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Production, Purification and Characterization of β-glucosidase from *Bacillus subtilis* strain PS Isolated from Sugarcane Bagasse

A.K. Verma*, Soniya Saini, S. Nishad, Vinod Kumar, Shalini Singh and Ashutosh Dubey

Department of Biochemistry, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar- 263 145, India.

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The effect of various carbon sources on extracellular β-glucosidase (BGL) production and its catalytic activity with different substrate was analyzed in *Bacillus subtilis* strain PS isolated from sugarcane bagasse. The highest extracellular enzyme activity was obtained in minimal medium with supplement of glucose was higher 0.42 U/ml, compared to other carbon sources such as maltose, cellobiose, lactose and xylose. Extracellular BGL was partially purified to 18.04 fold with specific activity 17.32 U/mg using Sephadex G-75 gel filtration chromatography after dialysis of 70% ammonium sulfate precipitation. In gel filteration fractions, three peaks having β-glucosidase activity and represents isozymes of β-glucosidase with molecular weight of 193 kDa, 64 kDa and 42 kDa, respectively. Purified BGL showed catalytic activity with lactose, cellobiose, pNPG and mellibiose.

Key words: β-Glucosidase production; Gel filteration; Carbon source; *Bacillus subtilis*.

Lignocellulosic biomass is a low cost substrate for ethanol production by chemical or biological process (Lynd *et al.,* 2002). Conversion of lignocellulose fraction to sugar monomers is time consuming and expensive (Prior and Day 2008). Biological conversion of cellulose to ethanol require endo-glucanase (endo-1,4-β–glucanase, EC 3.2.1.4), exo-glucanase or cellobio-hydrolase (exo-1,4-β-glucanase, EC 3.2.1.91) and β-glucosidase (β-D-glucoside glucohydrolase (EC 3.2.1.21). Cellobiose competitively inhibits the first two enzymes. This can be reverted by β-glucosidase (BGL) which converts cellobiose into glucose that in turn inhibits BGL (Woodward 1994; Gadgil *et al.,* 1995). This effect could be obviated when glucose is fermented into ethanol using simultaneous saccharification and fermentation process. In this process, enzyme production is still the most crucial and costly step (Wayman 1992). An efficient cellobiose hydrolysis requires a large amount of BGL for commercial utilization of cellulosic residues but until now, the saccharification yields are below economic viability. Beside the ethanol production BGLs are also the key enzymes in flavor industry to enhance the flavor in fruit juice and improve the organoleptic properties of citrus fruits and juices in which bitterness is in part due to a glucosidic compound Naringin, whose hydrolysis requires in succession, α-rhamnosidase and BGL (Riou *et al.,* 1998).

BGL from fungal has higher molecular weight from 60 kDa to 450 kDa while bacterial sources have lower molecular weight from 24 kDa to 200 kDa. The β-glucosidases isolated from various sources shows different temperature

* To whom all correspondence should be addressed. Tel.:+91-05944-2233310; Fax.+91-05944-233473; E-mail: akv72@rediffmail.com

optima in the range of 40°C - 70°C (Painbeni *et al.,* 1992; Watanabe *et al.,* 1992; Christakopoulos 1994; Yoon *et al.,* 2008). The advantages of production of temperature resistant BGL in conventional system includes, reduced operating costs with respect to maintaining growth temperature in large-scale systems, increase rate of productivity and ease to recover the product, particularly at the later stages in batch and fed batch reactor systems (Nolan *et al.,* 1994). Other advantages may include biocatalyst recycling and rapid product separation (Banat *et al.,* 1996). The major bottlenecks for industrial application of BGL are their hydrolytic efficiency and the high cost of enzyme production (Gusakov *et al.,* 2007). Substantial cost reduction can result from choosing microorganisms with high rates of enzyme production, which can be grown on cheap and easily available substrates. Indeed, several studies indicate that the carbon source is one of the most important factors affecting the production cost and yield of BGL (Gao *et al.,* 2008). Hence recently we have isolated and characterized thermostable extracellular BGL producing *Bacillus subtilis* strain PS from sugarcane bagasse (Shah 2008). Present study was carried out to determine effect of various carbon sources on thermostable BGL production followed by purification and characterization from *Bacillus* strain PS.

