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Properties of a xylanase from *Streptomyces matensis* being suitable for xylooligosaccharides production

Qiaojuan Yan[a], Shanshan Hao[b], Zhengqiang Jiang[b,*], Qian Zhai[b], Weiwei Chen[b]

[a] *Bioresource Utilization Laboratory, College of Engineering, China Agricultural University, Beijing 100083, China*
[b] *Department of Biotechnology, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China*

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ABSTRACT

An extracellular xylanase produced by *Streptomyces matensis* DW67 was purified from the culture supernatant by ammonium sulfate precipitation, ion exchange and gel filtration chromatography and characterized. The xylanase was purified to 14.5-fold to homogeneity with a recovery yield of 14.1%. The purified xylanase appeared as a single protein band on SDS-PAGE with a molecular mass of 21.2 kDa. However, it had a very low apparent molecular mass of 3.3 kDa as determined by gel filtration chromatography. The N-terminal sequence of first 15 amino acid residues was determined as ATTITTNQTGYDGMY. The optimal temperature and pH for purified xylanase was 65 °C and pH 7.0, respectively. The enzyme was stable within the pH range of 4.5–8.0 and was up to 55 °C. The xylanase showed specific activity towards different xylans and no activity towards other substrates tested. Hydrolysis of birchwood xylan by the xylanase yielded xylobiose and xylotriose as principal products. The enzyme hardly hydrolyzed xylobiose and xylotriose, but it could hydrolyze xylotetraose and xylopentaose to produce mainly xylobiose and xylotriose through transglycosylation. These unique properties of the purified xylanase make this enzyme attractive for biotechnological applications, such as bioblenching in paper and pulp industries, production of xylooligosaccharides. This is the first report of the xylanase from *S. matensis*.

1. Introduction

Xylan, a major component of hemicellulose in plant cell walls, is generally composed of a linear backbone of 1,4-β-linked D-xylose units [1]. β-1,4-Endoxylanases (EC 3.2.1.8) are crucial for depolymerization of xylan. The heterogeneity and complexity of xylan has resulted in an abundance of diverse xylanases with varying specificities, primary sequences and folds [2]. Xylanases are produced by many microorganisms such as bacteria, fungi and actinomycetes [3,4]. Microbial xylanases have attracted considerable research interest in recent years because of their potential application in the food, animal feed, paper and pulp industries [2,3,5,6]. *Actinomycetes* are emerging as an important source of enzymes involved in xylan degradation and among these microorganisms, information is largely derived from *Streptomyces* [1,3,7,8]. Xylanases from *Streptomyces* species are frequently reported to be extracellular and cellulase-free [7,9–11].

Till date, xylanolytic activity has been reported from different *Streptomyces* species such as *S. exfoliates*, *S. flavogriseus*, *S. lividans*, *S. xylophagus*, *S. olivaceoviridis*, *S. actuosus*, and *S. cyaneus* [1,11–13]. Some xylanases from different *Streptomyces* species have been purified and characterized [5,11–14]. *Streptomyces matensis* has been reported to produce β-1,3-glucanase [15]. Recently, we have isolated a novel xylanase-producing strain, i.e. *S. matensis* DW67. In the present study, we are reporting for the first time the purification and characterization of a xylanase from this strain.

2. Materials and methods

2.1. Chemicals

Birchwood xylan, beechwood xylan, oat-spelt xylan, locust bean gum and carboxymethylcellulose (low viscosity) were purchased from Sigma (St. Louis, USA). Artificial substrates (*p*-nitrophenyl (*p*NP) derivatives) such as *p*NP-β-D-xylopyranoside, *p*NP-β-D-glucopyranoside, *p*NP-β-D-galactopyranoside and *p*NP-β-D-mannopyranoside were also obtained from Sigma. Sephadex G-50 and Superdex™ 75 were from Pharmacia (Pharmacia, Uppsala,

Abbreviations: BSA, bovine serum albumin; CAPS, (cyclohexylamino)-1-propanesulfonic acid; CHES, 2-(cyclohexylamino)ethanesulfonic acid; CMC, carboxymethylcellulose; DNS, dinitrosalicylic acid; MES, 2-(N-morpholino)ethane sulfonic acid; MOPS, 3-(N-morpholino)-propane sulfonic acid; *p*NP, *p*-nitrophenyl; TLC, thin-layer chromatography; X1, xylose; X2, xylobiose; X3, xylotriose; X4, xylotetraose; X5, xylopentaose.

* Corresponding author at: Department of Biotechnology, College of Food Science and Nutritional Engineering, China Agricultural University, PO Box 294, Beijing 100083, China. Tel.: +86 10 62737689; fax: +86 10 82388508.
E-mail address: zhqjiang@cau.edu.cn (Z. Jiang).

