J. Microbiol. Biotechnol. (2006), **16**(8), 1255–1261

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Hydrolysis of Agricultural Residues and Kraft Pulps by Xylanolytic Enzymes from Alkaliphilic *Bacillus* sp. Strain BK

KAEWINTAJUK, KUSUMA[1], GIL HYONG CHON[4], JIN-SANG LEE[4], JIRASAK KONGKIATTIKAJORN[1], KHANOK RATANAKHANOKCHAI[1], KHIN LAY KYU[1], JOHN HWA LEE[2], MIN SUK ROH[3], YUN YOUNG CHOI[4], HYUN PARK[4], AND YUN SIK LEE[4]*

[1]*School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok, Thailand*
[2]*College of Veterinary Medicine, Chonbuk National University, Jeonju 561-756, Korea*
[3]*Department of Applied Biochemistry, Konkuk University, Chunju, Korea*
[4]*Department of Infection Biology, College of Medicine, Wonkwang University, Iksan, Jeonbuk 570-749, Korea*

Received: January 6, 2006
Accepted: April 24, 2006

Abstract An alkaliphilic bacterium, *Bacillus* sp. strain BK, was found to produce extracellular cellulase-free xylanolytic enzymes with xylan-binding activity. Since the pellet-bound xylanase is eluted with 2% TEA from the pellet of the culture, they contain a xylan-binding region that is stronger than the xylan-binding xylanase of the extracellular enzyme. The xylanases had a different molecular weight and xylan-binding ability. The enzyme activity of xylanase in the extracellular fraction was 6 times higher than in the pellet-bound enzyme. Among the enzymes, xylanase had the highest enzyme activity. When *Bacillus* sp. strain BK was grown in pH 10.5 alkaline medium containing xylan as the sole carbon source, the bacterium produced xylanase, arabinofuranosidase, acetyl esterase, and β-xylosidase with specific activities of 1.23, 0.11, 0.06, and 0.04 unit per mg of protein, respectively. However, there was no cellulase activity detected in the crude enzyme preparation. The hydrolysis of agricultural residues and kraft pulps by the xylanolytic enzymes was examined at 50°C and pH 7.0. The rate of xylan hydrolysis in corn hull was higher than those of sugarcane bagasse, rice straw, corn cop, rice husk, and rice bran. In contrast, the rate of xylan hydrolysis in sugarcane pulp was 2.01 and 3.52 times higher than those of eucalyptus and pine pulp, respectively. In conclusion, this enzyme can be used to hydrolyze xylan in agricultural residues and kraft pulps to breach and regenerate paper from recycled environmental resources.

Key words: Xylanolytic enzymes, agricultural residue, alkaliphilic *Bacillus* sp. strain BK, kraft pulp, xylan-binding ability

*Corresponding author
Phone: 82-63-850-6769; Fax: 82-63-857-0342;
E-mail: yusikmj@gmail.com

One of the aims of biotechnology is to utilize agricultural and food industry wastes for the production of energy, chemicals, and animal feed, and combat environmental degradation at the same time. For this reason, many researchers have focused their attention on the use of microbial enzymes for fuel production and industrial applications [1, 23, 27, 33]. Since hemicellulose is the second most abundant polysaccharide in higher plants, hemicellulases and cellulose-degrading enzymes are becoming increasingly important in the enzymatic saccharification of lignocellulose in agricultural waste.

Xylan is the most abundant hemicellulose. It has a linear backbone structure consisting of β-1,4-linked xylosyl residues that may contain branches of L-arabinofuranosyl, acetyl, glucuronosyl, and 4-*O*-methylglucuronosyl residues, depending on its origin [6, 28]. Xylan-degrading enzymes include xylanase (1,4-β-xylan xylanohydrolase; EC 3.2.1.8) and β-xylosidase (1,4-β-xylan xylohydrolase; EC 3.2.1.37), and the side chain-cleaving enzymes include α-L-arabinofuranosidase (EC 3.2.1.55), α-glucuronidase (EC 3.2.1.139), and acetylxylan esterase (EC 3.1.1.72) [6, 28]. Xylan-degrading enzymes, in addition to their possible use in recovering fermentable sugars from hemicellulose, have found new applications in facilitating the bleaching of kraft pulps as well as improving their fiber properties. Xylan hydrolysis was carried out by a treatment with endoxylanase and beta-xylosidase expressed in yeast [11], and surface immobilization was carried out on the silica plate of the endoxylanase produced from recombinant *Bacillus subtilis* [14]. Xylanase gene, *xyn A*, was cloned from *Streptomyces thermocyaneoviolaceus* to produce thermostable recombinant xylanase [5]. *Trichoderma harzianum* was also isolated and found to produce xylanase [18]. Some

plant cell wall hydrolases contain both catalytic and non-catalytic polysaccharide-binding domains [7, 8, 17]. The non-catalytic polysaccharide-binding domains play an important role in the efficient hydrolysis of cellulose-type substances [9, 16]. Until recently, xylanase from a few microorganisms has been believed to comprise non-catalytic, specific xylan-binding regions. The *Bacillus* sp. BK produced extracellular xylanolytic enzymes with xylan-binding ability, which are active and stable in alkaline conditions and have no cellulase activity [29].

