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# Purification and Characterization of the *Bacillus* sp. KK-1 β-Xylosidase from a Recombinant *Escherichia coli*

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**Abstract** β-Xylosidase was purified from the recombinant *Escherichia coli* carrying the *Bacillus* sp. KK-1 β-xylosidase gene (*xylB*). The molecular mass of the purified enzyme was estimated to be 62 kDa by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. However, the apparent molecular mass of the β-xylosidase was 140 kDa, indicating that the native β-xylosidase has an oligomeric structure composed of two identical subunits. The isoelectric point was determined to be pH 5.5. The enzyme was highly active on *p*-nitrophenyl-β-D-xylopyranoside but it barely hydrolyzed xylan substrates, and did not exhibit activity towards carboxymethylcellulose and *p*-nitrophenyl-β-D-glucopyranoside. The enzyme had a pH optimum for its activity at pH 6.5 and a temperature optimum at 40°C. The enzyme activity was completely inhibited by the presence of $Hg^{++}$, and also markedly inhibited by D-xylose and D-glucose.

Key words: *Bacillus*, β-xylosidase, purification

Xylan, a major component of hemicellulose, is a highly branched β-1,4-linked D-xylose polymer with substituents that include acetyl, arabinosyl, and glucuronyl groups [5, 20]. Complete degradation of xylan requires the action of several types of enzymes; endo-β-1,4-xylanase (EC 3.2.1.8), β-xylosidase (EC 3.2.1.37), α-arabinofuranosidase (EC 3.2.1.55), α-glucuronidase (EC 3.2.1.31), and acetylxylan esterase (EC 3.2.1.6). Among these enzymes, endoxylanase and β-xylosidase have the most important activities in the degradation of xylan. Endoxylanase degrades xylan into xylo-oligosaccharides by attacking internal xylosidic linkages on the xylan backbone, and β-xylosidase subsequently hydrolyzes xylobiose and short oligosaccharides to D-xylose by endwise attack. Xylose is a fermentative sugar for the production of valuable products, including xylitol, xylulose, and ethanol [5, 22]. β-Xylosidase has been reported to be a rate-limiting enzyme in xylan hydrolysis [17] and it is therefore important for the complete hydrolysis of xylan.

β-Xylosidases are produced by a variety of bacteria and fungi [20]. With some exceptions [2, 13], most bacterial β-xylosidases are intracellular, whereas those of fungi remain cell-associated during early stages of growth but are secreted during later stages [7, 22]. Whereas many fungal β-xylosidases have been characterized, only several bacterial β-xylosidases have been purified and characterized from *Bacillus pumilus* [23], *B. stearothermophilus* [16], *B. subtilis* [2], *Caldocellum saccharolyticum* [8], *Clostridium acetobutylicum* [13], *Clostridium stercorarium* [18], *Thermoanaerobacter ethanolicus* [19], *Thermoanaerobacterium saccharolyticum* [14], and *Thermomonospora fusca* [1].

We have previously reported the isolation of a thermophilic *Bacillus* sp. KK-1 from soil [11] and the cloning of the *Bacillus* sp. KK-1 β-xylosidase gene, designated *xylB* [4]. In this paper, we describe the purification and characterization of the *xylB* gene product from a recombinant *Escherichia coli*.

## MATERIALS AND METHODS

### Bacterial Strain and Plasmid

Plasmid pBX45 is a derivative of pUC19 with a 3.2-kb insert DNA containing the *Bacillus* sp. KK-1 β-xylosidase gene (*xylB*) [4]. *E. coli* XL-1 blue (*supE*44 *hsdR*17 *recA*1 *endA*1 *gyrA*46 *relA*1 *thi lac*$^-$ F'[*proAB*$^+$

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*lacI*$^q$ *lacZ*Δ M15 Tn*10*(*tet*$^r$)]) was used as a host for expression of the *xylB* gene.

