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Purification and Biochemical Characterization of a β-glucosidase from *Penicillium commune* ITV01

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Abstract: β-glucosidases have attracted considerable attention in recent years due to their important roles in various biotechnological processes such as hydrolysis of isoflavone glucosides, the production of fuel ethanol from agricultural residues, the release of aromatic compounds from flavorless precursors, among others. In this study, extracellular β-glucosidase induced by cellulose from *Penicillium commune* ITV01 was purified to homogeneity by electrofocusing (IEF) and Sephadex G-100 gel filtration. The enzyme was characterized and the molecular weight was 144.2 kDa as estimated by SDS-PAGE. The isoelectric point determined by IEF was 4.73 and the enzyme was able to hydrolyze cellobiose and cellulose to glucose but not laminarine, xylan, starch, pullulan, colloidal chitin and carboxymethyl-cellulose. Optimal pH and temperature were detected at 5.0 and 50°C, respectively. Stability was observed at temperatures 30 to 50°C and pH values between 5 and 7 for 24 h. Enzyme activity was activated by K^{+}, Cu^{+}, Mn^{++}, Fe^{++}, Cu^{++}, Ca^{++} ions and significantly by Co^{++}. β-glucosidase was completely inhibited by Hg^{++}. In conclusion, the novel β-glucosidase purified from *P. commune* shows great potential for biotechnological uses.

Key words: Purification · Glucosidase · *Peniclillium* · Hydrolase · Fungi · Cellulolytic activity

INTRODUCTION

β-Glucosidases (β-D-glucoside glucohydrolase, EC 3.2.1.21) are an essential component of the cellulase complex [1]. That is widely distributed in nature acting as intermediary of several metabolic routes in animals, plants, yeast and bacteria. This enzyme belong to the glycosyl hydrolases GH1 and GH3 families and several β-glucosidases constitute critical components in enzymatic mixtures which also include endoglucanases and cellobiohydrolases intended for industrial saccharification [2].

β-glucosidases are capable of carrying out the hydrolysis of the β-glycosidic linkages or the aryl and alkyl substituents of the β-glucosides and in the oligosaccharides. β-glucosidases act on the β-link releasing glucose molecules as a byproduct [3]. Due to their hydrolytic characteristics their applications in biotechnological processes, β-glucosidases have a great potential for preparing products with high commercial value; for example, in the production of biofuels, the washed-out of dyes and in the paper industry [4]. In the food industry this enzyme is used to obtain glucosides for the release of aromatic compounds from fruits, to increase the isoflavone content during the fermentation process of soymilk [5] and in the conversion of phenolic antioxidants from defatted residues from the same soymilk [6]. In the pharmaceutical industry β-glucosidases are employed to hydrolyze the glucoside-isoflavones

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originating aglicones, which are simple phenols that act as natural antioxidants and at the same time promote their absorption [7].

Reports on the identification and characterization of β-glucosidases in fungi to investigate their potential use are limited. Relevant studies found on fungal β-glucosidases include: *Aspergillus terreus* [8], *Penicillium purpurogenum* [4], *Ceriporiopsis subvermispora* [9] and *Trichoderma harzianum* [3].

Moreover, a fungal source with the ability to produce enzyme with high hydrolytic activity are commonly microorganisms which have mycoparasitism activity [10]. Mycoparasitism happens when one fungus attacks another fungus and lytic enzymes have a fundamental role to hydrolyze the cell wall of the later one [11]. In a previous study, with the purpose of obtain fungal strains with high antagonistic activity on phytopathogenic fungi in our laboratory, we isolated nearly 80 strains from soil samples and from various parts of agricultural crop plants at the Cotaxtla Experimental Station near the city of Veracruz, Mexico. From these strains, we selected fungi with high cellulolytic and mycoparasitism activities. One of these strains (ITV01) with high cellulolytic activity was identified through the sequencing of its ribosomal DNA ITS1-5.8s-ITS2 region as *Penicillium commune*. In this study, we describe the purification and biochemical characterization of the β-glucosidase produced by this strain.

MATERIALS AND METHODS

Strain, Maintenance and Propagation: *Penicillium commune* ITV01 was isolated from soil samples from tomato crops in Cotaxtla Experimental Station (Cotaxtla, Ver. Mexico). For maintenance and propagation of the strain YPD medium (glucose 20 g/L, yeast extract 10 g/L, peptone 20 g/L) was used. To maintain viable cultures, the strain was replanted every 30 days.

