J. Microbiol. Biotechnol. (2006), **16**(11), 1753–1759

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Characterization of an Extracellular Xylanase in *Paenibacillus* sp. HY-8 Isolated from an Herbivorous Longicorn Beetle

HEO, SUNYEON[1], JANGYUL KWAK[1], HYUN-WOO OH[1], DOO-SANG PARK[1], KYUNG SOOK BAE[1], DONG HA SHIN[2], AND HO-YONG PARK[1]*

[1]*Insect Resources Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-806, Korea*
[2]*Insect Biotech Co. Ltd., Daejeon 305-811, Korea*

Received: May 23, 2006
Accepted: August 14, 2006

Abstract *Paenibacillus* sp. HY-8 isolated from the digestive tracts of the longicorn beetle, *Moechotypa diphysis*, produced an extracellular endoxylanase with a molecular weight of 20 kDa estimated by SDS-PAGE. The xylanase was purified to near electrophoretic homogeneity from the culture supernatant after ammonium sulfate precipitation, gel filtration, and ion-exchange chromatography. The purified xylanase exhibited the highest activities at pH 6.0 and 50°C. The K_m and V_{max} values were 7.2 mg/ml and 16.3 U/mg, respectively, for birchwood xylan as the substrate. Nucleotide sequence of the PCR-cloned gene was determined to have the open reading frame encoding a polypeptide of 212 amino acids. The N-terminal amino acid sequence and the nucleotide sequence analyses predicted that the precursor xylanase contained a signal peptide composed of 28 amino acids and a catalytically active 19.9-kDa peptide fragment. The deduced amino acid sequence shared extensive similarity with those of the glycoside hydrolase family 11 of xylanases from other bacteria. The predicted amino acid sequence contained two glutamate residues, previously identified as essential and conserved for active sites in other xylanases of the glycoside hydrolase family 11.

Key words: *Paenibacillus*, xylanase, purification, xylan

Xylan is a major component of plant hemicellulose and the second most abundant renewable polysaccharide after cellulose [9]. The heterogeneous polysaccharide consists of homopolymeric backbones of β-D-1,4-linked xylopyranoside substituted with *O*-acetyl, α-L-arabinofuranosyl, and α-D-glucuronyl residues [17, 40]. Xylan backbones are hydrolyzed by xylanolytic enzymes, such as β-1,4-xylanases, β-xylosidase, α-glucuronidases, α-arabinofuranosidases, and esterases [12, 38]. The major potential application of xylanase is in the pulp and paper industries, since the enzyme facilitates the release of lignin from paper pulp [2, 37, 41]. Xylanases are also used in poultry as food additives, in wheat flour for improving dough handling and the quality of baked products, and in improvement of nutritional properties of agricultural silage and grain feed [2, 25, 38, 43].

The majority of xylanases fall into glycoside hydrolase (GH) families 10 and 11 according to the similarities of amino acid sequences, whereas some other xylanases are classified into GH families 5, 7, 8, and 43. The GH family 11 consists of xylanases with a relatively low molecular weight, ranging from 19 to 25 kDa, and have higher specific activities [22]. The 3D structure resolution by crystallography showed that the xylanases consist mainly of two β-sheets forming large pockets where the catalytic sites are located [20]. The GH family 10 xylanases, having higher molecular weights than 30 kDa, more efficiently hydrolyze a wider variety of substrates that are substituted with various branching chemical moieties [32]. The family 10 xylanases have eight-fold α/β-barrel structures, on the basis of the 3D structures resolved by crystallography [20].

The digestive tracts of insects contain a complex biota comprising both resident and transient members from bacterial, fungal, protozoan, and archaeal strains, relying on the microbes for various metabolic functions, including food digestion and essential vitamins. We have studied several herbivorous and xylophagous insects whose intestines contain various symbiotic microorganisms that produce cellulolytic and xylanolytic enzymes. We have isolated a bacterial strain, retaining high xylanolytic activities, on xylanase agar media, from the digestive tracts of adult longicorn beetles [14]. The strains fall into *Paenibacillus* species on the basis of the phylogenetic analyses following determination of the 16S rDNA sequences and chemotaxonomic characterization [14]. In this study, we purified an extracellular xylanolytic enzyme from the bacterial strain

*Corresponding author
Phone: 82-42-860-4650; Fax: 82-42-860-4659;
E-mail: hypark@kribb.re.kr

and characterized the biochemical features. The enzyme had enzymatic properties found in xylanases of GH family 11. The xylanase was similar to GH family 11 in deduced amino acid sequences.

