Isolation, Purification, and Characterization of a Thermostable Xylanase from a Novel Strain, *Paenibacillus campinasensis* G1-1

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High levels of xylanase activity (143.98 IU/ml) produced by the newly isolated *Paenibacillus campinasensis* G1-1 were detected when it was cultivated in a synthetic medium. A thermostable xylanase, designated XynG1-1, from *P. campinasensis* G1-1 was purified to homogeneity by Octyl-Sepharose hydrophobic-interaction chromatography, Sephadex G75 gel-filter chromatography, and Q-Sepharose ion-exchange chromatography, consecutively. By multistep purification, the specific activity of XynG1-1 was up to 1,865.5 IU/mg with a 9.1-fold purification. The molecular mass of purified XynG1-1 was about 41.3 kDa as estimated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Sequence analysis revealed that XynG1-1 containing 377 amino acids encoded by 1,134 bp genomic sequences of *P. campinasensis* G1-1 shared 96% homology with XylX from *Paenibacillus campinasensis* BL11 and 77%–78% homology with xylanases from *Bacillus* sp. YA-335 and *Bacillus* sp. 41M-1, respectively. The activity of XynG1-1 was stimulated by Ca2+, Ba2+, DTT, and β-mercaptoethanol, but was inhibited by Ni2+, Fe3+, Fe2+, and EDTA. The purified XynG1-1 displayed a greater affinity for birchwood xylan, with an optimal temperature of 60°C and an optimal pH of 7.5. The fact that XynG1-1 is cellulose-free, thermostable (stability at high temperature of 70°C–80°C), and active over a wide pH range (pH 5.0–9.0) suggests that the enzyme is potentially valuable for various industrial applications, especially for pulp bleaching pretreatment.

**Keywords:** *Paenibacillus campinasensis*, thermostable xylanase, isolation, purification, characterization

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mill in China. In order to characterize the xylanase from *P. campinasensis* G1-1, it was purified to homogeneity from culture supernatants by three kinds of chromatography. The attractive attributes of this xylanase suggest that it is potentially valuable for various industrial applications, especially for pulp bleaching pretreatment. Additionally, the xylanase-encoding gene from *P. campinasensis* G1-1 was cloned and heterologous expression of XynG1-1 in *E. coli* BL21 (DE3) was conducted for further study of this enzyme.

**MATERIALS AND METHODS**

**Strains, Vectors, and Chemicals**

*E. coli* DH5α (preserved in our laboratory) and *E. coli* BL21 (DE3) (preserved in our laboratory) were used for gene cloning, sequencing, and expression, respectively. As a cloning and expression vector, pET-22b(+) was from Novagen (Madison, WI, USA). Birchwood xylan, oat spelt xylan, carboxymethyl cellulose, Avicel, β-mercaptoethanol, sodium dodecyl sulfate (SDS), 2,2-dithiodipiridine, 1,4-dithiothreitol (DTT), and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Rice hull xylan and bran xylan were provided by Guanfu Fine Chemistry Ltd (Tianjin, China). Octyl-Sepharose, Sephadex G75, and Q-Sepharose were purchased from GE Healthcare Life Sciences (Beijing Branch Office, China). All other chemicals were of analytical reagent grade purity and obtained from commercial sources.

**Isolation of Xylan-Degrading Bacteria**

Cotton stalk stockpile samples were collected from Tianjin Guangjiuyuan paper mill in China and transported to the laboratory. Samples of 1.0 g were dispersed in 100 ml of sterile water, and 1 ml of the suspension was transferred to 5 ml of enriched medium [birch xylan 1.0% (w/v), Bacto-tryptone 0.5% (w/v), NaCl 0.5% (w/v), pH 9.0], incubated at 37°C for 48 h. The enriched bacterial broth was serially diluted and plated on selective plates [LB plates adding 1.0% (w/v) birch xylan], and incubated at 37°C for 48 h. Colonies harboring xylanase activity were determined using the xylan-Congo red clearance plate assay [26] based on their ability to produce a transparent zone on the selective plates. The positive colonies were further purified and stored in 15% glycerol.

