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A β-xylosidase from cell wall of maize: Purification, properties and its use in hydrolysis of plant cell wall

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ABSTRACT

To study the potential of plant glycoside hydrolase for hemicelluloses hydrolysis, a β-xylosidase with molecular weight of 68.5 kDa was purified from the maize during senescent stage. The optimal conditions for the β-xylosidase were 37 °C and pH 4.5. In absence of substrate, the β-xylosidase was comparatively stable at 37 °C and pH 4.5–5.5. At the optimum condition, the K_m and k_{cat} values of the β-xylosidase against *p*-nitrophenyl-xyloside were 2.5 mM and 6.5 s^{-1}, respectively. The enzyme activity was promoted by LiCl, $CaCl_2$, $MnCl_2$, $MgCl_2$, KCl, and NaCl, however severely inhibited by $CuCl_2$, $ZnCl_2$, $AgNO_3$, $HgCl_2$, and $NiCl_2$. The purified β-xylosidase was active against xylobiose, xylotriose, xylotetraose, and xylopentaose. In hydrolysis of corn stover hemicellulose, the xylose production increased by 94.9% and 140% when *Trichoderma reesei* hemicellulase supplemented with purified β-xylosidase and crude cell wall proteins of corn stover, respectively. The biochemical characterization of the maize β-xylosidase makes it a promising candidate enzyme additive for hemicelluloses hydrolysis.

1. Introduction

Xylan is the major component of plant hemicellulose and the second most abundant renewable polysaccharide in nature [1]. As a potentially renewable energy resource that could be converted to biofuel, xylan hydrolysis has gained growing interest. Xylan is a heterogeneous polysaccharide consisting of a main chain of β-1,4-linked D-xylose residues that often carry arabinosyl, acetyl, and glucuronosyl substituents.

The main enzymes involved in xylanbackbone hydrolysis are endo-1,4-β-xylanase and β-xylosidase. Endo-1,4-β-xylanase (EC 3.2.1.8) cleaves xylan into β-D-xylopyranosyl oligosaccharides [2,3]. β-Xylosidase (EC 3.2.1.37) cleaves these xylo-oligomers into xylose monomers [4,5]. β-Xylosidase activity is rate limiting in xylan hydrolysis [6] and addition of pure β-xylosidase to commercial hemicellulolytic enzyme mixture are usually applied to boost xylan hydrolysis [3]. In present, the β-xylosidases used for hemicelluloses hydrolysis are generally produced by fungi [7–9] and bacteria [10].

Cell wall proteins (CWPs) are important component of plant cell wall, which comprise about 5–10% of cell wall dry weight [11–13]. To now, around 400 CWPs, representing about one fourth of estimated cell wall proteome, have been described from Arabidopsis [14]. As key components of CWPs, glycoside hydrolase plays crucial roles in cell wall structure and architecture, cell enlargement, and many other physiological processes [15,16]. To now, plant β-xylosidases have been reported and characterized in maize [11], barley [17], potato tubers [18], tobacco microspores [19], and ripening strawberry fruit [20].

However, to our knowledge β-xylosidase has not been studied in the senescent stage of maize, and the application of plant β-xylosidase for biomass conversion has not yet been reported either. Xylan such as arabinoxylan from corn stover is highly substituted and recalcitrant to degradation by microbial enzymes [21,22], therefore new enzyme are needed for hemicellulose completely hydrolysis. As one of the key enzyme for plant cell wall modification, β-xylosidase of maize may be uniquely suited to hydrolyze the hemicelluloses of corn stover.

To seek new sources of β-xylosidase with improved specificity hydrolysis and reduce the enzyme cost in the agricultural residues bioconversion process, current study was initiated to characterize the maize β-xylosidase and the application in hemicelluloses hydrolysis. With the advantage of rich in resource, fresh corn stover was used as the material for β-xylosidase purification and characterization.

2. Materials and methods

2.1. Materials and enzymes

Fresh corn (NongDa 108, China Agricultural University, Beijing, China) stover without visible microbial contamination was

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harvested, washed with distilled water for 5 times and stored at −20 °C for β-xylosidase extraction. For enzymatic hydrolysis, the corn stover was stored dry for 7 months at ambient temperature. The dry corn stover was pretreated with steam explosion as described previously [23]. The steam explosion pretreated corn stover including the water soluble part was collected and air dried, then used for hemicellullose hydrolysis. The composition of corn stover was determined according to the method of Goering and Van Soest [24]. After the pretreatment, the composition of steam exploded corn stover (SECS) was 42.2% cellulose, 26.8% lignin, 12.1% hemicellulose and others 18.9% by dry weight. The SECS was milled to pass through 60-mesh filter, the screened fraction was kept at 4 °C as the source for hydrolysis.