MATERIALS AND METHODS

Bacterial Strains and Culture Conditions

The *Paenibacillus* strain HY-8 previously isolated from the adult longicorn beetle, *Moechotypa diphysis*, was used in this work [14]. The bacterium was routinely grown at 25°C in Luria-Bertani (LB), TY, or xylanase medium (XM; 0.7% KH_2PO_4, 0.2% KH_2PO_4, 0.1% $(NH_4)_2SO_4$, 0.1% $MgSO_4 \cdot 7H_2O$, 0.6% yeast extract, 0.3% birchwood xylan, and 1.5% agar). *Paenibacillus* sp. HY-8 was grown in an M9 minimal medium (Sigma, St. Louis, U.S.A.) supplemented with 0.5% birchwood xylan (Sigma) and 0.3% yeast extract, by shaking on a rotary shaker (170 rpm). The culture supernatant was harvested by centrifugation (6,000 ×*g*, 20 min) after 48 h of incubation at 25°C.

Enzyme Assays

The xylanase activity was determined by measuring the release of reducing sugars from 1% birchwood xylan (w/v) solution prepared in 50 mM phosphate buffer (pH 6.0). Reaction mixtures were incubated for 30 min at 50°C. Reducing sugars were measured by reading absorbance at 600 nm, after adding 1 ml of dinitrosalicylic acid (DNS) reagent to the 0.4 ml assay mixture and boiling the samples for 15 min [11, 28]. Absorbance was converted into moles of reducing sugars produced with a standard curve generated with D-xylose. One unit of the enzyme activity was defined as the amount of enzyme that liberates 1 μmole of xylose equivalent per minute at 50°C. The protein concentration was determined by the method of Bradford [4] using the Bio-Rad protein assay with bovine serum albumin as a standard. SDS-PAGE was performed using 12.5% (w/v) acrylamide gel, as described by Laemmli [21]. Proteins on the gel were stained with Coomassie brilliant blue R-250.

Enzyme Purification

All purification steps were performed at 4°C. Crude extracellular fractions were obtained by centrifugation at 10,000 ×*g* for 10 min. The extracellular proteins were precipitated by adding ammonium sulfate at 70% saturation. The precipitates were collected by centrifugation at 10,000 ×*g* for 20 min, dissolved in 50 mM sodium phosphate buffer, pH 6.0, and dialyzed overnight with the same buffer. Gel filtration chromatography was performed on a Hiload Superdex 200 HR (Amersham Pharmacia Biotech., Sweden) column (26×60 cm) equilibrated with the same buffer, with a FPLC system (Amersham Pharmacia Biotech., Sweden). Bound proteins were eluted with 50 mM sodium phosphate buffer, pH 7.0, at a flow rate of 2.5 ml/min. The active fraction was applied to a Mono S HR (Amersham Pharmacia Biotech., Sweden) column (5×5 cm) equilibrated with 50 mM MES buffer (pH 6.0). The bound xylanase was eluted with a gradient of sodium chloride ranging from 0 to 0.5 M at a flow rate of 1 ml/min. The active fractions were pooled, concentrated, and used for further study.

Characterization of Enzyme Activities

The optimum reaction temperature of the purified enzyme was determined by measuring the xylanolytic activity in the standard enzyme reaction conditions at selected temperature from 30 to 90°C. For the optimum pH determination, the xylanolytic activity was measured at various pH values ranging from 3.0 to 10.0 in the same reaction conditions, using 25 mM citrate-phosphate buffer for pH 3.0–6.0, 25 mM sodium phosphate buffer for pH 6.0–7.0, 25 mM Tris-HCl for pH 7.0–8.0, and glycine-caustic soda for pH 8.0–10.0. For thermal stability determination, the xylanase was incubated in 50 mM sodium phosphate buffer, pH 6.0, at the different temperatures for 30 min. After cooling the treated enzymes on ice for 10 min, the residual xylanase activities were measured. The effects of metal ions and chemical agents on the xylanase activity were determined by incubating the enzyme with various metal ions and inhibitors at room temperature for 30 min, and the residual activities were then measured.

Analysis of Hydrolysis Products

Hydrolysis of xylan by the xylanase was analyzed by thin-layer chromatography (TLC). The purified xylanase was added to 2% xylobiose, xylotriose, xylotetraose, or birchwood xylan in 50 mM of phosphate buffer, pH 6.0, and incubated at 37°C. The reaction was stopped by heating at 100°C for 5 min. Products from hydrolysis were fractionated along with xylose, xylobiose, xylotriose, and xylotetraose as a control on the silica gel 60 F24 plates (Merck, Germany). The solvent used was chloroform-acetic acid-water (6:7:1). The products were detected by spraying the plates with an ethanol-concentrated sulfuric acid mixture (95:5).