In this study, we investigated the production and location of xylanolytic enzymes such as xylanase, β-xylosidase, arabinofuranosidase, and acetyl esterase in alkaliphilic *Bacillus* sp. BK, when grown on xylan. We also studied the hydrolysis of xylan, lignocellulosic materials, and kraft pulps by these xylanolytic enzymes.

MATERIALS AND METHODS

Microorganism and Culture

Alkaliphilic *Bacillus* sp. BK was isolated from a pond used for shrimp cultivation, Bangkok, Thailand. *Bacillus* sp. BK was grown in Berg's mineral salts medium [2] containing 0.5% xylan. The pH of the medium was initially adjusted to 10.5 with 1% Na_2CO_3 after autoclaving. The culture was incubated in a rotary shaker at 200 rpm and 37°C for 2 days.

Xylan-Binding Assay

The insoluble xylan was prepared using the method previously reported by Irwin *et al.* [12]. The binding assay was carried out by adding 5 mg of the protein from the culture supernatant to 50 mg of insoluble xylan (oat spelt) in 1.0 ml of 50 mM sodium phosphate buffer (pH 7.0). The samples were shaken for 30 min at 4°C at different intervals before subjection to centrifugation. The amount of enzyme remaining in the supernatant was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), which was performed by the method reported by Laemmli [22]. The prestained protein marker was purchased from Pierce (ColorMeRanger product # 26671, Rockford, IL, U.S.A.).

Isolation of Xylanolytic Enzymes

After the bacterium had been grown in the alkaline xylan (0.5%) medium for 3 days at 37°C, the culture was centrifuged at 10,000 rpm for 10 min (4°C). The supernatant was assayed for any extracellular enzyme activity. The pellets (cells+residual xylan) were washed with a large amount of phosphate-buffered saline (PBS) (0.15 M sodium chloride in 0.1 M potassium phosphate buffer, pH 7.0) by centrifugation. The pellets were resuspended in 2% triethylamine (TEA) for 30 min at 4°C with constant stirring. After centrifugation, the supernatant was assayed for any enzyme activity freed from the pellet-bound. The pellets were washed again with PBS under the same conditions, resuspended in PBS, and incubated with lysozyme (10 mg/ml) at pH 8.0 (10 mM Tris-HCl buffer) for 20 min at 30°C. After centrifugation, the supernatant was assayed for any intracellular enzyme activity.

Enzyme Assay

The xylanase activity was assayed by determining the amount of reducing sugar released from oat spelt xylan (Sigma, St. Louis, MO, U.S.A.). The reaction mixture (0.6 ml) consisted of 0.5% xylan in 50 mM sodium phosphate buffer (pH 7.0) and enzyme [21]. After 10 min incubation at 50°C, the increase in reducing sugar was determined by the Somogyi-Nelson method [31]. One unit of enzyme activity was defined as the amount of enzyme that released 1 mol of reducing sugar in 1 min under the above conditions.

The cellulase activity was measured under the same conditions as described above using carboxymethylcellulose (Sigma) as a substrate. The β-xylosidase, arabinofuranosidase, β-glucosidase, and acetyl xylan esterase activities were assayed as previously reported by Ratanakhanokchai *et al.* [29]. Protein concentration was determined by the Lowry method [25] using bovine serum albumin as a standard.

Preparation of Substrates

The soluble and insoluble xylans were prepared by stirring a suspension (0.5%) of oat spelt xylan (Sigma) in deionized water for 1 h at room temperature. The mixture was centrifuged at 3,000 ×*g* for 10 min, and the supernatant containing the soluble fraction was removed and freeze dried. The pellet containing the insoluble fraction was washed twice with 20 volumes of deionized water and freeze dried. Lignocellulosic materials such as corn hull, sugarcane bagasse, eucalyptus wood, and rice straw were ground (40 mesh), washed several times in hot water to remove free reducing sugars remaining in these materials, and dried. The washed kraft pulps were also ground and dried.

Hydrolysis of Agricultural Residues

Agricultural residues such as corn hull, corn cop, sugarcane bagasse, rice straw, rice husk, and rice bran were ground (40 mesh) and washed several times in warm distilled water to remove any sugars remaining in these residues. Each material (0.5% dry weight) was hydrolyzed with crude enzyme at pH 7.0 (50 mM sodium phosphate buffer) and 50°C. After an appropriate incubation time, the samples were taken and the amount of reducing sugar produced was determined by the Somogyi-Nelson method with xylose as a standard.