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Sweden). DEAE-52 (Pre-swollen Microgranular Anion Exchange Celluloses) was from Whatman (Whatman Inc., Fairfield, NJ, USA). All other chemicals used were analytical grade reagents unless otherwise stated.

2.2. Strain and culture condition

S. matensis DW67, isolated from forest soil sample (Shandong province, China), was used in this investigation. The strain was identified by the Institute of Microbiology of Chinese Academy of Sciences and was deposited (under the number CGMCC 4.5509) at China General Microbiological Culture Collection Center. For xylanase production, the fermentation medium of culture contained (g/L): corncob xylan, 15; yeast extract, 4; tryptone, 8; $MgSO_4{\cdot}7H_2O$, 0.5; KH_2PO_4, 10; Tween-80, 5. A piece (1 cm^2) of growing 4–5-day-old culture of the strain was used to inoculate the fermentation medium (50 mL) in 250 mL Erlenmeyer flasks. Triplicate cultures were grown in an air-shaker at 150 rpm for 5 days at 30 °C. The cultures were centrifuged at 10,000 × *g* for 10 min at 4 °C, the supernatant was used as crude enzyme.

2.3. Enzyme and protein assays

Xylanase activity was determined according to Bailey et al. [16], using 1.0% (w/v) birchwood xylan in 50 mM citrate–phosphate buffer (pH 6.0). The assay mixture containing 0.9 mL substrate solution and 0.1 mL of suitably diluted enzyme solution in the above buffer was incubated at 55 °C for 10 min. The amount of reducing sugar liberated was determined by the DNS method using xylose as the standard [17]. One unit (U) of xylanase activity was defined as the amount of enzyme producing 1 μmole of reducing sugar (xylose equivalent) per min. The protein concentration was determined according to the method of Lowry et al. [18] using BSA (bovine serum albumin) as the standard.

2.4. Purification of xylanase

All purification steps were performed at 4 °C unless stated otherwise. Xylanase was purified by ammonium sulfate fractionation, ion exchange and gel filtration chromatography. Ammonium sulfate was added slowly with agitation at 20–50% saturation. The precipitate obtained by centrifugation (10,000 × *g*) was dissolved in 50 mM phosphate buffer (pH 6.5). Further purification was performed by ion exchange and gel filtration chromatography. A 50-mL solution was applied to a DEAE-52 column (10 cm × 1.0 cm) pre-equilibrated with 50 mM phosphate buffer (pH 7.2). The bound proteins were eluted with 0–1 M NaCl gradient at a flow rate of 1.0 mL/min. The unbound fractions were combined and concentrated to 0.8 mL by ultrafiltration using a 3-kDa membrane (Stirred Cell Model 8050, Millipore). The concentrated protein sample was subjected to gel filtration chromatography on a column (1.0 cm × 100 cm) of Sephadex G-50 pre-equilibrated with 50 mM phosphate buffer (pH 7.2). The sample was eluted at a flow rate of 0.1 mL/min using the same buffer that was used for equilibrating the column. The homogeneity was checked by SDS-PAGE.

2.5. SDS-PAGE, N-terminal sequence and gel filtration

Analysis of samples was done on a 12.5% (w/v) SDS-PAGE as described by Laemmli [19]. Proteins were visualized by Coomassie brilliant blue R-250 staining. A low molecular weight calibration kit for SDS electrophoresis (Amersham): phosphorylase b (97.0 kDa), albumin (66.0 kDa), ovalbumin (45.0 kDa), carbonic anhydrase (30.0 kDa), trypsin inhibitor (20.1 kDa), α-lactalbumin (14.4 kDa) was used as molecular weight standard. N-terminal sequence of the purified xylanase was determined by an automated Edman degradation apparatus (a model 477A protein sequencer) at Peking University, Beijing.

Molecular mass of the purified xylanase was determined by gel filtration on a Superdex 75 column (1.0 cm × 30.0 cm) previously equilibrated with 10 mM phosphate buffer (pH 7.0). The protein was eluted at a flow rate of 0.25 mL/min with the same buffer. Molecular weight standards from Sigma used to calibrate the column were albumin bovine V (68.0 kDa), albumin (45.0 kDa), chymotrypsinogen A (25.7 kDa) and cytochrome C (12.3 kDa).

2.6. Characterization of purified xylanase

In order to determine the effect of pH on xylanase activity, its activity was assayed at 55 °C in the pH range of 3.0–11.0. A 50-mM solution of different buffers was used: sodium citrate for pH 3.0–6.5; sodium acetate for pH 4.0–5.5; MES for pH 5.0–7.0; MOPS for pH 6.5–8.5; Tris–HCl for pH 7.0–9.0; CHES for pH 8.0–11.0; CAPS for pH 9.0–11.0. To determine pH stability of the xylanase, the purified xylanase was diluted in appropriate buffers (final concentration 50 mM) of different pH (as mentioned above) and incubated at 55 °C for 30 min, and then the remaining activities of these treated enzymes were measured by the standard assay procedure.