Determination of N-Terminal Amino Acid Sequence

To obtain the N-terminal sequence of the enzyme, the purified enzyme was fractionated by SDS-PAGE and transferred onto polyvinylidene difluoride (PVDF) membranes (Bio-Rad, U.S.A.) using 10 mM CAPS-NaOH, pH 11.0, and 10% methanol as a transfer buffer. Protein bands were excised and analyzed using Applied Biosystems Procise 491 at the Korea Basic Science Institute (Daejeon, Korea).

Nucleotide Sequence Determination

The chromosomal DNA of *Paenibacillus* sp. HY-8 was isolated and used as the PCR template. To amplify the gene fragment encoding XynA from the genomic DNA, we used the degenerate oligonucleotides. The forward PCR primer (TAYTGGCARTAYTGGAC; where N=A, G, T, C; Y=T, C; R=A, G) was designed to correspond to the

amino acid sequence, YWQNWT, from the N-terminal amino sequence. The reverse PCR primer (TGGGGNAC-NTAYCGNCC; where N=A, G, T, C; Y=T, C; R=A, G) was designed to be complementary to the amino acid sequence, WGTYRP, from the highly conserved region among bacterial GH family 11 xylanases. The amplified fragment was cloned into pGEM-T Easy (Promega, U.S.A.) and the nucleotide sequence was determined to confirm the identity of the partial gene fragment. The whole DNA fragment containing the xylanase-encoding gene was amplified by PCR using jthe DNA Walking SpeedUp Premix Kit (Seegene, U.S.A.) according to the manufacturer's recommendation. The nucleotide sequence was determined from the amplified fragment by Genotech Incorporation (Daejeon, Korea). The nucleotide sequence of *xynA* from *Paenibacillus* sp. HY-8 was assigned GenBank accession number DQ412045.

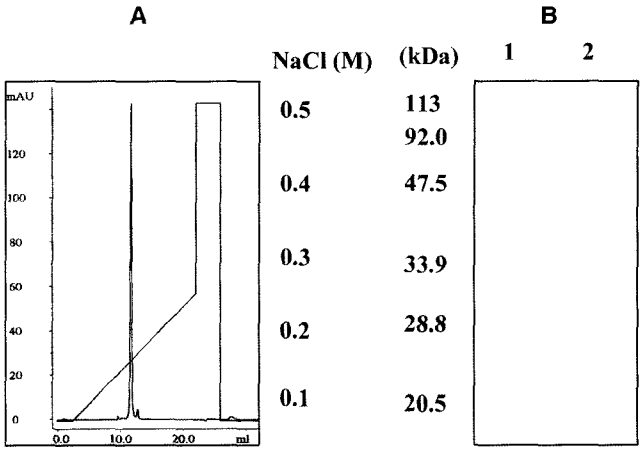

Fig. 1. **A.** Elution profile of the xylanase from Mono S column. **B.** SDS-PAGE of the purified xylanase from *Paenibacillus* sp. HY-8.
Lane 1, molecular mass marker (the molecular weights are marked at the left side of the gel); lane 2, purified xylanase. The gels were stained with Coomassie Brilliant Blue R-250.

RESULTS

Xylanase Purification

In a previous study, a bacterial strain retaining high xylanolytic activities on agar XM was isolated from the digestive tracts of adult longicorn beetles [14]. Phylogenetic analyses using the 16S rDNA sequence determination and chemotaxonomic characterization demonstrated that the strain belongs to *Paenibacillus* sp. [14]. In the present study, we purified the xylanase from the culture supernatant of the early stationary phase in XM containing yeast extract through ammonium sulfate precipitation, gel filtration, and ion-exchange chromatography (Table 1). The culture supernatant containing 5.49 unit/mg of xylanolytic activity was enriched after ammonium sulfate precipitation at 70% saturation. The gel filtration of the active fractions resulted in a 4-fold increase in the specific activity. After ion-exchange chromatography on a Mono-S column, the specific activity of the enzyme preparation was 147.8 U/mg and the final fold was 26.9 relative to the supernatant, with a recovery yield of 19.5% (Table 1). The enzyme was purified as a single protein band with a molecular mass of 22 kDa on an SDS-PAGE gel (Fig. 1). We named the enzyme XynA.

Enzymatic Properties of XynA

The purified xylanase exhibited the maximum activity at pH 6.0 (Fig. 2A). In the effect of pH on enzyme stability, almost 100% of relative activities were detected after the preincubation between pH ranges 5.0 to 7.5, compared with the enzyme activity without preincubation (data not shown). In the effect of temperature, the highest activity was shown at 50°C and more than 90% of relative activity was retained at 60°C (Fig. 2B). In the thermostability test, the enzyme retained 90% of its activity up to 50°C. After preincubation at 60°C for 30 min, the enzyme exhibited 50% of the relative activity, indicating that the half-life of enzyme activity was 30 min at 60°C (Fig. 2B). The apparent K_m and V_{max} values of the purified xylanase were 7.4 mg/ml and 16.3 U/mg of protein, respectively (data not shown), using birchwood xylan as the substrate.