β-glucosidase Production: To 100 mL of YPD medium contained in a 250 mL flask was added an inoculum of 1 x 10^6 spores. The inoculated medium was incubated in a Lab-Line rotary shaker at 200 rpm and 28°C for 50 hours. Mycelium from this flask was washed with sterile distilled water and was used to inoculate 1 L of minimal medium with 1% cellulose (Sigma). Minimal medium composition expressed as g/L was as follows: 2 K_2HPO_4, 14 $(NH_4)2SO_4$, $MgSO_4$.7HO 0.3 at pH 5.0 [12]. The medium was incubated in a 2 L flask at 150 rpm and 28°C for 75 hours.

β-glucosidase Activity: Standard assay for β-glucosidase activity was performed at 40°C in 150 mL of sodium citrate buffer 50 mM (pH 4.8) containing *p*-nitrophenyl-β-D-glucopyranoside (*Np*-β-Glu, Sigma) 1 mM and 50 µL of enzyme. The reaction was stopped by adding 50 µL of Na_2CO_3 (JT Baker) 1M after 15 minutes of incubation. The amount of *p*-nitrophenol generated was measured by reading the absorbance at 420 nm. One unit of enzyme activity (UI) was defined as the amount of enzyme that releases 1 µmol of *p*-nitrophenol equivalents per minute [3].

Protein Determination: The amount of soluble protein was determined by the method of Bradford, using bovine serum albumin as a standard [13]. Each assay was performed in duplicate.

Clarification of the Enzymatic Extract and Ultrafiltration: At the end of fermentation, the mycelium was separated from the supernatant by filtering with Whatman filter paper No. 1 and subsequently microfiltered through a 0.45µm membrane (Millipore). The filtrate was subjected to ultrafiltration (Amicon cell capacity: 350 mL) using a cutoff membrane of 500 kDa (Polyethersulfone, Millipore) according to manufacturer's instructions. The obtained filtrate was passed through another membrane cutoff of 10 kDa (Polyethersulfone, Millipore) and the concentrate was recovered. Ultrafiltration was carried out at 4°C with constant magnetic stirring and a pressure of 20 psi using nitrogen gas. The electrical conductivity of the concentrate was preserved in values lower than 780 µS/cm by adding deionized water and ultrafiltering with a 10 kDa membrane.

Isoelectrofocus: The concentrated enzyme obtained was injected into an isoelectrofocus cell of 58 mL capacity (Rotofor ® System, Bio-Rad). The applied power was 15 watts that remain constant during the separation. The temperature inside the cell was kept at 4°C and the separation required 5.5 hours. At the conclusion of the procedures, 20 fractions were recovered, to which were measured the final pH, protein content and β-glucosidase activity was determined. The isoelectric point (pI) of the enzyme was estimated by measuring the pH of the separated fractions by isoelectric focusing that showed β-glucosidase activity.

Gel Filtration Chromatography Sephadex G-100: Fractions with β-glucosidase activity were submitted to a fractionation by gel filtration chromatography using

Sephadex G-100 (Sigma). The gel was packed in a Bio-Rad column of 1.5 cm internal diameter and 120 cm length. The volume occupied by the packing was 190 mL. The column was equilibrated and eluted with 50 mM sodium citrate buffer at pH 4.8. The dead volume of the column was 56 mL, which was calculated using blue dextran 2000 (Sigma) at a concentration of 2 mg/mL. Separation was conducted at room temperature at a flow rate of 0.4332 mL/min. The buffer was fed into the column using a peristaltic pump (Millipore) and a flow adapter (Bio-Rad). The sample was applied using a micropipette onto the drained surface of the gel; after loading the sample a small amount of buffer was applied, drained and followed by filling the surface of the column with more buffer. The volume of the collected fractions was 2 mL. The protein content of each fraction was estimated by measuring their absorbance at 280 nm and interpolating on a graph of absorbance *vs* protein concentration of bovine serum albumin (Sigma) as standard. Fractions with β-glucosidase activity were lyophilized and analyzed by SDS-PAGE.