Effect of temperature on xylanase activity was determined by assaying its activity at different temperatures (30–80 °C) in 50 mM MOPS buffer (pH 7.0). For thermostability determination, the purified xylanase in 50 mM MOPS buffer (pH 7.0) was incubated for 30 min at different temperatures. After cooling the treated enzymes on ice for 30 min, the residual xylanase activity was measured according to the standard assay method.

The influence of metal ions, a chelating agent (EDTA), and some other agents on xylanase activity was investigated under the standard assay conditions. The purified xylanase was incubated in the presence of 1 mM of metal ions and other agents and its activity was compared to control without metal ions or other agents.

2.7. Substrate specificity

Substrate specificity was studied using different substrates. For the natural substrates, reactions were carried out in 50 mM MOPS buffer (pH 7.0) containing 10 mg/mL of each substrate at 55 °C for 10 min. The amount of reducing sugars produced was estimated using the DNS method as described above [17]. Activities towards *p*-nitrophenyl derivatives were measured by the rate of *p*-nitrophenol formed from 1 mM of the substrates in 50 mM MOPS buffer (pH 7.0) at 55 °C for 10 min during hydrolysis, and detected by spectrophotometry at 410 nm. One unit (U) of activity was defined as the amount of enzyme releasing 1 μmole of reducing sugar or *p*-nitrophenol per min under the above conditions.

2.8. Analyses of the hydrolytic products of birchwood xylan and xylooligosaccharides

For enzymatic hydrolysis of birchwood xylan and xylooligosaccharides, the reaction mixture consisted of 10 mg of each substrate in 2.0 mL of 50 mM MOPS buffer (pH 7.0) incubated with 10 U of enzyme at 50 °C for 24 h. Aliquots (100 μL) were withdrawn during hydrolysis, and samples were spotted on the silica gel Plates 60F 254 (E. Merck, Germany). The plates were developed with two runs of butanol–acetic acid–water (2:1:1, v/v/v) solvent system followed by heating for few minutes at 130 °C in an oven, after spraying the plates with a methanol–sulfuric acid mixture (95:5, v/v). A mixture of xylooligosaccharides consisting of xylose (X1), xylobiose (X2), xylotriose (X3), xylotetraose (X4) and xylopentaose (X5) was used as the standard.

Table 1
Summary of purification of the xylanase from *S. matensis* DW67.

Purification step	Total activity (U)[a]	Protein (mg)[b]	Specific activity (U/mg)	Purification factor (fold)	Recovery (%)
Crude supernatant	10,900	248	44.0	1	100
Ammonium sulfate precipitation (20–50%)	7,560	76.3	99.1	2.3	69.4
DEAE-52	4,920	19.5	252	5.7	45.1
Sephadex G-50	1,530	2.4	638	14.5	14

[a] Xylanase activity was measured in 50 mM citrate buffer (pH 6.0) at 55 °C using 1.0% (w/v) birchwood xylan as substrate by the DNS method [17].
[b] The protein was measured by the method of Lowry et al. [18], using BSA as the standard.

3. Results

3.1. Purification of the xylanase

A summary of the xylanase purification from the cell-free culture supernatant of *S. matensis* DW67 is given in Table 1. Xylanase was purified to 14.5-fold with a recovery yield of 14%. Analysis of xylanase on SDS-PAGE shows that the enzyme migrated as a single band with a molecular mass of 21.2 kDa confirming its purity to homogeneity (Fig. 1). The molecular mass of native xylanase was found to be about 3.3 kDa on gel filtration analysis (Fig. 2). N-terminal sequence of the first 15 residues of the enzyme was found to be ATTITTNQTGYDGMY(Ala-Thr-Thr-Ile-Thr-Thr-Asn-Gln-Thr-Gly-Tyr-Asp-Gly-Met-Tyr-).

3.2. Enzymatic properties of the purified xylanase

Effect of pH on the purified xylanase was determined within the pH range of 3.0–11.0. Xylanase activity remained high (more than 80%) at pH range of 5.5–8.0, and it reached maximum at pH 7.0 (Fig. 3A). Xylanase was stable over the range of pH 4.5–8.0 (Fig. 3B). The effect of temperature on xylanase activity was studied which showed that xylanase exhibited maximum activity at 65 °C (Fig. 4A), and was stable up to 55 °C at pH 7.0 (Fig. 4B).

