Journal of Bioscience and Bioengineering
VOL. xx No. xx, 1–5, 2014

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Putative endoglucanase PcGH5 from *Phanerochaete chrysosporium* is a β-xylosidase that cleaves xylans in synergistic action with endo-xylanase

Nguyen Duc Huy,[1] Cu Le Nguyen,[1] Jeong-Woo Seo,[2] Dae-Hyuk Kim,[3] and Seung-Moon Park[1,*]

Division of Biotechnology, College of Environmental and Bioresource Sciences, Chonbuk National University, Iksan, Jeonbuk 570-752, Republic of Korea,[1] Applied Microbiology Research Center, Bio-Materials Research Institute, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Jeongeup, Jeonbuk 580-185, Republic of Korea,[2] and Department of Molecular Biology, College of Natural Sciences, Chonbuk National University, Jeonju, Jeonbuk 561-756, Republic of Korea[3]

Received 27 May 2014; accepted 14 September 2014
Available online xxx

A predicted endoglucanase gene (*PcGH5*) was cloned from *Phanerochaete chysosporium*, and expressed in *Pichia pastoris*. Although *PcGH5* showed similarity with the conserved domains of a cellulase superfamily GH5, a β-glucosidase/6-phospho-β-glucosidase/β-galactosidase superfamily, and an endoglucanase, recombinant PcGH5 exhibited a β-xylosidase activity, rather than endoglucanase activity. Therefore, the predicted gene was named as *PcXyl5*. Further characterization of recombinant PcXyl5 showed not only catalysis of the hydrolysis of xylo-oligomers to xylose, but also displayed transglycosylation activity using alcohol as a receptor. Optimum pH of rPcXyl5 was found to be 5.5, whereas optimum temperature was 50°C. rPcXyl5 increased reducing sugar release of birchwood xylan, beechwood xylan, and arabinoxylan by 6.4%, 13%, 15.8%, respectively, in synergistic action with endo-xylanase. Interestingly, the late addition of rPcXyl5 into reaction with endo-xylanase resulted in a larger increase of reducing sugar release from pretreated barley straw that addition at the start or by treatment with endo-xylanases alone. The increases observed were 6.3% and 13.8%, respectively, showing a great potential application for hemicellulose saccharification.

[**Key words:** Xylosidase; Glycoside hydrolase family 5; Xylan; Hemicellulose; *Phanerochaete chrysosporium*]

β-Xylosidases (E.C 3.2.1.37) are an *exo*-type of xylan degrading enzymes, which catalyze the hydrolysis of β-1,4 linkage of short xylo-oligomer, releasing single units of xylose (1). According to the Carbohydrate Active Enzyme (CAZy) database (www.cazy.org), β-xylosidases are classified into ten families 1, 3, 30, 39, 43, 51, 52, 54, 116, and 120. This classification is based on the similarities of their amino acid sequences (2). All these families perform hydrolysis with retention of the anomeric configuration, except for GH 43, which is an inverting GH (3). β-Xylosidases are important accessory enzymes in lignicellulose utilization, because they not only produce xylose sugar, but also prevent the inhibition of xylanases by their end hydrolysis of the products (4). It has been shown that β-xylosidases, along with endo-xylanases are major extracellular hemicelluases when growing fungus on agroindustrial residues such as corn cob (5), promising a great synergy between the two for the saccharification of agroindustrial residues materials.

White-rot fungus, *Phanerochaete chrysosporium* is known to grow well on lignocelluloses substrates by secreting a complex enzyme to degrade lignin, cellulose and hemicelluloses. The prediction of genes from its genome reveals at least 87 glycoside hydrolases, belonging to 33 families (6). On medium containing cellulose or xylan substrates, *P. chrysosporium* secretes over 30 glycoside hydrolases including a xylan-degrading enzyme system involving endo-xylanase, β-xylosidase, α-L-arabinofuranosidase, and acetyl xylan esterase (7,8). However, the functions of most of these glycoside hydrolases are still uncharacterized.

In this study, we first characterized an unknown functional enzyme which belongs to glycosyl hydrolase family 5 (*PcGH5*). The cDNA was amplified with a specific primer, then cloned and expressed into *Pichia pastoris*. Enzyme function was studied with the use of broad substrates, and a mode of action was proposed. Presence of the enzyme did not exhibit endo-glucanase activity, but hydrolyzed xylo-oligomers to xylose, hence, the enzyme was named as a glycoside hydrolase family 5 β-xylosidase (rPcXyl5). We also investigated the synergistic effect with endo-xylanase on xylan substrates and pretreated barley straw saccharification.

