Expression, Characterization and Its Deinking Potential of a Thermostable Xylanase From Planomicrobium glaciei CHR43

Zhaoxing Liu, Tingting Shao, Yan Li, Bin Wu, Honghua Jia* and Ning Hao*

College of Biotechnology and Pharmaceutical Engineering, Nanjing Tech University, Nanjing, China

Genome mining is more and more widely used in identifying new enzymes from database. In the present study, we reported a putative xylanase, Pg-Xyn (WP_053166147.1), which originated from a psychrotolerant strain Planomicrobium glaciei CHR 43, and was identified from Genbank by genome mining. Sequence analysis and homology modeling showed that Pg-Xyn belongs to glycosyl hydrolase family 10. On the basis of heterologous expression in E. coli and biochemical characterization, we found Pg-Xyn was most active at pH 9.0 and 80°C and exhibited good stability from pH 5.0 to 12.0 and below 90°C. Pg-Xyn was slightly activated in the presence of Ca²⁺ and Mg²⁺, while it was strongly inhibited by Mn²⁺. The analysis of hydrolysis products showed that Pg-Xyn was an endo-β-1,4-xylanase. In addition, Pg-Xyn performed good deinking ability in a paper deinking test. In consideration of its unique properties, Pg-Xyn might be a promising candidate for application in the paper and pulp industries.

Keywords: endo-xylanase, thermo-alkali-stable, thermostable, psychrotolerant bacterium, enzymatic deinking

INTRODUCTION

Xylanase (EC 3.2.1.8) catalyzes the hydrolysis of β-1,4-glycosidic linkages within xylan, playing the most important role in complete breakdown of xylan (Collins et al., 2005). In nature, xylanases exist in a variety of animals, plants, and microorganisms (Uday et al., 2018), and they are widely used in food, textile, ethanol production, waste treatment, and pulp and paper industries (Kumar et al., 2016; Kumar P. S. et al., 2018).

In the past years, much effort have been made to isolate xylanase producers, and a variety of microorganisms including bacteria, yeasts, and filamentous fungi have been found as xylanase producers, among which fungi have the ability to secrete xylanases with much higher yield than bacteria or yeast (Polizeli et al., 2005; Ahmed et al., 2009; Lombard et al., 2014). However, xylanases from bacterial sources show higher thermal stability than those from fungi (Mamo et al., 2006; Shrinivas et al., 2010; Zhang et al., 2012; Mhiri et al., 2020).

Nowadays, more and more genomic sequences have been deposited in the sequence databases, which afford huge treasure for mining xylanase candidates. Planomicrobium glaciei CHR43 is a psychrotolerant bacterium isolated from the cold desert region (Salwan et al., 2014), and its genome sequence was reported in 2014 (GenBank accession number: AUYR0000000.1). Until now, no record of a xylanase originating from P. glaciei CHR43 has been identified. Since the mesophilic and extremophilic bacteria are generally considered to be potent producers of industrial xylanases,
when we used the sequence O30700 of a highly thermostable endo-xylanase isolated from mesophilic, alkalophilic *Bacillus* sp. NG-27 as a template that is most active at 70°C and pH 8.4 (Gupta et al., 2000) to run blastp job against UniParc database, a putative xylanase sequence from *P. glaciei* CHR43 (WP_053166147.1) was found to rank in low E-values. Considering that the selected sequence is highly identical to a xylanase from *Bacillus* sp. NG-27, we wonder whether the putative xylanase mined from the psychrotolerant bacterium would show similar properties. In the present study, the putative xylanase sequence from *P. glaciei* CHR43 was identified, heterologously expressed, and characterized, and its potential use for enzymatic deinking was also evaluated preliminarily.