The effect of various cations and compounds at a concentration of 1 mM was tested on the activity of xylanase (Table 2). Xylanase activity was strongly inhibited by Hg^{2+} (2.1%), Mn^{2+} (43.5%), and Na^{+} (66.3%), whereas it was moderately inhibited by Ni^{2+} (91.2%), Li^{+} (90.8%), Sr^{2+} (89.5%), Ca^{2+} (89.2%), and EDTA (87.9%). On the contrary, the enzyme was activated by Mg^{2+} (133%), Fe^{2+} (123%), Co^{2+} (121%), β-mercaptoethanol (119%), Zn^{2+} (118%), and Fe^{3+} (117%). The enzyme showed no significant inhibition in the presence of DTT, SDS and K^{+}.

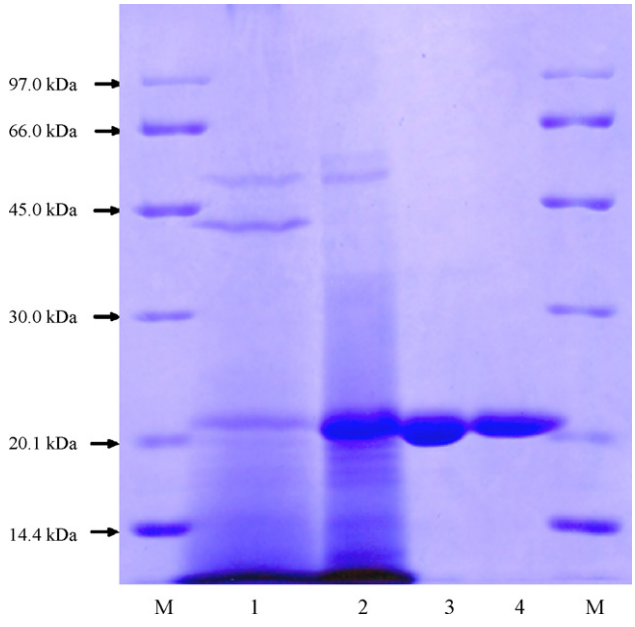

Fig. 1. SDS-PAGE of xylanase at different steps of purification. Lane M, low molecular weight calibration kit; lane 1, crude xylanase; lane 2, ammonium sulfate precipitation; lane 3, DEAE-52 ion exchange chromatography; lane 4, Sephadex G-50 chromatography.

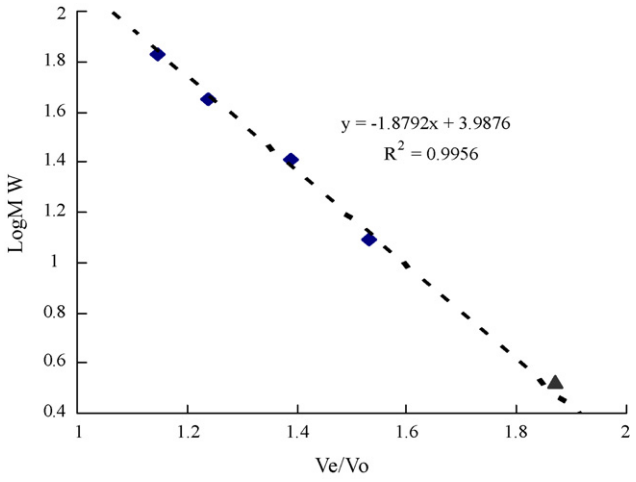

Fig. 2. Molecular mass estimation of the xylanase by gel filtration on Superdex 75. V_e, volume of eluted protein; V_0, volume of eluted blue dextran. The standards used (◆) were albumin bovine V (68.0 kDa), albumin (45.0 kDa), chymotrypsinogen A (25.7 kDa) and cytochrome C (12.3 kDa). The intact xylanase (▲) is indicated.

3.3. Substrate specificity of the xylanase

The hydrolytic activity of the purified xylanase on various natural and artificial substrates was examined (Table 3). Xylanase showed high specificity towards different xylans tested. Among them, the highest activity was observed with soluble oat-spelt xylan

Table 2
Effect of various reagents on the xylanase from *S. matensis* DW67[a].

Reagents (at 1 mM)	Specific activity (U/mg)	Relative activity (%)
Control	726 ± 26	100
Fe^{2+}	895 ± 32	123
Fe^{3+}	847 ± 30	117
Co^{2+}	877 ± 31	121
Ni^{2+}	662 ± 21	91.2
Hg^{2+}	15 ± 0.6	2.1
Mn^{2+}	316 ± 12	43.5
Zn^{2+}	857 ± 29	118
Sr^{2+}	650 ± 22	89.5
Ca^{2+}	647 ± 23	89.2
Mg^{2+}	962 ± 36	133
Li^{+}	659 ± 23	90.8
K^{+}	731 ± 27	101
Na^{+}	481 ± 15	66.3
EDTA	638 ± 19	87.9
SDS	716 ± 26	98.6
β-Mercaptoethanol	808 ± 30	111
DTT	736 ± 26	101

[a] The results presented are the average of three different experiments.