MATERIALS AND METHODS

Chemicals and Bacterial strain

All chemicals, media of analytical grade and obtained from Sigma chemicals Ltd., HiMedia Laboratories Ltd., GeNei, SRL, Glaxo, Merck Pvt. Ltd. Bacterial isolate *Bacillus subtilis* strain PS was previously isolated from bagasse in our laboratory and identified using 16S rDNA sequence analysis.

Bacterial growth and BGL production

The bacterial growth and enzyme production was achieved using minimal media described by Kumar *et al.,* (2007). The composition of media (g/l in distilled water) was: K_2HPO_4 - 5.8, KH_2PO_4 - 4.5, $(NH_4)_2SO_4$ - 2.0, $MgSO_4$ - 0.16, Na_2MoO_4 - 0.002, $CaCl_2$ - 0.02, $FeSO_4$ - 0.001, $MnCl_2$ - 0.001. For determining effect of carbon source on bacterial growth and enzyme production, media was supplemented with different carbon sources (glucose, maltose, cellobiose, lactose, and xylose). Production media inoculated with 1% primary culture was kept at 37°C, 120 rpm for 28 h in incubator shaker. Crude enzyme was obtained by centrifugation of bacterial culture for 10 min at 10000 rpm, 4°C.

Enzyme assay

β-Glucosidase activity was determined spectrophotometrically according to the method of Jager et al (2001). The reaction mixture containing 500 µl of β-glucosidase as culture supernatant and 500 µl of 10 mM *p*NPG in 100mM sodium acetate buffer, pH 5.0, was incubated for 30 min at 60°C and then the reaction was stopped with 1ml of 1.0 mol/L sodium carbonate solution. The absorbance was measured at λ_{405} nm. One unit (U) of *p*NPG hydrolyzing activity was defined as the amount of enzyme required to liberate 1 µmol p-nitrophenol per min under the assay conditions.

Protein estimation

Bradford method was used for measuring the total protein by using bovine serum albumin (BSA) as the standard (Bradford 1976). The protein concentration was estimated by observing the absorbance at λ_{660} nm.

Purification of β-glucosidase

Crude enzyme preparation was subjected to ammonium sulfate precipitation to obtain different saturation levels (10-80%). Precipitated fractions were dissolved in small quantity of acetate buffer (100mM, pH 5.0). β-Glucosidase activity was determined in different fractions and dialysis was performed to concentrate the pooled fractions having significant β-glucosidase activity. The concentrated enzyme solution was applied on Sephadex G75 gel filtration column equilibrated with 100 mM sodium acetate buffer, pH 5.0 and a flow rate of 3 ml/min. The protein was eluted with the same buffer.

Characterization of partially purified enzyme

The standard molecular weight marker proteins Catalase (240 kDa), Bovine Serum Albumin (66 kDa), Ovalbumin (43 kDa) and Lysozyme (14.3 kDa) were used for the determination of molecular weight using gel filteration chromatography.

The partially purified BGL through gel filtration chromatography was characterized on 10% Native polyacrylamide gel electrophoresis (PAGE). Purity of enzyme was checked using Native-PAGE (Laemmli 1970). The gel was run under constant voltage at 100 V and stained in

Coomassie brilliant blue R 250 (Manniatis 1989).

Substrate specificity

BGL activity for different disaccharides (10mM) i.e., Cellobiose, Mellibiose, Lactose, Sucrose, Xylan and pNPG was determined by glucose test kit, glucose oxidase-peroxidase (GOD-POD) method procured from Autospan Company (Watanabe *et al.,* 1992). Reaction mixture was incubated at 50°C for 30 min. Absorbance was taken at λ_{505} nm.