## MATERIALS AND METHODS

### Strains, Culture, and Chemicals

*E. coli* BL21(DE3), used as a host for the heterologous expression of xylanase, was purchased from TransGen Biotech (Beijing, China). Luria–Bertani (LB) medium was used to cultivate *E. coli* BL21(DE3) at 37°C. High-affinity Ni-NTA resins (L00250-C) were bought from GenScript (Nanjing, China). Xylans from bagasse, beechwood, birchwood, corncob and oat-spelt, as well as sodium carboxymethyl cellulose (CMC) and Avicel, were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade and commercially available.

### Gene Screening and Plasmid Synthesis

The sequence *Pg-Xyn* (WP_053166147.1) was aligned by BLAST search from the National Center for Biotechnology Information database using the xylanase sequence of *Bacillus* sp. NG-27 (O30700) as a template (Gupta et al., 2000) and a phylogenetic tree as reference. Plasmid was generated by inserting the potent xylanase gene, which was synthesized by GenScript (Nanjing, China), into the *Bam*HI-*Xho*I sites of pETDuet-1, which results in the addition of histidine tags to *Pg-Xyn* at N-terminus. The plasmid pETDuet-*Pg-Xyn* was transformed into *E. coli* BL21(DE3), and their construction was confirmed by sequencing.

### Sequence and Putative Structure Analysis

Protein sequence similarity was evaluated using the BLASTp program (Altschul et al., 1990). Molecular mass was predicted using ProtParam tool (Gasteiger et al., 2005). Multiple sequence alignment was conducted with ClustalW (Thompson et al., 1994). Phylogenetic tree construction was performed using MEGA5.1 software according to the neighbor-joining algorithm (Tamura et al., 2007). Structural model was developed through homology modeling using SWISS-MODEL (Biasini et al., 2014). The crystal structure of a xylanase from *Bacillus* sp. NG-27 (PDB accession code 2FGL) was used as the template for *Pg-Xyn*. The visualization of the constructed model structure and the generation of graphical figure was performed with the PyMol molecular graphics system (http://www.pymol.org).

## Expression and Purification of *Pg-Xyn*

Plasmid pETDuet-1-*Pg-Xyn* was transformed into *E. coli* BL21(DE3) competent cells, and pETDuet-1 served as a control. Positive transformants harboring pETDuet-1-*Pg-Xyn* were harvested in 5 mL of LB medium containing 100 µg ml⁻¹ ampicillin and grown overnight at 37 °C. Then, the overnight culture was shifted into 500 mL of LB medium containing ampicillin (100 µg ml⁻¹) until the absorbance at 600 nm (*A*₆₀₀) was 0.6. To express *Pg-Xyn*, isopropyl-β-D-1-thiogalactopyranoside (IPTG) was added to a final concentration of 1 mM, and the cultures were incubated for 12 h with vigorous agitation at 30 °C. Then, the cultures were centrifuged at 10,000 × g at 4°C for 15 min. The cells were washed twice with ice-cold phosphate-buffered saline (PBS, 100 mM, pH 7.0), resuspended in the same buffer, and lysed by ultrasonication (220 W) in an ice bath. The lysate supernatant was obtained by centrifuge at 10,000 × g at 4°C for 20 min.

To purify the histidine-tagged recombinant *Pg-Xyn*, the clarified supernatant was incubated at 50°C for 30 min and then centrifuged at 10,000 × g at 4°C for 10 min to collect the supernatant. The supernatant was loaded onto a Ni-NTA column and eluted with a linear imidazole gradient (50–200 mM). The purity and apparent molecular mass of purified *Pg-Xyn* was determined using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Protein concentration was measured via the Bradford method (Bradford, 1976).

### Enzyme Activity Assay

The determination of *Pg-Xyn* activity was based on the amount of reducing sugar produced from substrates, as determined by the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959). The mixture consisted of 0.2 mL of properly diluted enzyme and 1.8 mL of 1% (w/v) birchwood xylan (Sigma) in PBS buffer (pH 4.8), and it was incubated at 50 °C for 10 min, and then 2 mL of DNS was added to stop the reaction. The mixture was boiled for 10 min and then kept at 4°C for 1 h. The absorbance at 540 nm (*A*₅₄₀) until the absorbance at 540 nm (*A*₅₄₀) was measured after cooling the mixture to room temperature. A solution with the same amount of heat-denatured *Pg-Xyn* and DNS was used as a control. All activity measurements were carried out in triplicate. One unit of enzyme activity was defined as the amount of enzyme necessary to produce 1 µmol of reducing sugar per minute.

