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The recombinant xylanase B of *Thermotoga maritima* is highly xylan specific and produces exclusively xylobiose from xylans, a unique character for industrial applications

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Abstract

The *xynB* of a hyperthermophilic Eubacterium, *Thermotoga maritima* MSB8, coding xylanase B (XynB) was previously expressed in *E. coli* and the recombinant protein was characterized using the synthetic substrates [J. Biosci. Bioeng. 92 (2001) 423]. In this study, the same xylanase B was purified to homogeneity with a recovery yield of about 43% using heat treatment followed by the Ni-NTA affinity chromatography. The specificity of XynB towards different natural substrates was evaluated. XynB was highly specific towards xylans tested but exhibited low activities towards lichenan (19%), gellan gum (7.3%), laminarin (3.4%) and carboxymethylcellulose (CMC, 1.4%). The apparent K_m values of birchwood xylan and soluble oat-spelt xylan was 0.11 and 0.079 mg/ml, respectively. The XynB hydrolyzed xylooligosaccharides to yield predominantly xylobiose (X_2) and a small amount of xylose (X_1), suggesting that XynB was possibly an *endo*-acting xylanase. Analysis of the products from birchwood xylan degradation confirmed that the enzyme was an *endo*-xylanase with xylobiose and xylose as the main degradation products. HPLC results showed that hydrolyzed products of birchwood xylan by XynB yielded up to 66% of the total reaction product as xylobiose. These results clearly indicated that xylobiose could be mass-produced efficiently by the recombinant hyperthermostable XynB of *T. maritima*. Additionally, conversion of xylobiose (50 mM) to xylose was observed, while xylotriose (X_3) and xylotetraose (X_4) were detected in small amounts, indicating that the enzyme converted xylobiose to xylose based on the transglycosylation reaction. The increased binding ability of XynB to Avicel and/or insoluble xylan was also observed indicating the possibilities of roles of surface-aromatic amino acid residues for such action. However, further investigations are required to prove this speculation.

Keywords: *Thermotoga maritima*; *endo*-Xylanase; Hyperthermophilic; Catalytic properties; Xylobiose

1. Introduction

Xylan, a major component of hemicelluloses, is a heterogeneous polysaccharide with a backbone consisting of β-(1,4)-linked D-xylosyl residues with several side-groups attached to the main chain. In general, depolymerization of xylan is accomplished by the action of *endo*-xylanases and β-xylosidases. *endo*-1,4-β-Xylanases (EC 3.2.1.8) hydrolyze β-1,4-glycosidic linkages of the xylan backbone to produce short chain xylooligosaccharides of various lengths. Hence, *endo*-xylanases are the crucial enzyme components of the microbial xylanolytic systems [1]. In recent years, xylanases have attracted considerable research interest because of their potential industrial applications, such as improvements in bleaching ability of kraft pulp, upgradation of the feeds for better digestibility as well as the commercial production of xylooligosaccharides. In particular, the major interest in thermostable xylanases is found in enzyme-aided bleaching of paper [1,2].

Thermotoga maritima is a fermentative marine hyperthermophilic Eubacterium that grows optimally around 80 °C [3,4]. The enzymes isolated to date from *Thermotoga* species

Abbreviations: XynB, the recombinant xylanase B of a hyperthermophilic *Thermotoga maritima* MSB8; PHBAH, *p*-hydroxybenzoic acid hydrazide; X_1, xylose; X_2, xylobiose; X_3, xylotriose; X_4, xylotetraose; X_5, xylopentaose; ORF, open reading frame; MES, 2-(*N*-morpholino)ethane sulfonic acid; CMC, carboxymethylcellulose

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are proved to be extremely thermostable [5]. Generally, the catalytic properties of the xylanases are very important for their potential applications. Though most of the xylanases from mesophilic fungi and bacteria have been studied for their catalytic properties, little is known about the thermostable xylanases from different species of *Thermotoga* [2,6–14]. Until the present study, the catalytic properties of xylanase B (XynB) from *T. maritima* have not been investigated in detail. We have reported earlier the biochemical properties of the XynB from *T. maritima* MSB8 using synthetic substrates [15]. In the present study, we report an improved purification protocol for XynB as well as the catalytic properties of this specific enzyme using various natural substrates, which are of real commercial importance for industrial applications. The unique properties of xylobiose (X_2) production and xylobiose to xylose (X_1) conversion through transglycosylation by the hyperthermostable xylanase of *T. maritima* are also reported.