The glucose content was calculated as per following formula:

Glucose (mg/dl) = Absorbance of test x 100 / Absorbance of standard

Enzyme activity (U/ml) was calculated as per following formula:

$$\text{Enzyme activity}\,(\text{U/ml}) = \frac{\text{Glucose liberated}\ (\text{mg/ml})\ \times \text{dilution factor}\ \times 10^3}{180\times 30\times 20}$$

RESULTS

Bacterial growth and production of BGL

Bacterial growth study reveals that *Bacillus subtilis* strain PS showed maximum growth when glucose served as carbon source in minimal medium in comparison to other carbon sources such as maltose, lactose, cellobiose and xylose (Fig. 1). In the presence of xylose and lactose, bacterial isolate PS showed almost same growth rate. The growth of *Bacillus* strain PS was in order of glucose> maltose> cellobiose> lactose> xylose.

An increased level of extracellular BGL production was achieved by *Bacillus subtilis* strain PS when grown in minimal medium with glucose as a carbon source while decreases in presence of disaccharide (maltose, cellobiose and lactose). The pattern of BGL production (U/ml) with glucose, xylose, maltose, cellobiose and lactose as carbon source was 0.42, 0.1, 0.13, 0.1 and 0.05, respectively.

Growth of *Bacillus* strain PS versus specific activity of BGL was observed in different time interval. The specific activity of BGL was higher in presence of glucose (0.28 U/mg) compared to lactose (0.007 U/mg). When minimal medium was supplemented with maltose or cellobiose and glucose or xylose, almost similar specific activity i.e., 0.02 U/mg and 0.028 U/mg, respectively, was obtained. Crude cell free extract showed less BGL production with xylose in comparison to glucose. This could be due to less protein secretion when xylose was supplemented in minimal medium. Hence, the *Bacillus subtilis* strain PS was grown in minimal medium supplemented with glucose for BGL production and partial purification.

Partial purification and characterization

Partial purification of enzyme was achieved using ammonium sulfate fractionation followed by dialysis and gel filteration chromatography. Purification fold of 1.83 was achieved at 70% ammonium sulfate concentration with maximum activity (2.3 U/ml) and 37.5% yield. Sephadex G-75 gel filteration chromatography based purification of concentrated enzyme results 18.04 fold purified enzyme with 28% retention of total BGL activity and 1.5% retention of total protein. The specific activity of the purified enzyme after gel filtration chromatography was 17.32 U/mg of protein (Table 1).

Native-PAGE analysis of protein reveals three bands of size 193 kD, 64 kD, and 42 kD (Fig 2). Three peaks contain the BGL activity after gel filtration (fraction number 2-12, 58-65 and 81-87) (Fig 3a). These three peaks of different molecular weight might represent the three isozymes of BGL. The molecular weight of BGL enzyme was determined from the standard calibration curve which was approximately 193 kDa, 64kDa, 42 kDa

Table 1. Partial purification of BGL from culture filtrate by ammonium sulfate precipitation and gel filtration chromatography

Purification Step	Total Protein (mg)	Total Activity (U)	Specific activity (U/mg)	Purification fold	Yield %
Crude extract	96.25	92.5	0.96	1	100
Ammonium sulfate precipitation	19.5	34.5	1.76	1.83	37.5
Gel filtration	1.5	25.98	17.32	18.04	28