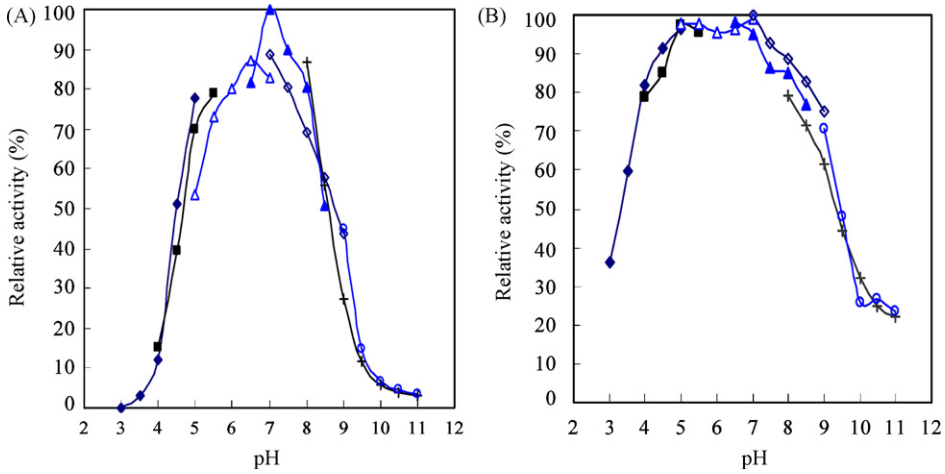

Fig. 3. Optimal pH (A) and pH stability (B) of the purified xylanase from *S. matensis*. The optimal pH of xylanase was determined at 55 °C using 50 mM of different buffers. To determine pH stability, the purified xylanase was diluted in 50 mM buffers of different pH and incubated at 55 °C for 30 min. Buffers used: sodium citrate (♦); sodium acetate (■); MES (△); MOPS (▲); Tris–HCl (◊); CHES (+); CAPS (○).

Table 3
Substrate specificity of the xylanase from *S. matensis* DW67.

Substrate[a]	Specific activity (U/mg)	Relative activity (%)[b]
Birchwood xylan	726 ± 26	100
Beechwood xylan	927 ± 35	128
Oat-spelt xylan	862 ± 28	119
Soluble oat-spelt xylan	1260 ± 45	174
Insoluble oat-spelt xylan	754 ± 28	104

[a] No activity was observed for Avicel, CMC, filter paper, and locust bean gum (galactomannan).

[b] The activity for birchwood xylan was defined as 100%. The results are expressed in mean ± S.D. from three separate experiments.

(174%) followed by beechwood xylan (128%). However, it did not show any activity towards Avicel, CMC, filter paper, and locust bean gum (galactomannan). Under these conditions, no detectable activity towards *p*NP-β-D-xylopyranoside, *p*NP-β-D-glucopyranoside, or *p*NP-β-D-mannopyranoside and *p*NP-α-L-arabinofuranoside was observed. These results indicate that the xylanase we isolated and purified from *S. matensis* is a true xylanase.

3.4. Enzymatic hydrolysis of birchwood xylan and xylooligosaccharides

Fig. 5 shows the time-course of hydrolysis of birchwood xylan and xylooligosaccharides by the purified xylanase. The xylanase degraded birchwood xylan at random (Fig. 5A). After 15 min, the enzyme liberated mainly xylotriose (X3) and xylotetraose (X4). After prolonged incubation, the level of X4 gradually reduced and the level of xylobiose (X2) and X3 increased markedly. X2 and X3 were the main products after 6 h of hydrolysis. After 24 h, the main products were X2 and X3 only and no xylose was formed. Thus, hydrolysis of birchwood xylan was found to yield xylobiose and xylotriose as the major products. Its mechanism of action on xylan indicates that it is a β-1,4-endoxylanase. The enzyme hardly hydrolyzed X2 or X3. But, it could hydrolyze X4 and xylopentaose (X5) to yield X2 and X3. No xylose was detected among the products. The main hydrolysis products of X5 were X2, X3 and X4 and a small amount of higher xylooligosaccharides (>X5) was observed (Fig. 5B). This result suggests that the xylanase requires at least four xylose residues for catalytic activity and has transxylosidase activity.

