing enzyme production in significant amount. Nurullah **[12]** has reported xylose and yeast extract as better carbon and nitrogen sources for β-galactosidase production for *Bacillus licheniformis*.

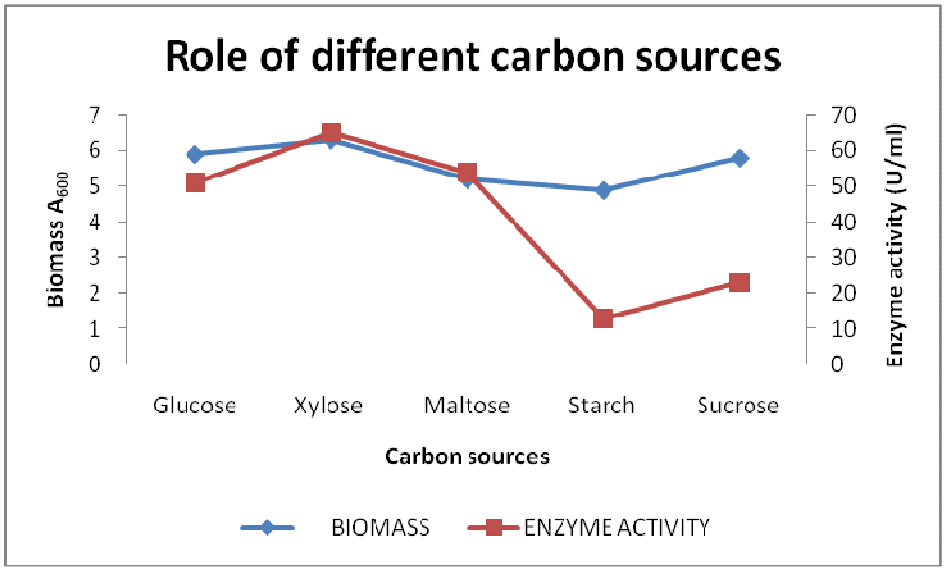

Fig. 4: Role of different carbon sources on β-galactosidase production

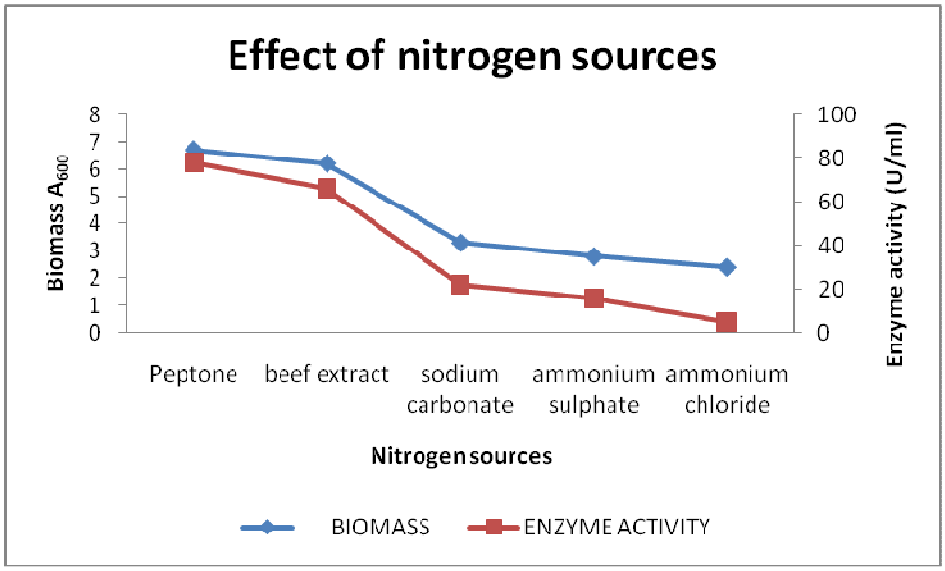

Fig. 5: Role of different nitrogen sources on β-galactosidase production

The production of β-galactosidase was increased when the production medium was supplemented with $MgCl_2$ (Fig. 6). This indicated the necessary of Mg^{2+} for the stabilization of the enzyme. The positive effect of Mg^{2+} and Mn^{2+} on β-galactosidase has been reported by Rao and Dutta **[25]**.

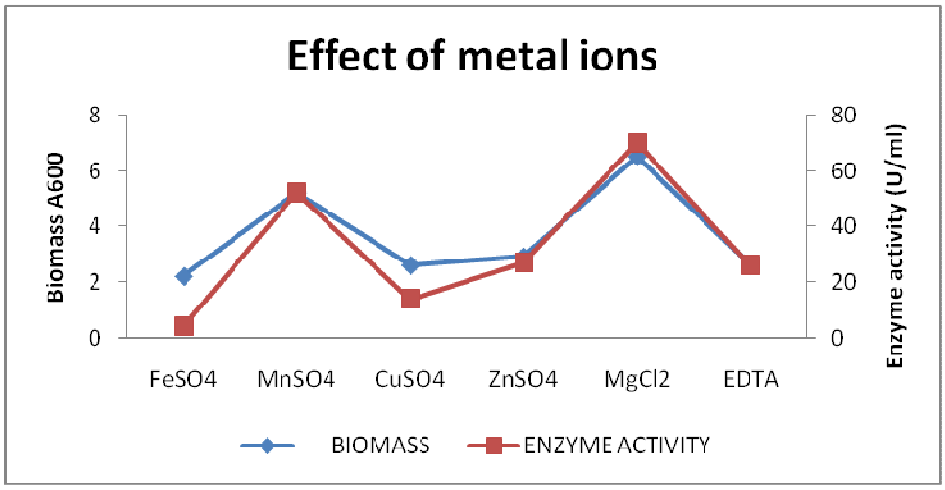

Fig. 6: Role of different metal ions on β-galactosidase production

The downstream processing was considered as the obstacle in the total production cost and hence needed a cheaper extraction and purification method **[26]**. The enzyme was purified using supherose 12 column chromatography followed by Ammonium sulphate precipitation. Sephadex G-25 and DEAE-cellulose columns can also be used for the purification of β-galactosidase **[27]**. The purified enzyme was stored at 4°C for further use.