SDS-PAGE Gel Electrophoresis Staining and Molecular Weight Estimation: Electrophoresis was carried out according to Laemmli [14]. The sample was mixed in a 1:2 ratio with the buffer (Tris-HCl 0.5 M pH 6.8, β-mercaptoethanol, bromophenol blue, SDS 10% and glycerol) and loaded into a discontinuous gel: packing gel, 4% resolving gel, 12% polyacrylamide. Molecular weight markers used were: Kaleidoscope Pre-stained Standard (Bio-Rad) and Pre-stained SDS-PAGE Standards, Low Range (Bio-Rad). A Mini-Protean® 3 Cell (Bio-Rad) was employed and 100 V were applied for 120 minutes. After electrophoresis, the proteins were fixed with a solution of methanol (40%) and acetic acid (10%) with stirring for 30 min. Staining was performed using the revealed kit with silver nitrate and Coomassie (Bio-Rad). The molecular weight was calculated by the interpolation of the sample in the regression curve of the log molecular weight of each marker (log PM) against relative mobility R_f. For the estimation of molecular weight by gel filtration chromatography was applied 0.5 mL of marker (Gel Filtration Standard 151.1901, Bio-Rad) on the surface of the column and eluted with sodium citrate buffer 50 mM pH 4.8 at a rate of 0.4332 mL/min. and fractions of 2 mL were collected.

β-glucosidase Characterization

Effect of pH and Temperature on Enzymatic Activity: The effect of pH on enzymatic activity was assessed by incubating 21 mU of enzyme in 50 mM citrate-phosphate buffer at a pH range of 3.0 to 7.0 for 1 h at 50 °C. Enzyme activity was expressed as relative activity (%) in relation to the maximum enzyme activity value obtained. Temperature effect was evaluated by incubating 21 mU of enzyme in the same buffer at pH 5.0, at temperatures of 30 to 70°C at 10°C intervals. Subsequently, β-glucosidase activity was determined.

Effect of pH on enzyme stability: The enzyme (21 mU) was incubated at 50°C in 50 mM citrate-phosphate buffer in a pH range of 3.0 to 7.0 at one pH unit intervals during 24 h. To determine the stability of the enzyme, samples were incubated (21 mU) in the same buffer at pH 5.0 at a temperature range of 30 to 70°C. Subsequently, β-glucosidase activity was determined.

Effect of Metal Ions, SDS and EDTA on Enzymatic Activity: The solutions of the following metal ions were prepared in 50 mM sodium citrate buffer (pH 4.8): Hg^{2+}, Zn^{2+}, Ca^{2+}, Mg^{2+}, Co^{2+}, Mn^{2+}, K^{+}, Cu^{3+}, Fe^{2+}, SDS and EDTA. Final concentration was 5 mM. Enzyme (15.5 mU) was pre-incubated with each of these metal ions for 30 min at room temperature. Residual enzyme activity was evaluated by incubating the enzyme at 50°C and pH 5.0 in a 1% cellulose solution for 30 min. Each assay was performed in duplicate. After incubation, the reaction was stopped by adding 3 mL of the DNS reagent and the amount of reducing sugars released at 540 nm was determined [15]. ANOVA was applied followed of Tukey's tests. All the analyses were conducted using Statistix 9.0 software [16].

RESULTS AND DISCUSSION

β-glucosidase Enzyme Production: After 50 hours of incubation of *P. commune* ITV01 in 100 mL flask, abundant growth was observed in form of pellets. The culture was transferred to minimal medium with cellulose as the sole carbon source (1000 mL) and after 75 hours of incubation the enzymatic activity in the supernatant was determined. A specific activity of 79.72 U of β-glucosidase/mg protein in the fermentation product was measured. Table 1 shows the enzyme purification steps; the enzyme was partially purified with

Table 1: Purification steps of β-glucosidase enzyme isolated from *Penicillium commune* ITV01

Purification step	Volume (mL)	Total Protein (mg)	Total activity (mU)	Specific activity (mU/mg)	Purification fold	Yield (%)
Crude extract	1000	57.17	4,557	79.72	1	100
Clarified extract	950	54.31	4,329	79.72	1	95
Ultrafiltration (10-500 kDa)	58	28.8	4,264	148	1.86	93.56
Isoelectric-focusing (F7)	2	0.453	959	2,120	26.59	21.04
Gel filtration (F30)	2	0.35	873	2,496	31.3	19.16