Commercial hemicellulase mixture (xylanase: 10.1 IU/mL, β-xylosidase: 0.5 IU/mL) produced by *Trichoderma reesei* was purchased from Ningxia Xiasheng Co., Ltd., China. Aminex Hpx 87H column (300 mm × 7.8 mm) was purchased from Bio-Rad Laboratories, Inc., Hercules, CA. Xylan from birch wood was purchased from Sigma. Biochemical reagents were of analytical grade, and obtained from Beijing Chemical Co., Ltd.

2.2. Extraction and purification of β-xylosidase from corn stover

All of the extraction purification steps were carried out at 4 °C. Protein in chromatographic column effluent was monitored at $A_{280\,nm}$. Fresh corn stover (1.5 kg, i.e. dry weight about 300 g) was used as material for β-xylosidase extraction. The crude cell wall proteins were extracted as described previously [25,26]. Briefly, the corn stover was homogenized in blender with freshly prepared grinding buffer (20 mM sodium acetate, 1 mM EDTA, pH 4.5), then filtered through 200-mesh screen, and the residue was washed thoroughly using the same buffer. The residue was then suspended in freshly prepared extracting buffer (20 mM Hepes, 2 mM EDTA, 3 mM $Na_2S_2O_5$, 1 M NaCl, 1 mM PMSF, pH 6.8), the homogenate was stirred with a magnetic stirrer for 24 h, and the cell wall fragments were removed by filtering through 200-mesh screen. The cell wall proteins were included in the salt soluble fraction, which was used for the following β-xylosidase purification.

The β-xylosidase activity versus *p*-nitrophenyl-xyloside (*p*-NPX) was measured during the extraction and purification. In β-xylosidase activity assay, 90 μL *p*-NPX (2 mM in 50 mM sodium acetate buffer, pH 4.5) and 10 μL enzyme were incubated at 40 °C for 10 min. The reaction was terminated by adding 200 μL Na_2CO_3 (180 mM), the released *p*-nitrophenol was measured at 400 nm with a spectrophotometer.

2.3. Ammonium sulfate fractionation

The crude cell wall protein was first precipitated by ammonium sulfate at 40% saturation, then centrifuged (15,000 × *g*) at 4 °C for 15 min. The ammonium sulfate was increased by 10% each step to 80%. The precipitate collected was dialyzed against acetate buffer (20 mM, pH 4.5) for 12 h, then subjected to β-xylosidase activity assay. The fractions precipitated with ammonium sulfate between its 60% and 80% saturation were pooled together, and concentrated with Amicon PM-10 units (Millipore, USA) for following purification.

2.4. CM-sephadex cation-exchange chromatography

The pooled fractions were loaded on a CM-sephadex cation-exchange column G75 equilibrated with sodium acetate buffer (20 mM, pH 4.5) [27]. Proteins were eluted with the same buffer, followed by a 0.0–1.0 M NaCl discontinuous gradient at a flow rate of 0.3 mL/min, 3.0 mL of fractions were collected and assayed for β-xylosidase activity. Peak fractions showing β-xylosidase activity were pooled concentrated by Amicon PM-10 units.

2.5. Preparative polyacrylamide gel electrophoresis (PAGE)

The protein with β-xylosidase activity was loaded on a preparative PAGE (8%, w/v) using Bio-rad electrophoresis system (16 cm × 20 cm), and run at 60 V for 12 h [28]. After electrophoresis, the gel was detected with ultraviolet light at $\lambda_{300\,nm}$, bans were cut immediately, similar bands were collected and eluted in sodium acetate buffer (20 mM, pH 4.5), and the parts with β-xylosidase activity were collected together.

2.6. Characterization of the corn stover β-xylosidase

2.6.1. Molecular weight (M_w) determination by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The molecular weight of the β-xylosidase was checked with SDS-PAGE, using 12% polyacrylamide in resolution gels and 5% in stacking gel [29].

2.6.2. Protein digestion and analysis with reverse-phase capillary liquid chromatography–electrospray ionization tandem mass spectrometry (LC–ESI/MS)

The band of β-xylosidase was cut from SDS-PAGE and digested in gel overnight with trypsin (Sigma) according to Shevchenko et al.'s method [30]. The trypsin digestion sample was analyzed on an ion-trap mass spectrometer model ESI-QUAD-TOF (Micromass, Manchester, UK) to generate the peptide sequence information. Thereafter, a database search was conducted using the MS/MS ion search (MASCOT, http://www.matrixscience.com/) against all plant entries to find any potential homology-based matches.