### Optimal pH and pH Stability

The optimal pH of *Pg-Xyn* was investigated by measuring its activity at 37°C for 10 min using the following buffer solutions between pH 3 and 12. Buffers were citrate-Na₂HPO₄ (pH 3–8), glycine-NaOH (pH 9–10), and Na₂HPO₄-NaOH (pH 11–12). To examine their pH stabilities, *Pg-Xyn* was added into various buffers ranging from pH 3 to 12, and incubated with birchwood xylan at 37°C for 12 h.

### Optimal Temperature and Thermal Stability

The optimal temperature for *Pg-Xyn* activity was determined from 30 to 90°C at the optimal pH. Enzyme thermostability was determined at temperatures ranging from 50 to 90°C for 1, 2, 3, 4, 5, and 6 h at the optimal pH by measuring the residual activity.
FIGURE 1 | Sequence comparison of Pg-Xyn to the other xylanases. Amino acid sequence identical to at least four of the aligned sequences are shaded in yellow, this area is highly conservative. Three boxed regions stretches that are unique to alkalophilic xylanases. The residues marked with *** are the putative active site in family 10 xylanase. Sequence name, microbial source and UniProt Knowledgebase accession number were given as follows: Pg-Xyn Planomicrobium glaciei CHR43 (WP_053166147.1); xyn1_geose Geobacillus stearothermophilus (P40943); xynA_bachd Bacillus halodurans (P07528); xynB Clostridium stercorarium (P40942); xynA_calsa Caldicellulosiruptor saccharolyticus (P23556).

FIGURE 2 | Phylogenetic tree constructed using the neighbor-joining method (MEGA 5.1). Microbial source and UniProt Knowledgebase accession number for the selected protein sequences were given as follows: Bacillus sp. NG-27 (O30700), Bacillus sp. N16-5 (D7RA44), Planomicrobium glaciei CHR43 WP 053166147.1, Clostridium stercorarium P40942.1, Thermobacillus composti LOEGW5, Paenibacillus barcinonensis O69231 and Paenibacillus barcinonensis O69230.

Effect of Metal Ions and Chemical Reagents
To determine the effect of various metal ions and chemical reagents on Pg-Xyn activity, metal ions (Cu$^{2+}$, Zn$^{2+}$, Fe$^{2+}$, Fe$^{3+}$, Ba$^{2+}$, Mg$^{2+}$, Mn$^{2+}$, and Ca$^{2+}$) and chemical reagents (EDTA and SDS) were mixed with the purified Pg-Xyn at 1 mM. Pg-Xyn activity was evaluated under the optimal pH and temperature, and a reaction with no additive was used as a control.

Kinetic Studies and Substrate Specificity
The kinetic constants of Pg-Xyn were determined by a Lineweaver–Burk plot. Reactions were conducted using the birchwood xylan as the substrate under the optimal conditions. To probe into its substrate specificity, Pg-Xyn was incubated with one of the following substrates (1%, w/v): xylans from beechwood, corncob, oat-spelt, bagasse, CMC-Na, starch, and Avicel. Pg-Xyn activity was also examined.

Analysis of Xylan Hydrolysis Products
Reactions, including 10 mL of 1% (w/v) substrate (beechwood xylan, corncob xylan, oat-spelt xylan, or bagasse xylan) and 50 µg of the purified Pg-Xyn were incubated at 50°C for 24 h. The hydrolysis products were detected by HPLC using a Zorbax NH$_2$ column (Agilent, USA) and a refractive index detector. The mobile phase consisted of 80% acetonitrile and 20% water, and
the flow rate was 1.0 ml min\(^{-1}\). Xylose, xylobiose, xylotriose, xylotetraose, and xylohexaose were used as the standards.