2. Materials and methods

2.1. Bacterial strains and plasmid vectors

The genomic DNA of the type strain *T. maritima* MSB8 was kindly provided by Professor Dr. K.O. Stetter (University of Regensburg, Germany). The plasmid, Topo-XL TOP 10 vector (Invitrogen, USA) was used to insert the PCR amplified product of the *xynB* gene of *T. maritima*. TOP 10 electrocompetent cells were used as the host for the *xynB* gene. Plasmid vector pET 28a(+) (Novogen, USA) was employed for the subcloning of the gene orienting the $(His)_6$ epitope tag at the gene's C-terminal end. *E. coli* BL21 (DE3) was used for the heterologous expression of the *xynB* gene. All the recombinant DNA techniques were performed as described by Sambrook et al. [16].

2.2. Enzyme substrates

The substrates: birchwood xylan, oat-spelt xylan, beechwood xylan, carboxymethylcellulose (CMC), lichenan, laminarin, gellan gum (GELRITE), pullulan, konjac gum, and locust bean gum were purchased from Sigma Chemical Company, St. Louis, USA. Avicel (cellulose microcrystalline) was purchased from Merck (Darmstadt, Germany). Purified β-1,4-xylooligosaccharides were kindly provided by Dr. Kusakabe, Tsukuba University (Japan).

2.3. Cloning, expression of xynB in E. coli and purification of the enzyme

The ORF TM0070 which codes the xylanase B of *T. maritima* MSB8 was cloned into pET 28a and this plasmid was then transformed into BL21 *E. coli* competent cells. The transformed cells were cultured by the method of Jiang et al. [15]. The cells were harvested by centrifugation at 12,000 × *g* for 10 min at 4 °C and the cell pellet was suspended in 50 mM sodium phosphate buffer (pH 8.0) consisted of 300 mM NaCl and 10 mM imidazole (buffer A). The cells were then lysed by sonification. The sonified sample was centrifuged at 16,000 × *g* for 10 min at 4 °C to remove cell debris. The supernatant containing crude enzyme was subjected to heat treatment at different temperatures for 10 min and centrifugation to remove the heat-denatured proteins. The recombinant xylanase B was purified by two steps: heat treatment and Ni-NTA affinity chromatography. After heating at 80 °C for 10 min, the supernatant was mixed with 2 ml of fresh Ni-NTA resin (Qiagen, Germany) and kept on ice under gentle shaking for 15–20 min. The enzyme-bound resin was then packed into a column and eluted the enzyme with a linear gradient 10–200 mM of imidazole in buffer A. The active fractions were pooled and used as a purified XynB.

2.4. Determination of protein purity, concentration and enzyme activity

The purity of the protein, XynB at each stage of purification was monitored by SDS–PAGE analyses [17]. The concentration of the protein was determined by the Bradford method with bovine serum albumin as a standard [18]. The activity of xylanase was measured by *p*-hydroxybenzoic acid hydrazide (PHBAH) method [19]. Unless mentioned otherwise, the 200 μl standard assay reaction mixture contained 0.25% (w/v) oat-spelt xylan in 50 mM MES buffer (pH 6.1), and a suitable amount of enzyme protein. The reaction was carried out at 70 °C for 10 min and the enzyme activity was stopped by adding 400 μl of ice-cold *p*-hydroxybenzoic acid hydrazide reagent followed by boiling for 6 min. The color developed was measured at 405 nm. One unit (U) of enzyme activity was defined as the amount of enzyme that produced 1 μmol of xylose per minute.