2.6.3. Substrate specificity assays

Substrate specificity of the β-xylosidase was assayed in sodium acetate buffer (50 mM, pH 4.5) at 37 °C. Besides *p*-NPX, the enzyme activity against xylan, *p*NP-β-D-cellobiose (*p*NPC), *p*NP-α-L-arabinofuranoside (*p*NPA), and carboxymethycellulose-Na (CMC-Na) were also checked. The reducing sugar from xylan and CMC-Na were measured by dinitrosalicylic acid (DNS) method. The activity on *p*-NPX, *p*NPA and *p*NPC were checked as in Section 2.2. To evaluate possible synergistic activity between xylanase (*T. reesei*) and corn stover β-xylosidase in xylan (1%) hydrolysis were analyzed as follows: (1) xylanase (*T. reesei*, 0.1 IU); (2) corn stover β-xylosidase (0.1 IU); (3) xylanase (*T. reesei*, 0.1 IU) and corn stover β-xylosidase (0.1 IU).

2.6.4. Optimum temperature and thermal stability

The temperature dependence of the corn stover β-xylosidase was carried in ranged of 20–70 °C in sodium acetate buffer (pH 4.5, 50 mM). In thermal stability analysis, the purified β-xylosidase was preincubated in absence of substrate at 37 °C, 45 °C, and 50 °C, respectively. Aliquots were withdrawn at various times, and the residual activities were measured with *p*-NPX as substrate.

2.6.5. Optimal pH and pH stability

In pH optimum determination, the activity of the β-xylosidase against *p*-NPX was checked in pH range of 3.0–7.5 at 37 °C. The buffer for pH 3.0–5.5 is sodium acetate (50 mM), pH 6.0–7.5 is sodium phosphate buffer (50 mM). In pH stability determination, the β-xylosidase was incubated in different buffer (50 mM) of pH series (3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6, 6.5, 7.0 and 7.5) for 60 min at 37 °C, then adjusting pH to 4.5, the residual activity with checked with *p*-NPX as substrate.

2.6.6. Kinetic parameters

Kinetic parameters of purified β-xylosidase were determined against *p*-NPX in a concentration range of 0.05–4.0 mM in sodium acetate buffer (pH 4.5, 50 mM) at 37 °C. Kinetic data were processed using GraphPad program based on Michaelis–Menten enzyme kinetics.

2.6.7. Effect of metal ions and chemical reagents on β-xylosidase activity

The corn stover β-xylosidase was incubated in sodium acetate buffer (pH 4.5, 50 mM), with addition of the following metal ion salts and chemical reagents to final concentration of 10 mM: $HgCl_2$, $AgNO_3$, $MgCl_2$, $CaCl_2$, $BaCl_2$, NaCl, KCl, LiCl, $CuCl_2$, $ZnCl_2$, $MnCl_2$, $FeCl_2$, $NiCl_2$, β-mercaptoethanol, and EDTA. After preincubating for 30 min at room temperature, the residual enzyme activities versus *p*-NPX were determined thereafter.

2.6.8. Hydrolysis of xylo-oligosaccharides with corn stover β-xylosidase

Xylo-oligosaccharides (xylobiose, xylotriose, xylotetraose, xylopentaose) were prepared in sodium acetate (50 mM) buffer with concentration of 1.25 mg/mL. The xylo-oligosaccharides (final concentration 1.0 mg/mL) were incubated with the purified β-xylosidase (5.0 IU/mL) to final volume of 100 μL in optimum conditions for 6 h. The reaction mixture was freeze dried and dissolved in 10 μL of ddH_2O, and spotted onto a silica plate (1 μL for each reaction mixture, thin-layer chromatography aluminum sheets; Merck), and developed with solvent (n-butanol/water, v/v, 85:15). The plates were stained by spraying with a solution containing H_2SO_4/methanol (v/v, 9/1) and 0.2% orcinol, then heating at 70 °C for 10 min.

2.6.9. Transxylosylation activity assay with xylobiose as substrate

Xylobiose (final concentration 30 mg/mL) was incubated with the purified corn stover β-xylosidase (5.0 IU/mL) at 37 °C for 60 min in sodium acetate buffer (50 mM, pH 4.5). The mixture was then boiled for 5 min to terminate the reaction, and subjected to high performance liquid chromatography (HPLC) analysis.