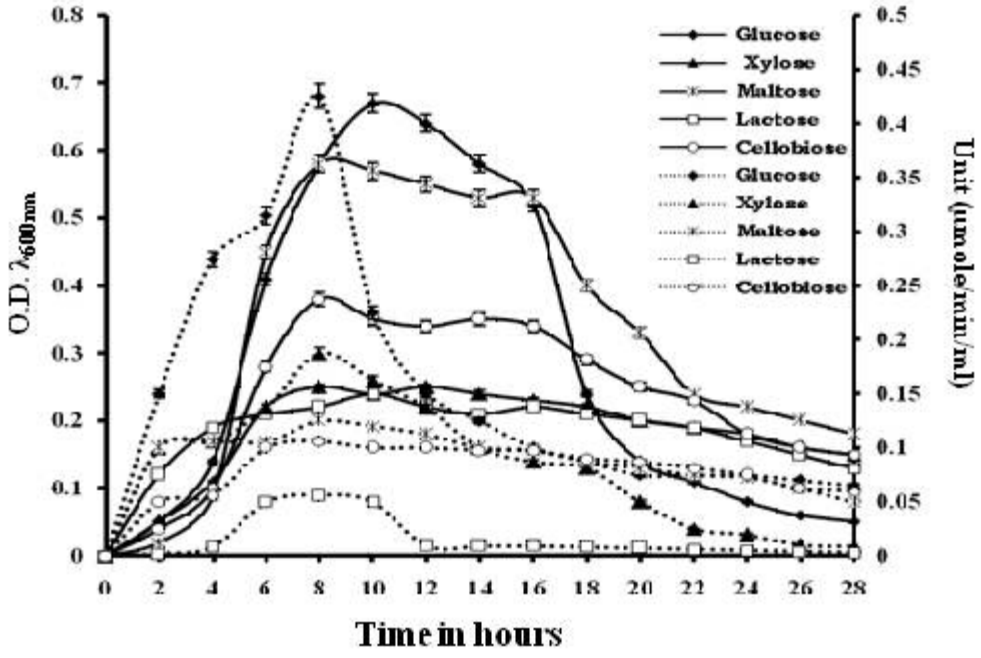

Fig. 1. Bacterial growth and β-glucosidase activity in minimal medium supplemented with 1% (w/v) different carbon sources. Bar represents the value of ± standard error at P > 0.05 of three replicates. (_____) bold line denoted the growth (……) dotted line denoted the enzyme activity

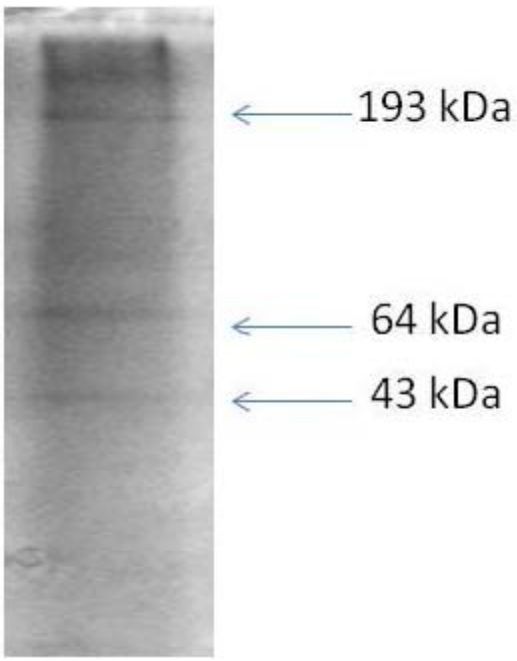

Fig. 2. Native PAGE of 70 % ammonium sulfate fraction of beta glucosidase

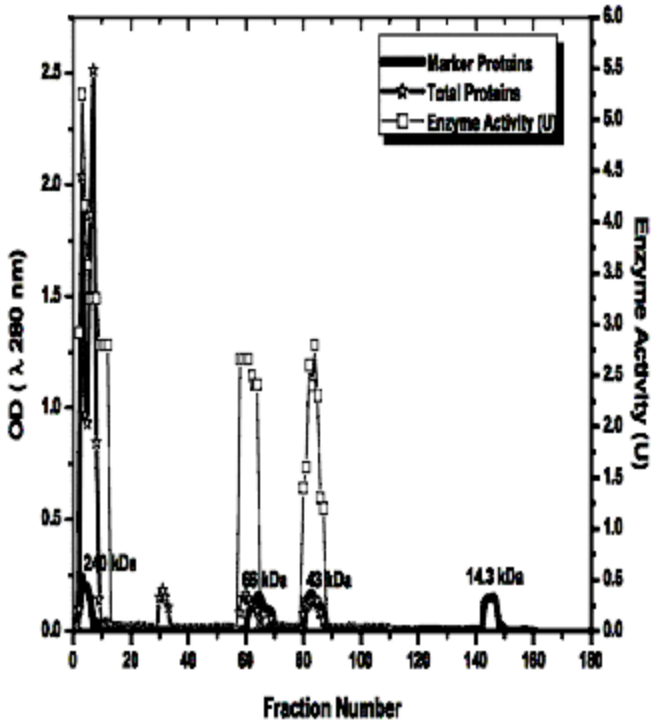

Fig. 3(a). Elution patterns of marker proteins, total proteins and β-glucosidase through Sephadex G-75 gel filtration column