**Deinking of Laser-Printed Paper Using Pg-Xyn**

A printed copy page (A4 size) from a Hewlett-Packard laser printer was used for deinking. The paper was printed with black ink. A fixed amount (100 mg) of cut paper (3 × 3 cm) was stripped. The paper strip was dipped into phosphate buffer (pH 7.0) containing Pg-Xyn (1 U mg\(^{-1}\)) and incubated overnight at the optimal temperature. A control with heat-denatured Pg-Xyn was also used. The deinking of the laser-printed paper was measured by testing the absorbance of the released color in the filtrate at 596 nm. All experiments were conducted in triplicate. Enzymatically treated pulp was further observed by microscopy, and laser paper without enzymatic treatment was used as a control.

**RESULTS**

**Sequence Analysis of Pg-Xyn**

The complete coding sequence for Pg-Xyn from *P. glaciei* CHR43 was 1,152 bp. The calculated molecular mass of Pg-Xyn is 44.06 kDa. Sequence analysis showed that Pg-Xyn belongs to GH10, showing 43–65% identity with those xylanases from *Geobacillus stearothermophilus* (P40943), *B. halodurans* (P07528), and *Clostridium stercorarium* (P40942), *Caldicellulosiruptor saccharolyticus* (P23556) using UniProt Knowledgebase searches. Pg-Xyn has two catalytic residues, Glu (179) and Glu (289) according to the sequence alignment of Pg-Xyn with the other 4 family 10 xylanases (Figure 1).

A phylogenetic tree was built on the basis of the amino acid sequence of Pg-Xyn (Figure 2). The closest homolog for Pg-Xyn is xylanase from *Bacillus* sp. NG-27 (O30700), indicating that it could have higher structural similarity. The higher structural similarity can endow Pg-Xyn similar enzymatic properties with thermostable xylanases from some *Bacillus* genera.

Pg-Xyn has 64.2% sequence identity with xylanase O30700 from *Bacillus* sp. NG-27. After Swiss-model modeling, it is found that although the detailed interaction sites between Pg-Xyn and Xylan are different, the superposition shows that their characteristic motifs and corresponding active regions are highly similar in structure, and the active pocket regions are quite conservative. Homology modeling revealed that Pg-Xyn has the same 8-fold (β/α) structure as thermostable F/10 xylanase O30700 (PDB 2FGL). This 8-fold (β/α) or TIM barrel fold typically found in all GH10 xylanases. Pg-Xyn and 2FGL have similar conserved sites Asp and Glu, which are conserved in alkalophilic xylanases, but less conserved in non-alkalophilic xylanases (Manikandan et al., 2006). On the basis of the 3 D-structure of Pg-Xyn, two glutamic acid residues, Glu 179 and Glu 289, were identified as putative catalytic residues (Figure 3).

![FIGURE 3](image-url) Structure prediction of Pg-Xyn by SWISS-Model. Glu 179 and Glu 289 are marked.

**TABLE 1** Summary of the purification of Pg-Xyn.

| Step         | Total protein (mg) | Total activity (U) | Specific activity (U mg\(^{-1}\)) | Purification (fold) | Yield (%) |
|--------------|--------------------|--------------------|-----------------------------------|--------------------|-----------|
| Crude extraction | 163                | 1.76 × 10^6        | 1.08 × 10^4                       | 1                  | 100       |
| Ni-NTA       | 75                 | 1.46 × 10^6        | 1.95 × 10^4                       | 2.17               | 83        |

![FIGURE 4](image-url) SDS-PAGE of the purified Pg-Xyn. M, Low protein marker; 1, Pg-Xyn purified enzyme; 2, Pg-Xyn crude enzyme.
Expression and Characterization of a Thermostable Xylanase