Effect of Metal Ions and Chemicals on Enzyme Activity

Previous studies reported that the activity of a bacterial xylanase is partially inhibited by Cu^{2+} and Fe^{2+} ions [29] and the enzyme is activated in the presence of Co^{2+} and Ca^{2+} ions [1]. A tryptophan-modifying reagent, *N*-bromosuccinimide (*N*-BS), also abolishes activities of xylanase A from *Schizophyllum commune* [8] and Xyl II from an extremophilic *Bacillus* [30]. To investigate the effects of metal ions and chemicals on XynA activity, we added 10 mM of various metal ions (Ca^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, K^{+}, Mg^{2+}, Mn^{2+}, Na^{+}, and Zn^{2+}) and 5 mM of *N*-BS, DTT,

Table 1. Purification of xylanase from the culture supernatant of *Paenibacillus* sp. HY-8.

Purification steps	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification (fold)	Yield (%)
Culture supernatant	4,398	801	5.4	1.0	100
Ammonium sulfate fraction	3,263	163	20	3.6	74.2
Hiload Superdex 200	1,330	17	78.2	14.2	40.7
Mono S	857.6	5.8	147.8	26.9	19.5

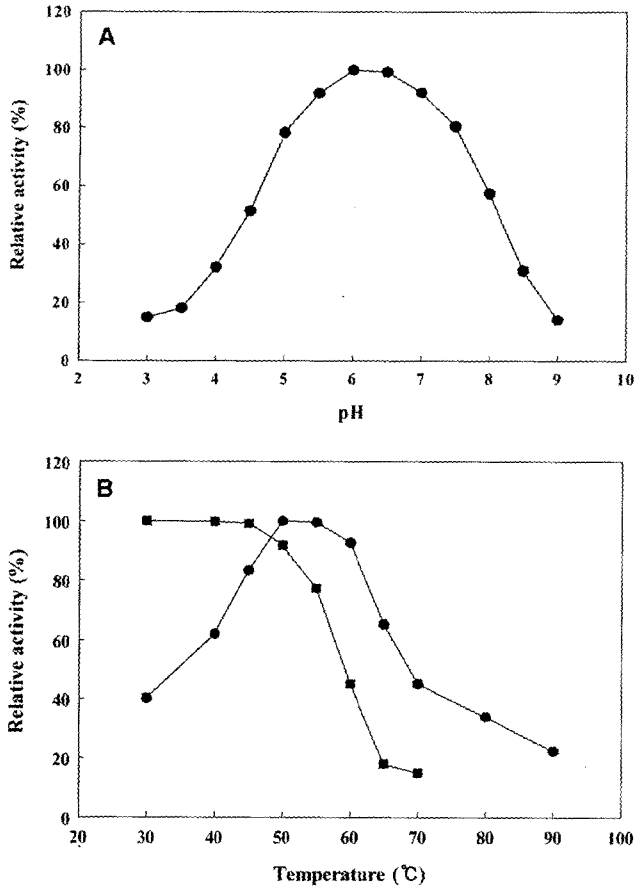

Fig. 2. Effect of pH and temperature on the activity of the purified xylanase.
A. Effect of pH on the activity of the purified xylanase. **B.** Effect of temperature on the activity of the purified xylanase (●). The residual activity was measured after incubation of the enzyme at various temperatures for 30 min (■).

and EDTA in reaction mixtures. Co^{2+} increased the xylanolytic activity by approximately 100% and considerable decreases

Table 2. Effect of various chemicals on the xylanase from *Paenibacillus* sp. HY-8.

Chemical	Relative activity (%)
Control	100.0
Ca^{2+}	135.1
Zn^{2+}	147.0
Fe^{2+}	39.5
K^{+}	125.5
Mg^{2+}	111.8
Cu^{2+}	40.0
Na^{+}	121.4
Mn^{2+}	98.5
Co^{2+}	207.4
Dithiothreitol (DTT)	170.5
N-Bromosuccinimide (*N*-BS)	19.2
EDTA	72.3