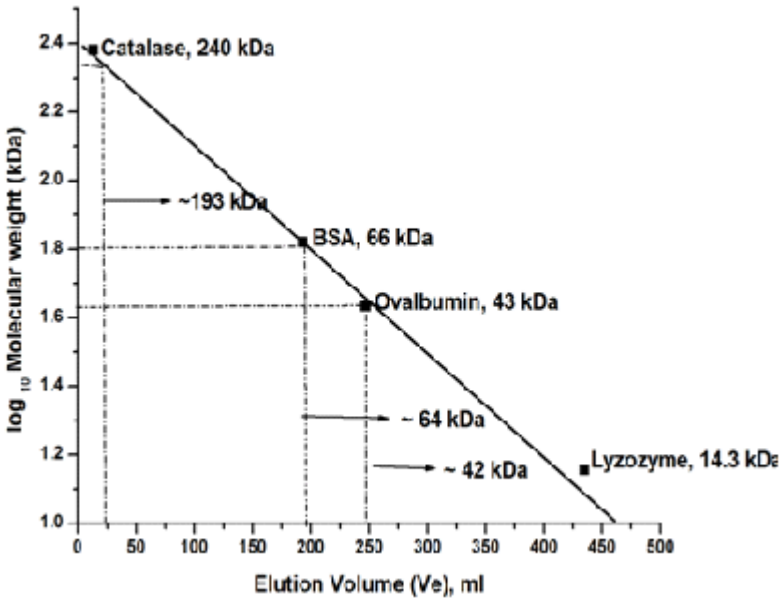

Fig. 3(b). Molecular weight determination of β-glucosidase from the standard curve drawn between $\log_{10}$ molecular weight v/s elution volumes (Ve)

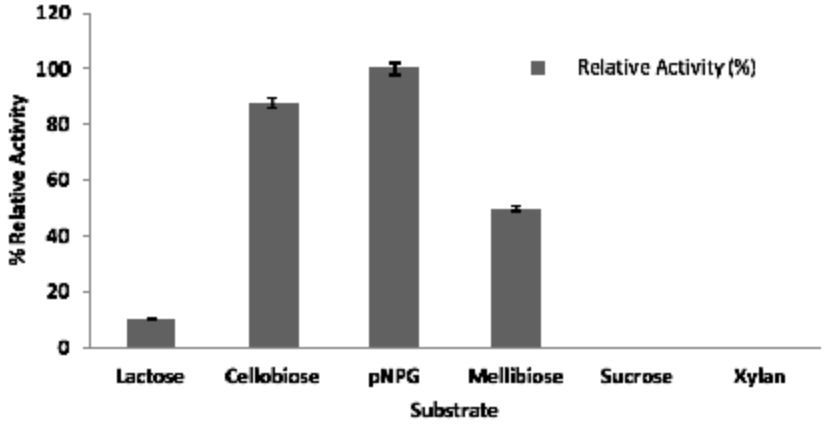

Fig. 4. Substrate specificity of β-glucosidase from *Bacillus* sp. PS

for three peaks respectively (Fig 3b).

Substrate specificity of BGL

The relative rate of hydrolysis of various substrates by the partially purified BGL is presented. The BGL hydrolyzed PNPG (100%) and cellobiose (87.7%) effectively. It acted slowly on lactose (10%) and mellibiose (49.9%) **(Fig 4).**