2.5. Substrate specificity and kinetic parameters

The method of Tenkanen et al. [20] was applied to prepare the soluble- and insoluble oat-spelt xylans. Substrate specificity of the enzyme was studied using various derivatives of the cellulose- and hemicellulose substrates. The reaction was carried out in 50 mM MES buffer (pH 6.1) containing 2.5 mg/ml of each substrate at 50 °C for 10 min. Amounts of the reducing sugars produced were computed using the PHBAH method [19]. For each assay, six different substrate concentrations were prepared in 50 mM MES buffer (pH 6.1), and incubated with the purified XynB at 50 °C for 5 min. The K_m and k_{cat} values were calculated from the kinetics data using the "Grafit" software [21].

2.6. Analyses of XynB hydrolytic products of xylooligosaccharides and xylans

In order to determine the mode of action of XynB, 25 mM of different xylooligosaccharides were incubated separately

at 50 °C for 4 h with 1–2 U of xylanase in a reaction volume of 0.2 ml containing 50 mM MES buffer (pH 6.1). At different time intervals, samples were analyzed for hydrolyzed products by the thin-layer chromatography (TLC) using silica gel plates 60F 254 (E. Merck, Germany). For enzymatic hydrolysis of xylan, the reaction mixture consisted of 40 mg of birch wood xylan in 2.0 ml of 50 mM MES buffer (pH 6.1) with 4 U of the enzyme, was incubated at 90 °C for 12 h. Aliquots (100 μl) from the samples were withdrawn at 10, 30 min, 1, 2, 4, 8, and 12 h of the incubation period and 1 μl of the aliquot was spotted on the TLC plates. The plates were developed with two runs of acetonitrile–water (85:15, v/v) 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 xylooligosaccharide mixture obtained from Suntory Ltd. (Japan) consisting of xylose, xylobiose, and xylotriose (X_3) was used as the standard. The hydrolyzed products of xylan were also analyzed by HPLC (Waters), with SHODEX Ks-802 Column (∅8 mm × 300 mm), Milli Q water as mobile phase (0.8 ml/min) and injection volumes of 20 μl. The retention time for xylose, xylobiose, xylotriose, xylotetraose (X_4), and xylopentaose (X_5) was found to be at 12.1, 11.0, 10.5, 9.8, and 9.2 min, respectively.

2.7. Polysaccharide-binding assay

The polysaccharide-binding ability of XynB was tested by the method described by Tenkanen et al. [20]. XynB (25 μg) was incubated with 1–20 mg/ml crystalline cellulose (Avicel) or insoluble oat-spelt xylan in 50 mM MES buffer (pH 6.1) at various concentrations, at 4 °C for 1 h with slow shaking and centrifuged to remove the insoluble polysaccharide complex. After centrifugation (10,000 × *g*, 5 min), the supernatant was collected and tested for its xylanase activity. Unbound enzyme was quantified by measuring residual activity in the supernatant.

3. Results and discussion

3.1. Purification of XynB

A summary of the purification of XynB is presented in Table 1. As XynB was found to be thermostable up to 100 °C [15], heat treatment was useful for the purification of this enzyme. Therefore, heat precipitation by different temperatures was used to purify the enzyme. Removal of host protein by heat precipitation at 65, 75, 80, and 90 °C for 10 min resulted in the increased specific activity from 30.3 to 115 U/mg protein. After heat treatment at 80 °C for 10 min more than 70% of the host proteins were removed and the specific activity of the protein increased to 115 U/mg protein. Finally, Ni-NTA affinity chromatography resulted in a homogenous protein with specific activity of 379 U/mg protein. Overall, the protein was purified to about 12.5-fold. And, this purified protein gave a single band at approximately 42 kDa on a 10% SDS–PAGE gel. The size of the protein was in agreement with the molecular weight calculated based on the primary structure of XynB (Fig. 1).