### Enzyme Purification

The recombinant *E. coli* cells harboring pBX45 were grown overnight at 37°C in 1 liter of LB medium containing 50 μg/ml of ampicillin. Cells were harvested by centrifugation at 6,000×g for 15 min. Harvested cells were washed once, suspended in 50 mM Tris-HCl buffer (pH 7.5), and then disrupted by sonication with a Branson sonifier. Cell debris was removed by centrifugation at 10,000×g for 30 min. Then, the cell-free extract was precipitated with ammonium sulfate (35~75% saturation) and dialyzed against 50 mM Tris-HCl buffer (pH 7.5) containing 1 M $(NH_4)_2SO_4$. The dialyzed sample was applied to a phenyl-Sepharose CL-4B column (2.5×27 cm) equilibrated with the same buffer and the proteins were eluted with a linear $(NH_4)_2SO_4$ gradient (1 to 0 M) at a flow rate of 60 ml/h. The active fractions were pooled, concentrated by ultrafiltration and dialyzed against 25 mM Tris-HCl buffer (pH 7.5) containing 0.4 M $(NH_4)_2SO_4$. The dialyzed sample was applied again to a phenyl-Sepharose CL-4B column (2.5×27 cm) and proteins were eluted with a linear $(NH_4)_2SO_4$ gradient (0.4 to 0 M) at a flow rate of 30 ml/h. The concentrated active fractions were dialyzed against 20 mM MES buffer (pH 6.0). The enzyme solution thus obtained was loaded onto a Resource-S column equilibrated with 20 mM MES buffer (pH 6.0) and proteins were eluted with a linear NaCl gradient (0 to 0.4 M) at a flow rate of 180 ml/h. The concentrated sample was dialyzed against 50 mM sodium phosphate buffer (pH 7.5) containing 150 mM NaCl. The dialyzed enzyme solution was loaded on a Superose 12 HR 10/30 column (1×30 cm) and proteins were eluted with the same buffer at a flow rate of 12 ml/h. The concentrated active fractions were dialyzed against 10 mM potassium phosphate buffer (pH 7.0). The dialyzed enzyme solution was loaded onto a hydroxyapatite column (1.75×13 cm) and the proteins were then eluted with a linear gradient of 10 to 400 mM sodium phosphate buffer (pH 7.0) at a flow rate of 12 ml/h. The active fractions were pooled and concentrated by ultrafiltration. Proteins were analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) as described by Laemmli [12].

### Determination of Molecular Weight

Molecular weight of the purified enzyme was determined by 10% (w/v) SDS-PAGE. The apparent molecular weight of the native enzyme was determined by fast protein liquid chromatography (FPLC) with Superose 12 HR 10/30, a type of gel filtration column. Protein peaks were monitored as absorbance at 280 nm.

### Enzyme Assays

β-Xylosidase activity was determined by measuring the amount of *p*-nitrophenol released from *p*-nitrophenyl-β-D-xylopyranoside (pNPX). Reaction mixtures of 0.5 ml containing 1 mM pNPX in 20 mM sodium phosphate buffer, pH 6.5, were incubated at 40°C for 15 min. The reaction was stopped by adding 1 ml of 1 M sodium carbonate. The absorbance was measured at 400 nm. Xylanase and carboxymethylcellulase activities were determined by measuring the amount of reducing sugars using the dinitrosalicylic acid reagent [15]. One unit of the enzyme activity was defined as the amount of enzyme that produced 1 μmole of *p*-nitrophenol or reducing sugar per min. Protein concentrations were determined by the Bradford method [3].

### Isoelectric Focusing

Isoelectric focusing was performed using Phastsystem with PhastGel IEF 3~9 media (Pharmacia Co., Sweden). After gel running, the protein in the gel was fixed by soaking the gel in 20% trichloroacetate. Then, the gel was stained with Coomassie Brilliant Blue and destained with destaining solution (30% methanol and 10% acetic acid in distilled water).

## RESULTS AND DISCUSSION

### Enzyme Purification

The *Bacillus* sp. KK-1 β-xylosidase was purified from the cell-free extract of *E. coli* (pBX45) to determine its physicochemical properties. The purification procedure