**Bacterial Characterization and Sequence Analysis of 16S rRNA Gene**

Strain G1-1 with the highest capability of producing xylanase was identified by biochemical and morphological tests as outlined by the *Bergey’s Manual of Determinative Bacteriology* [5] and by its 16S rRNA gene sequence analysis. The strain was deposited in the China General Microbiological Culture Collection Center (CGMCC; No. 5023), Beijing, China. Genomic DNA was extracted using the phenol-chloroform method [19] and the 16S rRNA gene was amplified using the bacterial universal primers [23] 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-GTT AAC TAC GGG ACT T-3'). PCR amplification was performed using the following cycling profile: 1 cycle of 95°C for 5 min; 30 cycles of 94°C for 45 s, 55°C for 45 s, and 72°C for 90 s; and a cycle of 72°C for 10 min. The purified PCR product was cloned into the pUCm-T vector and sequenced. Homologous analysis was then performed online with the NCBI BLAST software (http://blast.ncbi.nlm.nih.gov). The target sequence was compared with 16S rRNA sequences in the GenBank, and aligned with close relatives using the ClustalW 2.1 software. A phylogenetic tree was constructed with MEGA 5.05 using the neighbor-joining method [24]. Bootstrap analysis for 100 replicates was performed to estimate reproducibility of the tree topologies.

**Growth and Xylanase Production Conditions of *P. campinasensis* G1-1**

The strain was routinely cultured on Luria–Bertani (LB) agar plates containing 10 g Bacto-tryptone, 5 g yeast extract, 10 g NaCl, and 15 g agar per liter at pH 7.0, at 37°C for 16–24 h. For xylanase production, *P. campinasensis* G1-1 grew in a complex medium [4% (w/v) bran powder, 0.5% (w/v) peptone, and 0.5% (w/v) K₂HPO₄, pH 7.0] or a modified Berg’s mineral salt medium [3] (synthetic medium) at pH 7.0 containing 0.5% (w/v) NaNO₃, 0.3% (w/v) K₂HPO₄, 0.03% (w/v) MgSO₄·7H₂O, 0.002% (w/v) MnSO₄·H₂O, 0.002% (w/v) FeSO₄·7H₂O, 0.002% (w/v) CaCl₂·2H₂O, and supplemented with 0.5% (w/v) commercial birch xylan (Sigma) at 37°C with shaking at 180 rpm. Culture samples were collected at 6 h intervals over the period of 96 h cultivation. The culture growth was monitored by measuring the optical density at 600 nm. All experiments were conducted in triplicates.

**Purification of Extracellular Xylanase**

The culture supernatants of *P. campinasensis* G1-1 were collected by centrifugation at 8,000 rpm for 10 min after 48 h cultivation andsalted out with ammonium sulfate at 70% saturation. After that, the precipitates were dissolved in 20 mM sodium phosphate buffer (pH 7.0; buffer A) and loaded onto a Octyl-Sepharose FF column (180 × 26 mm) pre-equilibrated with buffer A containing 40% saturation of ammonium sulfate. The target protein was eluted with a linear gradient (40%~0% saturation) of ammonium sulfate in the same buffer. Active fractions were loaded onto a Sephadex G75 column (500 × 16 mm) equilibrated with 20 mM sodium phosphate buffer (pH 8.0, buffer B). The xylanase active fractions were collected for further purification by a Q-Sepharose HP column (100 × 26 mm) using linear gradient elution from 0~1 M NaCl in buffer B. Finally, the purified xylanase was desalted by dialysis against deionized water and concentrated by freeze-drying. The protein content of each chromatographic fraction was determined by measuring the absorbance at 280 nm using a Bio-Rad purifier system. Protein concentration was determined according to the Bradford method [4] using bovine serum albumin as a standard.

**SDS-PAGE and Zymogram Analysis**

SDS-PAGE was performed based on the method of Laemmli [12] with the stacking and separating gel concentrations of 5% and 12%, respectively, and protein bands were visualized by staining with Coomassie Brilliant Blue G250 (Sigma). The zymogram for xylanase was performed by PAGE using a 0.1% (w/v) of birch xylan incorporated into the polyacrylamide [15]. After electrophoresis, the enzyme was activated by 25% (v/v) 2-propanol, and the gel was incubated at 60°C for 30 min in 20 mM sodium phosphate buffer (pH 7.5), followed by staining for residual carbohydrates with Congo red solution (1 mg/ml), destaining with 1 M NaCl, and fixing with 5% (v/v) acetic acid. Clear zones in the zymogram indicate
enzymatic activity. Unstained protein molecular weight standards were purchased from Fermentas.