2.7. Application of corn stover β-xylosidase in hemicelluloses hydrolysis

Enzymatic hydrolysis of SECS hemicellulose (20 mg) was performed in sodium acetate buffer (50 mM, pH 4.5, 200 μL) at 37 °C for 12 h with gentle agitation. Different enzyme mixtures were applied, respectively: (1) hemicellulase mixture (*T. reesei*, xylanase 1.0 IU and β-xylosidase 1.0 IU); (2) crude CWPs of fresh corn stover (β-xylosidase 1.0 IU); (3) hemicellulase mixture (*T. reesei*) and crude CWPs; (4) corn stover β-xylosidase (1.0 IU); (5) hemicellulase mixture (*T. reesei*) and corn stover β-xylosidase. After hydrolysis, the samples were boiled for 10 min to terminate the reaction and stored at −20 °C for xylose analysis. Xylose of the hydrolysate was determined by high performance liquid chromatography (HPLC) method as described previously [31].

2.8. Analytical methods

The compositions of SECS were determined according to the method of Goering and Van Soest [24]. The amount of xylose and xylobiose in the reaction mixture was quantified by a high performance liquid chromatography system (Agilent technology 1200 series, Palo Alto, CA). Samples were filtered through a 0.45 μm filter and diluted properly by eluent (5 mM H_2SO_4). The separation was performed on an Aminex Hpx-87H ion exclusion column (Bio-Rad, Sunnyvale, CA) with 5 mM H_2SO_4 as eluent at a flow rate of 0.6 mL/min. Protein concentrations were determined according to the Coomassie Brilliant Blue method using bovine serum albumin as a standard [32].

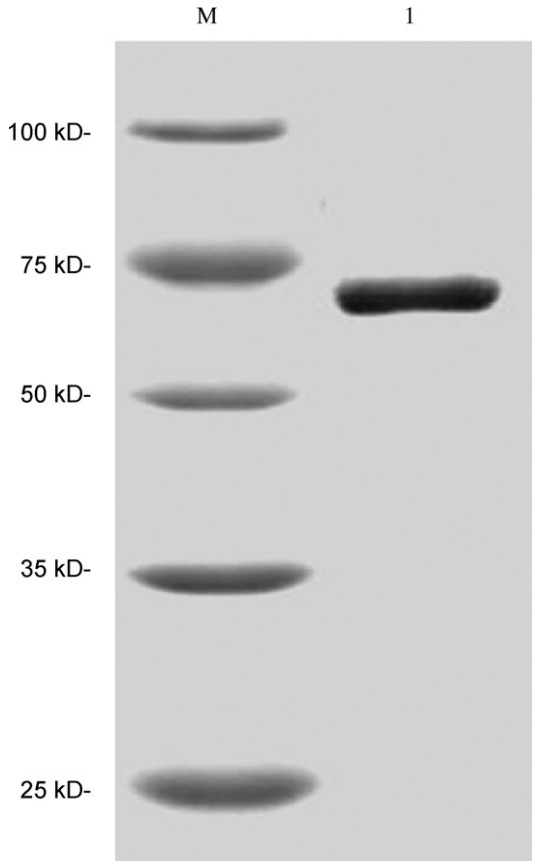

Fig. 1. SDS-PAGE of the purified β-xylosidase from fresh corn stover. Lane M: protein markers. Lane 1: β-xylosidase.

3. Results and discussion

3.1. Extraction and purification of β-xylosidase from corn stover

Stem tissues of maize at the senescent stage were used for cell wall proteins (CWPs) extraction and enzyme activity analysis. The crude CWPs of fresh corn stover were extracted with high salt concentration buffer. The β-xylosidase of the crude CWPs was purified through three steps as described below (Table 1). The crude CWPs were firstly fractionated by bulk ammonium sulfate precipitation, the 60–80% saturated fraction was collected and dialyzed against sodium acetate buffer (20 mM, pH 4.5) for CM-sephadex G75 column purification. The specific activity increased to 16.65 IU/mg after CM-sephadex chromatography. The fraction with β-xylosidase was further purified with preparative PAGE, the final specific β-xylosidase activity reached 24.36 IU/mg. In SDS-PAGE, the β-xylosidase revealed as a single band with M_w of 68.5 kDa (Fig. 1).

3.2. Characterization of the purified β-xylosidase

3.2.1. Peptide sequence determination and protein identification

To identify the protein, the purified β-xylosidase was further analyzed with capillary LC–ESI/MS in data-dependent acquisition mode. The resulting data obtained was processed using the Mascot MS/MS Ion search engine. The results indicated that the corn stover β-xylosidase has high homology with a β-xylosidase of *Oryza sativa* (GenBank accession no. BAB55751). The matched peptide frag-

Table 1
Purification of β-xylosidase from fresh corn stover.