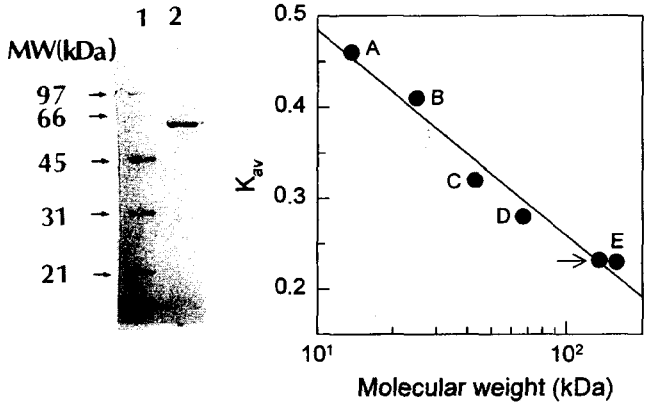
**Table 1.** Purification of the *Bacillus* sp. KK-1 β-xylosidase from *E. coli* (pBX45).

| Purification step | Total protein (mg) | Total activity (U) | Specific activity (U/mg) | Yield (%) | Purification (fold) |
|---|---|---|---|---|---|
| Cell-free extract | 483.2 | 351.6 | 0.7 | 100.0 | 1.0 |
| $(NH_4)_2SO_4$ precipitation | 269.3 | 231.1 | 0.9 | 65.7 | 1.3 |
| 1st Phenyl-Sepharose | 61.3 | 199.2 | 3.2 | 56.7 | 4.6 |
| 2nd Phenyl-Sepharose | 10.7 | 65.3 | 6.1 | 18.6 | 8.7 |
| Resource-S | 5.2 | 36.4 | 7.0 | 10.4 | 10.0 |
| Superose 12 HR 10/30 | 1.3 | 19.3 | 14.8 | 5.5 | 21.1 |
| Hydroxyapatite | 0.8 | 12.4 | 15.5 | 3.5 | 22.1 |

is summarized in Table 1. The enzyme was purified 22-fold with a recovery yield of 3.5%. Gel filtration was found to be a particularly effective step and the low overall yield was mainly due to the great losses in activity occurred during the two purification steps by phenyl-Sepharose column chromatography.

The final preparation gave a single protein band on SDS-PAGE and the molecular mass of the purified enzyme was estimated to be 62 kDa (Fig. 1A). This value was consistent with the molecular mass, 61.6 kDa, calculated from the DNA sequence [4]. On the other hand, the molecular mass of the purified enzyme estimated by gel filtration chromatography was about 140 kDa (Fig. 1B). In addition, zymogram analysis on a SDS-polyacrylamide gel showed that the protein having the molecular weight of 62 kDa did not show activity towards 4-methylumbelliferyl-β-D-xylopyranoside (MUX) whereas the protein having the molecular weight of 140 kDa was active towards MUX (data not shown). These results suggest that the native enzyme has an oligomeric structure composed of two identical subunits.

Isoelectric point (pI) of the β-xylosidase was determined to be pH 5.5 (Fig. 2). This is similar to the pI values reported for β-xylosidases from *B. subtilis* and




(A) (B)

**Fig. 1.** Determination of molecular weight of the purified β-xylosidase.

(A): SDS-PAGE; Lane 1, molecular weight standard markers; Lane 2, purified enzyme. (B): Gel filtration chromatography. Molecular weight standard markers; A, ribonuclease A (13.7 kDa); B, chymotrypsinogen A (25 kDa); C, ovalbumin (43 kDa); D, albumin (67 kDa); E, aldolase (158 kDa). →, the purified β-xylosidase from *E. coli* (pBX45).

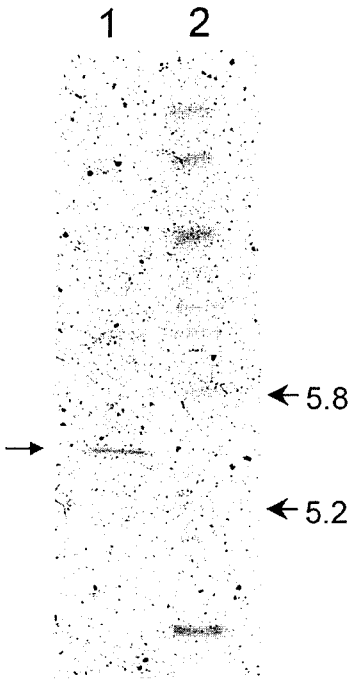



**Fig. 2.** Analytical isoelectric focusing of the purified β-xylosidase using PhastGel IEF 3-9.

Lane 1, standard pI markers (trypsinogen, 9.3; lentil lectin (basic), 8.65; lentil lectin (middle), 8.45; lentil lectin (acidic), 8.15; horse myoglobin (basic), 7.35; horse myoglobin (acidic), 6.85; human carbonic anhydrase B, 6.55; bovine carbonic anhydrase B, 5.85; α-lactoglobulin A, 5.20; soybean trypsin inhibitor, 4.55; amyloglucosidase, 3.5). Lane 2, the purified β-xylosidase from *E. coli* (pBX45). →, the purified β-xylosidase from *E. coli* (pBX45).