**Xylanase Assay**

Xylanase activity was measured by determining the amount of reducing sugars (xylose) released from birch xylan (Sigma) using the 3,5-dinitrosalicylic acid method [1]. The reaction mixture containing 100 µL of 1% (w/v) birch xylan as substrate and 100 µL of appropriately diluted enzyme extract was incubated at 60°C and pH 7.5 for 10 min. The reaction was terminated by adding 600 µL of 3,5-dinitrosalicylic acid and placing in a boiling-water bath for 10 min. A control containing the same reagents was incubated simultaneously except that the reaction was terminated before the addition of the enzyme. After cooling, the absorbance of the reaction mixtures was measured against the control at 540 nm in a spectrophotometer. One international unit (IU) of xylanase activity is defined as the amount of enzyme that releases 1 µmol of reducing sugars (xylose) per minute under the above conditions.

**Effects of Temperature and pH on Xylanase Activity and Stability**

The optimum temperature of the xylanase was measured by incubating the reaction mixtures at different temperatures (40–80°C) and pH 7.5 for 10 min. The relative enzymatic activity was calculated as a percentage of the maximal activity. To assess its pH stability, the xylanase was pre-incubated in buffers at various pH values at 60°C for 180 min. The residual enzyme activity was calculated as a percentage of the starting activity.

The optimum pH of xylanase activity was measured at 60°C using the following three buffer systems, all at a concentration of 20 mM: sodium phosphate (pH 5.0–7.5), Tris-HCl (pH 8.0–9.0), and glycine-NaOH (pH 9.5–10.0). The relative enzymatic activity was calculated as a percentage of the maximal activity. To assess its pH stability, the xylanase was pre-incubated in buffers at various pH values at 60°C for 60 min prior to determination of the residual activities. Remaining activities were calculated as percentages of the starting activity.

**Effects of Various Metal Ions and Other Additives on Xylanase Activity**

The effects of various metal ions on xylanase activity were evaluated by incubating the reaction mixtures with KCl, CaCl₂, BaCl₂, MgCl₂, MnCl₂, NiCl₂, FeCl₃, ZnCl₂, and CuSO₄ at the concentration of 2, 5, and 10 mM, respectively, and the enzyme activity assayed in the absence of metal ions was defined as 100%. To evaluate the effects of metal chelators and surfactants on xylanase activity, EDTA, DTT, β-mercaptoethanol, and SDS at various concentrations were tested. Enzyme solutions were incubated at 60°C for 60 min before the residual activity was measured. The activity of the enzyme assayed under the same condition without additives was defined as 100%. All of the presented values are the averages of triplicate assays.

**Substrate Specificity and Kinetic Studies**

Specificities of purified XynG1-1 against birchwood xylan, oat spelt xylan, rice hull xylan, bran xylan, carboxymethyl cellulose, and Avigel at concentrations of 1% (w/v) under optimal reaction conditions were assayed. The enzyme preparation was incubated with birchwood xylan and oat spelt xylan at concentrations between 0.1% (w/v) and 1% (w/v). The kinetic parameters (Kₘ and Vₘₚₓ) were estimated from a Lineweaver–Burk double reciprocal plot of xylanase activity at its optimum reaction conditions.