MATERIALS AND METHODS

cDNA synthesis The white rot fungus *P. chrysosporium* BKM-F-1767 was obtained from the Korean Collection for Type Culture and maintained in medium for seven days as described by Tien et al. (9). Mycelia were harvested, and total mRNA was isolated using the Oligotex mRNA Mini kit (Qiagen, Valencia, CA, USA). The cDNA library was synthesized using the SMARTer PCR cDNA Synthesis kit following the manufacturer's instructions (Clontech, Mountain View, CA, USA).

Gene identification and manipulation Based on data from the genome database on *P. chrysosporium* RP78 (http://genome.jgi-psf.org/Phchr1/Phchr1.home.html), the predicted endo-glucanase glycoside hydrolase 5 gene which corresponds to the protein assignment number 121774, was selected for this study. Two specific primers, 5′-ATCGATGGCCTACCTCAAGGTTTCT-3′ and 5′-TCTAGA-TACGCGCCCTGCGCAACA-3′ were designed to amplify the coding sequence. The amplicon procedure was carried out as described in our previous study (10). Gene sequence was confirmed by sequencing with three different colonies, then aligning the sequence with *P. chrysosporium* RP78 endo-glucanase GH5 template. The gene with the highest match was selected for cloning and expression in

* Corresponding author. Tel.: +82 63 850 0837; fax: +82 63 850 0834.
E-mail address: smpark@chonbuk.ac.kr (S.-M. Park).

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http://dx.doi.org/10.1016/j.jbiosc.2014.09.012

P. pastoris GS115 (Invitrogen, Carlsbad, CA, USA) using pPICZαC vector (Invitrogen). Gene sequencing was performed using AOX primers (5′-GACTGGTTCCAATTGACAAGC-3′ and 5′-GCAAATGGCATTCTGACATCC-3′) to avoid expression errors as manufacturer's instructions.

The construction of pPICZαC-*PcGH5* was linearized with *Pme*I, then transformed into *P. pastoris* GS115 using an electroporation method, as recommended by the manufacturer (Bio-Rad, Hercules, CA, USA). Transformed cells were selected on YPD agar plates (1% yeast extract, 2% peptone, 2% potato dextrose, and 1.5% agar) containing 100 μg ml^{-1} of zeocin and 1 M sorbitol at 30°C, until colonies were observed (2–3 days). The integration of *PcGH5* into the *P. pastoris* genome was confirmed by PCR using AOX1 primers.

Enzyme expression and purification To screen for gene expression, twenty positive *P. pastoris* transformants were examined by dot blot analysis using His-tag antibodies (11). The transformant showing the highest hybridization significant was selected for enzyme induction in a 100 ml scale. Briefly, the single selected colony was picked and inoculated in 5 ml YPD medium for 1 day. The cells were harvested by centrifuging at 2000 rpm, 5 min, 4°C, then was transferred to 50 ml YPG medium (1% yeast extract, 2% peptone, and 1% glycerol), and inoculated for 1 day. The cells were harvested by centrifuging at 2000 rpm, 5 min, 4°C, and resuspended in 10 ml YP medium (1% yeast extract, 2% peptone). Then, the suspensions were added, slowly, to 90 ml of fresh YP medium until the OD reached 1; finally, fresh YP medium was added to make up a final volume of 100 ml. For enzyme induction, 1 ml 100% methanol was added every 24 h, to a final concentration of 1%, for three days cultivation at 30°C (OD ~ 14.2). The supernatant was harvested by centrifuging for 10 min at 2000 rpm, then filtered using 0.45 μm membranes. In total, 90 ml of filtered supernatant was mixed with 10 ml of 10× binding buffer (20 mM sodium phosphate, 0.5 M NaCl, and 20 mM imidazole; pH 7.4). The mixture was applied to a Ni^{2+} His-tag column (Histrap, GE Healthcare, Piscataway, NJ, USA) using the ÄKTA fast protein liquid chromatography purification system (GE Healthcare, Piscataway, NJ, USA). Protein was eluted with an elution buffer (20 mM sodium phosphate, 0.5 M NaCl and 500 mM imidazole; pH 7.4) and was collected as 15 ml fractions. All fractions containing purified enzyme were dialyzed in distilled water to remove salt and imidazole overnight at 4°C. Protein concentration was estimated by the Bradford method, using a Thermo Scientific Protein Assay kit (Rockford, IL, USA), taking serum albumin as the standard. The molecular mass of purified enzyme was qualified by SDS-PAGE.