**Table 2.** Properties of purified bacterial β-xylosidases.

| Organism | MW (kDa) | Subunit (kDa) | Structure | pI | Opt. Temp (°C) | Opt. pH | Reference |
|---|---|---|---|---|---|---|---|
| *Bacillus* sp. KK-1 | 140 | 62 | dimer (or trimer) | 5.5 | 40 | 6.5 | This work |
| *B. pumilus* | 190 | 56 | tetramer (or trimer) | - | 40 | 7.0 | [23] |
| *B. stearothermophilus* | 150 | 75 | dimer | 4.2 | 70 | 6.0 | [16] |
| *B. subtilis* | 240 | 65 | tetramer | 5.4 | 50 | 7.0 | [2] |
| *Caldocellum saccharolyticum* | - | 53 | - | 4.3 | 70 | 5.7 | [8] |
| *Clostridium acetobutylicum* | 224 | 85<br>63 | trimer | 5.9 | 45 | 6.0~6.5 | [13] |
| *Clostridium stercorarium* | 220 | 53 | tetramer | - | 65 | 7.0 | [18] |
| *Thermoanaerobacter ethanolicus* | 165 | 85 | dimer | 4.6 | 82 | 5.0 | [19] |
| *Thermoanaerobacterium saccharolyticum* | 60 | 55 | monomer | - | 70 | 5.5 | [14] |
| *Thermomonospora fusca* | 168 | 56 | trimer | 4.4 | 40~60 | 5.0~9.0 | [1] |

-, not determined.

*Clostridium acetobutylicum* [2, 13]. The physicochemical properties of other bacterial β-xylosidases are summarized in Table 2.

## Substrate Specificity

Xylanolytic and cellulolytic enzymes are inextricably linked and both cleave β-1,4-glycosidic bonds by similar catalytic mechanisms. They are therefore closely related at the functional level [5, 20]. A few β-xylosidases with an additional β-glucosidase activity have been reported, although the ratios of the two activities may differ considerably depending on the enzymes examined [21]. The β-xylosidase was found to be active only on pNPX which is known to be a representative chromogenic substrate for β-xylosidase (Table 3). However, the purified enzyme did not exhibit hydrolyzing activity against *p*-nitrophenyl-β-D-glucopyranoside, a substrate for β-glucosidase.

It was found previously that the *xylB* gene product hydrolyzed xylobiose and short xylo-oligosaccharides to xylose as the end product of the enzymic reaction [4]. However, the affinity of most β-xylosidases towards xylo-oligosaccharides usually decreases with the increasing degree of polymerization. Nevertheless, there are some reports of β-xylosidases that are able to attack xylan slowly to produce xylose [20], and this was also found with the *Bacillus* sp. KK-1 β-xylosidase. As shown in Table 3, the purified enzyme showed low levels of enzyme activity towards oat spelt xylan and birchwood xylan. But, no significant activity was detected on carboxymethylcellulose.

**Table 3.** Substrate specificity of the purified β-xylosidase.

| Substrate | Specific activity (U/mg protein) |
|---|---|
| *p*-Nitrophenyl-β-D-xylopyranoside | 4.74 |
| *p*-Nitrophenyl-β-D-glucopyranoside | - |
| *p*-Nitrophenyl-β-D-cellobioside | - |
| *p*-Nitrophenyl-β-D-arabinopyranoside | - |
| *p*-Nitrophenyl-β-D-glucuronic acid | - |
| *p*-Nitrophenyl-β-D-galactopyranoside | - |
| Oat spelt xylan | 0.14 |
| Birchwood xylan | 0.10 |
| Carboxymethylcellulose | - |

-, not detected.