Table 1. Location of the xylanolytic enzymes in *Bacillus* sp. BK.

Source of enzymes	Enzyme activity (U/mg protein)			
	Xylanase	β-Xylosidase	Arabinofuranosidase	Acetyl-esterase
Extracellular	10.14	0.51	0.78	0.98
Pellet-bound	1.69	ND	ND	ND
Intracellular	ND	0.64	2.25	ND

ND, were not be able to detect under the assay condition.

Hydrolysis of Xylan, Lignocellulosic Materials, and Unbleached Kraft Pulps

Oat spelt xylans (Sigma) were hydrolyzed with the crude enzyme (30 mg protein) at pH 7.0 and 50°C. At appropriate intervals, the concentration of reducing sugars released was determined by the Somogyi-Nelson method. The soluble and insoluble oat spelt xylans (1% dry weight) were also hydrolyzed using the same amount of enzyme at pH 7.0 and 50°C for 20 min.

All of the prepared lignocellulosic materials (1% dry weight) were hydrolyzed with the xylanolytic enzymes (1 U) at pH 7.0 and 50°C for 1 h, but the unbleached kraft pulps (1% dry weight) were hydrolyzed at pH 9.0 and 40°C for 1 h. The reducing sugars released were determined.

RESULTS AND DISCUSSION

Enzyme Production and Location

The xylanolytic enzyme activities such as xylanase, β-xylosidase, arabinofuranosidase, and acetyl esterase were evaluated in three fractions: extracellular, intracellular, and pellet-bound (Table 1). Xylanase activity was detected in the extracellular and the pellet-bound fractions, whereas β-xylosidase and arabinofuranosidase were found in both the extracellular and intracellular fractions. Acetyl esterase was detected only in the extracellular fraction. The enzyme activity of xylanase in the extracellular fraction was 6 times higher than that in the pellet-bound fraction. Among the enzymes, xylanase had the highest activity. When *Bacillus* sp. strain BK was grown in pH 10.5 alkaline medium containing xylan as the sole carbon source, the bacterium produced xylanase, arabinofuranosidase, acetyl esterase, and β-xylosidase with specific activities of 1.23, 0.11, 0.06, and 0.04 units per mg of protein, respectively. However, there was no cellulase activity detected in the crude enzyme preparations.

Production of Xylanolytic Enzymes

Xylanase, β-xylosidase, arabinofuranosidase, and acetyl esterase were detected in the extracellular fraction, and reached the highest level after 3 days of incubation when the bacteria were grown in the alkaline xylan medium. Only the xylanase activity was detected in the pellet-bound fraction that had been eluted from the pellet of the culture with TEA (2%). It has been reported that the crude enzyme preparation shows two major protein bands of xylanases on the SDS-PAGE and zymogram, and their molecular weights were estimated to be approximately 29 and 40 kDa. The low molecular weight xylanase (29 kDa) has a xylan-binding region [26]. In the present study, there were protein bands of the pellet-bound xylanase enzyme on SDS-PAGE with molecular weights of 29 and 43 kDa, indicating that these pellet-bound xylanases were different from the extracellular xylanases in SDS-PAGE. Since the pellet-bound xylanase was eluted with 2% TEA from the pellet of the culture, they also contain a xylan-binding

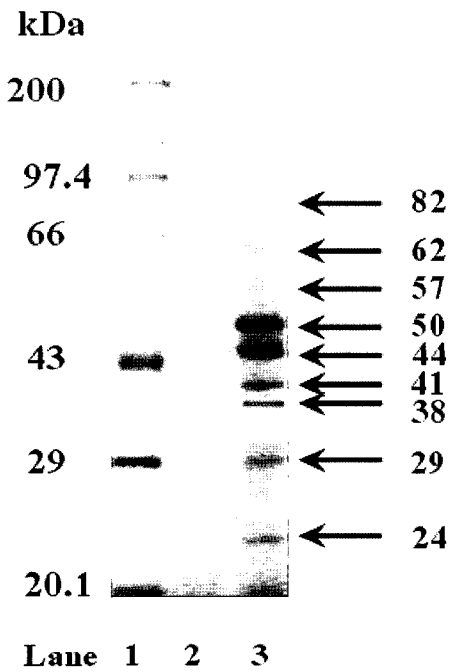

Fig. 1. Production of the extracellular xylanolytic enzymes from *Bacillus* sp. BK.