Enzyme determination and biochemical characterization The endo-activity of the recombinant enzyme was determined by measuring its performance on different polysaccharide substrates, including carboxymethylcellulose (CMC), laminarin, birchwood xylan, beechwood xylan, locust bean gum (Sigma, St. Louis, MO, USA), xyloglucan, glucomannan, barley glucan, arabinoxylan, and debranched arabinan (Megazyme, Bray, Co. Wicklow, Ireland), whereas exo-activity was investigated using *p*-nitrophenyl-β-D-xylopyranoside (pNPβX), *p*-nitrophenyl-β-D-glucopyranoside (pNPβG), *p*-nitrophenyl-α-D-xylopyranoside (pNPαX), *p*-nitrophenyl-α-L-arabinofuranoside (pNPαA), *p*-nitrophenyl-α-D-mannopyranoside (pNPαM) and *p*-nitrophenyl-β-D-mannoside (pNPβM) (Sigma). Briefly, 30 μg of the purified enzyme was incubated with 200 μl of 1% polysaccharide substrate in a reaction solution with a total volume of 400 μl, containing 50 mM sodium acetate buffer, pH 5.5. The reaction was carried out at 50°C for 60 min. Reducing sugar release was measured using 3,5-dinitrosalicylic acid (DNSA) (12). One unit of enzymatic activity was defined as the amount of enzyme that produced 1 μmol of the reducing sugar per minute. To determine exo-activity, 3 μg of purified enzyme was mixed with 200 μl of 50 mM sodium acetate, pH 5.5, at 50°C. The reaction was initiated by adding 10 μl of 50 mM pNPβX, pNPβG, pNPαX, pNPαA, pNPαM or pNPβM and incubated for 10 min at 50°C. The reaction was terminated by addition of 1 ml of 0.5 M sodium bicarbonate. The amount of *p*-nitrophenol (pNP) released was determined at a wavelength of 410 nm, using a TCC-240A UV spectrophotometer (Shimadzu, Kyoto, Japan). A standard curve was generated using pNP as a substrate, and the absorbance was converted into moles of pNP released. One unit of xylosidase activity was defined as the release of 1 μmol per minute of pNP under experimental conditions.

The optimum pH of the recombinant enzyme was determined by assaying for enzyme activity at 40°C at the different pH values of 3.0–6.0 using 50 mM sodium acetate and 7.0–8.0 using 50 mM sodium phosphate buffer, while the optimum temperature was quantified by testing at temperatures of 30–70°C at the optimal pH which had been obtained. The experiment was carried out using pNPβX as substrate. The released pNP was qualified as described above.

The thermostability of the recombinant enzyme was examined by incubating at different temperature (40°C, 50°C, 55°C, 60°C) for 30 min, 1 h, 2 h, 6 h, and 12 h. Whereas pH stability was investigated with pH range of 3–8 for 30 min, 1 h, 2 h, 6 h, and 12 h. The residual activity of recombinant enzyme was qualified as described above.

Mode of action The hydrolysis activity of the recombinant enzyme on oligosaccharides was studied using xylobiose, xylotriose, and xylopentaose. Approximately twenty micrograms of purified enzyme was incubated with 50 μM xylobiose, 33.3 μM xylotriose, or 20 μM xylopentaose in 100 μl of 50 mM sodium acetate buffer, pH 5.5, at 50°C for 3 h. Hydrolytic products were analyzed using thin layer chromatography (TLC) using chloroform/acetic acid/H_2O (6:7:1) as a mobile phase and visualized by spraying TLC plates with sulfuric acid/ethanol (5:95, v/v) containing 1 mg/ml of orcinol, followed by baking at 110°C for 5 min. Xylose, xylobiose, xylotriose, and xylopentaose were used as standards.

Transglycosylation activity was further studied using ethanol, methanol, and 2-propanol as receptors. Three micrograms of purified enzyme was mixed with 10 μl of 50 mM pNPβX in the presence of 10% of each receptor. Hydrolytic products were visualized on TLC as described above.

Synergistic action with endo-xylanase Xylans and biomass saccharification of the recombinant enzyme was examined in synergy with endo-xylanase (rPcXynC) (10). Recombinant enzyme was initially added or sequentially supplemented in the reaction mixture containing rPcXynC. After an incubation period of 60 min and 16 h for xylans and NaOH-pretreated barley straw, respectively, the reaction was terminated by boiling for 10 min. The reducing sugar release was measured as described above.