Purification Step	Total protein (mg)	Total activity (IU)	Specific activity (IU/mg)	Purification (fold)	Recovery (%)
Crude extract	836.58	920.24	1.1	1	100
$(NH_4)_2SO_4$ precipitation	248.36	797.24	3.21	2.92	86.63
CM-sephadex	32.31	537.96	16.65	15.14	58.46
Preparative PAGE	13.56	363.41	26.80	24.36	39.49

ments (VRDLVGR, RDPRWGR, NDAALLPLSPEK) were located at the position of 75–81, 184–190, 442–453 of *O. sativa* β-xylosidase. The matched *O. sativa* β-xylosidase belongs to glycoside hydrolase (GH) 3, and has two conserved domain at the N-terminal and C-terminal. The molecular weight of the matched *O. sativa* β-xylosidase is 87 kDa. The enzyme characterization and the origin indicating the two β-xylosidases are different.

3.2.2. Optimum temperature and thermal stability

The optimum temperature and thermal stability of the β-xylosidase were shown in Fig. 2. As shown in Fig. 2A, the β-xylosidase was sensitive to temperature, and showed maximal activity at about 37 °C. The activity decreased significantly above 50 °C and below 30 °C. The optimum temperature of the enzyme was similar to the β-xylosidase of barley [17], but different from Arabidopsis β-xylosidases, which showed temperature optima around 60 °C [33]. β-Xylosidase from potatoes showed an optimal activity at a temperature of 50 °C [18]. The temperature optimum of the purified β-xylosidase was also different from most microbial β-xylosidases, which showed optimal temperature above 50 °C and depressed activity at 37 °C. *T. reesei* β-xylosidase has a temperature optimum at 60 °C [6], and the temperature optimum for *Aspergillus niger* β-xylosidase is as high as 70 °C [34].

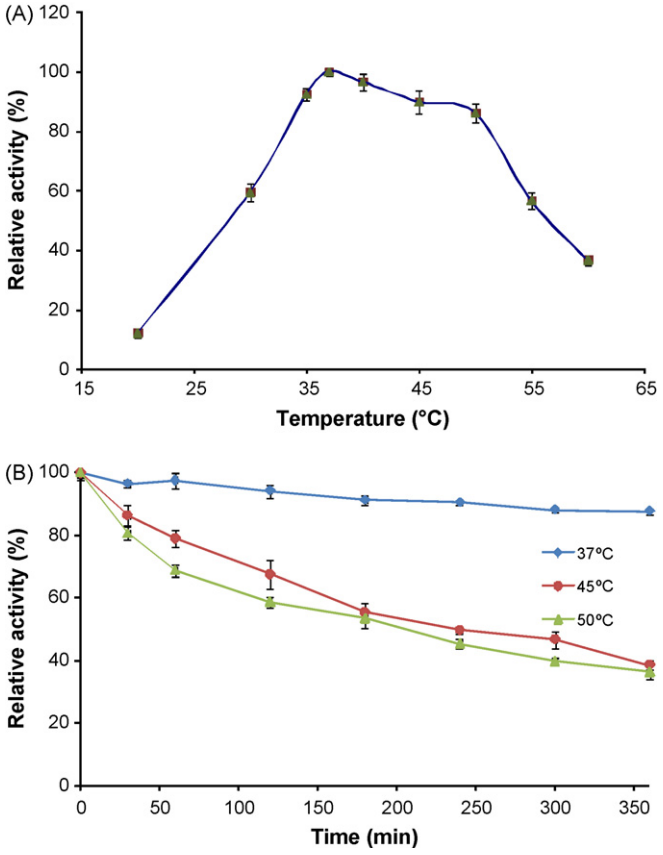

Fig. 2. Effect of temperature on β-xylosidase activity (A) and thermal stability (B). For temperature optimum analysis, the β-xylosidase activity against *p*-NPX was assayed at different temperatures (20–60 °C) in sodium acetate buffer (pH 4.5, 50 mM). In thermal stability analysis, the purified β-xylosidase was preincubated in absence of substrate at 37 °C, 45 °C, and 50 °C. After various times, aliquots were withdrawn and the residual activities were measured with *p*-NPX as substrate.

The thermal stability studies of the purified β-xylosidase was analyzed at 37 °C, 45 °C and 50 °C incubated for up to 360 min. The results showed that the activity of the enzyme decreases slowly with the increase of incubation time. Taken the relative initial activity as 100%, the residual activity at 30 min and 360 min decreased to 96.4% and 87.6% at 37 °C. The stability decreased rapidly at 45 °C, the enzyme lost 13.6% of its activity incubated for 30 min and 61.4% of its activity for 360 min. At 50 °C, the relative activity decreased to 80.6% and 36.4% when incubated for 30 min and 360 min, respectively. It could be seen that the corn stover β-xylosidase is active and stable around 37 °C, and showed depressed activity and thermal stability at 50 °C. The optimum temperature for most microbial β-xylosidases is higher than 50 °C, for example, the β-xylosidase of *T. reesei* was a relatively thermostable enzyme, which is stable up to 55 °C when incubated for 24 h [6].