The extracellular xylanolytic enzymes were shown by SDS-PAGE of pellet-bound xylanase in *Bacillus* sp. BK. The protein bands on 10% acrylamide gel from SDS-PAGE were dyed with Coomassie brilliant blue R-250. Lanes 2 and 3 show the pellet-bound xylanase and crude mixture of xylanase, respectively. The prestained protein marker shows a wide range of protein molecular weights: myosin, 200 K; phosphorylase B, 97.4 K; bovine serum albumin, 66 K; ovalbumin, 43 K; carbonic anhydrase, 29 K; soybean trypsin inhibitor, 20.1 K.

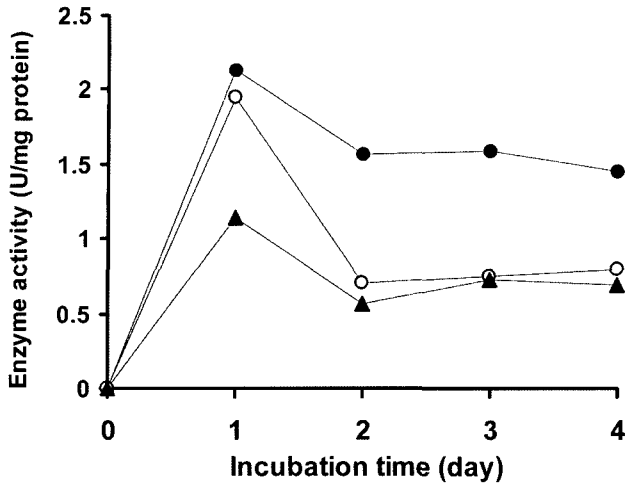

Fig. 2. Time course for the production of the xylanolytic enzymes in *Bacillus* sp. BK.
The production of pellet-bound xylanase from the residual xylan of the culture and intracellular enzymes, β-xylosidase and arabinofuranosidase, in alkaline xylan medium were assayed. The yield of pellet-bound xylanase (●) and intracellular enzymes, β-xylosidase (▲) and arabinofuranosidase (○), are shown with their corresponding enzyme activities (U/mg of protein). The data represent the mean of three independent experiments.

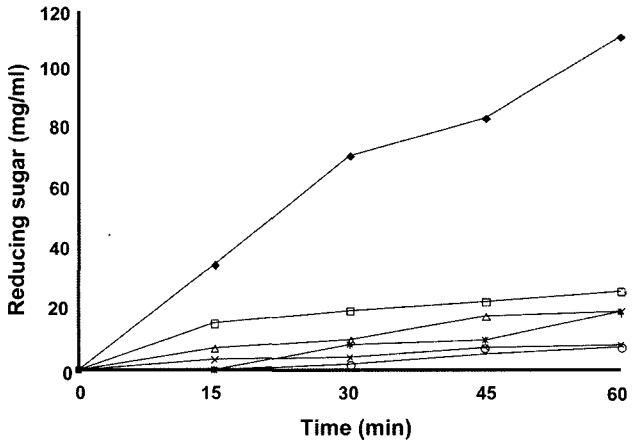

Fig. 3. Reducing sugar released from agricultural residues.
Time course of the hydrolysis of soluble xylans from the xylanolytic enzymes showed the initial hydrolysis of soluble xylans in the agricultural residues. Amount of hydrolysis was analyzed with the culture supernatant after incubating them with the crude enzyme at 50°C and pH 7.0, and showed a certain quantity of reducing sugar (mg/ml) at the indicated time. Corn hull (◆), corn cop (●), sugarcane bagasse (□), rice straw (△), rice husk (×), and rice bran (○) were used as the agricultural residues. The data represent the mean of three independent experiments.

region that is stronger than the xylan-binding xylanase of the extracellular enzyme. The latter was eluted with 1% TEA. The ability of the xylanolytic enzyme to bind to insoluble xylan was examined, and the unbound fraction was subjected to SDS-PAGE. The result showed almost complete binding of the insoluble xylan in the crude xylanolytic enzymes. Among the crude enzymes, the 50 kDa and 44 kDa enzymes appeared to have the highest activity (Fig. 1).

The time course of the xylanolytic enzyme activity was examined at one-day intervals. The highest enzyme activity was observed after incubation for 1 day and then decreased slightly as shown in Fig. 2. Arabinofuranosidase and β-xylosidase were detected in the intracellular fraction, and the pellet-bound xylanase reached a maximum after incubation for 1 day. However, the level of these enzymes decreased after incubation for 2 days.