RESULTS AND DISCUSSION

Sequence analysis The predicted endo-glucanase GH5, which corresponds to protein assign number 121774, has not yet been characterized. Moreover, we found that the functions of the most of the similar genes that have been deposited on NCBI, are unknown. Thus, we aimed to investigate this gene functionality. We designed two specific primers to amplify the gene from the cDNA library. As expected, we obtained a PCR product with size of 1.4 kb which corresponds to the *P. chrysosporium* RP78 gene (*PcGH5*). Sequence analysis indicated that *PcGH5* contains 1431 nucleotides which encodes for a chain of 476 peptides. However, no secretion signal peptide could be predicted using the SignalP 4.1 Server (http://www.cbs.dtu.dk/services/SignalP/), whereas one potential *N*-glycosylation site (Asn-Ala-Ser) was found in the *PcGH5*sequence. *PcGH5* amino acid sequence revealed a high similarity to glycoside hydrolase family 5 from *P. carnosa* (99%) (EKM51309), *Dichomitus squalens* (98%) (EJF56933), *Trametes versicolor* (98%) (EIW52790), and *Ceriporiopsis subvermispora* (98%) (EMD35774). To date, none of these proteins have been characterized. *PcGH5* also showed similarity with a conserved domain of cellulase superfamily glycoside hydrolase 5, a β-glucosidase/6-phospho-β-glucosidase/β-galactosidase superfamily, and an endoglucanase. Moreover, *PcGH5* did not show similarity to *P. chrysosporium* β-xylosidase/α-L-arabinofuranosidase (13) or other xylosidases. The nucleotide sequence of *PcGH5* was deposited in the GenBank database (accession number KF977410). Despite the classification of xylosidases into various glycoside hydrolase families, most have been characterized to belong to family 3, 39, 43, 52 and 120 (3). However, Shao et al. (14) recently reported a novel β-xylosidase from *Thermoanaerobacterium saccharolyticum* which was not identified as one of the known β-xylosidases. Similarly, our gene did not reveal any significant identity to reported β-xylosidase sequences, suggesting it would be assigned as a novel β-xylosidase.

Purification and identification of rPcGH5 function Due to the lack of a predicted leader signal peptide in the *PcGH5* sequence, we fused *PcGH5* with pPICZαC which contains an alpha secretion signal peptide, then transferred it into *P. pastoris*. Dot blot analysis indicated recombinant *P. pastoris* secreted rPcGH5 (data not shown). rPcGH5 was induced in a 100 ml scale, then purified with a his-tag column. As shown in Fig. 1, purified rPcGH5 exhibited a single protein band at approximately 54 kDa which is considered similar with the predicted size calculated for the amino acid chain. The purified rPcGH5 was subjected for N-terminal sequencing. The result showed that the fifteen first amino acids were Glu-Ala-Glu-Ala-Ser-Met-Ala-Tyr-Leu-Lys-Val-Ser-Gly-Thr-Lys, indicating the sequence of expressed protein matches with *PcGH5* sequence.

As our gene was expected to be an endo-glucanase, we tested endo-glucanase activity using CMC, barley glucan, laminarin; however, no significant activity was detected on these substrates.

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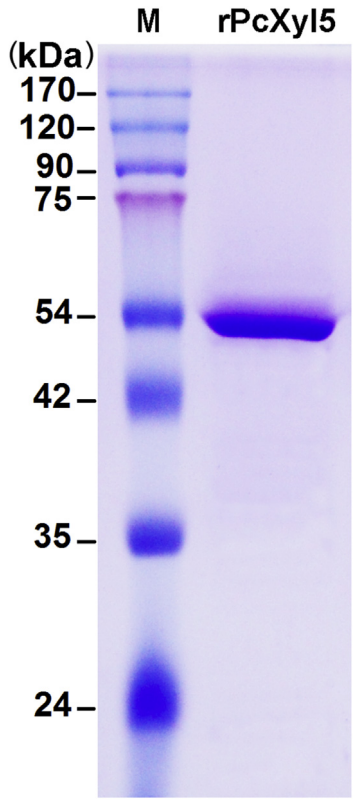

FIG. 1. SDS-PAGE analysis of recombinant β-xylosidase 5 (rPcXyl5). M, protein standard molecular maker; rPcXyl5, purified of recombinant β-xylosidase 5.