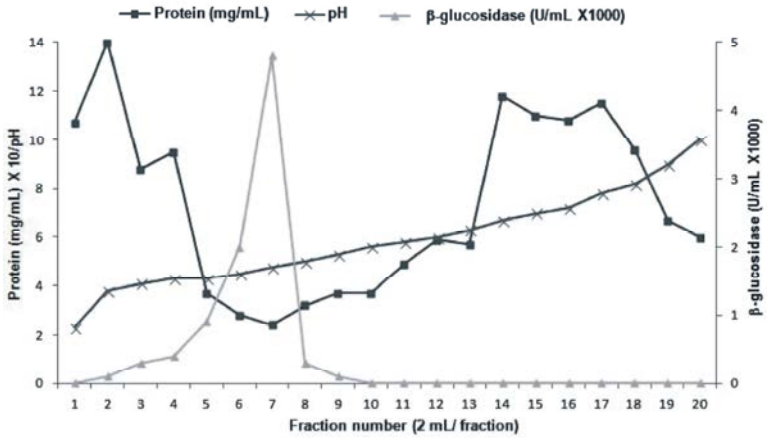

Fig 1: Profile of separation by electrofocusing of β-glucosidase from *P. commune* ITV01

a recovery of 19.16% and a purification factor of 31.3. The results for the β-glucosidase production suggest that this method of purification is favorable for the enzyme. β-glucosidases are not dependent on other enzymes to carry out their activity, as is the case of endoglucanases and exoglucanases, which act synergistically on cellulose degradation; therefore, its total activity was not drastically decreased as the purification advanced.

Ultrafiltration: In the ultrafiltration step the enzymatic activity and protein concentration were determined in fractions smaller than 10 kDa, in the 10-500 kDa fractions and in the fraction with molecular sizes greater than 500 kDa. Neither enzymatic activity nor proteins were detected in the fraction of less than 10 kDa. The highest enzymatic activity was detected in the 10-500 kDa fractions (4,254 U/mL and 0.497 mg protein/mL). However, in the highest fraction (500 kDa) 14% of the total activity of β-glucosidase was retained (600 U/mL, 0.210 mg protein/mL). It is suggested that the activity in the retainded is due to the formation of a layer on the surface of the membrane that prevents the enzymes with molecular weights smaller than 500 kDa pass through.

Isoelectric Focusing Fractionation (IEF): An enzyme concentrate of 58 mL was loaded to an isoelectric focusing fractionation equipment. The pH gradient formed by ampholytes was 2.2 to 10.0 (Figure 1). At this stage, 20 fractions were obtained of which three had β-glucosidase activity (F5, F6 and F7). Figure 1 shows that the distribution of protein is concentrated at both ends of the pH gradient. In the case of fractions with β-glucosidase activity, the protein content was low. According to the pH gradient obtained, the isoelectric point of the F7 (β-glucosidase) is 4.73. For isoelectric focusing fractionation, there are few studies that report the pI of this enzyme. In all of these, the values were low and consistent with our data. Accordingly, the pI value reported for β-glucosidase from *Aspergillus oryzae* is 4.9 [17] and for *Bacillus polymyxa* is 4.6 [18].

IEF Separation of Fraction 7 (F7) by Gel Filtration Chromatography with Sephadex G-100: Initially, the fraction 7(F7) of isoelectric focusing with high β-glucosidase activity, was separated on a gel filtration chromatography column Sephadex G-75. However, a significant amount of protein with β-glucosidase activity was detected in the void volume, so that separation was not efficient. These data suggests that the protein was greater than 80 kDa, which is the maximum separation limit of Sephadex G75. For this reason, a longer column was prepared loaded with a resin with a greater range for separation (Sephadex G-100 which can separate from 4 to 150 kDa, according to manufacturer's information). Therefore, F7 with β-glucosidase activity was applied