DISCUSSION

Major bottlenecks for industrial application of cellulases and BGL are the cost of enzyme production (Gusakov *et al.,* 2007). Several studies indicate that the carbon source is one of the most important factors affecting the production cost and yield of BGL (Gao *et al.,* 2008). In the present study out of various carbon sources supplemented in minimal medium, glucose 1% (w/v) resulted in optimal BGL productivity 0.42 U/ml in 8 hours at 37°C. Mahajan et al (2010) have obtained similar results with 2% (w/v) glucose for maximum production of membrane bound BGL in *Lactobacillus acidophilus*. Jager et al (2001) also reported maximum BGL production in different *Aspergillus* strains with glucose as carbon source. The pattern of enzyme production in presence of different carbon sources was Glucose> Xylose> Maltosee" Cellobiose> Lactose. However, Samiullah *et al.,* (2009) reported that sucrose was the best from amongst carbon sources in *Bacillus* sp. The use of commercial cellobiose as a substrate for large scale production of BGL is uneconomical (Rajoka *et al.,* 2004). Studies on *Kluyveromyces marxianus* the induction of BGL indicated that cellobiose medium was essential to induce high BGL activities (Samiullah *et al.,* 2009). It is known that glucose plays an important role in the solubilization and reconstitution of biological membrane (Gunata *et al.,* 1994; Shinoyama *et al.,* 1991). This could be the reason that BGL production was induced in presence of monosaccharide (glucose and xylose) compared to the disaccharide. The difference in the complexity of the carbon sources could account for the disparity in the growth of the organism in the different medium (Nowodo-Chinedu *et al.,* 2007).

Fernandez et al (2002) reported three isozymes of BGL from the fungus *Thermoascus aurantiacus* and partially purified two of them using gel filtration chromatography. Saibi et al (2007) reported five BGL from the fungus *Stachybotrys* sp and after purification of one BGL, a monomeric protein of 78 kDa was found. Bhiri *et al.,* (2008) isolated and purified two forms of BGL (β-glu 1 and β-glu 2) from *Penicillium occitanis.* Riou *et al.,* (1998) reported two isozymes of BGL from the *Aspergillus oryzae* with molecular weight 130 kDa and 43 kDa respectively. The molecular weight of two isozymes was 175 kDa (Gl-2) and 157 kDa (Gl-3) as analyzed by gel filtration chromatography and polyacrylamide gel electrophoresis.

However this is first study on three isozymes of BGL in *Bacillus* sp. strain PS. Several *Bacillus* sp. having BGL activity are *Bacillus*

circulans (87 kDa) (Aono *et al.,* 1992), *Bacillus polymyxa* (50 kDa) (Painbeni *et al.,* 1992) and *Lactobacillus casei* (480 kDa) (Coulon *et al.,* 1998). Thus the partially purified BGL came under the different range of molecular weight.

Multisubstrate catalysis by BGL was found in *Bacillus* strain PS. Catalytic activities of partially purified BGL with lactose as substrate was 10%. The BGL also catalyzed cellobiose (87.7%), PNPG (100%) and mellibiose (49.9%). Multisubstrate catalysis by BGL has also been reported by Wolosowska and Synowiecki (2004). BGL from *Xylaria regalis* have also showed maximum catalytic activity towards cellobiose and PNPG (Wei *et al.,* 1996). The catalytic activity of partially purified BGL was maximum with PNPG and cellobiose and could not act on sucrose and xylan, which reveals catalytic activity of the isolated enzyme towards â1-4 linkage. BGL activity have been seen with different carbohydrate in *Humicola geisea* (Benoliel *et al.,* 2010), *A. niger* (Kuwahana *et al.,* 1992), *A. oryzae* (Lecas *et al.,* 1998) and in thermophilic bacterium (Morgan *et al.,* 1987).

Production of extracellular BGL from *Bacillus* strain PS with low cost carbon source glucose in minimal medium can be exploited for the biotechnological application in several industries such as in ethanol and wine production.