Hydrolysis of Agricultural Residues

Corn hull, corn cop, sugarcane bagasse, rice straw, rice husk, and rice bran were used as agricultural residues. Figure 3 shows the hydrolysis of these agricultural residues as a function of time. The result showed that corn hull had the highest rate of hydrolysis, followed by sugarcane bagasse, rice straw, corn cop, rice husk, and rice bran. *Bacillus* sp. strain BK produced extracellular cellulase-free xylanolytic enzymes with xylan-binding ability. Therefore, the crude enzyme could hydrolyze agricultural residues synergistically [4]. The different resistances of these agricultural residues to enzymatic hydrolysis have been attributed to their lignin content [10]. Furthermore, the content, position, and structure of xylan are factors associated with a different hydrolysis resistance. However, the enzyme appeared to hydrolyze xylan in the cell wall of these materials and produced appreciable amounts of reducing sugars from all of these materials. Therefore, the crude enzyme from this

Table 2. Hydrolysis of the insoluble and soluble xylans by the extracellular enzymes and pellet-bound enzyme from *Bacillus* sp. strain BK.

Oat spelt xylan	Enzymes	Reducing sugars (mg/ml)	Relative reducing sugars (%)
Insoluble xylan	Crude extracellular enzyme	0.81	100.00
	Unbound extracellular enzyme	0.30	37.04
	Bound extracellular xylanase	0.61	75.31
	Pellet-bound xylanase	0.34	41.97
Soluble xylan	Crude extracellular enzyme	0.79	100.00
	Unbound extracellular enzyme	0.42	53.16
	Bound extracellular xylanase	0.29	36.71
	Pellet-bound xylanase	0.14	17.72

Table 3. Hydrolysis of the lignocellulosic materials and unbleached kraft pulps by the xylanolytic enzymes from *Bacillus* sp. BK.

Substrates	Reducing sugars (μg/U enzyme)
Lignocellulosic materials	
Corn hull	198.8
Sugarcane bagasse	95.9
Rice straw	52.1
Eucalyptus wood	46.2
Unbleached kraft pulps	
Sugarcane bagasse pulp	159.8
Eucalyptus pulp	79.5
Pine pulp	45.4

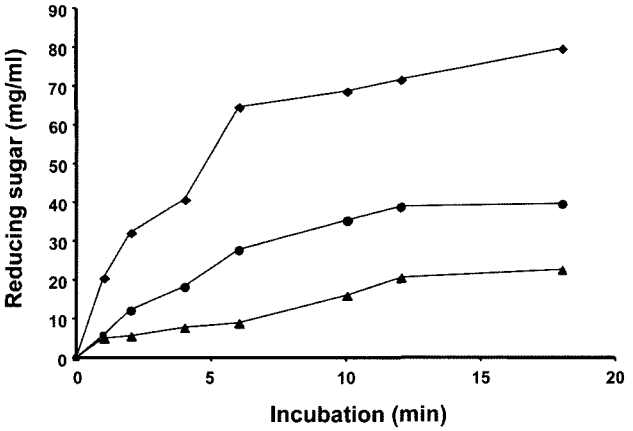

Fig. 4. Hydrolysis of unbleached kraft pulps.
Time course of hydrolysis of the soluble xylans of xylanolytic enzymes showed the initial hydrolysis of kraft pulps of soluble xylans. The amount of hydrolysis was analyzed with the culture supernatant after incubating them with the crude enzyme at 50°C and pH 7.0, and showed a certain level of the reducing sugar (mg/ml) at the indicated time. Sugarcane bagasse pulp (◆), eucalyptus pulp (●), and pine pulp (▲) were used as substrates. The data represent the mean of three independent experiments.

bacterium could be used to hydrolyze agricultural residues to xylose and xylooligosaccharides for ethanol production [38].

Table 2 shows the effects of extracellular crude, unbound, bound, and pellet-bound xylanases on the hydrolysis of soluble and insoluble oat spelt xylans. The bound extracellular enzyme and pellet-bound xylanase appeared to effectively hydrolyze the insoluble xylan because of its xylan-binding ability. The amount of reducing sugars by the bound enzyme was 75.3% of that by the crude enzyme. The unbound enzyme (53.2%) was better in hydrolyzing the soluble xylan than the bound enzyme (36.7%) because of the presence of the high molecular weight xylanase (43 kDa) together with the xylan debreaching enzymes. Therefore, the xylan-binding region is not important for hydrolyzing soluble xylan.

Hydrolysis of Xylan in Kraft Pulps by Crude Enzyme

Table 3 shows the effect of the crude enzyme on the hydrolysis of lignocellulosic materials and unbleached kraft pulps. The enzyme was found to hydrolyze xylan in these materials. The amount of reducing sugars released from corn hull and sugarcane was higher than that of the other materials tested. This might be due to the xylan content and the structural complexity. The enzymes also appeared to hydrolyze xylan in the pulps of sugarcane bagasse, eucalyptus, and pine. A possible explanation might be the presence of xylan in sugarcane bagasse pulp (15.7%), more than those in eucalyptus and pine kraft pulps with 8.3 and 8.0%, respectively [20]. In addition, the chemical structure of xylan in these pulps may not be uniform and can vary across the fibers [37].