**Cloning and Expression of the Xylanase Gene (XynG1-1)**

The xylanase gene was amplified using the genomic DNA of *P. campinasensis* G1-1 as template and the following primers: 5'-CCCAAGGCTATGGAAATTCAGGGAGAAGGAA-3' and 5'-CCGCTCTGAGTCCACCGGACTCAGTAATGCAATG-3' (underlined and bold letters indicate HinIII and XhoI sites, respectively) designed based on the conserved sequences revealed by sequence alignment and comparative analysis of *Paenibacillus* sp. xylanase homologous genes from the GenBank database. PCR was performed at 95°C for 5 min, followed by 30 cycles for 45 s at 94°C, 45 s at 55°C, and 90 s at 72°C, and a final extension of 10 min at 72°C, respectively. The product was digested with HinIII and XhoI, and then cloned into the corresponding sites of pET-22b(+), resulting in expression vector pET-G1-1. The cloned xylanase gene XynG1-1 was sent for sequencing. Alignment of the putative amino acid sequence of XynG1-1 with other related xylanases was conducted using the Clustalω software. After that, pET-G1-1 was transformed into *E. coli* BL21 (DE3) and the expression of XynG1-1 examined by detecting xylanase activity of the culture broth of the recombinant strain.

**Nucleotide and Protein Sequences Accession Numbers**

The 16S rRNA sequence of strain G1-1 has been submitted to the GenBank database under Accession No. JF830004.1. The nucleotide and protein sequences of XynG1-1 have been submitted to the GenBank database under accession No. JF830005.1 and AE54132.1, respectively.

**RESULTS and DISCUSSION**

**Isolation and Identification of Xylan-Degrading Bacteria**

Based on transparent zones on the selective plates (described in Materials and Methods), 35 xylan-degrading isolates were observed from a total of 342 bacterial colonies, and 11 positive colonies forming relative larger transparent zones were selected and identified to be *Bacillus* or *Paenibacillus*. One of these isolates, named G1-1, possessed the highest xylan degrading activity in the assay conditions. It grew between 25°C and 65°C with an optimal temperature of 37°C. The pH range for its growth was between 4.0 and 10.0 with an optimal pH of 7.0 (data not shown). Physiological and biochemical characteristics of strain G1-1 are listed in Table 1, and characteristics of *P. campinasensis* BL11 [10] that served as a control. Strain G1-1 is a Gram-positive, aerobic, and rod-shaped bacterium. It displayed characteristics similar to *P. campinasensis* BL11 as listed in Table 1 except for some aspects of its physiology. For example, strain G1-1 could not use d(+)-mannose or glycerol as carbon source and KNO₃ as nitrogen source, whereas *P. campinasensis* BL11 could. In contrast to *P. campinasensis* BL11, strain G1-1 not only produced multiple polysaccharide hydrolases but also proteases. However, neither strain was capable of producing H₂S and indole. BLAST search of the obtained partial 16S RNA
sequence of strain G1-1 using the NCBI software identified 12 organisms closely related to strain G1-1. A phylogenetic tree analysis (Fig. 1) revealed that strain G1-1 is most closely related to *P. campinasensis* IB-411 and *P. campinasensis* BL11, sharing 99% sequence identity. Thus, based on the results of physiological and biochemical tests as well as 16S rRNA gene sequence analysis, the strain G1-1 was identified as a new subspecies of *P. campinasensis*, which had some significantly different physiological characteristics compared with *P. campinasensis* BL11 [10].

### Production of Extracellular Xylanase by *P. campinasensis* G1-1

Xylanase production by *P. campinasensis* G1-1 was investigated during 96 h of cultivation in the synthetic medium and the complex medium (detailed in Materials and Methods), respectively. After a 6 h lag phase, the exponential growth began and lasted for 24 h, followed by the stationary phase when *P. campinasensis* G1-1 was grown in the synthetic medium (Fig. 2). In contrast, a shorter lag phase and exponential growth phase were observed when it was cultivated in the complex medium in which stationary phase was reached after 24 h (Fig. 2). Interestingly, xylanase production reached the maximal level of 143.98 IU/ml in the synthetic medium, which were about 2-fold higher than that of 68.22 IU/ml produced in the complex medium (Fig. 2). Moreover, the xylanase secretion pattern exhibited slight differences in that extracellular xylanase was secreted at the start of the exponential phase in the synthetic medium, whereas secretion in the complex medium did not occur until the beginning of the stationary phase. However, the maximal xylanase production by *P. campinasensis* G1-1 in both media was achieved at the end of their stationary phases. The above results clearly show that xylanase production by *P. campinasensis* G1-1 in the synthetic medium is more cost effective compared with that in the complex medium. In addition, the synthetic medium contains only mineral salts and lack many complex proteins contained in the complex medium, thus greatly facilitating purification of the extracellular xylanase. Above all, *P. campinasensis* G1-1 produced high levels of xylanase (143.98 IU/ml) when cultivated in the synthetic medium (Fig. 2), which had an approximate 13.7-fold higher activity than xylanase (10.5 IU/ml) produced by *P. campinasensis* BL11 [10].