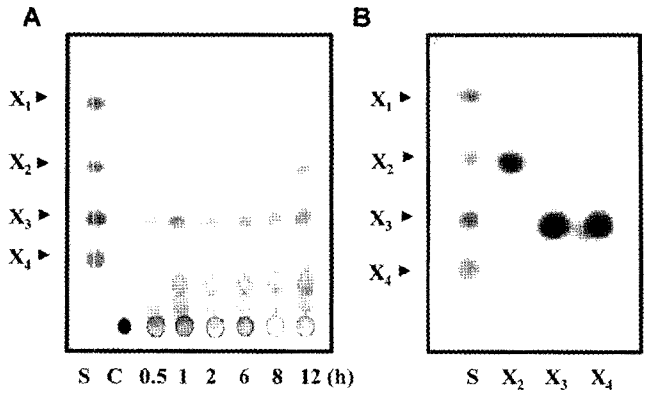

Fig. 3. TLC analysis of hydrolysis products of birchwood xylan (**A**) and xylooligosaccharides (**B**) by the purified xylanase.
The reaction mixture consisted of 2% birchwood xylan in 50 mM sodium phosphate buffer (pH 6.0) with 5 U/ml of xylanase. S, standard; C, xylan without enzymes. The numbers below the gel picture indicate the numbers of incubation time in hours. The standards (lane S) used were xylose (X_1), xylobiose (X_2), xylotriose (X_3), and xylotetraose (X_4).

were observed in the presence of Fe^{2+} and Cu^{2+} (Table 2). *N*-BS highly inhibited the enzyme activities (Table 2).

Characterization of Hydrolysis Products

To understand the action mode of the xylanase, we analyzed the hydrolysis products of birchwood xylan by the purified xylanase by thin-layer chromatography. When birchwood xylan was incubated with the enzyme for 12 h, the major end-products were xylobiose, xylotriose, and higher oligosaccharides (Fig. 3). The hydrolysis product profile of the enzyme for xylooligosaccharides was also investigated. The xylanase had no obvious activity toward xylobiose or xylotriose, and hydrolyzed xylotetraose more efficiently than xylobiose or xylotriose (Fig. 3). The trace amounts of xylobiose in the reactions with xylotriose or xylotetraose suggested that the xylanase has transxylosidase activity, as suggested in previous other findings with several other xylanases from *Paenibacillus* sp. [23], *Bacillus pumilus* [31], and *Cryptococcus albidus* [3].

Cloning and Sequencing of the Xylanase Gene

We identified the N-terminal amino acid sequence of the purified XynA as ATDYWQNWTD. We isolated the partial gene fragment of *xynA* by PCR amplification with degenerate oligonucleotide primers and isolated a 1,140-bp DNA fragment by chromosomal walking, as described in Materials and Methods. Nucleotide sequence determination showed that an open reading frame was present in the DNA fragment. The open reading frame encoded a polypeptide of 211 amino acids, whose deduced molecular weight and pI values were 22,900 and 9.37, respectively. A putative ribosomal binding site (GGAGG) was present at 7 bp upstream from the translation initiation codon. The N-terminal amino acid sequence of the purified XynA was exactly the same as the 29^{th} to 38^{th} amino acid sequence

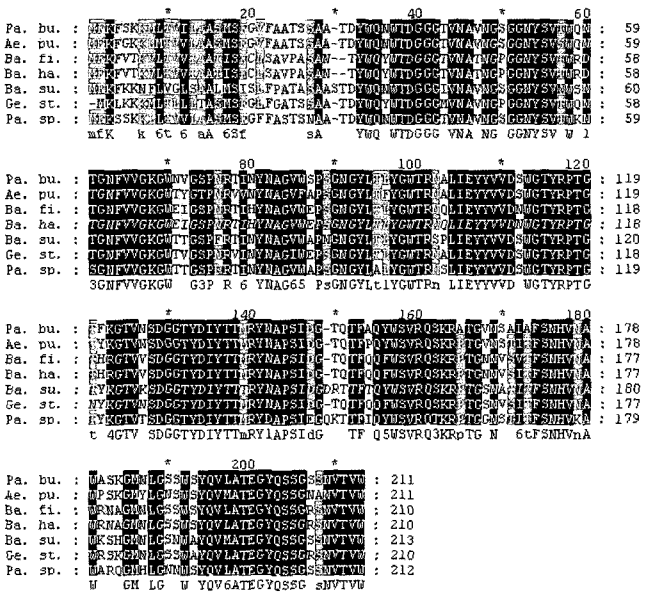

Fig. 4. Alignment of the deduced amino acid sequence of xylanase with those of family 11 bacterial xylanases.
Abbreviations and GenBank accession numbers: Pa. bu., *Paenibacillus* sp. HY-8 (DQ412045, this study); Ae. pu., *Aeromonas punctata* (D32065); Ba. fi., *Bacillus firmus* (AY376352); Ba. ha., *Bacillus halodurans* (AY170624); Ba. su., *Bacillus subtilis* (Z34519); Ge. st., *Geobacillus stearothermophilus* (P45705); Pa. sp., *Paenibacillus* sp. KCTC8848P (AF195421).

