Journal of Microbiology and Biotechnology
Vol. 7. No. 2, 114-120, 1997

Characterization of an Endoxylanase Produced by an Isolated Strain of *Bacillus* sp.

LEE, JAY J., KYOUNG-SOO HAHM[1], KI-YOUNG LEE[2], AND SUNG-TAIK LEE*

Department of Biological Sciences, Korea Advanced Institute of Science and Technology, 373-1 Kusung-Dong, Yusung-Gu, Taejon 305-701, Korea
[1]Peptide Engineering Research Unit, Korea Research Institute of Bioscience and Biotechnology, Korea Institute of Science and Technology, P.O. Box 115, Yusung-Gu, Taejon 305-600, Korea
[2]Department of Food and Nutrition, Hoseo University, 29-1 Sechul-Ri, Baebang-Myun, Asan-Kun, Chungnam 337-850, Korea

Microorganisms producing xylanase were screened for the enzymatic production of xylo-oligosaccharides from xylan. One of the bacteria isolated from compost produced an endoxylanase extracellularly. The bacterium was identified as *Bacillus* sp. according to its taxonomic characteristics examined. Xylanase production reached upto 5 U/ml after 22 h of culture in LB medium at 30°C. The xylanase was purified by ammonium sulfate precipitation and gel filtration. The molecular weight of the xylanase was estimated to be 20,400 by SDS-PAGE. Optimal temperature and pH for the xylanase activity was 60°C and 6.5, respectively. The enzyme was stable at temperatures upto 40°C and pH values from 4 to 10. The xylanase was completely inhibited by the addition of 2 mM mercury ion. Apparent K_m and V_{max} values for oat spelt xylan were 9.2 mg/ml and 1954 U/mg protein, respectively. For birchwood xylan, the values were 6.3 mg/ml and 1009 U/mg protein. The predominant products of the xylan hydrolysis were xylobiose, xylotriose and xylotetraose, indicating that the enzyme is an endoxylanase. Upto 85% of the initially added enzyme (2 U/ml) was bound to 50 mg/ml of the insoluble fraction of oat spelt xylan after incubation at 30°C for 30 min.

Plant cell walls are consist of three major polymeric constituents: cellulose (insoluble fibers of β-1,4-glucan), hemicellulose (non cellulosic polysaccharides including glucans, mannans and xylans) and lignin (polyphenolic complex). Xylan is the major component of hemicellulose having a β-1,4-linked xylopyranose backbone with a high degree of polymerization and branches (16). Bacteria and fungi have evolved xylanolytic systems consisting of a number of different enzymes with different specificities (13). Among these, endo-1,4-β-xylanases and β-xylosidases are the best characterized enzymes. Endo-1,4-β-xylanase is considered to be the most important enzyme as it initiates the degradation of xylan into xylooligosaccharides (2, 18). One of the most recent and exciting application of xylanases can be found in the production of xylooligosaccharides from plant materials. There have been reports on the stimulative effect of xylooligosaccharides on the selective growth of human intestinal bifidobacteria that are regarded to be important for the maintenance of healthy intestinal microflora (11). Among the xylooligosaccharides, those with low molecular weight such as xylobiose and xylotriose are known to have the best physiological effect. Xylooligosaccharides produced by microbial xylanases could be used as high quality food materials.

In this study, we have screened xylanase producing microorganisms from compost and soil, isolated a bacterial strain identified as *Bacillus* sp. and also characterized properties of the xylanase.

MATERIALS AND METHODS

Chemicals

Oat spelt xylan, birchwood xylan, cellulose, carboxymethylcellulose, β-glucan, amylose, amylopectin, lichenan, *p*-nitrophenyl-β-D-xylopyranoside, *p*-nitrophenyl-β-D-glucopyranoside and chemicals for electrophoresis were purchased from Sigma (U.S.A.). Yeast extract and tryptone were from Difco (U.S.A.). Cibacron Blue 3G-A was from Hoechst (Germany). All other chemicals used

*Corresponding author
Phone: 82-42-869-2617. Fax: 82-42-869-2610.
E-mail: stlee@sorak.kaist.ac.kr.
Key words: xylanase, *Bacillus*, xylooligosaccharide

were of reagent grade.