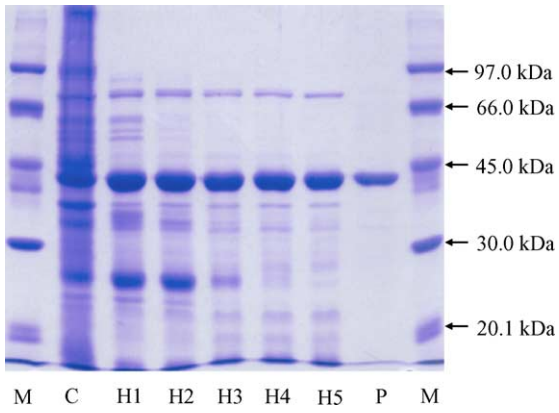

Fig. 1. SDS-PAGE analysis of the recombinant XynB during different purification steps. Lanes: M, low molecular weight standards, phosphorylase b (97 kDa), albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa), α-lactalbumin (14.4 kDa); lane C, crude extract; lane H_1, H_2, H_3, H_4, and H_5, are different heat (65, 70, 75, 80, and 90 °C for 10 min, respectively) precipitation steps; lane P, Ni-NTA fraction.

3.2. Substrate specificity and kinetic parameters

The hydrolytic activity of the purified XynB on various substrates was examined. The enzyme showed a high specificity towards different xylans tested (Table 2). The highest activity (127%) was observed with the beech wood xylan

Table 1
Summary of purification of the recombinant XynB from *T. maritima*

Purification method	Total activity[a] (U)	Protein (mg)	Specific activity (U/mg)	Purification factor (*X*-fold)	Recovery (%)
Crude extract	1581	52.2	30.3	1	100
65 °C, 10 min	1515	28.4	53.3	1.8	96
70 °C, 10 min	1494	20.6	72.5	2.4	95
75 °C, 10 min	1469	14.8	99	3.3	93
80 °C, 10 min[b]	1443	12.5	115	3.8	91
90 °C, 10 min	1397	12.2	115	3.8	88
Ni-NTA	683	1.8	379	12.5	43

[a] Activity was measured at 70 °C using oat-spelt xylan as substrate by the PHBAH method.
[b] After heating at 80 °C for 10 min, the supernatant was applied for Ni-NTA affinity chromatography.

Table 2
Substrate specificity of the recombinant XynB from *T. maritima*

Substrate[a]	Specific activity (U/mg)	Relative activity[b] (%)
Birchwood xylan	867	100
Beechwood xylan	1099	127
Oat-spelt xylan	1022	118
Insoluble oat-spelt xylan	808	93
Soluble oat-spelt xylan	718	83
Lichenan	164	19
Gellan gum	64	7.3
Laminarin	30	3.4
CMC (low viscosity)	12	1.4

[a] No activity was observed for Avicel, filter paper, starch, amylopectin, pullulan, konjac gum (galactoglucomannan), and locust bean gum (galactomannan).
[b] The activity for birchwood xylan was defined as 100%.

followed by the oat-spelt xylan (118%). XynB did not act towards Avicel, filter paper, starch, amylopectin, pullulan, konjac gum, locust bean gum, and guar gum. The enzyme also exhibited activity towards cellulosic substrates tested, but with lower activities towards lichenan (19%), gellan gum (7.3%), laminarin (3.4%), and CMC (1.4%).

There is currently much interest in the use of the hyperthermophilic xylanases for the prebleaching of kraft pulps [2,8,12]. The low activity of XynB of *T. maritima* on carboxymethyl cellulose is an advantage for the potential application in biobleaching pulp for high-quality paper. It is noteworthy that the XynB was also more active toward the beechwood xylan (127%). Because of its high thermostability and activity over a wide pH range (5.0–11.4) [15], as well as its ability to effectively degrade beechwood xylan, the XynB of *T. maritima* might become an attractive enzyme in industrial bleaching of pulp in the near future [22].

The Michaelis–Menten constants were determined for the birchwood xylan and the soluble oat-spelt xylan (Table 3).

Table 3
Kinetic constants for the recombinant XynB

Substrate	K_m (mg/ml)	k_{cat} (s^{-1})	k_{cat}/K_m (ml/mg s)
Birchwood xylan	0.11 ± 0.021	106.5 ± 7.9	968
Soluble oat-spelt xylan	0.079 ± 0.011	89.7 ± 4.1	1135

Enzymatic reactions were carried out for 5 min at 50 °C in 50 mM MES buffer, pH 6.1.