### Purification of Xylanase XynG1-1

In order to elucidate characteristics of this novel xylanase, purification was carried out as detailed in Materials and Methods using supernatants from culture broth of *P. campinasensis* G1-1 grown in the synthetic medium. The purification procedure and relevant data are summarized in Table 2. The specific activity of the purified XynG1-1 was 1,865.5 IU/mg with a 9.1-fold purification and the final yield for the enzyme was 6.2%. As a single band on SDS-PAGE with an apparent molecular mass of about 41.3 kDa, the purified xylanase from culture supernatants was designated as XynG1-1 (Fig. 3A, lane 4), whereas zymogram analysis of purified XynG1-1 exhibited a clear activity band on the corresponding zone (Fig. 3B, lane 2). Moreover, in contrast to several protein bands of the crude enzyme...
A thermostable xylanase from a novel *P. campinasensis*. Preparation that appeared on the SDS-PAGE gel (Fig. 3A, lane 2), only a single clear zone corresponding to xylanase activity was visualized using zymography (Fig. 3B, lane 1). The results indicate that *P. campinasensis* G1-1 produces only a single xylanase with high enzyme activity, unlike a number of microorganisms producing multiple xylanases to achieve effective hydrolysis of xylan [9, 17, 18].

Effects of Temperature and pH on Enzyme Activity and Stability

The purified XynG1-1 showed maximum enzymatic activity at 60°C, which is higher than that of *Paenibacillus* sp. 12-11 (55°C) [27], and retained 33% of its maximum activity at 80°C (Fig. 4A). The thermal stability of XynG1-1 was evaluated by incubation at temperatures from 40 to 80°C for 180 min. The enzymatic activity retained at least 80.7% of the original activity after incubation at 40°C to 60°C for 180 min, and still retained about 77.1% and 50.2% of the original activity after incubation at 70°C and 80°C for 60 min, respectively (Fig. 4B). This thermal stability is better than earlier reports on most of the bacterial xylanases [2, 22, 27]. Meanwhile, the purified XynG1-1 showed the
maximum activity at pH 7.5, which is higher than that of XyliX (7.0) from *P. campinasensis* BL11 [11] and retained 50% of its maximum activity at pH 10.0 (Fig. 5A). It was stable between pH 5.0 and pH 9.0 for 60 min, retaining about 60% and 70% of its activity at pH 7.5 (Fig. 5B), manifesting much greater pH stability than those of most bacterial xylanases reviewed [2, 22]. Its stability at high temperature (70°C~80°C) and activity over a wide pH range (pH 5.0~9.0) (Fig. 4 and 5) make XynG1-1 valuable for various industrial applications, especially for pulp bleaching pretreatment.

### Effects of Various Metal Ions and Additives on Xylanase Activity

The effects of various cations at concentrations of 2, 5, and 10 mM on the activity of XynG1-1 are summarized in Table 3. The purified XynG1-1 was activated by Ca\(^{2+}\) and Ba\(^{2+}\) at each concentration examined, and their stimulatory effects were observed on *Penicillium sclerotiorum* xylanase Xyl I [2], whereas Ca\(^{2+}\) inhibited xylanase activity from *P. campinasensis* BL11 [11]. However, Ni\(^{2+}\), Fe\(^{2+}\), Fe\(^{3+}\), and Zn\(^{2+}\) displayed different levels of inhibitory effects on the activity of the purified XynG1-1, and the detrimental effects of Cu\(^{2+}\) were gradually enhanced with increased

