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Purification and Characterization of β-Xylosidase from *Trichoderma* sp. SY

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Abstract A β-xylosidase was purified from the culture of *Trichoderma* sp. SY. The ten-day-old culture filtrate was concentrated, followed by ion-exchange chromatography and gel filtration chromatography. As a result, β-xylosidase was finally purified about 53-fold and appeared as a single band by SDS-PAGE. The optimum pH and temperature were 5.0 and 55°C, respectively, and the molecular weight about 80 kDa. The purified β-xylosidase was found to be inhibited by various metal ions but not inhibited by xylose.

Key words: β-Xylosidase, *Trichoderma*

Xylan is one of the most abundant carbohydrate resources found in nature and a complex polysaccharide consisting of a backbone of β-D-1,4-linked xylopyranoside units substituted with acetyl, glucuronosyl, and arabinosyl side chains [15]. For the complete degradation of xylan, endo-β-xylanase and β-D-xylosidase are required, where endo-β-xylanases (EC 3.2.1.8) act on xylans and xylooligosaccharides, producing mainly mixtures of xylooligosaccharides, while β-D-xylosidases (EC 3.2.1.37) hydrolyze xylooligosaccharides, produced through the action of β-xylanases, to D-xylose [3]. Interest in β-xylanases has increased due to their application in biobleaching and the food and animal feed industries [1]. A lots of previous researchers has focused on finding thermophilic xylanases and cellulose-free xylanases [6–8]. In addition, the importance of β-xylosidase as a constituent of cell wall degrading enzymes in plant pathogenicity has been evaluated [13, 15]. Previously, the current authors reported on the enzymatic characterization of endo-β-xylanase from *Trichoderma* sp. SY. However, the current study purified and characterized the β-xylosidase from *Trichoderma* sp. SY, as β-xylosidase cooperates with endoxylanase for the complete degradation of xylan into a monomer.

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The *Trichoderma* sp. SY, previously isolated from the soil [11], was grown on HMT-medium with cellulose as a carbon source. β-Xylosidase activity was measured by using nitrophenyl-xylofuranoside as a substrate [13]. Also, xylan and oat meal were used to induce the production of β-xylosidase. However, the addition of 2% sucrose as a carbon source repressed the production of β-xylosidase in *T.* sp. SY. as with production of β-xylanase in *T.* sp. SY. Carbon catabolite repression, which is known to be involved in CREA in filamentous fungi such as *Aspergillus nidulans* and *Neurospora crassa*, was also observed in the production of other enzymes [4, 12].

The β-xylosidase activity reached a maximum after 10-day culture. Ten-day-old culture filtrate was concentrated by ultrafiltration (MW CO 10 K). The filtrates were dialyzed in 10 mM Tris/HCl (pH 7.5) and loaded onto DEAE-cellulose chromatography column (2 cm×10 cm) equilibrated with the dialysis buffer. The bound proteins were then eluted by a 30-min linear gradient from dialysis buffer to dialysis buffer plus 0.5 M NaCl at a flow rate of 2 ml/min. β-Xylosidase activity was detected in both unbound and bound fractions, suggesting that at least two xylosidases were present in the culture filtrate. Although most fungi produce single xylosidase, *Aspergillus pulverulentus* produce two β-xylosidases whose molecular weights are 180 kD and 190 kDa, [14] and *Penicillium wortmanni* produce four β-xylosidases [10]. The unbound fractions were collected, dialyzed in 10 mM sodium acetate buffer (pH 4.0), and was resolved again with HQ/20 anion-exchange Fast Performance Liquid Chromatography (FPLC) column (4.6×100 mm, Poros, Pharmacia, Piscataway, NJ, U.S.A.). The fractions containing β-xylosidase activity were collected, concentrated by ultracentrifugation though a 10 kDa molecular weight cut-off ultracentrifugation membrane, and applied to a Superdex 200 HR 10/30 FPLC column.

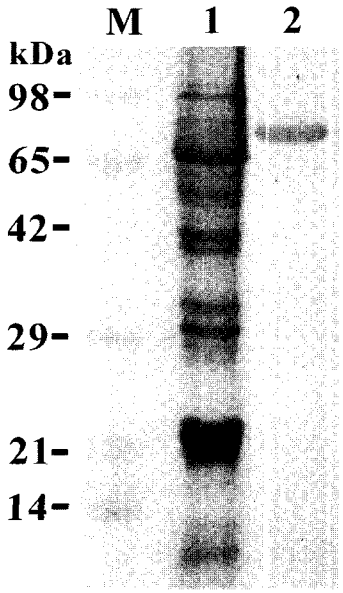

Fig. 1. SDS-PAGE analysis of β-xylosidase from *Trichoderma* sp. SY.
Lane 1: Culture filtrates; Lane 2: Purified β-xylosidase.