Expression and Purification of Pg-Xyn
After being expressed in E. coli BL21 (DE3), Pg-Xyn showed the highest specific activity of \(1.08 \times 10^4\) U mg\(^{-1}\) in crude cell extracts. Pg-Xyn was treated at 50\(^\circ\)C for 30 min and then purified by Ni-NTA column chromatography. Pg-Xyn exhibited a yield of 83\% after purification from the crude extract. The specific activity of the purified Pg-Xyn reached \(1.95 \times 10^4\) U mg\(^{-1}\). The purified Pg-Xyn was analyzed by SDS-PAGE. There was a single band of \(\sim 44.3\) kDa, which agreed with its theoretical molecular weight (Table 1; Figure 4).

Biochemical Characterization of the Purified Pg-Xyn
The optimal pH for Pg-Xyn was pH 9, presenting high activity between pH 5 and 11. After incubation at 37\(^\circ\)C for 12 h, Pg-Xyn retained more than 80\% of its activity (Figure 5A). The pH characteristic of Pg-Xyn was similar to that of a xylanase from Bacillus sp. NG-27 (Gupta et al., 2000). The optimal temperature for Pg-Xyn was 80\(^\circ\)C at pH 9.0 (Figure 5B). Pg-Xyn retained >50\% of its activity between 50 and 90\(^\circ\)C after 6 h (Figure 5C). The optimal temperature for Pg-Xyn was higher than those of earlier reports for most bacterial xylanases, as the optimal temperatures of Bacillus sp. PKD-9, Bacillus arseniciselenatis DSM 15340, Bacillus pumilus, and Paenibacillus campinasensis G1-1 range from 50 to 60\(^\circ\)C (Kamble and Jadhav, 2012; Zhang et al., 2012; Thomas et al., 2014). These results suggested that Pg-Xyn is an alkali-thermostable xylanase.

The purified Pg-Xyn activity was tested in the presence of metal ions and chemical agents (Table 2). Pg-Xyn was slightly activated in the presence of Ca\(^{2+}\) and Mg\(^{2+}\), while it was inhibited by the addition of Cu\(^{2+}\), Fe\(^{2+}\), Fe\(^{3+}\), and EDTA. In particular, Pg-Xyn was strongly inactivated by Mn\(^{2+}\). These results were similar to the other xylanases in previous publications (Ratanakhanokchai et al., 1999; Ping et al., 2018).

Kinetic Analysis
Kinetic parameters of Pg-Xyn were determined according to Lineweaver–Burk plots (Figure 6), and the \(K_m\) and \(V_{max}\) values of the purified Pg-Xyn were 1.8 mg mL\(^{-1}\) and 683 U mg\(^{-1}\), respectively.

The catalytic constant \(k_{cat}/K_m\) can be used to compare the catalytic ability of enzymes (Zhao et al., 2013; Table 3). Compared with other bacterial xylanases, the \(k_{cat}/K_m\) of Pg-Xyn was 318.9 mL mg\(^{-1}\) s\(^{-1}\), which is higher than that of other bacterial xylanases (Qiu et al., 2010; Verma and Satyanarayana, 2012; Ribeiro et al., 2014; An et al., 2015).

FIGURE 5 | Biochemical characterization of the purified Pg-Xyn. (A) Effect of pH on Pg-Xyn activity and pH stability of Pg-Xyn; (B) Effect of temperature on Pg-Xyn activity; (C) Thermal stability of Pg-Xyn.
TABLE 2 | Effect of metal ions and chemical reagents on the activity of the purified Pg-Xyn.

| Relative activity (%) | Pg-Xyn |
|-----------------------|--------|
| Control               | 100    |
| Cu²⁺                  | 91.3   |
| Zn²⁺                  | 101    |
| Fe³⁺                  | 88.0   |
| Ba²⁺                  | 91.3   |
| Mg²⁺                  | 104    |
| Mn²⁺                  | 58.0   |
| Ca²⁺                  | 105    |
| EDTA                   | 86.7   |
| SDS                    | 98.0   |

FIGURE 6 | Lineweaver-Burk plots of Pg-Xyn kinetic analysis.