## Effects of pH and Temperature on Enzyme Activity

The purified enzyme had a narrow pH activity curve and the optimal pH for pNPX hydrolysis was about 6.5 (Fig. 3A). Half of the maximal activity was detected around pH 6.0 or 8.0, but the enzyme stability was less affected than the enzyme activity above pH 6.5. The enzyme was inactive below pH 5.0. The pH optima of most bacterial β-xylosidases are reported to be close to neutrality, whereas fungal enzymes are usually acidic, below pH 5 [20].

The optimum temperature for pNPX hydrolysis was 40°C (Fig. 3B). To examine thermostability, the purified enzyme was incubated for 1 h at various temperatures and the residual activities were assayed. As shown in Fig. 3B, the enzyme was stable up to 40°C but the enzyme activity decreased rapidly at temperatures over 40°C.

The amino acid sequence of the *xylB* gene product is highly homologous to those of the (83% identity) *B. subtilis* and *B. pumilus* (73% identity) β-xylosidases [4], and the pH and temperature optima for the *Bacillus* sp. KK-1 β-xylosidase are also similar to those for the *B. subtilis* and *B. pumilus* β-xylosidases (see Table 2). However, thermophilic *Bacillus* sp. KK-1 produces a thermostable cellulase-free xylanase comparable with that of *B. stearothermophilus* [11]. In addition, we found recently that this bacterium secreted another cellulase-free xylanase, active at 40°C [24]. It is interesting that

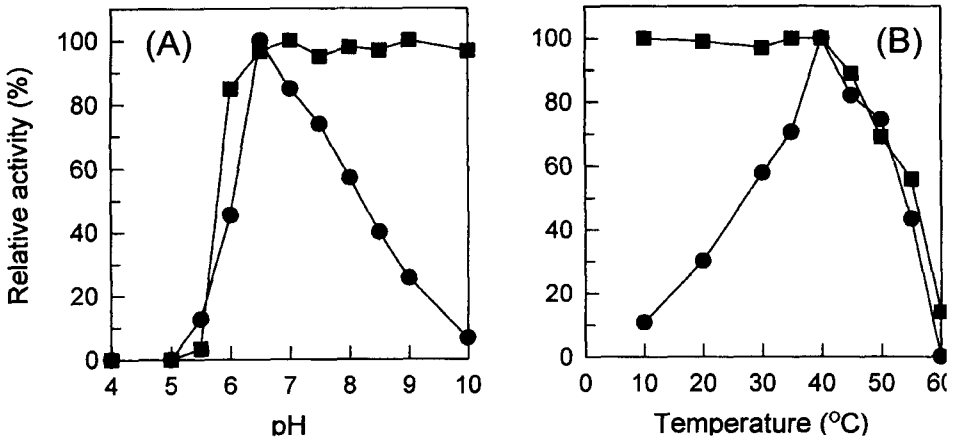



**Fig. 3.** Effects of pH (A) and temperature (B) on the pNPX hydrolyzing activity.
The enzyme reactions were performed with 20 mM sodium phosphate buffer (pH 6.5) at 40°C for 15 min. Stabilities were determined by measuring the residual activities after pre-incubations for 1 h at various pHs and temperatures. Symbols: ●, activity; ■, stability.

**Tabel 4.** Effect of ions and other reagents on enzyme activity.

| Efector | Relative activity (%) | |
|---|---|---|
| | 1 mM | 10 mM |
| None | 100 | 100 |
| $CaCl_2$ | 83 | 96 |
| $MnCl_2$ | 91 | 78 |
| $MgCl_2$ | 61 | 100 |
| $CuCl_2$ | 13 | - |
| $HgCl_2$ | 0 | 0 |
| NaCl | 70 | 91 |
| KCl | 91 | 100 |
| EDTA | 100 | 100 |
| SDS | 83 | 78 |

-, not determined.

this thermophilic bacterium produces xylanolytic enzymes active at mesophilic temperatures, even though the strain does not grow below 45°C [11].