deduced from the nucleotide sequence. These results indicated that the N-terminal signal peptide was split between the 28th and 29th Ala residues. The mature enzyme is composed of 183 amino acid residues with a predicted molecular weight of 19,951. Database search revealed that the deduced amino acid sequence showed significant similarity with xylanases belonging to family 11 glycosyl hydrolases as classified by Gilkes *et al.* [13] (Fig. 4). The similarity level was 88% with *Aeromonas punctata* [19], 86% with *Geobacillus stearothermophilus* [7], 84% with *Paenibacillus* sp. KCTC8848P (unpublished), 80% with *Bacillus halodurans* [26], and 80% with *Bacillus subtilis* [42]. The amino acids Glu-106 and Glu-200 corresponded to two highly conserved residues required for catalytic activity in family 11 glycoside hydrolases [18].

DISCUSSION

A new xylanase was identified from the culture supernatant of *Paenibacillus* sp. isolated from the digestive tract of a longicorn beetle. The xylanase seems to fall into the GH 11 family on the basis of several pieces of evidence. The molecular weights estimated from the purified enzyme and the deduced amino acid sequence were approximately 20 kDa, and the calculated isoelectric point was highly basic, like those of other xylanases belonging to the same family of xylanases. The amino acid sequences deduced from the nucleotide sequence were also highly similar to those of the GH family 11 of xylanases from other bacteria. Two glutamate residues required for catalytic activity were also present in the open reading frame of *xynA*.

The XynA from *Paenibacillus* sp. seems to be secreted to the external medium through processing of the signal peptide that is localized at the N-terminal region. The peptide contained a significant stretch of hydrophobic amino acids and two lysine residues and like most signal peptides are composed of three domains; an N-domain containing charged residues, a hydrophobic H-domain, and a short C-domain containing the signal peptidase recognition site [24]. Because of its occurrence in culture supernatant, broad pH stability, moderate thermal stability, and xylooligosaccharide production profile, the xylanase is an attractive candidate for various industrial applications. The potential use of the enzyme may be in the pulp and paper industries, in which alkaline pH and moderate to high temperatures of around 55–70°C are employed [1, 2, 41]. The low apparent molecular weight of these enzymes could also be advantageous for pulp treatment application, as such enzymes would have greater access to the xylan component of the wood matrix [36]. The inhibition and activation of some metal ions and chemicals on the enzyme activity provides plausible backgrounds in the use of the chemicals during industrial processes.

In the present study to isolate a novel xylanase, we exploited the dietary system of an herbivorous insect thriving on a lignocellulose-rich diet and digesting up to 65% of the plant fibers. We isolated a xylanolytic bacterium, *Paenibacillus* sp., from intestines of the adult longicorn beetle *M. diphysis*, successfully isolated a xylanase, and characterized the biochemical features. We speculate that the bacterium may have a mutualistic or synergistic relationship with the insect. Previously, xylanases have been reported to be present in bacteria isolated from diverse sources [15, 16, 33–35] and several lignocellulose-degrading activities have been reported from termites [6] and cockroaches [10, 27, 39]. Direct DNA isolation from insect guts and consecutive cloning of xylanase-encoding genes have also facilitated isolation of novel xylanases and their expression in heterologous systems [5].

Acknowledgment

This work was supported by grant (MGM0900524) from the 21C Frontier Microbial Genomics and Applications Center Program of the Korean Ministry of Science and Technology.

REFERENCES

1. Bae, S. H. and Y. J. Choi. 1991. Purification and characterization of an extracellular xylanase of *Bacillus stearothermophilus*. *Kor. J. Appl. Microbiol. Biotechnol.* **19:** 592–597.