Substrate Specificity
The substrate specificity of the purified Pg-Xyn is displayed in Table 4. The purified Pg-Xyn can hydrolyze corncob xylan effectively, and it showed slightly lower activity on oat-spelt and birchwood xylans, while it exhibited lower activity on bagasse and beechwood xylans. No activity was detected on CMC-Na, starch, and Avicel, indicating that Pg-Xyn is a cellulose-free xylanase.

Analysis of Corncob Xylan Hydrolysis Products
Pg-Xyn has the highest specificity activity to corncob xylan, so we chose corncob xylan as the substrate of the hydrolysis reaction for HPLC analysis. The products of corncob xylan hydrolyzed with the purified Pg-Xyn were determined by HPLC analysis of the reaction solution (Table 5). The major products are xylose, xylobiose and xylotriose, but no xylotetraose was observed (Supplemental Figure 1). According to these results, we confirm that Pg-Xyn is an endo-β-1,4-xylanase.

Enzymatic Deinking
Xylanase can increase the relative hydrophobicity of the surface of paper, and facilitate the detachment of adsorbed ink particles. The evaluation of the paper deinking by the newly characterized xylanase revealed that Pg-Xyn had 298% deinking activity, which is higher than 239.58% by a purified xylanase from Bacillus sp. CKBx1D (Maity et al., 2012). The effect of Pg-Xyn on deinking was further investigated by microscopy, as is shown in Figure 7. The results clearly show that the paper lost its fiber compactness and became brighter after enzymatic treatment.

DISCUSSION
Psychrophiles and psychrotolerants are found widely in nature, producing a lot of enzymes to be cold active and general thermostabile. However, thermostable enzymes deriving from psychrophiles are also not uncommon. For example, a thermostable xylose isomerase originating from the psychrophilic soil microorganism, Paenibacillus sp. appears to be most active at 60°C (Park et al., 2019). Oikawa et al. found an aldehyde dehydrogenase and an aspartase with the highest activity at 55°C deriving from Cytophaga sp. KUC-1 (Oikawa et al., 2003). The recombinant L-haloacid dehalogenase isolated from a marine bacterium Psychromonas ingrahamii is most active at 45°C and retains above 70% of its activity after being incubated at 65°C for 90 min (Novak et al., 2013). In the present study, we mined a thermophilic xylanase Pg-Xyn (WP_053166147.1) from the genome sequence of a psychrotolerant bacteria P. glaciei CHR43. The deduced amino acid sequence for Pg-Xyn has strong similarity to some other family 10 xylanases from bacteria. Biochemical characterization showed that Pg-Xyn was thermostable and alkaline preference with the highest activity at pH 9.0 and 80°C. According to previous publications, similar xylanases active at elevated temperatures and alkaline pH have ever been reported. For instance, XynDZ5, a newly reported thermostable xylanolytic enzyme derived from Thermoanaerobacterium thermosaccharolyticum, performs best at 65–75°C and pH 7.5 (Zarafeta et al., 2020). Another xylanase XynHB originating from B. pumilus HB8 was extracellular secreted expressed in E. coli, which have showed high activity under alkaline conditions at 70°C (Zhang et al., 2020). Additionally, a novel xylanase identified from an extreme temperature hot spring metagenome was most active at pH 7 and 80°C and exhibited significant pH stability at pH ranging from 6 to 9 (Joshi et al., 2020).