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REFERENCES

1. Aono, R., Sato, M., Yamamoto, M., Horikoshi, K., Cloning and structural analysis of bglM gene coding for the fungal cell wall-lytic β-1, 3-glucan-hydrolase BglM of Bacillus circulans IAM1165. *Biosci. Biotech. biochem.,* 2002; **66**(6):1246-1255.
2. Banat, I., Singh, D., Marchant, R., The use of a thermotolerant fermentative Kluyveromyces marxianus IMB3 yeast strain for ethanol production. *Acta biotech.,* 1996; **16**(2 3):215-223.
3. Benoliel, B., Poças-Fonseca, M. J., Torres, F. A., de Moraes., L. M., Expression of a glucose-tolerant beta-glucosidase from Humicola grisea var. thermoidea in Saccharomyces cerevisiae. *Appl. Biochem. Biotechnol*., 2010; **7**:2036-44.
4. Bhiri, F., Chaabouni, S. E., Limam, F., Ghrir, R., Marzouki, N., Purification and biochemical characterization of extracellular β-glucosidases from the hypercellulolytic Pol6 mutant of Penicillium occitanis. *Appl. Biochem. Biotechnol*., 2008; **149**(2):169-182.
5. Bradford, M. M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analyt. biochem.,* 1976; **72**(1-2)248-254.
6. Coulon, S., Chemardin, P., Gueguen, Y., Arnaud, A., Galzy, P., Purification and characterization of an intracellular β-glucosidase from Lactobacillus casei ATCC 393. *Appl. Biochem. Biotechnol*., 1998; 74(2):105-114.
7. de Palma-Fernandez, E., Gomes, E., Da Silva, R., Purification and characterization of two β-glucosidases from the thermophilic fungusThermoascus aurantiacus. *Folia microbiologica* 2002; **47**(6):685-690.
8. Gadgil, N., Daginawala, H., Chakrabarti, T., Khanna, P., Enhanced cellulase production by a mutant of Trichoderma reesei. *Enz. Microb. Technol.,* 1995; **17**(10):942-946.
9. Gao, J., Weng, H., Zhu, D., Yuan, M., Guan, F., Xi, Y., Production and characterization of cellulolytic enzymes from the thermoacidophilic fungal Aspergillus terreus M11 under solid-state cultivation of corn stover. *Biores. technol.,* 2008; **99**(16):7623-7629.
10. Gunata, Y., Bayonove, C., Baumes, R., Cordonnier, R., The aroma of grapes I. Extraction and determination of free and glycosidically bound fractions of some grape aroma components. *J.Chromat., A* 1985; 331:83-90.
11. Gusakov, A., Sinitsyn, A., Manenkova, J., Protas, O., Enzymatic saccharification of industrial and agricultural lignocellulosic wastes. *Appl. Biochem. Biotechnol*., 1992; **34**(1):625-637.
12. Jager. S., Brumbauer. A., Feher. E., Kati. R., Kiss. L., Production and characterization of β-glucosidase from different Aspergillus strains. *World J. Microbiol. Biotechnol.,* 2001; **17**: 455–461.
13. Kumar, K., Devi, S. S., Krishnamurthi, K., Kanade, G. S., Chakrabarti, T., Enrichment and isolation of endosulfan degrading and detoxifying bacteria. *Chemosphere* 2007; **68**(2):317-322.
14. Kuwahana, S. M., Seviour, R. J., Mcdougall, B.

M., Purification and characterization of an extracellular β-glucosidase from the filamentous fungus *Acremonium persicinum* and its probable role in β- glucan degradation. *Microb Technol.,* 1992; **21**:182-190