2. Beg, Q. K., M. Kapoor, L. Mahajan, and G. S. Hoondal. 2001. Microbial xylanases and their industrial applications: A review. *Appl. Microbiol. Biotechnol.* **56:** 326–338.
3. Biely, P., Z. Kratky, and M. Vrsanska. 1981. Substrate-binding site of endo-1,4-beta-xylanase of the yeast *Cryptococcus albidus*. *Eur. J. Biochem.* **119:** 559–564.
4. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72:** 248–254.
5. Brennan, Y., W. N. Callen, L. Christoffersen, P. Dupree, F. Goubet, S. Healey, M. Hernandez, M. Keller, K. Li, N. Palackal, A. Sittenfeld, G. Tamayo, S. Wells, G. P. Hazlewood, E. J. Mathur, J. M. Short, D. E. Robertson, and B. A. Steer. 2004. Unusual microbial xylanases from insect guts. *Appl. Environ. Microbiol.* **70:** 3609–3617.
6. Brune, A., E. Miambi, and J. A. Breznak. 1995. Roles of oxygen and the intestinal microflora in the metabolism of lignin-derived phenylpropanoids and other monoaromatic compounds by termites. *Appl. Environ. Microbiol.* **61:** 2688–2695.
7. Cho, S. G. and Y. J. C. 1995. Nucleotide sequence analysis of an endo-xylanase gene (xynA) from *Bacillus stearothermophilus*. *J. Microbiol. Biotechnol.* **5:** 117–124.
8. Clarke, A. J. 1987. Essential tryptophan residues in the function of cellulase from *Schizophyllum commune*. *Biochim. Biophys. Acta* **912:** 424–431.
9. Coughlan, M. P. and G. P. Hazlewood. 1993. Beta-1,4-D-xylan-degrading enzyme systems: Biochemistry, molecular biology and applications. *Biotechnol. Appl. Biochem.* **17(Pt 3):** 259–289.
10. Cruden, D. L. and A. J. Markovetz. 1984. Microbial aspects of the cockroach hindgut. *Arch. Microbiol.* **138:** 131–139.
11. Ghose, T. K. 1987. Measurement of cellulase activities. *Pure Appl. Chem.* **59:** 257–268.
12. Gilbert, H. J. and G. P. Hazlewood. 1993. Bacterial celluloses and xylanases. *J. Gen. Microbiol.* **139:** 187–194.
13. Gilkes, N. R., B. Henrissat, D. G. Kilburn, R. C. Miller Jr., and R. A. Warren. 1991. Domains in microbial beta-1,4-glycanases: Sequence conservation, function, and enzyme families. *Microbiol. Rev.* **55:** 303–315.
14. Heo, S., H. W. Oh, D. S. Park, K. S. Bae, H. Y. Park, and J. Kwak. 2006. Polyphasic assignment of highly xylanolytic bacteria isolated from a longicorn beetle. (In preparation).
15. Heo, S. Y., J. K. Kim, Y. M. Kim, and S. W. Nam. 2004. Xylan hydrolysis by treatment with endoxylanase and β-xylosidase expressed in yeast. *J. Microbiol. Biotechnol.* **14:** 171–177.
16. Kim, B. G., B. R. Jung, J. G. Jung, H. G. Hur, and J. H. Ahn. 2004. Purification and characterization of β-xylosidase from *Trichoderma* strain SY. *J. Microbiol. Biotechnol.* **14:** 643–645.
17. Kim, K. J., K. N. Kim, and Y. J. Choi. 2004. Characterization of the arfA gene from *Bacillus stearothermophilus* No. 236 and its protein product, α-L-arabinofuranosidase. *J. Microbiol. Biotechnol.* **14:** 474–482.
18. Ko, E. P., H. Akatsuka, H. Moriyama, A. Shinmyo, Y. Hata, Y. Katsube, I. Urabe, and H. Okada. 1992. Site-directed mutagenesis at aspartate and glutamate residues of xylanase from *Bacillus pumilus*. *Biochem. J.* **288:** 117–121.
19. Kubata, K. B., T. Suzuki, H. Horitsu, K. Kawai, and K. Takamizawa. 1992. Xylanase I of *Aeromonas caviae* ME-1 isolated from the intestine of a herbivorous insect (*Samia cynthia pryeri*). *Biosci. Biotechnol. Biochem.* **56:** 1463–1464.
20. Kulkarni, N., A. Shendye, and M. Rao. 1999. Molecular and biotechnological aspects of xylanases. *FEMS Microbiol. Rev.* **23:** 411–456.
21. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227:** 680–685.
22. Lee, C. C., M. Smith, R. E. Kibblewhite-Accinelli, T. G. Williams, K. Wagschal, G. H. Robertson, and D. W. Wong. 2006. Isolation and characterization of a cold-active xylanase enzyme from *Flavobacterium* sp. *Curr. Microbiol.* **52:** 112–116.
23. Lee, Y. E. and P. O. Lim. 2004. Purification and characterization of two thermostable xylanases from *Paenibacillus* sp. DG-22. *J. Microbiol. Biotechnol.* **14:** 1014–1021.
24. Li, H., D. Faury, and R. Morosoli. 2006. Impact of amino acid changes in the signal peptide on the secretion of the Tat-dependent xylanase C from *Streptomyces lividans*. *FEMS Microbiol. Lett.* **255:** 268–274.
25. Liab, K., P. Azadi, R. Collins, J. Tolan, J. S. Kim, and K. L. Eriksson. 2000. Relationships between activities of xylanases and xylan structures. *Enzyme Microb. Technol.* **27:** 89–94.
26. Martinez, M. A., O. D. Delgado, J. D. Breccia, M. D. Baigori, and F. Sineriz. 2002. Revision of the taxonomic position of the xylanolytic *Bacillus* sp. MIR32 reidentified as *Bacillus halodurans* and plasmid-mediated transformation of *B. halodurans*. *Extremophiles* **6:** 391–395.
27. Matoub, M. and C. Rouland. 1995. Purification and properties of the xylanases from the termite *Macrotermes bellicosus* and its symbiotic fungus *Termitomyces* sp. *Comp. Biol.* **112:** 629–635.
28. Miller, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* **31:** 426–428.
29. Nascimento, R. P., R. R. R. Coelho, S. Marques, L. Alves, F. M. Girio, E. P. S. Bon, and M. T. Amaral-Collaço. 2002. Production and partial characterization of xylanase from *Streptomyces* sp. strain AMT-3 isolated from Brazilian cerrado soil. *Enzyme Microb. Technol.* **31:** 549–555.
30. Nath, D. and M. Rao. 1998. Structural and functional role of tryptophan in xylanase from an extremophilic *Bacillus*: Assessment of the active site. *Biochem. Biophys. Res. Commun.* **249:** 207–212.
31. Panbangred, W., A. Shinmyo, S. Kinoshita, and H. Okada. 1983. Purification and properties of endoxylanase produced by *Bacillus pumilus*. *Agric. Biol. Chem.* **47:** 957–963.
32. Pell, G., E. J. Taylor, T. M. Gloster, J. P. Turkenburg, C. M. Fontes, L. M. Ferreira, T. Nagy, S. J. Clark, G. J. Davies, and H. J. Gilbert. 2004. The mechanisms by which family 10 glycoside hydrolases bind decorated substrates. *J. Biol. Chem.* **279:** 9597–9605.