4. Discussion

According to the glycoside hydrolase classification, xylanases mostly belong to families 10 and 11 [2,20]. We purified a family 11 xylanase of *S. matensis* DW67. Physico-chemical and enzymatic properties of the xylanase from *S. matensis* DW67 were compared with xylanases from other *Streptomyces* species xylanases as shown in Table 4. The molecular mass of denatured protein was found to be approx. 21.2 kDa, which is similar to the values of most family 11 xylanases [3,5,9,11,12,14,21,22]. Native molecular masses of some *Streptomyces* family 11 xylanases as determined by gel filtration

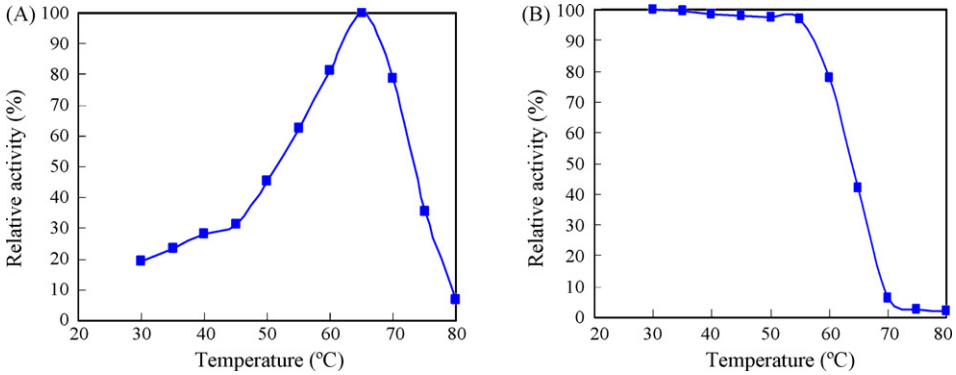

Fig. 4. Optimal temperature (A) and thermostability (B) of the purified xylanase from *S. matensis*. The optimal temperature was measured at different temperatures and pH 7.0, 50 MOPS buffer. For thermostability determination, the purified xylanase in 50 mM MOPS buffer (pH 7.0) was incubated for 30 min at different temperatures.

Table 4
Comparison of properties between *Streptomyces matensis* DW67 and other *Streptomyces* species xylanases.

Microorganism	MW (kDa)		Optimum temperature (°C)	Optimum pH	Hydrolysis products	Reference
	SDS-PAGE	Gel filtration				
Streptomyces matensis DW67	21.2	3.3	65	7.0	X2, X3 from birchwood xylan	Present work
Streptomyces sp. strain S38	24.5	ND[a]	55–60	6.0–6.5	ND	5
Streptomyces cyaneus SN32	20.5	ND	60–65	6.0	X1, X2 from birchwood xylan	11
Streptomyces olivaceoviridis E-86	23	ND	60	6.0	X2-X5 from oat spelts xylan	12
Streptomyces actuosus A-151	20	21	60	4.0	ND	13
Streptomyces roseiscleroticus NRRL B-11019	22.6	5.5	60	6.5–7.0	Arabinose, X2, trimeric product from arabinoxylan	14
Streptomyces sp. K37	26.4	24.3	60	6.0	ND	22

[a] Not determined.

were also found to be within the average Mr range of 20–26.5 kDa [13,22]. However, the enzyme we purified had a very low apparent molecular mass of 3.3 kDa as determined by gel filtration (Fig. 2). This finding is in agreement with study on the xylanase from *S. roseiscleroticus* which has a molecular mass of 5.5 kDa [14]. However, *S. roseiscleroticus* could produce the red pigments, interfering with purification steps [14]. This enzyme characteristic may render it valuable for enzyme treatment of wood pulp. The major current application of xylanases is in the pulp and paper industries [2,5]. Xylanases with low Mr as blenching agents are desirable in pulp and paper industry since they can easily penetrate into the re-precipitated xylan on the surface of kraft pulp [5,11]. According to BLAST homology search, the 15 N-terminal amino acid residues of xylanase was found to be 100% identical to two other family 11 xylanases i.e. β-1,4-endoxylanase 3 (Xyl3, 31 amino acids, accession no. AAB26280.1) from *S. roseiscleroticus* [23] and xylanase (precursor, 240 amino acids, accession no. CAA56935.1) from *S.* sp. EC3