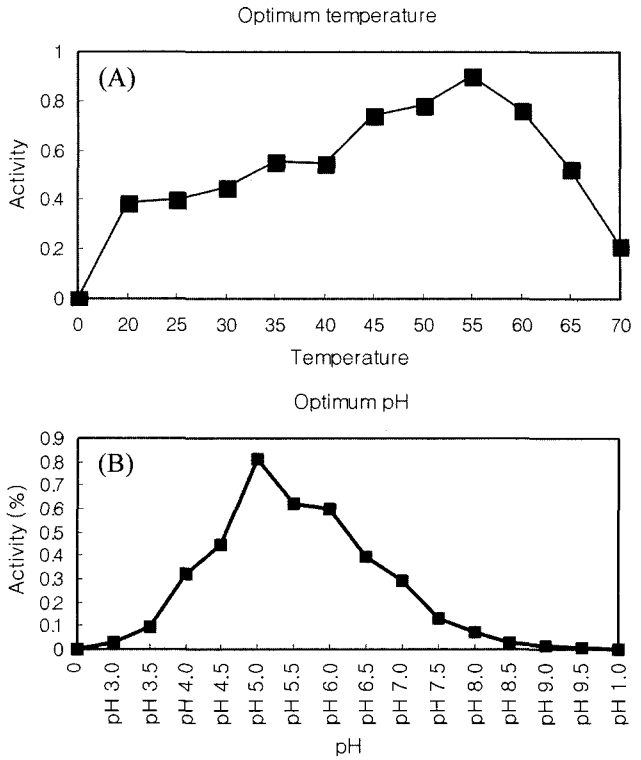

Fig. 2. Temperature dependence (A) and pH dependence (B) of purified β-xylosidase.
The enzyme activity was assayed at various temperatures. All reactions were run for 30 min with 5 mU of enzyme.

The proteins were then eluted with Tris/HCl (pH 8.0) buffer. The fraction containing β-xylosidase was resolved as a single peak, and the SDS-PAGE result showed that the β-xylosidase was puried to homogeneity with an estimated molecular weight of about 80 kDa (Fig. 1). The β-xylosidase was purified 53-fold with the final yield of 8.4% (Table 1). β-Xylosidases were purified from various fungi and most of their molecular weight ranged from 90-kDa to 210-kDa at optimum temperature of around 60°C [2].

The optimum temperature of β-xylosidase was determined by incubating the purified β-xylosidase between 20°C to 75°C. β-Xylosidase exhibited the maximum activity at 55°C (Fig. 2A). The pH optimum was determined with buffers composed of 50 mM sodium citrate buffer (pH 3.0-4.0), 50 mM potassium acetate buffer (pH 4.5-5.5), 50 mM potassium phosphate buffer (pH 6.0-8.0), and 50 mM Tris/HCl buffer (pH 8.5-10.0). The optimum pH was 5.0 (Fig. 2B). The effect of various metal ions and reagents on the β-xylosidase activity was examined by adding 1 mM of metal reagents. As a result, β-xylosidase was inhibited by Ca^{+2}, Cu^{+2}, Fe^{+2}, and Mg^{+2}. Also, EDTA and dithiothreitol (DTT) inhibited the β-xylosidase (Table 2).

To complete degradation of xylan into xylose, β-xylosidase is a critical enzyme. However, inhibition of β-xylosidase activity by xylose is a barrier that needs to be overcome. Thus, screening for a xylose-tolerant β-xylosidase is important for enzymatic saccharification of xylan. Therefore, current studies investigated whether the purified β-xylosidase was inhibited by xylose, and found that the presence of xylose at a concentration of 40 mM had no effect on the enzyme activity. Therefore, it would appear that the purified β-xylosidase is xylose-tolerant. The β-xylosidase from *Humicola grisear* var. *thermoidea*

Table 1. Purification of β-xylosidase from culture filtrates of *Trichoderma* sp. SY.

	Total protein (mg)	Total xylosidase (U)	Specific activity (U/mg protein)	Yield (%)	Purification (fold)
Culture filtrate	125	119	0.95	100	100
U. F. Retentate	18.8	95.4	5	80	5.3
Dialysis	14.2	74	5.2	62	5.5
DEAE-Cellulose eluant	2	14.6	7.3	12	7.7
Cation-exchange FPLC	0.3	11.5	38.3	10	40
Gel-filtration FPLC	0.2	10	50	8.4	52.6

Table 2. Comparison of various reagents as inhibitors of purified β-xylosidase from fungus *Trichoderma* sp. SY.

Effector	Concentration (mM)	Relative enzyme activity (%)
None	-	100
$CaCl_2$	5	72.8
$CuSO_4$	5	27.7
$FeSO_4$	5	72.0
$MgCl_2$	5	90.0
DTT	10	93.3
EDTA	10	85.2

was not inhibited by xylose at final concentration of 10 mM [10].

The purified β-xylosidase did not exhibit any activity against *p*-nitrophenyl arabinofuranoside or any xylanase activity, as it did not degrade oat spelt xylan or birchwood xylan. Some β-xylosidases only degrade xylooligosaccharides [2, 9, 13], while the β-xylosidase from *Trichoderma reesei* only is a multifunctional enzyme exhibiting α-L-arabinofuranosidase activity and xylanase activity as well as β-xylosidase activity [5].

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