### Table 2. Purification of xylanase G1-1 from *P. campinasensis* G1-1.

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Total activity (IU)</th>
<th>Total protein (mg)</th>
<th>Specific activity (IU/mg protein)</th>
<th>Yield (%)</th>
<th>Purification fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture filtrate</td>
<td>72,000</td>
<td>352.2</td>
<td>204.4</td>
<td>100.0</td>
<td>1.0</td>
</tr>
<tr>
<td>(NH(_4))SO(_4) precipitation</td>
<td>57,024</td>
<td>107.3</td>
<td>531.4</td>
<td>79.2</td>
<td>2.6</td>
</tr>
<tr>
<td>Octyl-Sepharose</td>
<td>24,840</td>
<td>27.0</td>
<td>919.8</td>
<td>34.5</td>
<td>4.5</td>
</tr>
<tr>
<td>Sephadex G75</td>
<td>11,232</td>
<td>8.3</td>
<td>1,349.0</td>
<td>15.6</td>
<td>6.6</td>
</tr>
<tr>
<td>Q-Sepharose</td>
<td>4,464</td>
<td>2.4</td>
<td>1,865.5</td>
<td>6.2</td>
<td>9.1</td>
</tr>
</tbody>
</table>

Fig. 4. Effects of temperature on activity (A) and stability (B) of XynG1-1 (Sp. activity = 1,865.5 U/mg).

(A) Relative activities at different temperatures; The maximum activity observed at 60°C was taken as 100%. (B) Residual activities after incubation for various times at 40°C (filled rhombus), 50°C (filled squares), 60°C (filled triangles), 70°C (empty rhombus), 80°C (fork); The original activity without incubation was taken as 100%. Data are presented as means ± SD (n = 3).

Fig. 5. Effects of pH on the activity (A) and stability (B) of XynG1-1 (Sp. activity = 1,865.5 U/mg).

(A) Relative activities at different pH values; The maximum activity observed at pH 7.5 was taken as 100%. (B) Stability at different pH values; The maximum residual activity observed at pH 7.5 was taken as 100%. Data are presented as means ± SD (n = 3).
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concentrations. In contrast, a thermostable xylanase from the thermophilic fungus *Talaromyces thermophilus* was shown to be activated by Cu$^{2+}$ [13]. No significant effects on XynG1-1 activity were detected for K$^+$, Mg$^{2+}$, and Mn$^{2+}$ at each concentration tested (Table 3), whereas the relative activity of Xyl II from *P. sclerotiorum* was shown to be enhanced up to 225.8 ± 2.9% by 10 mM of Mn$^{2+}$ [9]. DTT and β-mercaptoethanol could enhance the xylanase activity of XynG1-1. As strong reducing agents, they stimulated the enzymatic activities mainly by preventing the oxidation of sulfhydryl groups of the purified xylanase [9]. The purified XynG1-1 retained only 20.35% of its original activity after incubation with 10 mM SDS, suggesting that hydrophobic interactions may be important in maintaining the structures of xylanases. Being a metal chelator, EDTA decreased the XynG1-1 activity, indicating that the purified enzyme requires metal ions for its action [9]. Similarly, the xylanase activity of XylX from *P. campinasensis* BL11 was stimulated by the addition of 0.5% (v/v) β-mercaptoethanol and was obviously inhibited by the addition of 5 mM EDTA or 0.25% (w/v) SDS [11].

### Substrate Specificity and Kinetic Studies

Specificity studies indicate that XynG1-1 could hydrolyze all the tested xylans, but did not act on carboxymethyl cellulose and Avicel. The absence of cellulase activity required for selective removal of only the hemicellulose component with minimal damage to the cellulose pulp renders XynG1-1 an excellent candidate for pulp bleaching pretreatment [13]. The purified XynG1-1 showed $K_m$ values of 5.86 and 9.07 mg/ml and $V_{max}$ values of 1,696.90 and 941.15 µmol/min/mg of protein for birchwood and oat spelt xylans, respectively. The values of $K_m$ for these two substrates indicated that XynG1-1 had greater affinity for birchwood xylan. Strong specificity toward birchwood and oat spelt xylans was also verified for xylanases from *P. campinasensis* BL11 [11], *T. clypeatus* [21], and *P. sclerotiorum* [9].