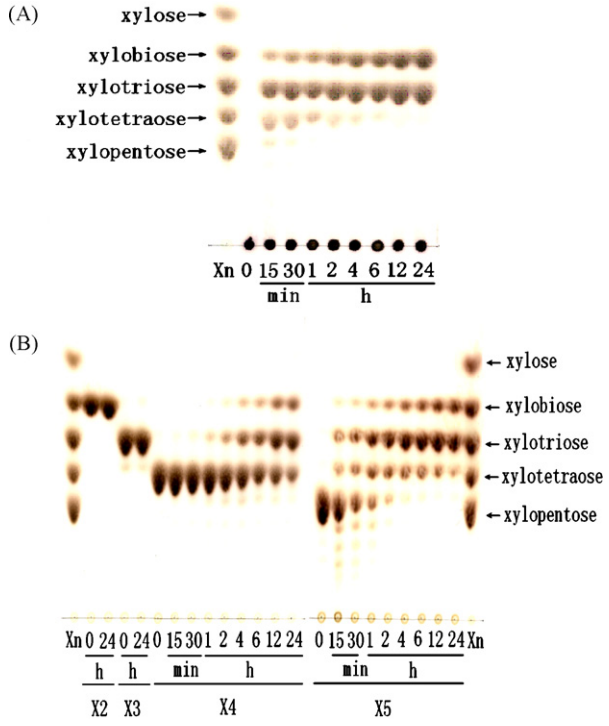

Fig. 5. Time-course of hydrolysis of birchwood xylan (A) and xylooligosaccharides (B) by the xylanase. The reaction mixture consisted of 20 mg of each substrate in 2.0 mL of 50 mM MOPS buffer (pH 7.0) and 10 U enzyme was incubated at 50 °C. Lanes Xn, authentic xylose to xylopentaose from top to bottom. Time of incubation (h or min) and xylooligosaccharides are indicated. X1, xylose; X2, xylobiose; X3, xylotriose; X4, xylotetraose; X5, xylopentaose.

[24]. Thus, it is apparent that the enzyme is a member of glycoside hydrolase family 11 [20].

Most of the family 11 xylanases that have been reported so far are optimally active at acidic pH values (pH 5.0–6.5) and in the range of 50–60 °C [3,5,7–13,21–23]. Our data indicates that the enzyme exhibited optimum xylanase activity at neutral pH value (pH 7.0). There are several xylanases from *Streptomyces* species which are optimally active at or above pH 7.0 [1,14,25]. A similar temperature optimum of 65 °C was reported for several xylanases from *Streptomyces* species [11,26]. Most of the xylanases were found to be strongly inhibited by Mn^{2+} and Hg^{2+} [4,9,26]. This indicates the presence of active site thiol group. SDS did not influence xylanase activity from some *Streptomyces* speices [9]. Inhibition of xylanase activity by EDTA has been reported earlier [3,9,13]. Unusually, the xylanase activity was stimulated by Mg^{2+}, Fe^{2+}, Co^{2+}, β-mercaptoethanol, Zn^{2+} and Fe^{3+} by 32.6%, 23.4%, 20.9%, 18.9%, 18.1%, and 16.7% (Table 2). Stimulation of the xylanase activity by β-mercaptoethanol was also observed by others [3,4].

Many xylanases have both xylanase and cellulase activities [8,9,27]. Some cellulase-free xylanases have been found in *Streptomyces* species [7,10,11,28]. Interestingly, the xylanase showed higher activity for the highly substituted xylans (such as oat-spelt xylan) than for the less branched beechwood and birchwood xylans. The specific activity of the xylanase was slightly higher on birchwood xylan than on oat-spelt xylan [5]. The purified xylanase only hydrolyzed xylan and was free from all other enzyme activities examined including those of carboxymethylcellulase, β-D-xylosidase, β-D-glucosidase, β-D-cellobiosidase, β-D-mannosidase and α-arabinofuranosidase. Cellulase-free xylanases are important in paper manufacture to avoid cellulose degradation [3,5,6]. This substrate specificity is another advantage, considering its potential application of this enzyme in paper and pulp industries.

Two families of xylanases differ considerably in their mode of action on various xylans and xylooligosaccharides [29]. Family 10 xylanases have smaller substrate binding sites than that of family 11 xylanases, which may be more than four subsites. The purified xylanase hydrolyzed birchwood xylan principally to X2 and X3, while many others produce mainly xylooligosaccharides with higher degree of polymerization (DP) (Dp ≥ 3) [8,9,11,12,21,23]. Several family 11 xylanases could release arabinose from arabinoxylan [1,14]. Some xylanases could not produce xylose from xylan [9,11,23]. Furthermore, the production of X2 and X3 without the formation of xylose from X4 or X5 suggested that the enzyme can carry out transglycosylation reactions. This suggests that X4 was split to X2 and that the majority of X3 was released from xylohexaose previously formed from X2 and X4 [30]. Some other xylanases also display catalytic activities of transglycosylation [9,27,30–32]. Hydrolysis by xylanase is the major process for the production of xylooligosaccharides [33]. Xylooligosaccharides with DP 2–4 were preferred for food-related applications [33]. On the basis of the

above hydrolysis properties, the purified xylanase can potentially be used in xylooligosaccharides production.

5. Conclusions

A family 11 xylanase from *S. matensis* DW67 was purified and characterized in this investigation. The molecular mass of enzyme in the native condition was found to be 3.3 kDa. The xylanase was optimally active at pH 7.0 and 65 °C. Birchwood xylan was hydrolyzed to produce mainly X2 and X3 as end products. Furthermore, X4 or X5 could be converted to X2 and X3 through transglycosylation reactions. These properties indicate the potential application of xylanase for bioblenching in paper and pulp industries as well as xylooligosaccharides production. This is the first report of a xylanase from *S. matensis*.