### Effects of Metal Ions and Other Reagents on Enzyme Activity

Evidence for the direct involvement of thiol groups in the β-xylosidase catalytic function has been reported at least in one case, a *B. pumilus* β-xylosidase [9]. The subunit of *B. pumilus* β-xylosidase contains four cysteinyl residues, and one sulfhydryl group is essential for its catalytic activity. Considering that the *xylB* gene product contains eight cysteinyl residues and is homologous to the *B. pumilus* β-xylosidase [4], some of the sulfhydryl groups of the *Bacillus* sp. KK-1 β-xylosidase are also supposed to be involved in its catalytic activity as observed for the *B. pumilus* β-xylosidase. Therefore, oxidation of the cysteine residue might be a primary cause of the enzyme inactivation at high temperatures [6].

As shown in Table 4, $Hg^{++}$ completely inhibited the pNPX hydrolyzing activity and $Cu^{++}$ decreased the activity by 13% from its maximum activity at 1 mM concentration. However, other metal ions including $Mg^{++}$, $Ca^{++}$, and $Mn^{++}$ exhibited no severe inhibition effect on the β-xylosidase activity in spite of the requirement of a free thiol for β-xylosidase activity. Probably, divalent metal ions react nonspecifically with the protein-hydrophobic groups, resulting in salting out of these cysteinyl residues from the surface into the interior of the enzyme macromolecules, as suggested earlier by Klibanov [10].

As expected, SDS exhibited a slight denaturating effect on the β-xylosidase at 10 mM concentration (Table 4).

### End Product Inhibition

The effects of xylose and glucose on the enzyme activity were investigated. As shown in Fig. 4, the activity of hydrolyzing pNPX was strongly inhibited by D-xylose and D-glucose. Most of the β-xylosidases studied so far were reported to be completely inhibited by xylose, an end-product of xylan hydrolysis catalyzed by xylanolytic enzymes, and also inhibited by other monosaccharides such as arabinose and galactose [5, 8].

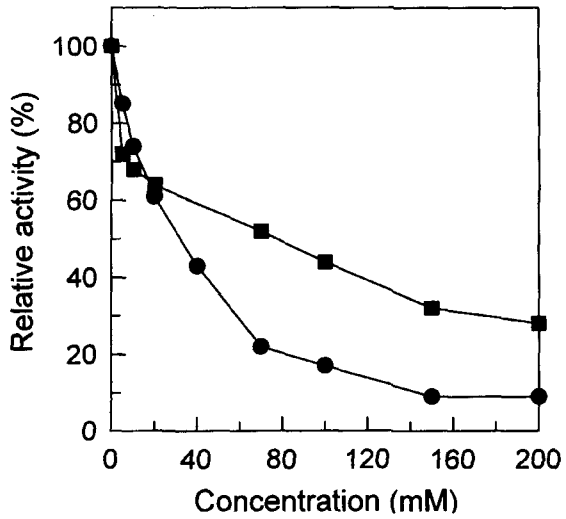



**Fig. 4.** Effects of xylose (●) and glucose (■) on the pNPX hydrolyzing activity.

## REFERENCES

1. Bachmann, S. L. and A. J. McCarthy. 1989. Purification and characterization of a thermostable β-xylosidase from *Thermomonospora fusca*. *J. Gen. Microbiol.* **135:** 293–295.
2. Bernier Jr., R., M. Desrochers, M. G. Paice, and M. Yaguchi. 1987. Isolation and characterization of β-xylosidase from a recombinant *Escherichia coli* strain. *J. Gen. Appl. Microbiol.* **33:** 409–419.
3. 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.
4. Chun, Y. C., K. H. Jung, J.-C. Lee, S.-H. Park, H. K. Chung, and K.-H. Yoon. 1998. Molecular cloning and the nucleotide sequence of the *Bacillus* sp. KK-1 β-xylosidase gene. *J. Microbiol. Biotechnol.* **8:** 28–33.
5. Coughlan, M. P. and G. P. Hazlewood. 1993. β-1,4-D-Xylan-degrading enzyme systems: Biochemistry, molecular biology and applications. *Biotechnol. Appl. Biochem.* **17:** 259–289.
6. Ghosh, M. and G. Nanda. 1993. Thermostability of β-xylosidase from *Aspergillus sydowii* MG49. *FEBS Lett.* **3:** 275–278.
7. Hebraud, M. and M. Fevre. 1990. Purification and characterization of an extracellular β-xylosidase from the rumen anaerobic fungus *Neocallimastix frontalis*. *FEMS Microbiol. Lett.* **72:** 11–16.
8. Hudson, R. C., L. R. Schofield, T. Coolbear, R. M. Daniel, and H. W. Morgan. 1991. Purification and properties of an aryl β-xylosidase from a cellulolytic