Therefore, we extended the investigation of catalytic activity by measuring endo-xylanase activity with birchwood xylan, beechwood xylan, and arabinoxylan, while endo-mannanase and endo-arabinanase were identified using locust bean gum, xyloglucan, glucomannan and debranched arabinan, respectively. As a result, rPcGH5 did not exhibit endo-mannanase or endo-arabinanase activities, while a slightly significant activity was observed on xylan substrates (Table 1). Thus, we further examined rPcGH5 *exo*-activity using pNPβX, pNPαX, pNPβG, pNPαA, pNPαM and pNPβM.

TABLE 1. Specific activity of recombinant β-xylosidase 5 (rPcXyl5) on various substrates.

Substrate	Specific activity (U/mg)
Carboxymethylcellulose (CMC)	nd
Barley glucan	nd
Glucomannan	nd
Laminarin	nd
Xyloglucan	<0.01
Locust bean gum	nd
Birchwood xylan	<0.09
Beechwood xylan	<0.17
Arabinoxylan	<0.02
Debranched arabinan	nd
p-Nitrophenyl-β-D-xylopyranoside (pNPβX)	25
p-Nitrophenyl-β-D-glucopyranoside (pNPβG)	nd
p-Nitrophenyl-α-D-xylopyranoside (pNPαX)	nd
p-Nitrophenyl-α-L-arabinofuranoside (pNPαA)	nd
p-Nitrophenyl-α-D-mannopyranoside (pNPαM)	nd
p-Nitrophenyl-β-D-mannoside (pNPβM)	nd

nd, not detectable. Specific activity of rPcXyl5 was assessed by inoculating 30 μg purified enzyme with 0.5% CMC, barley glucan, glucomannan, laminarin, xyloglucan, locust beam gum, birchwood xylan, beechwood xylan, or arabinoxylan for 60 min, whereas specific activity on pNPβX, pNPβG, pNPαX, pNPαA, pNPαM, or pNPβM was measured using 3 μg purified enzyme in a reaction containing 2.5 mM of each substrate for 5 min under optimal conditions. Reducing sugar and pNP release were qualified as described in Materials and methods.

The results indicated that rPcGH5 hydrolyzed only pNPβXy (Table 1), which is proposed that rPcGH5 could be β-xylosidase. Hence, the gene's name was changed to β-xylosidase GH5 (*PcXyl5*).

Enzymatic characterization Optimum pH and temperature of rPcXyl5 were found to be 5.5 and 50°C, respectively (Fig. 2A and C). rPcXyl5 activity dramatically decreased when the pH was raised above or dropped below 5.5, with retained activities of only 65% and 80% at pH 5.0 and 6.0, respectively. Enzyme activity was enhanced by increasing the temperature from 30°C to 50°C, but quickly decreased at temperatures above 60°C. rPcXyl5 optimum pH and temperature are in accordance with *P. chrysosporium* rPcXyl (13). Moreover, these characteristics are similar with *P. chrysosporium* endo-xylanases (15), supporting the synergistic action of these enzymes. rPcXyl5 showed high pH stable against pH 3–8 (Fig. 2B), which more than 80% retained activity was found after 12 h incubation. rPcXyl5 was stable at temperature ≤40°C, however, when temperature was ≥50°C rPcXyl5 activity was dramatically decreased to <50% after 12 h incubation (Fig. 2D).

Mode of action The mode of action of rPcXyl5 was assessed using xylo-oligomers as substrates. Fig. 3A clearly showed that rPcXyl5 hydrolyzed xylobiose, xylotriose, and xylopentaose to xylose. These results confirmed that rPcXyl5 is a β-xylosidase, and catalyzes the hydrolysis of xylo-oligomers to xylose units.

Transglycosylation ability of rPcXyl5 was examined with various alcohol receptors. Transglycosylated-xyloses were migrated and visualized on TLC silica gel, resulting in spots which differed from pNPβX or xylose. The results indicated that rPcXyl5 can perform transglycosylation of xylose and alcohol (Fig. 3B). Previous study showed that *P. chrysosporium* rPcXyl hydrolyzed xylo-oligomers but did not exhibit transglycosylation activity (13), suggesting that the mechanism of action of rPcXyl5 differs from rPcXyl.

rPcXynC is endo-xylanase which cleaves xylans to xylo-oligomers but not xylose. The addition of rPcXyl5 into reaction with rPcXynC resulted in a xylose release (Fig. 3C). This result indicated that rPcXyl5 could synergistically react with rPcXynC to produce xylose.