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References

[1] K.K.Y. Wong, L.U.L. Tan, J.N. Saddler, Microbiol. Rev. 52 (1988) 305.
[2] T. Collins, C. Gerday, G. Feller, FEMS Microbiol. Rev. 29 (2005) 3.
[3] Q.K. Beg, M. Kapoor, L. Mahajan, G.S. Hoondal, Appl. Microbiol. Biotechnol. 56 (2001) 326.
[4] T. Dutta, R. Sengupta, R. Sahoo, S. Sinha Ray, A. Bhattacharjee, S. Ghosh, Lett. Appl. Microbiol. 44 (2007) 206.
[5] J. Georis, F. Giannotta, E.D. Buyl, B. Granier, J.M. Frère, Enzyme Microbiol. Technol. 26 (2000) 178.
[6] M. Ayyachamy, T.M. Vatsala, Lett. Appl. Microbiol. 45 (2007) 467.
[7] M.E. Flores, R. Pérez, C. Huitrón, Lett. Appl. Microbiol. 24 (1997) 410.
[8] H.L. Cheng, P.M. Wang, Y.C. Chen, S.S. Yang, Y.C. Chen, Bioresour. Technol. 99 (2008) 227.
[9] G. Elegir, G. Szakács, T.W. Jeffries, Appl. Environ. Microbiol. 60 (1994) 2609.
[10] C. Techapun, T. Charoenrat, N. Poosaran, M. Watanabe, K. Sasak, J. Biosci. Bioeng. 93 (2002) 431.
[11] S. Ninawe, M. Kapoor, R.C. Kuhad, Bioresour. Technol. 99 (2008) 1252.
[12] S. Kaneko, A. Kuno, M. Muramatsu, S. Iwamatsu, I. Kusakabe, K. Hayashi, Biosci. Biotechnol. Biochem. 64 (2000) 447.
[13] S.L. Wang, Y.H. Yen, I.L. Shih, A.C. Chang, W.T. Chang, W.C. Wu, Y.D. Chai, Enzyme Microbiol. Technol. 33 (2003) 917.
[14] A.C. Grabski, T.W. Jeffries, Appl. Environ. Microbiol. 57 (1991) 987.
[15] M. Nakabayashi, T. Nishijima, G. Ehara, N. Nikaidou, H. Nishihashi, T. Watanabe, J. Ferment. Bioeng. 85 (1998) 459.
[16] M.J. Bailey, P. Biely, K. Poutanen, J. Biotechnol. 23 (1992) 257.
[17] G.L. Miller, Anal. Chem. 31 (1959) 426.
[18] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, J. Biol. Chem. 193 (1951) 265.
[19] U.K. Laemmli, Nature 227 (1970) 680.
[20] B. Henrissat, A. Bairoch, Biochem. J. 293 (1993) 781.
[21] L. He, G.F. Bickerstaff, A. Paterson, J.A. Buswell, Enzyme Microbiol. Technol. 15 (1993) 13.
[22] F.A. Mansour, A.A. Shereif, M.M. Nour el-Dein, M.I. Abou-Dobara, A.S. Ball, Acta Microbiol. Pol. 52 (2003) 159.
[23] A.C. Grabski, I.T. Forrester, R. Patel, T.W. Jeffries, Protein Exper. Purif. 4 (1993) 120.
[24] C. Mazy-Servais, A. Moreau, C. Gerard, J. Dusart, DNA Seq. 6 (1996) 147.
[25] H. Tsujibo, K. Miyamoto, T. Kuda, K. Minami, T. Sakamoto, T. Hasegawa, Y. Inamori, Appl. Environ. Microbiol. 58 (1992) 371.
[26] R.P. Nascimento, R.R.R. Coelho, S. Marques, L. Alves, F.M. Gírio, E.P.S. Bon, M.T. Amaral-Collaço, Enzyme Microbiol. Technol. 31 (2002) 549.
[27] P. Christakopoulos, D. Kekos, B.J. Macris, M. Claeyssens, M.K. Bhat, Carbohydr. Res. 289 (1996) 91.
[28] T.S. Magnuson, D.L. Crawford, Enzyme Microbiol. Technol. 21 (1997) 160.
[29] P. Biely, M. Vrsanská, M. Tenkanen, D. Kluepfel, J. Biotechnol. 57 (1997) 151.
[30] M.K. Kang, P.J. Maeng, Y.H. Rhee, Appl. Environ. Microbiol. 62 (1996) 3480.
[31] Z.Q. Jiang, W. Deng, Y.P. Zhu, L.T. Li, Y.J. Sheng, K. Hayashi, J. Mol. Catal. B: Enzym. 27 (2004) 207.
[32] H.Y. Fang, S.M. Chang, C.H. Lan, T.J. Fang, Process Biochem. 43 (2008) 49.
[33] M.J. Vázquez, J.L. Alonso, H. Domínguez, J.C. Parajó, Trends Food Sci. Technol. 11 (2000) 387.