### Table 3. Effects of metal ions and other additives on the activity of the purified XynG1-1.

<table>
<thead>
<tr>
<th>Agents</th>
<th>Relative activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 mM</td>
</tr>
<tr>
<td>Control</td>
<td>100 ± 1.5</td>
</tr>
<tr>
<td>K$^+$</td>
<td>95.5 ± 4.6</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>121.8 ± 7.2</td>
</tr>
<tr>
<td>Ba$^{2+}$</td>
<td>107.2 ± 3.4</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>95.5 ± 4.5</td>
</tr>
<tr>
<td>Mn$^{2+}$</td>
<td>95.7 ± 4.8</td>
</tr>
<tr>
<td>Ni$^{2+}$</td>
<td>68.4 ± 2.6</td>
</tr>
<tr>
<td>Fe$^{2+}$</td>
<td>64.4 ± 5.4</td>
</tr>
<tr>
<td>Fe$^{3+}$</td>
<td>26.5 ± 2.3</td>
</tr>
<tr>
<td>Zn$^{2+}$</td>
<td>35.8 ± 6.1</td>
</tr>
<tr>
<td>Cu$^{2+}$</td>
<td>95.5 ± 3.3</td>
</tr>
<tr>
<td>DTT</td>
<td>102.4 ± 2.7</td>
</tr>
<tr>
<td>β-Mercaptoethanol</td>
<td>110.5 ± 1.3</td>
</tr>
<tr>
<td>EDTA</td>
<td>65.2 ± 4.3</td>
</tr>
<tr>
<td>SDS</td>
<td>70.3 ± 2.1</td>
</tr>
</tbody>
</table>

The experiment were performed as mentioned in Materials and Methods. Data are presented as means ± SD (n = 3).

### Fig. 6. Alignment of the predicted amino acid sequence of XynG1-1 and its closest relatives from the GenBank databases.

The closest sequences shown are xylX from *Paenibacillus campinasensis* BL11 (GenBank No. ABB77852.1); endo-1, 4-beta-xylanase from *Bacillus* sp. YA-335 (GenBank No. CAA41784.1); xylanase J from *Bacillus* sp. 41M-1 (GenBank No. BAA82316.1); and xylanase from *Paenibacillus curdlanolyticus* B-6 (GenBank No. ADB54799.1). Gaps are indicated by dashes. Asterisks indicate identical amino acids.
Cloning, Sequence Analysis, and Expression of XynG1-1

The complete nucleotide sequence of the gene encoding XynG1-1 was determined. The sequence is 1,134 bp in length and is predicted to encode a protein of 377 amino acids. This putative amino acid sequence was 96% identical to that of XylX from Paenibacillus campinasensis BL11 with up to 14 base mutations, 78% and 77% identical to that of the endo-1,4-β-xylanase from Bacillus sp. YA-335 and xylanase J from Bacillus sp. 41M-1, respectively (Fig. 6). Although XynG1-1 was highly similar to XylX, it still harbored higher activity, higher optimum pH, and some other different characteristics comparing with XylX that have been discussed above. The 14 mutations are of great value as a basis for the futher study of the differences between the two xylanases by structural analysis. As previously reported, XylX is a thermostable xylanase, the endo-1, 4-β-xylanase and xylanase J are alkalophilic xylanases, and all three xylanases belong to glycosyl hydrolase family 11 [8, 11, 16]. Thus, both sequence analysis and further characterization suggest that the purified XynG1-1 from *P. campinasensis* G1-1 is a thermostable and alkaline-tolerant xylanase. To verify the cloned gene, the coding sequence of XynG1-1 was subcloned into the expression vector pET-22b and expressed in *E. coli* BL21 (DE3). Extracellular xylanase activity of 10.58 IU/ml and intracellular xylanase activity of 4.47 IU/ml were detected from the culture broth of the recombinant strain.

In conclusion, a cellulose-free and thermostable xylanase (XynG1-1) from *P. campinasensis* G1-1 newly isolated from samples in a Chinese paper mill, possessing some attractive attributes such as high optimal temperature (60°C), stability at high temperature of 70°C~80°C, and activity over a wide pH range (pH 5.0~9.0), was suitable for various industrial applications, especially for pulp bleaching pretreatment. Thus, strain G1-1 provides a valuable new enzyme source in industrial applications. Moreover, the successful cloning of the XynG1-1 gene has provided a platform for further developing XynG1-1 to be tailored for specific industrial applications.

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References