Although most of the β-xylosidases are belonging to glycoside hydrolase family 3, 39, 43, or 52 (3), recently reports expanded β-xylosidases to be included in glycoside hydrolase family 1 (16), 30 (17), 120 (14). However, there is no report of a β-xylosidase belonging to glycoside hydrolase family 5. It has been reported that *P. chrysosporium* secreted at least three endo-xylanases (15), and one bifunctional β-xylosidase/α-L-arabinofuranosidase (13). Herein, we also discovered a new β-xylosidase from this fungus, indicating that xylan or hemicelluloses metabolite plays an important role in its carbohydrate utilization system. rPcXyl5 displayed great activity toward xylo-oligomer (Fig. 3A), thus, it may be a major enzyme in order to degrade hemicelluloses in synergy with endo-xylanases.

Synergistic action with endo-xylanase In the present study, we examined rPcXyl5 applications on biomass saccharification in synergy with rPcXynC using xylans and pretreated barley straw. As we expected, rPcXyl5 enhanced the reducing sugar release from birchwood xylan, beechwood xylan, and arabinoxylan by 3.3%, 5.4%, and 11.7%, respectively (Fig. 4). We observed that rPcXyl5 had very low or non-existent hydrolyzation of xylan substrates (Table 1). Furthermore, TLC analysis indicated that rPcXyl5 increased xylose sugar in synergistic action with rPcXynC (Fig. 3C). Our study indicated that rPcXynC was initially inhibited by xylobiose, the main hydrolysis product by rPcXynC, at concentration of 5 mM rPcXynC activity decreased 18% in presence of 5 mM xylobiose whereas xylose did not affect on enzyme. Thus, the enhancement of xylan degradation by rPcXyl5 supplementation may be due to reduced rPcXynC inhibition by xylo-oligomers. To confirm this, we added rPcXyl5 to a xylan hydrolysis reaction after 30 min of reaction with rPcXynC. The

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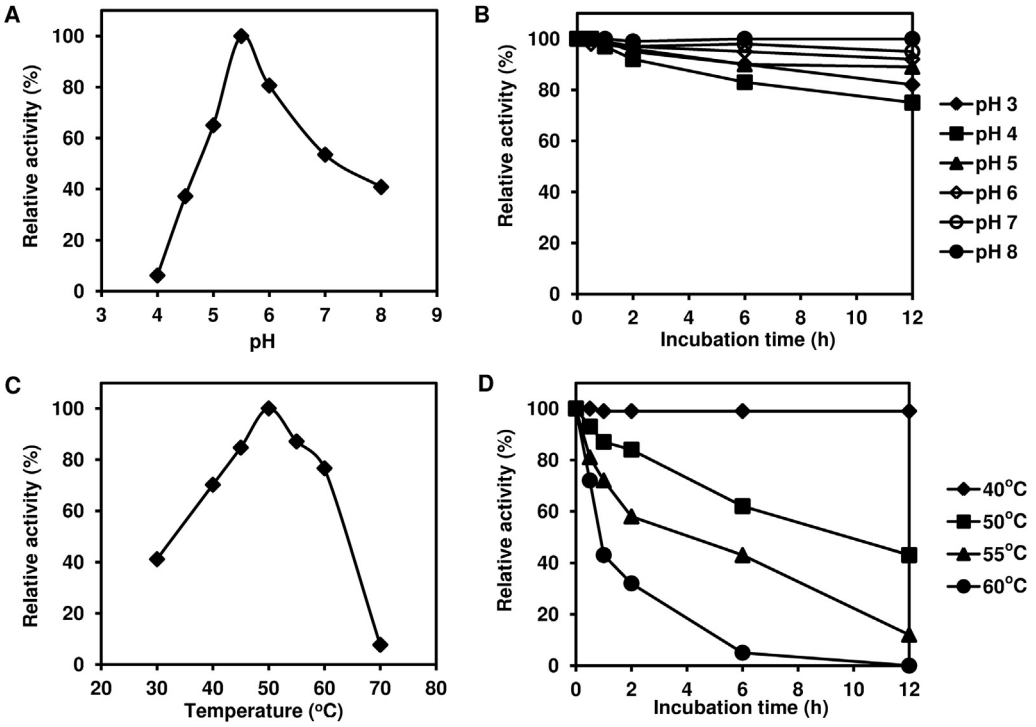

FIG. 2. Effect of pH (A) and temperature (C), and stability of pH (B) and thermal (D) of recombinant β-xylosidase 5 (rPcXyl5). Reaction was carried out using 3 μg purified enzyme in 200 μl of 50 mM sodium acetate or sodium phosphate buffer containing 2.5 mM pNPβX as substrate. pNP release was measured as described in Materials and methods.

results showed that rPcXyl5 supplementation after 30 min of rPcXynC reaction increased the reducing sugar release much more than simultaneous reaction with rPcXyl5 and rPcXynC, shown as an increase of 3%, 7.2%, and 3.7% on birchwood xylan, beechwood xylan, and arabinoxylan, respectively (Fig. 4).