3.2.3. Optimum pH and pH stability

As shown in Fig. 3, the optimum pH of the purified β-xylosidase was around 4.5, the activity decreased significantly below pH 4.0 and above pH 6.0. Based on the relative activity of 100% for the β-xylosidase at pH 4.5, the activity decreased to 67.2% and 50.8% at pH 6.0 and pH 7.0. Only 13.5% and 20.1% of the activity was retained at pH 3.0 and 3.5, respectively. The optimum pH is similar to that found in plant and microorganism, for example, β-xylosidase from potato has an optimal pH of 4.0–4.5 [18], *T. reesei* β-xylosidase has optimal pH of 4.0 [6].

In pH stability analysis, the β-xylosidase was incubated at different buffer without substrate. The results indicated that the enzyme was comparatively stable during 60 min incubation in the range of pH values 4.5–5.5. In contrast, the activity decreased markedly below pH 4.5 and above pH 6.5, only13.2% and 21.9% of its activity were retained at pH 3.5 and 7.5, respectively.

3.2.4. Substrate specificities

In specific activity analysis, the β-xylosidase has no activity against xylan, *p*NPC, *p*NPA or CMC-Na. However, the synergism between *T. reesei* xylanase and corn stover β-xylosidase in xylan (1%) hydrolysis was observed, the degree of synergism is 2.05.

3.2.5. Kinetic parameters

Kinetic parameters of the β-xylosidase for *p*-NPX were estimated at pH 4.5 and 37 °C. The K_m and k_{cat} values of the corn stover β-xylosidase for *p*-NPX were 2.5 mM and 6.5 s^{-1}. Several plant β-xylosidases have also been characterized. The K_m and k_{cat} values for β-xylosidase of barley against *p*-NPX were 1.7 mM and 15.5 s^{-1} [17]. The K_m of the corn stover β-xylosidase was higher than that the enzyme isolated from stem tissues of Arabidopsis [33].

3.2.6. Effects of metal ions and chemical reagents on the β-xylosidase activity

The β-xylosidase activity was tested with different metal ions or chemical reagents addition to the reaction mixture. As shown in Table 2, the enzyme showed enhanced activity in presence of LiCl, $CaCl_2$, $MnCl_2$, $MgCl_2$, KCl, and NaCl. However, heavy metal

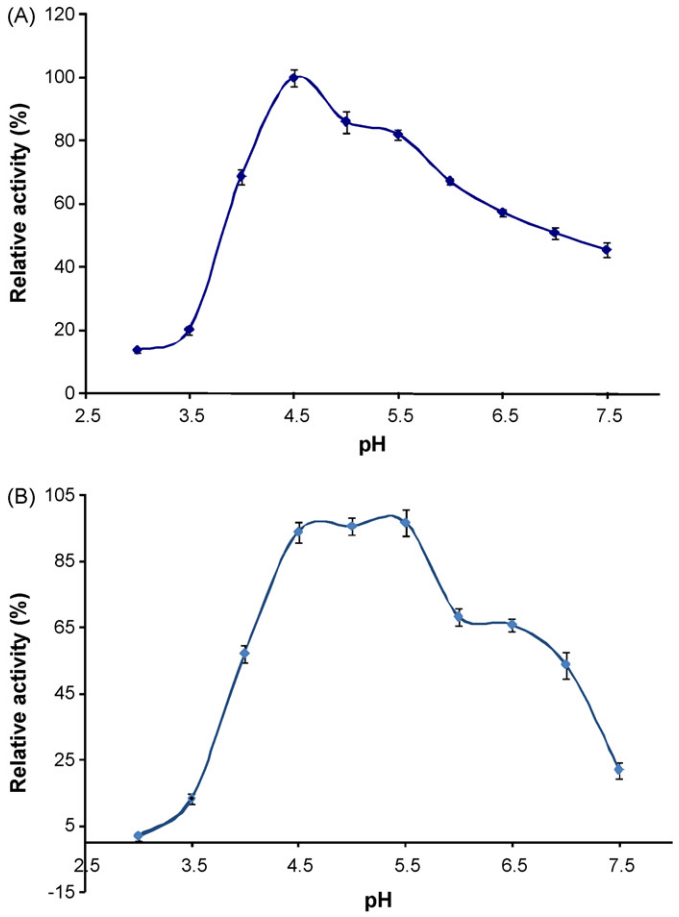

Fig. 3. Effect of pH on β-xylosidase activity (A) and pH stability (B). In pH optimum determination, the activity of the β-xylosidase against *p*-NPX was checked at 37 °C pH varied from 3.0 to 7.5. In pH stability determination, the β-xylosidase was incubated in different buffer (50 mM) of pH series (3.0–7.5) for 60 min at 37 °C, then adjusting pH to 4.5 for activity assay.