33. Rivas, R., P. F. Mateos, E. Martinez-Molina, and E. Velazquez. 2005. *Paenibacillus phyllosphaerae* sp. nov., a xylanolytic bacterium isolated from the phyllosphere of *Phoenix dactylifera*. *Int. J. Syst. Evol. Microbiol.* **55:** 743–746.
34. Rivas, R., P. F. Mateos, E. Martinez-Molina, and E. Velazquez. 2005. *Paenibacillus xylanilyticus* sp. nov., an airborne xylanolytic bacterium. *Int. J. Syst. Evol. Microbiol.* **55:** 405–408.
35. Rivas, R., M. E. Trujillo, P. Schumann, R. M. Kroppenstedt, M. Sanchez, P. F. Mateos, E. Martinez-Molina, and E. Velazquez. 2004. *Xylanibacterium ulmi* gen. nov., sp. nov., a novel xylanolytic member of the family Promicromonosporaceae. *Int. J. Syst. Evol. Microbiol.* **54:** 557–561.
36. Samain, E., P. Debeire, and J. P. Touzel. 1997. High level production of a cellulase-free xylanase in glucose-limited fed batch cultures of a thermophilic *Bacillus* strain. *J. Biotechnol.* **58:** 71–78.
37. Subramaniyan, S. and P. Prema. 2000. Cellulase-free xylanases from *Bacillus* and other microorganisms. *FEMS Microbiol. Lett.* **183:** 1–7.
38. Sunna, A. and G. Antranikian. 1997. Xylanolytic enzymes from fungi and bacteria. *Crit. Rev. Biotechnol.* **17:** 39–67.
39. Teunissen, M. J., A. A. Smits, H. J. Op den Camp, J. H. Huis in 't Veld, and G. D. Vogels. 1991. Fermentation of cellulose and production of cellulolytic and xylanolytic enzymes by anaerobic fungi from ruminant and non-ruminant herbivores. *Arch. Microbiol.* **156:** 290–296.
40. Thomson, J. A. 1993. Molecular biology of xylan degradation. *FEMS Microbiol. Rev.* **10:** 65–82.
41. Viikari, L., A. Kantelineo, J. Bundquist, and M. Linko. 1994. Xylanase in bleaching: From an idea to the industry. *FEMS Microbiol. Rev.* **13:** 335–350.
42. Wolf, M., A. Geczi, O. Simon, and R. Borriss. 1995. Genes encoding xylan and beta-glucan hydrolysing enzymes in *Bacillus subtilis*: Characterization, mapping and construction of strains deficient in lichenase, cellulase and xylanase. *Microbiology* **141:** 281–290.
43. Wong, K. K., L. U. Tan, and J. N. Saddler. 1988. Multiplicity of beta-1,4-xylanase in microorganisms: Functions and applications. *Microbiol. Rev.* **52:** 305–317.