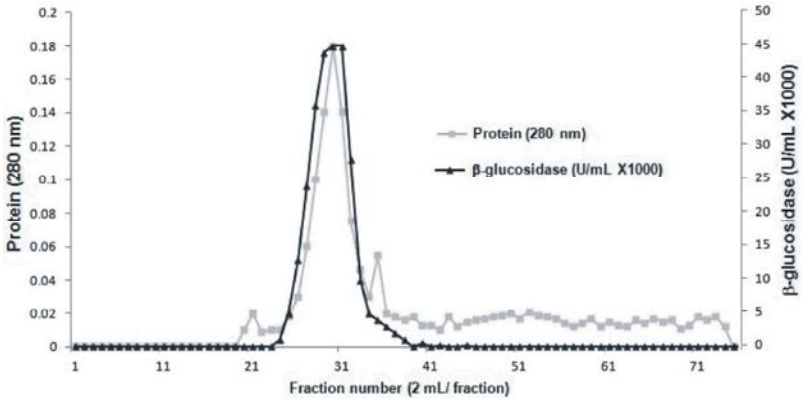

Fig 2: Profile of separation of β-glucosidase from *P. commune* ITV01 by gel filtration chromatography (Sephadex G100).

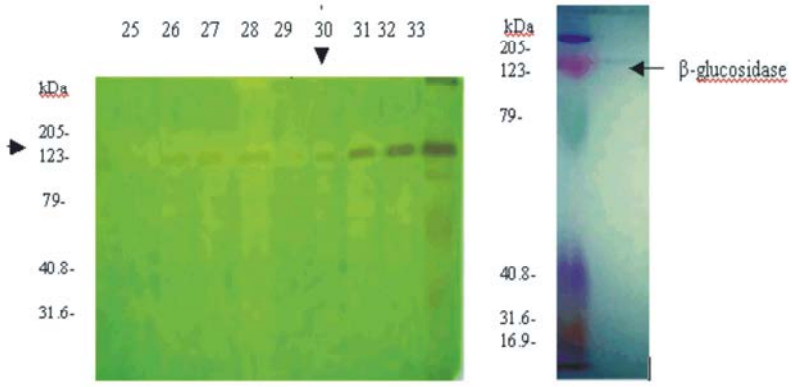

Fig 3: SDS-PAGE electrophoretic analysis of the β-glucosidase. (a) Silver staining gel of the fractions recovered from the filtration chromatography gel. The arrow indicates the fraction 30, which has the maximium β-glucosidase activity. (b) Gel revealed with Coomassie blue. The arrow indicate the purified β-glucosidase in lane 2. The marker used is the Kaleidospe of Bio-Rad (myosin, 205, β-galactosidase, 123; bovine serum albumin, 79; carbonic anhydrase, 40.8, soybean trypsin inhibitor, 31.6; lysozyme, 16.9; aprotinin, 6.6).

to this second gel filtration column (Sephadex G-100). The separation profile is shown in Figure 2. Starting from fraction 25, enzymatic activity was observed, reaching its maximum value in fraction 30.

Molecular Weight Estimation of β -glucosidase: The activity detected in fractions 25 to 33 collected from the column coincides with the approximate 140 kDa bands that were observed in the electrophoresis gel of the samples that was revealed with silver stain (Figure 3a). In the development of sample F30 with Coommassie blue (Figure 3b) a band corresponding to 144.2 kDa was found, as calculated with the Kaleidoscope marker (Bio-Rad). Furthermore, estimation of molecular weight using the gel filtration column was of 150.42 kDa (data not shown). The molecular weights estimated by both methods were very similar and the small differences can be attributed to the variability of each method. For the molecular weight estimation, values reported for β-glucosidase are 98kDa for *Trichoderma reesei* [19], 130kDa for *Aspergillus oryzae* [17] and 275kDa for *Pichia pastoris* [20]. A β-glucosidase 43kDa monomer of the fungus *Aspergillus oryzae*, is the smallest known β-glucosidase among the aerobic mushrooms [21]. In general, β-glucosidases of microbial origin have high molecular weights. By migration of the enzyme SDS-PAGE electrophoresis we suggest that the enzyme is monomeric. This result is consistent with those reported in the literature since the β-glucosidases of molecular weights of more than 200 kDa have more than one monomer. For example β-glucosidase from *Aureobasidium pullulans* with molecular weight of 340 kDa has two subunits [22], the enzyme of 275 kDa from *Pichia pastoris* has 4 subunits [20].