salts, such as $CuCl_2$, $ZnCl_2$, $AgNO_3$, $HgCl_2$ and $NiCl_2$ inhibited the β-xylosidase activity severely. The β-xylosidase activity was slightly inhibited with the presence of $BaCl_2$ and $ZnCl_2$, 87.09% and 93.71% of the original β-xylosidase activity were left. The β-xylosidase activity decreased markedly with EDTA and β-mercaptoethanol added. The effects of metal ions on enzyme activity were also analyzed for β-xylosidase from potato, the activity of which decreased with addition of Ni^{2+} and Cu^{2+} and slightly increased in the presence of Mn^{2+} or Ca^{2+} [18].

3.2.7. Hydrolysis of xylo-oligosaccharides with corn stover β-xylosidase

The catalytic activities of the purified β-xylosidase on xylo-oligosaccharides were shown in Fig. 4. The β-xylosidase was

Table 2
Effect of metal ions and chemicals on β-xylosidase activity.

Chemicals	Relative activity (%)	Chemicals	Relative activity (%)
Control	100	$MgCl_2$	121.7
EDTA	36.0	$ZnCl_2$	93.7
β-Mercaptoethanol	42.2	$MnCl_2$	56.8
$CuCl_2$	22.0	$AgNO_3$	10.4
LiCl	146.8	$HgCl_2$	7.9
$CaCl_2$	109.2	KCl	130.6
$BaCl_2$	87.1	NaCl	126.4
$FeCl_2$	43.3	$NiCl_2$	12.8

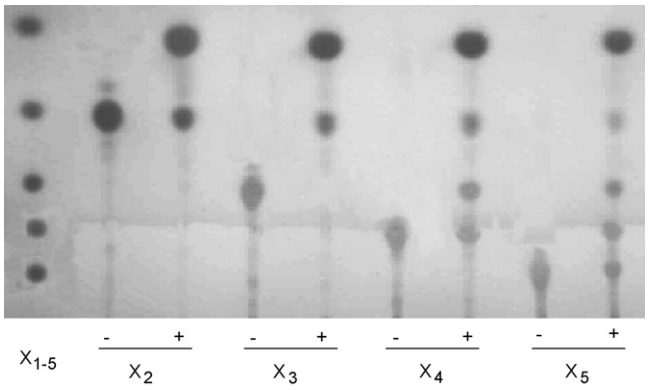

Fig. 4. Thin-layer chromatogram of xylo-oligosaccharides hydrolysis with corn stover β-xylosidase. Oligosaccharides (xylobiose, xylotriose, xylotetraose, xylopentaose, final concentration 1.0 mg/mL) were incubated with the purified β-xylosidase (5.0 IU/mL) in optimum conditions for 6 h. The products were freeze dried and dissolved in H_2O, then resolved with TLC. Lanes (−), no enzyme; lanes (+), β-xylosidase was added in the relation. Xylose (X_1) and xylo-oligosaccharides (X_2–X_5) were applied as standard.

active on xylobiose, xylotriose, xylotetraose, and xylopentaose. The results indicating that the β-xylosidase could convert the xylo-oligosaccharides into xylose monomers.

3.2.8. Transxylosylation activity assay with xylobiose as substrate

Besides hydrolysis of xylo-oligosaccharides to xylose, β-xylosidases can also transfer the β-xylosyl moiety from β-xylosyl donors to acceptors [35]. Some GH 3 β-xylosidases showed transxylosylation activity at high substrate concentrations, and xylo-oligosaccharides with a higher DP value are synthesized when xylobiose used as both substrate and acceptor [36]. To analyze transxylosylation activity, xylobiose (30 mg/mL) was incubated with the purified corn stover β-xylosidase for 60 min. HPLC analysis indicated that only xylose and xylobiose were found in the productions, no longer xylo-oligosaccharides (DP ≥ 3) were synthesized. The results indicating the corn stover β-xylosidase has no transxylosylation activity.