The K_m and k_{cat} values were 0.11 mg/ml and 106.5 s^{-1} for birchwood xylan and 0.079 mg/ml and 89.7 s^{-1} for oat-spelt xylan. XynB showed a higher affinity for xylans and its K_m values were found to be 10-fold lower than most of the reported xylanases [1,2,22–28].

3.3. Enzymatic hydrolysis of xylooligosaccharides

The mode of action of XynB was determined using different xylooligosaccharides. The enzyme rapidly hydrolyzed xylopentaose and xylotetraose, but it hydrolyzed xylotriose slowly. The degradation of xylobiose was very slow and the detectable activity was observed after 4 h of incubation (Fig. 2). During the early course of hydrolysis of X_4 and X_5, the main products formed were xylotriose and xylobiose. As the incubation time increased, X_3 was further hydrolyzed into X_2 and xylose. The presence of relatively high concentrations of X_2 and X_3 in the X_4 and X_5 reaction mixtures suggested that the enzyme preferentially cleaved the internal glycosidic bonds of the above oligosaccharides. Therefore, it was concluded that XynB is possibly a typical *endo*-β-1,4-xylanase.

The final products of hydrolyses of xylooligosaccharides tested were predominantly xylobiose and xylose and a smaller amount of xylotriose. Hence, XynB of *T. maritima* can be classified as a type II a *endo*-xylanase, similar to the xylanase from *Bacillus stearothermophilus* T-6 [24].

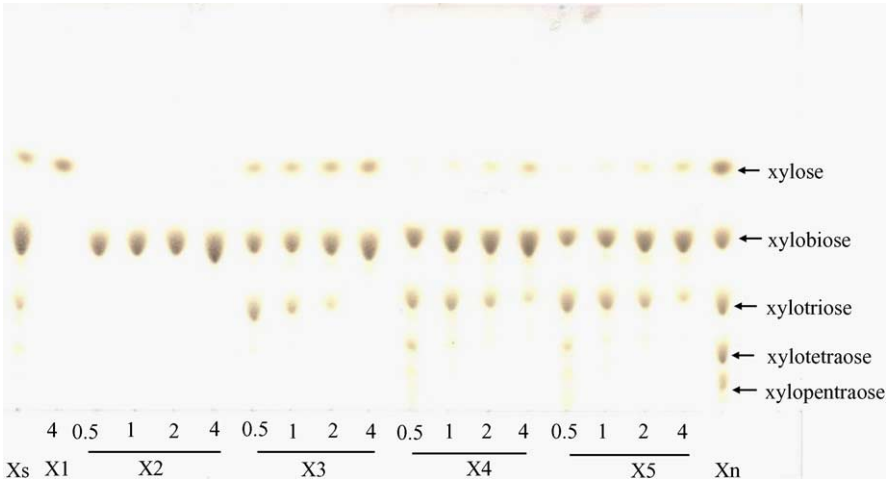

Fig. 2. TLC analyses of xylooligosaccharides hydrolyzed by the recombinant XynB. Each xylooligosaccharide (25 mM) was incubated with 1–2 U of XynB for 4 h at 50 °C, and hydrolyzates were analyzed by TLC. Lane Xs, a mixture of standard xylooligosaccharides from Suntory Co. Ltd.; lane X_1, xylose; lane X_2, xylobiose; lane X_3, xylotriose; lane X_4, xylotetraose; lane X_5, xylopentaose; lane Xn, a mixture X_1 to X_5; incubation time (h) is indicated.

3.4. Enzymatic hydrolysis of xylans

The products of hydrolysis of birchwood xylan were analyzed by TLC. Xylobiose was produced within 1 h of the reaction period (Fig. 3A) and it was the major end product yielded. As the reaction time increased, the xylobiose concentration decreased with a simultaneous increase of xylose concentration. These results also confirmed the *endo*-acting nature of XynB. Analysis of the degradation products of oat-spelt xylan and beechwood xylan also showed xylobiose and xylose were the main end products obtained (data not shown). In addition, birchwood xylan was rapidly and completely degraded at 90 °C. After the complete hydrolysis of birchwood xylan, its end products were only xylobiose and xylose. Xylose content increased steadily during the long-term incubation (>2 h), suggesting that some of the xylobiose had been converted into xylose.