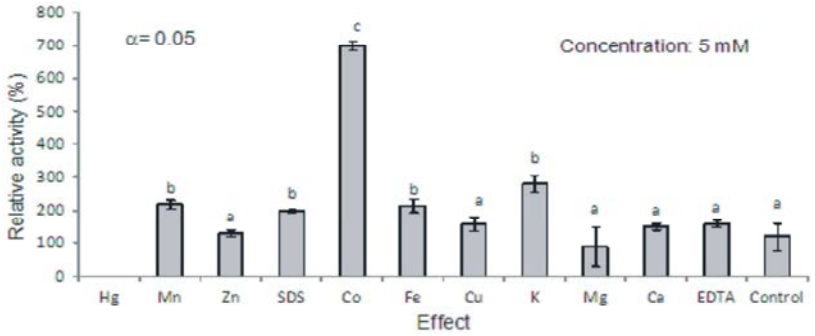

Fig 4: Effect of metal ions, EDTA and SDS on β-glucosidase activity

Effects of pH and Temperature on Enzymatic Activity and Stability: The influence of pH and temperature on β-glucosidase was determined. The optimum pH obtained was 5 after 24 h of incubation. At pH values of 5, 6 and 7, the enzymatic activity retained was greater than 70%, compared to the activity after 1 hour. With respect to temperature, the optimum was 50°C and the enzyme maintained stability greater than 70% at 30 and 40°C. β-glucosidase genes expressed in yeast are similar in pH and optimum temperatures; however, it may have more stability at higher temperatures. For example, the cloning of β-glucosidase in *P. pastoris* stabilizes the enzyme at pH values between 4 and 7 and is stable at 70°C [23].

Substrates, Effectors Effect on Enzymatic Activity of EG144 and Specificity Kinetic Parameters: β-glucosidase enzymes are heterogeneous; although there are no specific criteria for classification, substrate specificity and amino acid sequence can be used [24]. In this study β-glucosidase was tested on different substrates with α and β configurations. The enzyme preparation showed no activity on starch (α-1,4 and α-1,6 glucose), xylan (β-1,4 xylose), swarm (α-1,4), laminarin (β-1,3 glucose), colloidal chitin (β-1,4, N-acetylglucosamine) and CMC (β-1,4 CMC glucose). The substrates that showed activity were cellulose and cellobiose. According to Enari and Niku-Paavola [25] β-glucosidases are classified into three groups: (1) those with high affinity for aryl β-glucoside (2) those which hydrolyze oligosaccharides such as cellobiose and (3) those that are active on both substrates.

β-glucosidase of this study showed a higher activity in the presence of K^{+}, Cu^{+}, Mn^{++}, Fe^{++}, Cu^{++}, Ca^{++} ions and remarkably high with Co^{++}. EDTA did not significantly affect its activity, as also did not affect SDS and Mg^{++} and Zn^{++} ions (Figure 4). These results suggest that it is not a metalloenzyme, since it was not affected by EDTA [26]. Its activity decreased markedly in the presence of Hg^{++} ions. In numerous studies it has been reported that Hg^{++} ions have a negative effect on the activity of many enzymes [24]. The sensitivity to Hg^{++} suggests the existence of at least one catalytically important sulfhydryl group [27]. On the other hand, Co^{++} ions increased enzyme activity. In general cellulases are stimulated by these ions [28, 29].

The kinetic parameters, K_m and V_{max} values were determined using cellobiose as substrate. The estimated values for K_m and V_{max} were 2.3 mM and 670 U/mg, respectively. Joo *et al*. [30] reported values of K_m and V_{max} of 5.5 mM of 1120 U/mg of protein on cellulose. Ng *et al*. [31] reported a K_m of 1.2 mM and V_{max} of 85.93 U/mg of rice flour.

CONCLUSIONS

We purified and characterized a monomeric β-glucosidase from *P. commune* with important extracellular characteristics. It has good stability to pH and temperature, with a high affinity to cellulose, as shown by its low K_m value. These features could prove advantageous for easy recovery and shall provide potential uses in the food industry.

REFERENCES

1. Peshin, A and J.M.S. Mathur, 1999. Purification and characterization of β-glucosidase from Aspergillus niger strain 322, Letters in Applied Microbiology. 28(5): 401-404.
2. Bohlin, C., E. Praestgaard, M.J. Brauman, K. Borch, J. Praestgaard, N. Rune and M.P. Westh, 2013. A comparative study of hydrolysis and transglycosylation activities of fungal β-glucosidases. Applied Microbiology and Biotechnology, 97(1): 159-169.