The presence of individual band near the molecular weight of 70 kDa indicates the presence of β-galactosidase. It has been previously reported that, β-galactosidases are also having molecular weight of 75 kDa **[15]** and 67.5 kDa **[28]**.

CONCLUSION

The present study shows that there is appreciable production of extracellular β-galactosidase using the native *Bacillus* Sp. This suggests that *Bacillus* Sp. can be a potential producer of extracellular β-galactosidase which may have applications in both industry and biotechnology. Due to the importance of these findings, further studies will be done for the enhanced enzyme production.

REFERENCES

[1] P. Alliet, P. Scholtens, M. Raes, K. Hensen, H. Jongen, J.L. Rummens, G. Boehm, Y. Vandenplas, *Nutr*., **2007**, 23, 719-723.
[2] O. Juajun, PhD thesis, Suranaree University of Technology, Thail, **2009.**
[3] M.L. Richmond, J.I. Gray and C.M. Stine, *J. Dairy. Sci*., **1981,** 64, 1759-1771.
[4] A. Jokar, A. Karbassi, *J. Agric. Sci. Tech.*, **2009,** 11, 301-308.
[5] B. Stahl, Y. Zens, G. Boehm, *Compr. Glycosci*., **2007,** 4, 725-742.
[6] W.Y. Liu, Y.W. Shi, X.Q. WanG, K. Lou, *Czech J. Food Sci.*, **2008,** 26, 284–290.
[7] J.F. Mac Faddin; Media for isolation-Cultivation-identification-Manitenance of medical bacteria, Williams and Wilkins, Baltimore, MD, **1985,** 1.
[8] P.H.A. Sneath, Bergey's manual of systematic Bacteriology, Williams and Wilkins, Baltimore, **1986,** 2.
[9] B. Guieysse, M.D. Cirne, B. Mattiasson, *Appl Microbiol Biotechnol*, **2001,** 56,796–802.
[10] S. Altschul, W. Gish, W. Miller, E. Myers, D. Lipman, *J Mol Biol.*, **1990,** 215, 403–410.
[11] S. Chakraborti, R.K. Sani, U.C. Banerjee and R.C. Sobti, *Journal of Industrial Microbiology and Biotechnology***, 2000,** 24,58-63.
[12] Nurullah AKCAN, *African journal of Microbiology Research*, **2011,** 5(26), 4615-4621.
[13] C. Henroette, S. Zinebi, M.F. Aumaitre, E. Petitdemange and H. Petitdemange, *J. Industrial Microbiol*., **1993,** 12, 129-135.
[14] P. Nakkharat and D. Haltrich, *Journal of Biotechnology*, **2006,** 123, 304-313.

[15] Somyos Osiriphun and Phimchanok Jaturapiree, *Asian Journal of Food and Agro-Industry*, **2009,** 2(04), 135-143.
[16] T.K. Sundaram, *Journal of Chemical Technology and Biotechnology*, **1990,** 42, 308-313.
[17] Ammini Parvathi, Kiran Krishna, Jiya Jose, Neetha Joseph and Santha Nair, *Braz. J. Microbiol*., **2009**, 40, 269-275.
[18] A.M. Gupta and J.S. Nair, *Journal of scientific and industrial Research*, **2010,** 69, 85-859.
[19] M.A. Ku and Y.D .Hang, *Biotechnol letts*, **1992,** 14, 925-928.
[20] M.J. Artolozaga, R. Jones, A.L. Schneider, S.A. Furlan, M.F. Carvallo-Jones, *Bioseparation*, **1998,** 7, 137-143.
[21] M.J. Rojoka, S. Khan and R. Shahid, *Food Technol,Biotechnol*, **2003,** 41, 315-320.
[22] C. Hin, A. Chien, L. His, W. Yeh, C. Hsueh and F. Chin, *Applied and Environmental Microbiology.*, **1986,** 52(5), 1147-1152.
[23] R. Patel, M. Dodia, S.P. Singh, *Process Biochem.*, **2005,** 40, 3569-3575.
[24] Z. Konsoula, M. Liakopoulou-Kyriakides, *Bioresource Technol*., **2007,** 98, 150-157.
[25] R.M.V. Rao and S.M. Dutta, *Appl. Environ. Microb*., **1977,** 34, 185-188.
[26] R. Gupta, Q.K. Beg, P. Lorenz, *Appl. Microbiol. Biot.*, **2002,** 59, 15-32.
[27] S.K. Akolkar, A. Sajgure, S.S. Lele, *World J. Microb. Biot.*, **2005,** 21, 1119-1122.
[28] Kumar Shivam, Sarad Kumar Mishra, *Process Biochemistry*, **2010,** 45, 1088–1093.