The profile of xylooligosaccharides from birchwood xylan hydrolysis by XynB was monitored using HPLC. At 90 °C, xylobiose was the major product from birchwood xylan (Fig. 3B) and its production increased with the incubation time. After 1 h of incubation, about 66% of the total reaction products was xylobiose. Xylobiose has been found to have a stimulatory effect on the selective growth of human intestinal *Bifidobacteria*, which are important for the maintenance of a healthy intestinal microflora. But, the production of analytical grade xylobiose is a time consuming and expensive process [29,30]. Therefore, XynB of *T.*

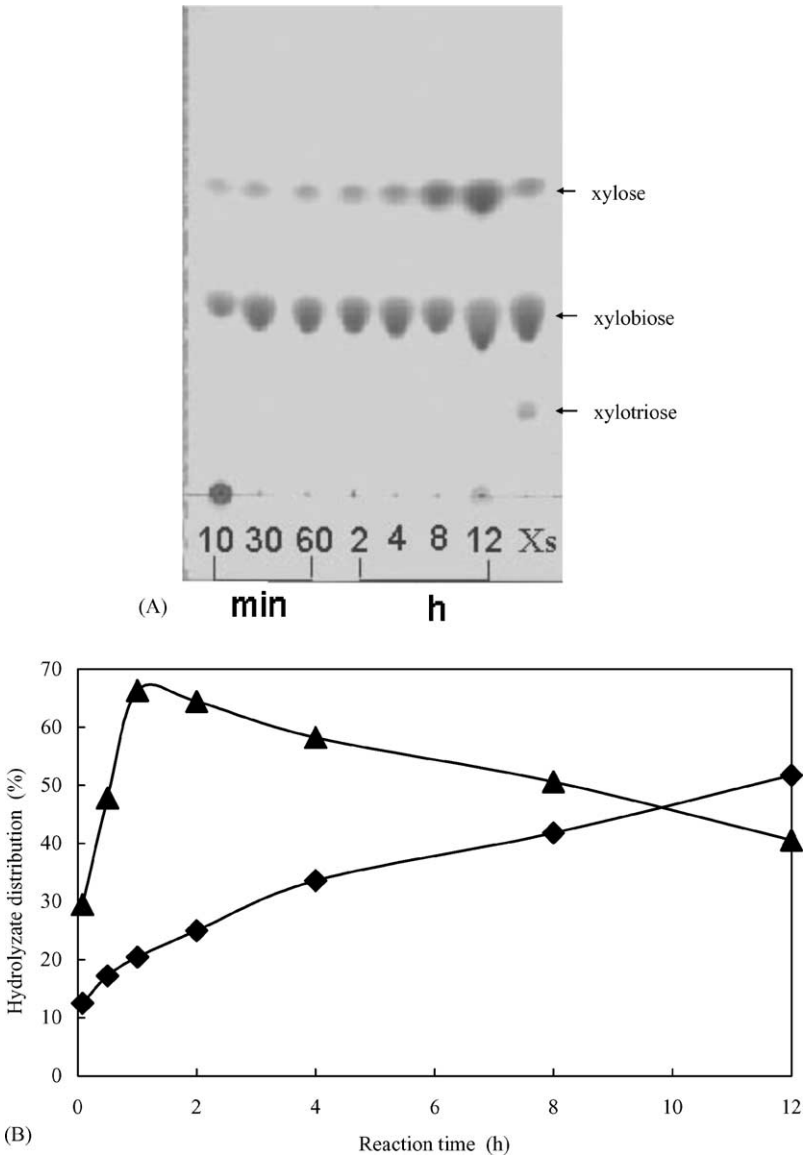

Fig. 3. Time course of hydrolysis of birchwood xylan by the recombinant XynB. Birchwood xylan (40 mg) was incubated with 4 U of the enzyme in 2 ml 50 mM MES buffer (pH 6.1) and the reaction was carried out at 90 °C for 12 h and then the hydrolyzates were analyzed by TLC (A) and HPLC (B), respectively. Incubation times (min or h) are indicated. A mixture of xylooligosaccharides from Suntory Co. Ltd. was as the standard (lane Xs). Symbols: (◆) xylose; (▲) xylobiose.