Xylanases are distributed among two GH families, family 10 and family 11, according to hydrophobic cluster analysis and sequence alignment (Henrissat et al., 1991; Davies and Henrissat, 1995). Most alkali-thermostable xylanases belonging to GH 10 were isolated from alkaliophilic, thermophilic or mesophilic bacteria, such as the genus Paenibacillus, Geobacillus, and Bacillus (Zhao et al., 2011; Canakci et al., 2012; Thomas et al., 2014). So far none of alkali-thermostable xylanases has been reported to be identified in the genus Planomicrobium inhabiting cold area. On the basis of the results, this study highlights a new resource.
TABLE 3 | Comparison of the enzymatic properties of Pg-Xyn with those of other bacterial xylanases.

| Xylanase  | Organism                  | pH  | Temperature (°C) | $V_{\text{max}}$ (U mg$^{-1}$) | $K_{\text{m}}$ (mg mL$^{-1}$) | $k_{\text{cat}}$ (s$^{-1}$) | $k_{\text{cat}}/K_{\text{m}}$ (mL mg$^{-1}$ s$^{-1}$) | References            |
|-----------|----------------------------|-----|------------------|--------------------------------|-------------------------------|----------------------------|--------------------------------|-----------------------|
| MpXyn10A  | Malbrancheapulchella       | 5.8 | 80               | 82                            | 4.6                           | 748                         | 162                           | Ribeiro et al., 2014  |
| Xyn10B    | Caldicellulosiruptorbesii | 7.2 | 70               | 450                           | 2.16                          | 321.6                       | 148.8                         | An et al., 2015       |
| XYNAM6    | Streptomyces megasporus    | 5.5 | 70               | 322.69                        | 2.33                          | 255.89                      | 109.82                        | Qiu et al., 2010      |
| xyl-gt    | Geobacillusthermoleovorans | 8.5 | 80               | 42.5                          | 2.1                           | 318.75                      | 151.78                        | Verma and Satyanarayana, 2012 |
| Pg-Xyn    | P. glacieCHRI43            | 9.0 | 80               | 683                           | 1.8                           | 574                         | 318.9                         | This study            |

TABLE 4 | Substrate specificity of Pg-Xyn.

| Substrate     | Concentration (%) | Relative activity (%) |
|---------------|-------------------|-----------------------|
| Corn cob xylan| 1                 | 100                   |
| Oat-spelt xylan| 1                 | 78.1                  |
| Bagasse xylan | 1                 | 31.2                  |
| Beechwood xylan| 1                | 40.6                  |
| Birchwood xylan| 1                 | 78.1                  |
| CMC-Na        | 1                 | 0                     |
| Avicel        | 1                 | 0                     |

TABLE 5 | Hydrolysis products of corn cob xylan by Pg-Xyn.

| Product            | Xylose Percentage (%) | Xylobiose Percentage (%) | Xylotriose Percentage (%) | Xylotetraose Percentage (%) |
|--------------------|-----------------------|--------------------------|---------------------------|-----------------------------|
| Percentage (%)     | 48.07 ± 0.03          | 36.89 ± 0.02             | 15.04 ± 0.02              | 0                           |

FIGURE 7 | Microscopic view of laser printed paper. (A) Control; (B) Treated by Pg-Xyn.

for thermostable xylanase producers. In the light of the good thermal performance, Pg-Xyn could attract the interests from many industrial processes. The application of xylanases has long been recognized as an effective bleaching pretreatment in the industries seeking to reduce the use of chlorine-containing bleach agents, thereby minimizing the negative environmental impact (Woolridge, 2014).

In the recent years, deinking of paper pulp employing enzymes has attracted more and more attention. Of these enzymes, cellulase, xylanase, and laccase have usually been used in deinking of paper waste. Xylanase isozymes from Bacillus sp. CKBx1D and cellulose-xylanase complex from E. coli SD5 have been found to exhibit deinking activity and to enhance the brightness of waste pulp (Maity et al., 2012; Kumarab N. V. et al., 2018). According to deinking test, Pg-Xyn showed the higher activity in enzymatic deinking. In view of its good thermal stability and higher activity, we believe Pg-Xyn might be a good candidate to be utilized in pulp and paper industries.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

HJ and NH conceived and designed the experiments. ZL and TS performed the experiments and analyzed the data. BW and YL contributed to experimental design and also critically revised the manuscript. ZL drafted the manuscript. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fbioe.2021.618979/full#supplementary-material
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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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