3. Yun, S.I., C.S. Jeong, D.K. Chung and H.S. Choi, 2001. Purification and some properties of a β-glucosidase from Trichoderma harzanium Type C-4. Bioscience Biotechnology and Biochemistry Journal, 65(9): 2028-2032.
4. Dhake, A.D. and M.B. Patil, 2005. Production of ß-Glucosidase by Penicillium purpurogenum. Brazilian Journal of Microbiology, 36(2): 170-176.
5. Otieno, D.O. and N.P. Shah, 2007. Endogenous β-glucosidase and β-galactosidase activities from selected probiotic microorganisms and their role in isoflavone biotransformation in soymilk. Journal of Applied Microbiology, 103(4): 910-917.
6. McCue, P. and K. Shetty, 2003. Role of carbohydrate-cleaving enzymes in phenolic antioxidant mobilization from whole soybean fermented with Rhizopus oligosporus. Food Biotechnology, 27(l): 27-37.
7. Hendrich, S., 2002. Bioavailability of isoflavones. Journal of Chromatography, 777(1-2): 203-210.
8. Sidik, N.M., A.H. Abdul-Manan, R.M. Yazid and S.A. Sharifuddin, 2012. Gene cloning and characterization of a new glycosyl hydrolase family 3-β-glucosidase from Aspergillus terreus SUK-1. Australian Journal of Basic and Applied Sciences, 6(3): 106-111.
9. Magalhaes, P.O., A. Ferraz and A.F.M. Milagres, 2006. Enzymatic properties of two β-glucosidases from Ceriporiopsis subvermispora produced in biopulping conditions. Journal of Applied Microbiology, 101(2): 480-486.
10. Atanasova, L., S. Le Crom, S. Gruber, F. Coulpier, V. Seidl-Seiboth, C.P. Kubicek and I.S. Druzhinina, 2013. Comparative transcriptomics reveals different strategies of Trichoderma mycoparasitism. BMC Genomics, 14: 121-136.
11. López-Mondéjar, R., M. Ros and J.A. Pascual, 2011. Mycoparasitism-related genes expression of Trichoderma harzianum isolates to evaluate their efficacy as biological control agent. Biological Control, 56(1): 59-66.
12. Marcello, C.M., A.S. Steindorff, S.P. da Silva, R.N. Silva, L.A.M. Bataus and C.J. Ulhoa, 2010. Expression analysis of the exo-β-1,3 glucanase from the mycoparasitic fungus Trichoderma asperellum. Microbiological Research, 165(1): 75-81.
13. Bradford M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72(1-2): 248-254.
14. Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227(5259): 680-685.
15. Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry, 31(3): 426-428.
16. Analytical Software, 2008. Statistix 9. Analytical Software. Tallahassee, FL.
17. Riou, C., J.M. Salmon, M.J. Vallier, Z. Günata and P. Barre, 1998. Purification, characterization and substrate specificity of a novel highly glucose-tolerant β-Glucosidase from Aspergillus oryzae. Applied and Environmental Microbiology, 64(10): 3607-3614.
18. Painbeni, E., S. Valles, J. Polaina and A. Flors, 1992. Purification and characterization of a Bacillus polymyxa beta-glucosidase expressed in Escherichia coli. Journal of Bacteriology, 174(9): 3087-3091.
19. Saloheimo, M, J. Kuja-Panula, E. Ylösmäki, M. Ward and M. Penttilä, 2002. Enzymatic properties and intracellular localization of the novel Trichoderma reesei β-glucosidase BGLII (Cel1A). Applied and Environmental Microbiology, 68(9): 4546-4553.
20. Turan, Y. and M. Zheng, 2005. Purification and characterization of an intracellular β-glucosidase from the methylotrophic yeast Pichia pastoris. Biochemistry, 70(12): 1363-1368.
21. Saha, B.C. and R.J. Bothast, 1996. Production, purification and characterization of a highly glucose-tolerant novel β-glucosidase from Candida peltata, Applied and Environmental Microbiology, 62(9): 3165-3170.
22. Saha, B.C., S.N. Freer and R.J. Bothast, 1994. Production, purification and properties of a thermostable β-glucosidase from a color variant strain of Aureobasidium pullulans. Applied and Environmental Microbiology, 60(10): 3774-3780.
23. Liu, D., R. Zhang, X. Yang, S. Song, Y. Miao and Q. Shen, 2012. Characterization of a thermostable β-glucosidase from Aspergillus fumigatus Z5 and its functional expression in Pichia pastoris X33. Microbial Cell Factories, 11:25. doi:10.1186/1475-2859-11-25.
24. Singhania, R.R., A.K. Patel, R.K. Sukumaran, C. Larroche and A. Pandey, 2013. Role and signi?cance of beta-glucosidases in the hydrolysis of cellulose for bioethanol production. Bioresource Technology, 127: 500-507.