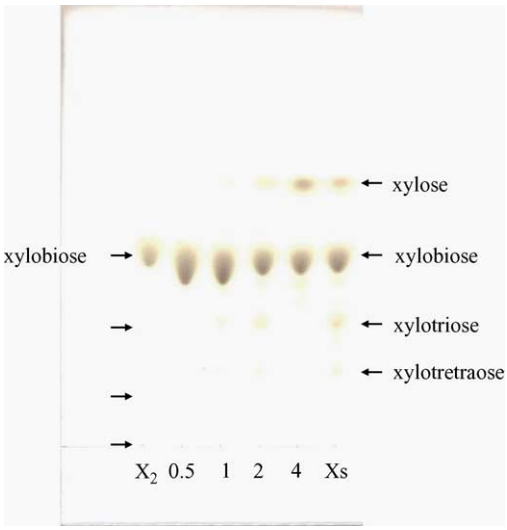

Fig. 4. TLC analyses of the hydrolyzed products of xylobiose. Xylobiose (50 mM) was hydrolyzed for 4 h with 2 U of XynB in 2.0 ml of 50 mM MES buffer (pH 6.1) at 90 °C. Lane Xs, a mixture of xylooligosaccharides standard from Suntory Co. Ltd.; lane X_2, xylobiose.

maritima could be used for the large-scale production of xylobiose.

Similar production of xylobiose and/or xylose could be obtained from xylan hydrolysis with the *endo*-acting xylanase from *Thermotoga neapolitana* [11], *B. stearothermophilus* T-6 [24], and *Aureobasidium pullulans* Y-2311-1 [25]. In addition, several xylanases like xylanase V of *Aeromonas caviae* ME-1 also produce xylobiose exclusively from xylan by the exomechanism [31].

The ability of XynB to hydrolyze xylobiose was studied using 50 mM of xylobiose and purified XynB at 90 °C. However, only a slow hydrolysis of xylobiose to xylose was observed as shown in Fig. 4. After 2 h of incubation, xylose and xylotriose as well as a trace amount of xylotetraose were produced in the reaction mixture. Surprisingly, it was found that xylobiose (a typical substrate for β-xylosidase) was also hydrolyzed by the same enzyme. None of the *Thermotaga* xylanases reported so far are able to hydrolyze xylobiose at an appreciable rate in regular enzyme–substrate mixtures [6]. Since XynB hardly hydrolyzed xylobiose under low substrate concentration, the formation of xylose was speculated to be a result of transglycosylation reactions. The overall reaction is then envisaged to be: $2X_2 + E = E(X_4)$; $E(X_4) = E(X_3) + X_1$; $E(Xyl_3) = Xyl_2 + X_1 + E$; where X_2 represents xylobiose; X_1, xylose; E, free *endo*-xylanase; $E(X_4)$, *endo*-xylanase xylotetraose complex; and $E(X_3)$, *endo*-xylanase xylotriose complex. The transglycosylation activity of XynB was prominent towards X_2, possibly because of the accessibility of the smaller substrates to the active center of the enzyme. This type of reaction may enable xylanase to split xylobiose after it has been incorporated into xylooligomers. To our knowledge, this is the first time xyobiose was found to be hydrolyzed by a thermostable xylanase through the transglycosylation mechanism.

Xylotriose was the smallest oligomer hydrolyzed by most of the characterized xylanases [1,2]. It is still novel that XynB catalyzed the hydrolysis of xylobiose. The ability of XynB to hydrolyze polymeric substrates such as birchwood xylan mainly to dimer and monomer may be important in view of its application for practical purposes, such as the saccharification of xylan-rich materials for subsequent fermentation and the preparation of xylose and xylobiose. Also, this xylanase could be useful for specific applications when short xylose fragments and high degree hydrolysis are required [2,26].