Since most agroindustrial residues, such as barley straw, wheat straw, rice straw, and corn cobs contain a large amount of xylose (18). We aimed to evaluate the efficiency of rPcXyl5 on pretreated barley straw in synergy with rPcXynC. We observed that rPcXynC alone produced 65.3 mg, whereas the addition of rPcXyl5 released 69.9 mg reducing sugar per 1 g substrate. This was similar to xylans degradation, where the addition of rPcXyl5 to the reaction mixture which was carried out with rPcXynC increased reducing sugar release up to 74.3 mg per 1 g substrate (Table 2). rPcXynC is a highly thermostable enzyme which shows an optimum temperature of 70°C, while rPcXyl5 was lower thermostable (Fig. 2D). Therefore, rPcXynC could retain great activity for long incubation times at 50°C when compared with rPcXyl5. Moreover, the velocity of the pretreated barley straw reaction was lower than the xylan reaction, resulting in lower accumulation of xylo-oligomers. This could explain why late rPcXyl5 addition showed a larger increase in reducing sugar release, suggesting that accessory enzymes such as

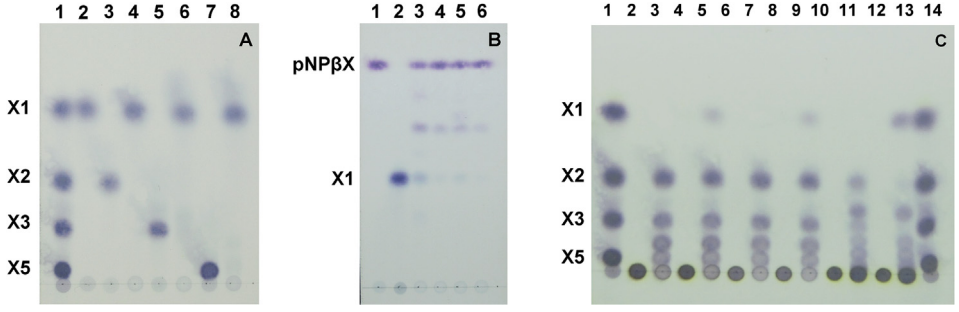

FIG. 3. Thin layer chromatogram of sugars produced during hydrolysis by recombinant β-xylosidase 5 (rPcXyl5). (A) Xylo-oligomers hydrolysis products: lane 1 represents xylo-oligomers; lanes 2, 3, 5 and 7 represent xylose (X1), xylobiose (X2), xylotriose (X3) and xylopentaose (X5), respectively; lanes 4, 6, and 8 represent the xylobiose, xylotriose and xylopentaose hydrolysis by rPcXyl5. Approximately 5 mM xylobiose, 3.3 mM xylotriose or 2 mM xylopentaose was incubated with 15 μg of rPcXyl5 for 1 h at 50°C in 100 μl of 50 mM sodium acetate buffer at pH 5.5. The reaction was terminated by heating at 100°C for 5 min, after which 1 μl of the reaction products was taken and loaded on a TLC silica gel. The hydrolysis products were separated and visualized on the TLC silica gel. (B) pNPbX hydrolysis products: lane 1 represents pNPβX; lane 2 represents xylose (X1); lanes 3, 4, 5, and 6 represent the hydrolysis of pNPbX by rPcXyl5 in 10% ethanol, 20 ethanol, 10% methanol, and 10% 2-propanol, respectively. Purified rPcXyl5 of 3 μg was incubated with each alcohol in 100 μl of 50 mM sodium acetate at pH 5.5 containing 5 mM pNPbX. The reaction was carried out at 50°C for 10 min, after which 1 μl of reaction products was taken and loaded on a TLC silica gel. The hydrolysis products were separated and visualized on the TLC silica gel. (C) Xylan hydrolysis products: lanes 1 and 14 represent xylo-oligomers of xylose (X1), xylobiose (X2), xylotriose (X3) and xylopentaose (X5); lanes 2, 6, and 10 represent birchwood xylan, beechwood xylan, and arabinoxylan; lanes 3, 7, and 11 represent the hydrolysis of birchwood xylan, beechwood xylan, and arabinoxylan by recombinant xylanase C (rPcXynC); lanes 4, 8, and 12 represent the hydrolysis of birchwood xylan, beechwood xylan, and arabinoxylan by rPcXyl5; lanes 5, 9, and 13 represent the hydrolysis of birchwood xylan, beechwood xylan, and arabinoxylan by the combination of rPcXyl5 and rPcXynC. Approximately 3 μg rPcXyl5 and 1 μg rPcXynC were incubated with 0.5% substrate in 100 μl of 50 mM sodium acetate at pH 5.5 and 50°C for 1 h. The reaction was terminated by heating at 100°C for 5 min, after which 1 μl of reaction products was taken and loaded on a TLC silica gel. The hydrolysis products were separated and visualized on the TLC silica gel.