15. Laemmli, U. K., Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *nature* 1970; **227**(5259):680-685.
16. Lecas, M., Gunata, Z. Y., Sapis, J. C., Bayonove, C. L., Purification and partial characterization of [beta]-glucosidase from grape. *Phytochemistry* 1991; **30**(2): 451-454.
17. Lynd, L. R.; Weimer, P. J.; Van Zyl, W. H.; Pretorius, I. S., Microbial cellulose utilization: fundamentals and biotechnology. *Microbiol. Mol. Biol. Rev.,* 2002; **66**(3):506-577.
18. Mahajan, P.; Desai, K.; Lele, S., Production of Cell Membrane-Bound α-and β-Glucosidase by Lactobacillus acidophilus. *Food Biopro. Technol.,* 2010; 1-13.
19. Maniatis, T., *Molecular cloning: a laboratory manual/J. Sambrook, EF Fritsch, T. Maniatis*. New York: Cold Spring Harbor Laboratory Press: 1989.
20. Morgan, R., Naimark, J., Morgenstern, E., Bayer, E. A., Specialized cell surface structures in cellulolytic bacteria. *J. Bacteriol.,* 1987; **169**: 3792-3800
21. Nolan, A. M., Barron, N., Brady, D., McAree, T., Smith, D., McHale, L., McHale, A. P., Ethanol production at 45°C by an alginate-immobilized strain of *Kluyveromyces marxianus* following growth on glucose-containing media. *Biotechnol. Letters*., 1994; 16:849-852.
22. Nowodo-Chinedu, S., Okochi, V. I., Smith, H., Omidiji, O., Effect of carbon soyrces on celllase production by *Penicillim chrysogenm*. *Afri. J. Biochem.,* 2007; 1: 6-10
23. Painbeni, E., Valles, S., Polaina, J., Flors, A., Purification and characterization of a *Bacillus polymyxa* α-Glucosidase Expressed in *Escherichia coli*. *J. Bacteriol.,* 1992; 174: 3087-3091
24. Prior, B. A., Day, D. F., Hydrolysis of ammonia-pretreated sugar cane bagasse with cellulase, β-glucosidase, and hemicellulase preparations. *Biotechnol. for Fuels Chem.* 2008; 271-284.
25. Rajoka, M., Latif, F., Khan, S., Shahid, R., Kinetics of improved productivity of β-galactosidase by a cycloheximide-resistant mutant of Kluyveromyces marxianus. *Biotechnol. letters,* 2004; 26(9):741-746.
26. Riou, C., Salmon, J. M., Vallier, M. J., Günata, Z., Barre, P., Purification, Characterization, and Substrate Specificity of a Novel Highly Glucose-Tolerant β-Glucosidase from Aspergillus oryzae. *Appl. Environ. Microbiol.,* 1998; **64**(10):3607-3614.
27. Saibi, W., Amouri, B., Gargouri, A., Purification and biochemical characterization of a transglucosilating β-glucosidase of Stachybotrys strain. *Appl. Microbiol. Biotechnol.,* 2007; **77**(2):293-300.
28. Samiullah, T. R., Bakhsh. A., Rao, A. Q., Naz, M., Saleem, M., Isolation, purification and characterization of extracellular [beta]-glucosidase from *Bacillus* sp. *Adv. in Environ. Biol.,* 2009; 3:269-277.
29. Shah. P., Characterization of β-glucosidase activity in microorganisms isolated from jaggery and bagasse. M.Sc. thesis. Department of Biochemistry, G. B. Pant University of Agriculture and Technology, Pantnagar, 2010
30. Shinoyama, H., Take, K., Ando, A., Fuilli, T., Sasaki, M., Doi, Y., Yasul, T., Enzymatic synthesis of useful alkyl- β-glucosidase. *Agri. Biol. Chem*., 1991; **55**:1679-1681
31. Watanabe, T., Sato, T., Yoshioka, S., Koshijima, T., Kuwahara, M., Purificication and properties of *Aspergillus niger* β-glucosidase. *Eur. J. Biochem.,* 1992; **209**:651 -659
32. Wayman, M., Chen, S., Doan, K., Bioconversion of waste paper to ethanol. *Process biochem.,* 1992; **27**(4):239-245.
33. Wei, D. L., Kirimura, K., Usami, S., Lin, T. H., Purification and characterization of an extracellular β-glucosidase from the wood-grown fungus Xylaria regalis. *Current Microbiol.,* 1996; **33**(5):297-301.
34. Wolosowska, S., Synowiecki, J., Thermostable β-glucosidase with a broad substrate speciûcity suitable for processing of lactose-containing products. *Food Chem*., 2004; **85**:181–187.
35. Woodward, J., Synergism in cellulase systems. *Biores. Technol.,* 1991; 36(1):67-75.
36. Yoon, J. J., Kim, K. Y., Cha, C. J., Purification and characterization of thermostable β-glucosidase from the brown-rot basidiomycete Fomitopsis palustris grown on microcrystalline cellulose. *J. Microbiol.,* 2008; **46**(1):51-55.