25. Enari, T.M. and M.L. Niku-Paavola, 1987. Enzymatic hydrolysis of cellulose: Is the current theory of the mechanism of hydrolysis valid? Critical Reviews in Biotechnology, 5(1): 67-87.
26. Elshafei, A.M., M.M. Hassan, M.A.E. Abouzeid, D.A. Mahmoud and D.H. Elghonemy, 2012. Purification, characterization and antitumor activity of L-asparaginase from Penicillium brevicompactum NRC829. British Microbiology Research Journal, 2(3): 158-174.
27. Singh, J., Batra, N. and R.S. Chander, 2004. Purification and characterization of alkaline cellulase produced by a novel isolate, Bacillus sphaericus JS1. Journal of Indian Microbiology and Biotechnology, 31(2): 51-56.
28. Story, S.V., C. Shah, F.E. Jr. Jenney and M.W.W. Adams, 2005. Characterization of a novel zinc-containing, lysine-specific aminopeptidase from the hyperthermophilic archaeon Pyrococcus furiosus. Journal of Bacteriology, 187(6): 2077-2083.
29. Saha, B.C., 2004. Production, purification and properties of endoglucanase from a newly isolated strain of Mucor circinelloides. Process Biochemistry, 39(12): 1871-1876. 67-87.
30. Joo, A.R., M. Jeya, K.M. Lee, H.J. Moon, Y.S. Kim and J.K. Lee, 2010. Production and characterization of β-1-4 glucosidase from a strain of Penicillium pinophilum. Process Biochemistry, 45(6): 851-858.
31. Ng, L.S., C.W. Li, S.P. Chan, J.L. Chir, P.T. Chen, C.G. Tong, S.M. Yu and H.T.K. David, 2010. High level production of a thermophilic β-glucosidase from Penicillium citrinum YS40-5 by solid-state fermentation with rice bran. Bioresource Technology, 101(4): 1310-1317.

Persian Abstract

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چکیده

بتا گلوکزیداز در سالهای اخیر توجه زیادی را با نقش مهم آن در فرآیندهای مختلف بیوتکنولوژی مانند هیدرولیز گلوزید ایزوفلاون ، تولید سوخت اتانولی از پسماندهای کشاورزی ، آزاد کردن ترکیبات آروماتیک از مواد اولیه طعم دار نشده و ... به خود جلب نموده است. در این مطالعه بتاگلوکزیداز خارج سلولی با تحریک *Penicillium commune ITV01* توسط سلولز تولید و توسط فرایند electrofocusing (IEF) با استفاده از فیلتراسیون ژل Sephadex G-100 ، خالص و همگن بدست آمده است. این آنزیم مشخصه یابی گردید و وزن مولکولی آن بوسیله SDS-PAGE برابر با ۱۴۴/۲ کیلودالتون تخمین زده شد. نقطه ایزوالکتریک با IEF ۴/۷۳ بدست آمد و همچنین این آنزیم قادر بود تا سلوبیوز و سلولز را به گلوکز هیدرولیز کند اما در مورد لامینارین ، زایلن ، نشاسته ، pullulan، کیتین کلوئیدی و کربوکسی متیل سلولز اینگونه نبود. pH و دمای بهینه به ترتیب ۵ و ۵۰ درجه سانتیگراد بود. همچنین در بازه دمایی ۳۰ تا ۵۰ درجه سانتیگراد و مقادیر pH بین ۵ تا ۷ به مدت ۲۴ ساعت پایدار بود. فعالیت آنزیمی توسط یونهای Ca^{++} , Cu^{++} , Fe^{++} , Mn^{++} , Cu^{+} ,K^{+} و بویژه Co^{++} فعال شد. بتاگلوکزیداز بطور کامل توسط Hg^{++} مهار گردید. در نتیجه بتاگلوکزیداز جدید خالص سازی شده از *P. commune* پتانسیل خوبی برای استفاده در بیوتکنولوژی دارد.