3.5. Polysaccharide-binding properties

The polysaccharide-binding capacity of XynB was analyzed by incubating the purified enzyme with insoluble polysaccharide substrates such as Avicel and insoluble oat-spelt xylan. As shown in Fig. 5, XynB could bind to both Avicel and insoluble xylan even though it had no activity on Avicel. On Avicel, the bound protein increased with higher cellulose–enzyme ratios. In contrast, the enzyme did show its capacity to bind to insoluble xylan, as about 60% enzyme activity still remained in the supernatant. Almost 40% of the adsorption took place on the insoluble oat-spelt xylan since increasing concentrations of this substrate hardly changed the xylanase activity in the supernatant, suggesting that a xylan-binding domain might not present.

Binding is generally mediated by several co-planar, solvent-exposed aromatic rings, which form stacking interactions with the sugars in the polysaccharide and also through hydrogen bonding [20]. The presence of a carbohydrate-binding domain in xylanases can increase the degradation efficiency of complex substrates. Some xylanases show only a limited level of activity toward insoluble xylan due to the lack of binding domain [32–34]. However, XynB showed a high activity towards insoluble oat-spelt xylan and could effectively hydrolyze insoluble substrates such as insoluble oat-spelt xylan, beech wood xylan, etc.

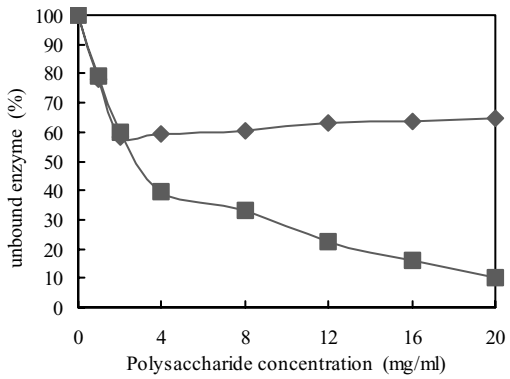

Fig. 5. Effect of different concentrations of cellulose (Avicel) and insoluble xylan on the binding ability of the recombinant XynB. XynB (25 μg) was incubated with 1–20 mg/ml crystalline cellulose (Avicel) or insoluble oat-spelt xylan in 50 mM MES buffer (pH 6.1) at 4 °C. Symbols: (◆) insoluble xylem; (■) advice.

According to the deduced amino acid sequence for XynB, a total of 47 aromatic residues (Tyr, 19; Phe, 18; Trp, 10;) are present in XynB molecule. These residues could be responsible for the carbohydrate binding to the protein. Some studies have shown that the aromatic residues play a key role in the carbohydrate-binding of xylanases. A single domain xylanase from the *Bacillus* D3 strain can bind insoluble xylan and Avicel, implying that a series of surface-aromatic residues form hydrophobic clusters [35]. XynA from *T. maritima* contains cellulose-binding domain and is capable of binding to crystalline cellulose [7,10]. However, a single domain of XynB also binds strongly to Avicel or partly to xylan. Therefore, binding of these above insoluble polysaccharides could be mediated by the surface-aromatic residues of XynB. However, further research is required to confirm these assumptions.

4. Conclusions

The recombinant xylanase B from a hyperthermophilic Eubacterium, *T. maritima* is not only an extremely thermostable enzyme, but also stable in the neutral to alkaline region. In this experiment, XynB exhibited the highest activity towards the beechwood xylan and a low activity towards CMC. These attractive features are essential for the prebleaching of kraft pulp in paper industries. XynB hydrolyzed xylooligosaccharides and xylans to yield predominantly xylobiose as end product, suggesting it was an *endo*-xylanase. Therefore, the enzyme could be used for the large-scale production of xylobiose from xylans. XynB was also found to slowly hydrolyze xylobiose by the transglycosylation mechanism. Additionally, a single domain XynB exhibited its capacity to bind to Avicel and insoluble xylan possibly owe to its surface-aromatic residues. In conclusion, the recombinant XynB from *T. maritima* could be of commercial interest in the near future.

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