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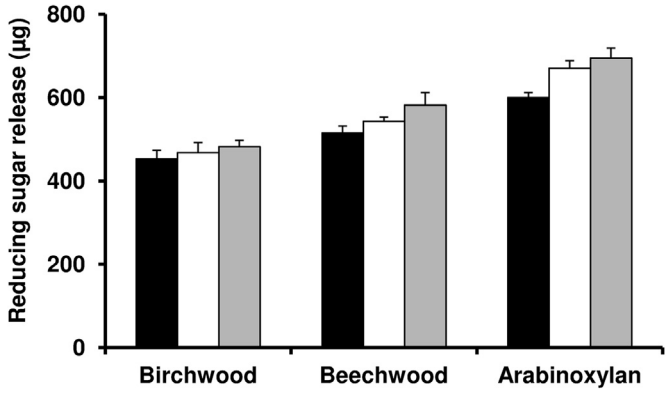

FIG. 4. Reducing sugar release of birchwood xylan, beechwood xylan, and arabinoxylan hydrolysis by the combination of recombinant β-xylosidase 5 (rPcXyl5) and recombinant xylanase C (rPcXynC). Blacked columns indicate reducing sugar release by rPcXynC for 1 h. Whited columns indicate reducing sugar release by rPcXyl5 and rPcXynC for 1 h. Grayed columns indicate reducing sugar release by rPcXynC for 30 min following the combination rPcXyl5 and rPcXynC for 30 min. The reactions were performed using 1 μg rPcXynC and 3 μg rPcXyl5 in 400 μl of 50 mM sodium acetate buffer, pH 5.5 at 50°C containing 0.5% each substrate. Reducing sugar release was determined using DNSA method.

TABLE 2. Synergistic action of recombinant β-xylosidase 5 (rPcXyl5) and recombinant xylanase C (rPcXynC) on NaOH-pretreated barley straw.

First reaction	Second reaction	Reducing sugar release (mg)
rPcXynC	rPcXyl5	74.3 ± 0.3
rPcXyl5 + rPcXynC		69.9 ± 0.5
rPcXynC		65.3 ± 0.5

Approximately 1 g of pretreated barley straw was incubated with 10 μg rPcXynC and 30 μg rPcXyl5 in 5 ml of 50 mM sodium acetate buffer pH 5.5 at 50°C. The first reaction was carried out for 10 h, whereas the second reaction was assessed for 6 h. Total reducing sugar was measured by taking out 200 μl supernatant and quantified using DNSA methods. All experiments were performed in triplicate, and average data were recorded.

rPcXyl5 should be added to a reaction mixture late, in synergistic with major enzymes such as endo-xylanase rPcXynC.

It has been well demonstrated that β-xylosidases play an important role in xylan degradation, enhancing reducing sugar release (19,20). However, the efficiency of β-xylosidases on hemicellulose saccharification of biomasses such as barley straw is still limited. We showed that rPcXyl5 assists an endo-xylanase, resulting in an improvement of 9.5% for barley straw saccharification. Thus, rPcXyl5 shows great promise for application in biomass saccharification.

ACKNOWLEDGMENTS

This research was supported by the Bio-industry Technology Development Program, Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea. This research was also supported in part by the Korea Research Council of Fundamental Science & Technology (Joint Degree and Research Center for Biorefinery). We would like to thank the Research Institute of Bioindustry at Chonbuk National University for kindly providing the facilities for which to conduct this research.

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