extreme thermophile expressed in *Escherichia coli*. *Biochem. J.* **273:** 645–650.
9. Kersters-Hilderson, H., E. van Doorslaer, M. Lippens, and C. K. De Bruyne. 1984. The pH dependence and group modification of β-D-xylosidase from *Bacillus pumilus*: evidence for sulfhydryl and histidyl groups. *Arch. Biochem. Biophys.* **234:** 61–72.
10. Klibanov, A. M. 1983. Stabilization of enzymes against thermal inactivation. *Adv. Appl. Microbiol.* **29:** 1–28.
11. Kim, D.-J., H.-J. Shin, B.-H. Min, and K.-H. Yoon. 1995. Isolation of a thermophilic *Bacillus* sp. producing the thermostable cellulase-free xylanase, and properties of the enzyme. *Kor. J. Appl. Microbiol. Biotechnol.* **23:** 304–310.
12. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* (London) **227:** 680–685.
13. Lee, S. F. and C. W. Forsberg. 1987. Isolation and some properties of a β-D-xylosidase from *Clostridium acetobutylicum* ATCC 824. *Appl. Environ. Microbiol.* **53:** 651–654.
14. Lee, Y.-E. and J. G. Zeikus. 1993. Genetic organization, sequence, and biochemical characterization of recombinant β-xylosidase from *Thermoanaerobacterium saccharolyticum* strain B6A-RI. *J. Gen. Microbiol.* **139:** 1235–1243.
15. Miller, M. L., R. Blum, W. E. Glennon, and A. L. Burton. 1960. Measurement of carboxymethylcellulase activity. *Anal. Biochem.* **2:** 127–132.
16. Nanmori, T., T. Watanabe, R. Shinke, A. Kohno, and Y. Kawamura. 1990. Purification and properties of thermostable xylanase and β-xylosidase produced by a newly isolated *Bacillus stearothermophilus* strain. *J. Bacteriol.* **172:** 6669–6672.
17. Poutanen, K. and J. Pulus. 1988. Characteristics of *Trichoderma reesei* β-xylosidase and its use in the hydrolysis of solubilized xylans. *Appl. Microbiol. Biotechnol.* **28:** 425–432.
18. Sakka, K., K. Yoshikawa, Y. Kojima, K. Ohmiya, and K. Shimada. 1993. Nucleotide sequence of the *Clostridium stercorarium xylA* gene encoding a bifunctional protein with β-D-xylosidase and α-L-arabinofuranosidase activities, and properties of the translated product. *Biosci. Biotechnol. Biochem.* **57:** 268–272.
19. Shao, W. and J. Wiegel. 1992. Purification and characterization of a thermostable β-xylosidase from *Thermoanaerobacter ethanolicus*. *J. Bacteriol.* **174:** 5848–5853.
20. Sunna, A. and G. Antranikian. 1997. Xylanolytic enzymes from fungi and bacteria. *Crit. Rev. Biotechnol.* **17:** 39–67.
21. Vroemen, S., J. Heldens, C. Boyd, B. Henrissat, and N. T. Keen. 1995. Cloning and characterization of the *bgxA* gene from *Erwinia chrysanthemi* D1 which encodes a β-glucosidase/xylosidase enzyme. *Mol. Gen. Genet.* **246:** 465–477.
22. Wong, K. K. Y., L. U. L. Tan, and J. N. Saddler. 1988. Multiplicity of β-1,4-xylanase in microorganism: Functions and applications. *Microbiol. Rev.* **52:** 305–317.
23. Xu, W.-Z., Y. Shima, S. Negoro, and I. Urabe. 1991. Sequence and properties of β-xylosidase from *Bacillus pumilus* IPO. Contradiction of the previous nucleotide sequence. *Eur. J. Biochem.* **202:** 1197–1203.
24. Yoon, K.-H., H. N. Yun., and K. H. Jung. 1998. Molecular cloning of a *Bacillus* sp. KK-1 xylanase gene and characterizaton of the gene product. *Biochem. Mol. Biol. Int.* in press.