Figure 4 shows the time course of the hydrolysis of sugarcane (grass), eucalyptus (hardwood), and pine (softwood) kraft pulps with crude enzyme. The result showed that xylan in sugarcane bagasse was most susceptible to hydrolysis, and there were 2.01 and 3.52 times higher levels of reducing sugars released than those of eucalyptus and pine at 180 min, respectively. The dissolution of pulp xylan has been the key parameter in the pulp prebleaching process [15, 30], and depends on the accessibility of the enzyme to the substrate. Reduced accessibility limits the hydrolysis of xylans in kraft pulps [36].

The xylanolytic enzymes produced by *Bacillus* sp. BK were detected in 3 fractions, indicating the intracellular, pellet-bound, and extracellular fractions. The extracellular xylanolytic enzymes produced by *Bacillus* sp. BK consisted of two types of xylanases; β-xylosidase and debranching enzymes such as arabinofuranosidase and acetyl esterase. One of these xylanases (23 kDa) was the xylan-binding endoxylanase [29], which appeared to be released from the residual xylan of the culture by the reducing sugars. It was found that the final hydrolysis product, xylose, could affect the adsorption of the polysaccharide binding region of xylanase A from *Clostridium stercorarium* to insoluble xylan. Moreover, desorption occurred when the xylose concentration was increased [32]. However, the pellet-bound xylanase (43 kDa) appeared to be bound strongly to the residual xylan, because it was eluted by 2% TEA instead of the usual 1% TEA. Some plant cell wall hydrolases, such as cellulase [8] and xylanase [26], contain both catalytic and non-catalytic polysaccharides-binding domains, and the non-catalytic polysaccharides-binding domains play an important role in efficient hydrolysis of cellulosic substances [9, 16].

Our data suggest that xylan-binding endoxylanase and pellet-bound xylanase hydrolyzed more insoluble xylan than the soluble xylan. The enzyme was also able to hydrolyze xylans in the lignocellulosic materials and kraft pulps in the alkaline condition. Until recently, the xylanases from only a few microorganisms, such as *Thermomonospora*

fusca [12], *Cellulomonas fimi* [4], *Streptomyces thermoviolaceus* [35], and *Bacillus* sp. K-1 [29] containing non-catalytic specific xylan-binding regions have been described. Most xylanolytic microorganisms synthesize isoenzymatic forms of xylanases, because of the structural complexity of xylan [35]. *Bacillus* sp. BK produced nine detectable isoforms of xylanases, which have different molecular weights, such as 23, 30, 39, and/or 40, 45, 56, 63, and 82 kDa. The function of these nine isoenzymes might be to synergistically hydrolyze the xylan substrate via different modes of action according to their substrate specificities. Intracellular β-xylosidase appeared to hydrolyze the short chain of xylooligosaccharides to xylose during the growth of cells. It has been briefly reported that xylobiose and xylotriose penetrate the cell of *Cryptococcus albidus* via an active transport system with β-xyloside permease [3]. β-Xylosidase hydrolyzes xylobiose and xylotriose to xylose in the cell [3]. However, the process for xylose uptake in the present bacterium is still unknown. In *Bacillus subtilis,* xylose needs to change to xylulose and then to xylulose-5-phosphate via the pentose phosphate pathway [19]. Intracellular arabinofuranosidase of *Aspergillus niger* 5–16 hydrolyzes the branched chain of xylooligosaccharide to increase the rate of hydrolysis of the short chain of xylooligosaccharides by β-xylosidase [13, 24]. The enzymes located in the different fractions are expected to have synergistic action on the hydrolysis of xylan to xylose. The crude extracellular enzymes from *Bacillus* sp. BK may be used in the hydrolysis of xylans in lignocellulosic materials and kraft pulps for paper industrial purposes.

Acknowledgments

The authors gratefully acknowledge the financial support given by the National Research Council of Thailand under the Thai-Japan Co-operative Research Program and by a grant No. RTI05-03-02 from the Regional Technology Innovation Program of the Ministry of Commerce, Industry and Energy (MOCIE), Korea.