3.3. Application of corn stover β-xylosidase in SECS hemicellulose hydrolysis

The effect of the β-xylosidase on SECS hemicelluloses hydrolysis was analyzed in different hydrolytic system (Fig. 5). When *T. reesei* hemicellulase mixture was applied in the reaction, the xylose production was 1.38 mg/mL. The final xylose concentration was 0.21 mg/mL when corn stover β-xylosidase was added alone. In contrast, the final xylose concentration reached 2.69 mg/mL when *T. reesei* hemicellulase mixture and corn stover β-xylosidase applied together. The degree of synergism for *T. reesei* hemicellulase mixture and corn stover β-xylosidase was 1.69. When crude CWPs with same β-xylosidase activity was added to SECS, the final produced xylose was 0.41 mg/mL. The xylose concentration was 3.31 mg/mL when *T. reesei* hemicellulase mixture and crude CWPs added together. It could be seen that the promotion of crude CWPs is higher than that of the purified β-xylosidase. As a protein mixture, there might be some glycoside hydrolases exist in plant CWPs besides β-xylosidase, such as xylanase, β-glucosidase, β-exoglucanase. The presence of different glycoside hydrolases in fresh corn stover might be the reason for higher promotion for hydrolysis. Beta-xylosidase has been reported in germinating and stem tissues of plant, and has been found to involve in cell elongation and other cellular processes [15,16]. This study indicated that β-xylosidase of fresh corn stover can be used for hemicellulose hydrolysis. With the advantage of large resource, the enzyme

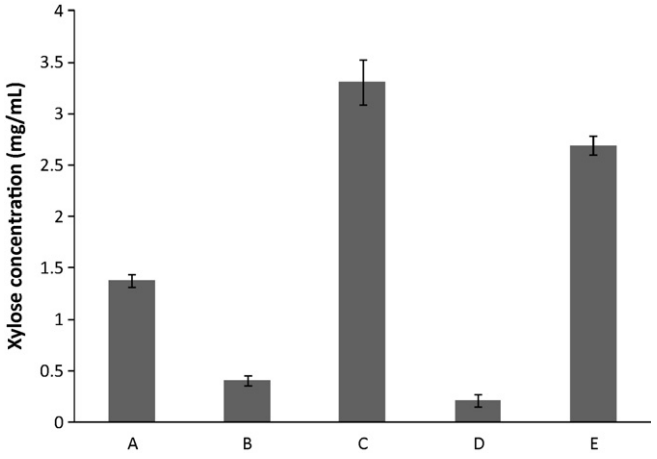

Fig. 5. Application of corn stover β-xylosidase in SECS hemicellulose hydrolysis. Enzymatic hydrolysis of SECS hemicellulose were performed in sodium acetate buffer (50 mM, pH 4.5), containing 20 mg SECS in final volume of 200 μL, at 37 °C with gentle agitation. Different enzyme mixtures were applied, respectively: (1) hemicellulase mixture (*T. reesei*, xylanase 1.0 IU, β-xylosidase 1.0 IU) (A); (2) crude CWPs of corn stover (β-xylosidase 1.0 IU) (B); (3) hemicellulase mixture (*T. reesei*) and crude CWPs (C); (4) corn stover β-xylosidase (β-xylosidase 1.0 IU) (D); (5) hemicellulase mixture (*T. reesei*) and corn stover β-xylosidase (E).

for biomass bioconversion could be reduced to some extent if the glycoside hydrolase of fresh corn stover could be applied effectively.

The synergism between β-xylanase and β-xylosidase in hydrolysis of xylan has been investigated extensively. The extent of xylan hydrolysis increased from 18% to 48% when *Neurospora crassa* β-xylanase supplemented with *N. crassa* β-xylosidase. The analysis of the production indicated that the amounts of lower xylo-oligosaccharides (X_2–X_4) and xylose were increased significantly compare with β-xylanase applied alone [37]. Delcheva et al. studied the synergism between *A. niger* β-xylanase and β-xylosidase in birchwood xylan. The amount of xylose produced was 0.2 mg/mL with *A. niger* β-xylanase, and increased to 2.1 mg/mL with *A. niger* β-xylosidase addition [9]. To improve the catalytic efficiency of β-xylosidase, thermostable *Sulfolobus solfataricus* β-xylosidase has been immobilized into alginate to synergize *Trichoderma viride* xylanase for birchwood xylan hydrolysis [38].

4. Conclusions

Xylan is a potential substrate for future energy resource; the effective and economical enzyme for xylose production has been studied extensive. A β-xylosidase was purified and characterized from harvested fresh corn stover. The β-xylosidase has optimum activity at pH 4.5 and 37 °C, was stable around 37 °C and pH 5.0. The corn stover β-xylosidase could synergize microbial xylanase to convert hemicellulose of SECS to xylose. The biochemical characteristics of the β-xylosidase and plentiful resource of fresh corn stover make it a promising enzyme additive for microbial hemicellulase in plant cell wall hydrolysis.

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