References

1. Baker, U., S. Yavascaoglu, F. Guvenc, and A. Ersayin. 2001. An endo-β-1,4-xylanase from *Rhizopus oryzae*: Production, partial purification and biochemical characterization. *Enzyme Microb. Technol.* **29:** 328–334.
2. Berg, B., B. V. Hofstan, and G. Petterson. 1972. Growth and cellulose fermentation by *Cellvibrio fulvus*. *J. Appl. Bacteriol.* **35:** 201–214.
3. Biely, P., J. Puls, and H. Schneider. 1985. Acetyl xylan esterase in fungal cellulolytic systems. *FEBS Lett.* **186:** 80–84.
4. Black, G. W., G. P. Hazlewood, S. J. Millward-Sadler, J. I. Laurie, and H. J. Gilbert. 1995. A modular xylanase containing a novel non-catalytic xylan-specific binding domain. *Biochem. J.* **307:** 191–195.
5. Choi, J. H., O. S. Lee, J. H. Shin, Y. Y. Kwak, Y. M. Kim, and I. K. Rhee. 2006. Thermostable xylanase encoded by xynA of *Streptomyces thermocyaneoviolaceus*: Cloning, purification, characterization and production of xylooligosaccharides. *J. Microbiol. Biotechnol.* **16:** 57–63.
6. Coughlan, M. P., M. G. Tuohy, E. X. F. Filho, J. Puls, M. Claeyssens, M. Vrsanska, and M. H. Hughes. 1993. Enzymological aspects of microbial hemicellulases with emphasis on fungal systems, pp. 53–84. *In* Coughlan, M. P. and G. P. Hazlewood (eds.), *Hemicelluloses and Hemicellulases.* Portland Press, London.
7. Ferrira, L. M. A., D. M. Wood, and G. Williamson. 1993. A modular esterase from *Pseudomonas fluorescens* subsp. *cellulosa* contains identical cellulose-binding domain. *Biochem. J.* **294:** 349–355.
8. Gilkes, N. R., B. Henrissat, D. G. Kilburn, Jr. R. C. Miller, and R. A. J. Warren. 1991. Domains in microbial β-1,4-glycanases: Sequence conservation, function and enzyme families. *Microbiol. Rev.* **55:** 303–315.
9. Hall, J., G. W. Black, L. M. A. Ferreira, S. H. Millward-Sadler, and B. R. S. Ali. 1995. The non-catalytic cellulose-binding domain of a novel cellulase from *Pseudomonas fluorescens* subsp. *cellulosa* is important for the efficient hydrolysis of avicel. *Biochem. J.* **309:** 749–756.
10. Hayn, M., W. Steiner, R. Kinger, H. Steinmuller, M. Sinner, and H. Esterbauer. 1993. Basic research and pilot studies on the enzymatic conversion of lignocellulosics, pp. 33–72. *In* Saddler, J. N. (ed.), *Bioconversion of Forest and Agricultural Plant Residues.* C. A. B. International Publishers, Wallingford.
11. Heo, S. Y., J. K. Kim, Y. M. Kim, and S. W. Nam. 2004. Xylan hydrolysis by treatment with endoxylanase and beta-xylosidase expressed in yeast. *J. Microbiol. Biotechnol.* **14:** 171–177
12. Irwin, D., E. D. Jung, and D. B. Wilson. 1994. Characterization and sequence of a *Thermomonospora fusca*. *Appl. Environ. Microbiol.* **60:** 763–770.
13. Kaneko, S., T. Shimasaki, and I. Kusakabe. 1993. Purification and some properties of intracellular α-L-arabinofuranosidase from *Aspergillus niger* 5-16. *Biosci. Biotechnol. Biochem.* **57:** 1161–1165.
14. Kang, S. C., H. J. Kim, S. W. Nam, and D. K. Oh. 2002. Surface immobilization on silica of endoxylanase produced from recombinant *Bacillus subtilis*. *J. Microbiol. Biotechnol.* **12:** 766–772.
15. Kantelinen, A., T. Rantanen, and J. Buchert. 1993. Enzymatic solubilization of fiber-bound and isolated birchwood xylans. *J. Biotechnol.* **28:** 219–228.
16. Karita, S., K. Sakka, and K. Ohmiya. 1996. Cellulose-binding domain confer an enhanced activity against insoluble cellulose to *Ruminococcus albus* endoglucanase IV. *J. Ferment. Bioeng.* **81:** 553–556.
17. Kelett, L. E., D. M. Poole, L. M. A. Ferreira, A. J. Durrant, G. P. Hazlewood, and H. J. Gilbert. 1990. Xylanase B and an arabinofuranosidase from *Pseudomonas fluorescens* subsp.

cellulosa contain identical cellulose-binding domains and are encoded by adjacent genes. *Biochem. J.* **272:** 369–376.
18. Kim, K. C., S. S. Yoo, Y. A. Oh, and S. J. Kim. 2003. Isolation and characteristics of *Trichoderma harzianum* FJ1 producing cellulases and xylanase. *J. Microbiol. Biotechnol.* **13:** 1–8.
19. Klier, A. F. and G. Rapoport. 1988. Genetic and regulation of carbohydrate catabolism in *Bacillus*. *Annu. Rev. Microbiol.* **42:** 65–95.
20. Kyu, K. L., K. Ratanakhanokchai, M. Tanticharoen, T. Ratanarojmongkol, and S. T. Chen. 2001. Hydrolysis of lignocellulosic materials and kraft pulps by xylanolytic enzymes from alkaliphilic *Bacillus* sp. K-1. *J. Natl. Res. Council Thailand* **33:** 39–54.
21. Kyu, K. L., K. Ratanakhanokchai, D. Uitapap, and M. Tanticharoen. 1994. Induction of xylanase in *Bacillus circulans* B6. *Bioresour. Technol.* **48:** 163–167.
22. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227:** 680–685.
23. Lee Y. E. and P. O. Lim. 2004. Purification and characterization of two thermostable xylanases from *Paenibacillus* sp. DG-22. *J. Microbiol. Biotechnol.* **12:** 1014–1021.
24. Lee, Y. E., S. E. Lowe, and G. Zeikus. 1993. Regulation and characterization of xylanolytic enzymes of *Thermoanaerobacterium saccharolyticum* B6A-RI. *Appl. Environ. Microbiol.* **59:** 763–771.
25. Lowry, O. H., N. H. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
26. Millward-Sadler, S. J., D. M. Poole, B. Henrissat, G. P. Hazlewood, J. H. Clarke, and H. J. Gilbert. 1994. Evidence for a general role for high-affinity non-catalytic cellulose binding domains in microbial plant cell wall hydrolases. *Molec. Microbiol.* **11:** 375–382.
27. Paik, H. D., S. K. Lee, S. Heo, S. Y. Kim, H. H. Lee, and T. J. Kwon. 2004. Purification and characterization of the fibrinolytic enzyme produced by *Bacillus subtilis* KCK-7 from *chungkookjang*. *J. Microbiol. Biotechnol.* **14**: 829–835.
28. Puls, J. and J. Schuseil. 1993. Chemistry of hemicelluloses: Relationship between hemicellulose structure and enzymes required for hydrolysis, pp. 1–28. *In* Coughlan, M. P. and G. P. Hazlewood. (eds.), *Hemicellulose and Hemicellulases*. Portland Press Ltd., London.
29. Ratanakhanokchai, K., K. L. Kyu, and M. Tanticharoen. 1999. Purification and properties of xylan-binding endoxylanase from alkaliphilic *Bacillus* sp. K-1. *Appl. Environ. Microbiol.* **65:** 694–697.
30. Senior, D. J., P. R. Meyers, D. Miller, R. Sutcliffe, L. Tan, and J. N. Saddler. 1988. Selective solubilization of xylan in pulp using a purified xylanase from *Trichoderma harzianum*. *Biotechnol. Lett.* **10:** 907–912.
31. Somogyi, M. 1952. Notes in sugar determination. *J. Biol. Chem.* **195:** 19–23.
32. Sun, J. L., K. Sakka, S. Karita, T. Kimura, and K. Ohmiya. 1998. Adsorption of *Clostridium stercorarium* xylanase A to insoluble xylan and the importance of the CBD to xylan hydrolysis. *J. Ferment. Bioeng.* **85:** 63–68.
33. Song, H. H., M. J. Gill, and C. Lee. 2005. Mass-spectral identification of an extracellular protease from *Bacillus subtilis* KCCM 10257, a producer of antibacterial peptide subtilein. *J. Microbiol. Biotechnol.* **15:** 1054–1059.
34. Sunna, A. and G. Antrankian. 1997. Xylanolytic enzymes from fungi and bacteria. *Crit. Rev. Biotechnol.* **17:** 39–67.
35. Tsujibo, H., T. Ohtsuki, T. Ilo, I. Yamazaki, K. Miyamoto, M. Sugiyama, and Y. Inamori. 1997. Cloning and sequence analysis of genes encoding xylanases and acetyl xylan esterase from *Streptomyces thermoviolaceus* OPC-520. *Appl. Environ. Microbiol.* **63:** 661–664.
36. Viikari, L., A. Kantellinen, J. Sundquist, and M. Linko. 1994. Xylanases in bleaching: From an idea to the industry. *FEMS Microbiol. Lett.* **13:** 335–350.
37. Viikari, L., M. Tenkanen, and J. Buchert. 1993. Hemicellulase from industrial applications, pp. 131–182. *In* Saddler, J. N. (ed.), *Bioconversion of Forest and Agricultural Plant Residues*. C. A. B. International Publishers, Wallingford.
38. Wheals, A. E., L. C. Basso, D. M. G. Alves, and H. V. Amorim. 1999. Fuel, ethanol after 25 years. *Trends Biotechnol.* **17:** 482–487.