Isolation, Identification and Cultivation of the Bacterium

Xylanase-producing *Bacillus* sp. used in this study was isolated from an ordinary farm compost sampled near Taejon. Compost samples were suspended to 0.9% NaCl solution, incubated at 30°C for 30 min with shaking (120 rpm) and the suspension was diluted and plated on a screening plate containing 5 g yeast extract, 10 g tryptone, 5 g sodium chloride and 3 g Cibacron Blue-xylan (8) per liter. Autoclaved medium was agitated mildly while pouring into plates to keep the insoluble particles be dispersed. Bacterial strains with a clear zone of hydrolysis on the screening plate were selected and used for further study. Identification of the bacterium was based on the taxonomic characteristics listed in Bergey's Manual of Determinative Bacteriology (6). The DNA base composition was determined by reversed phase HPLC using the method of Tomaoka and Komagata (15).

Rhodococcus sp. KCTC 3218 was used as a xylanase negative bacterium.

The *Bacillus* sp. was maintained on LB agar plates containing 5 g yeast extract, 10 g tryptone, 5 g sodium chloride and 15 g agar per liter and transferred every 4 weeks. Before cultivating in a fermentor the bacterium was pre-cultured in 250 ml Erlenmeyer flask containing 50 ml of LB medium for 20 h. A 5 liter jar fermentor containing 2 liters of LB medium was used for the production of xylanase. The bacterium was cultured at 30°C with an agitation speed of 150 rpm and air flow rate of 1 vvm. Fermentation was started by inoculating 40 ml (2%) of the precultured broth.

Enzyme Assays

Reductometric assay of xylanase by the dinitrosalicylic acid (DNS) method (3) was based on the measurement of the reducing groups of sugar released by the hydrolytic enzymes. Standard assays were performed at 50°C with 10 mg/ml oat spelt xylan solutions in 50 mM potassium phosphate buffer (pH 6.5). One hundred μl of the assay mixture was added to 1 ml of DNS reagent, heated in a boiling water bath for 10 min and absorbances were measured at 570 nm as xylose equivalent. One unit of xylanase was defined as the amount of enzyme that produced reducing power equivalent to 1 μM of xylose per min.

Xylanase assays in the enzyme purification steps and experiments for xylanase binding to insoluble xylan were carried out with Cibacron Blue-xylan (8) which was based on the measurement of the soluble dyed fragment released from the insoluble dyed substrate. Test tubes containing 2 ml of the substrate solution in 50 mM potassium phosphate buffer (pH 6.5) were preincubated at the reaction temperatures indicated in the text for 5 min. Then enzyme solutions were added and shaken in a horizontal shaking water bath (180 rpm). Reactions were terminated by adding 1/5 volume of 1 N NaOH. Released soluble fractions were separated from the insoluble substrate by centrifugation (9,600 g, 1 min) and absorbances of the supernatant solution were measured at 625 nm.

Hydrolytic activities toward polysaccharides such as cellulose, carboxymethylcellulose (CMC), β-glucan, amylose, amylopectin and lichenan were determined by measuring the reducing power of the reaction mixture according to the DNS method. Substrate solutions (10 mg/ml in 50 mM phosphate buffer, pH 6.5) were mixed with the enzyme solution and incubated at 50°C for 20 min. Assays for β-xylosidase and β-glucosidase activities are based on the measurement of *p*-nitrophenol released from *p*-nitrophenyl-β-D-xylopyranoside (pNPX) and *p*-nitrophenyl-β-D-glucopyranoside (pNPG) at 405 nm. Reactions were carried out in 1 ml of 5 mM substrate in 50 mM phosphate buffer (pH 6.5). The reaction mixtures were incubated at 50°C for 20 min and released *p*-nitrophenol was measured at 405 nm.

Enzyme Purification

All purification steps were carried out at 4°C. Culture supernatant was collected at the early stationary phase of cell growth by centrifugation (6,000 g, 30 min). Proteins were precipitated by adding ammonium sulfate to the supernatant at 70% saturation. The solution was stood overnight and the precipitate formed was collected by centrifugation (6,000 g, 30 min). The precipitate was dissolved in 20 mM phosphate buffer (pH 7.0) and dialysed against the same buffer in a dialysis membrane that has a MWCO (molecular weight cut off) size of 3,500 with 4 changes of buffer at 6 h intervals. The dialysate (6 ml) was then applied to a Sephacryl S-200 column (2.5 × 98 cm) pre-equilibrated with 20 mM phosphate buffer (pH 7.0) and eluted with the same buffer at a flow rate of 0.6 ml/min. Active fractions were collected and ultrafiltered through an ultrafiltration membrane (Amicon; MWCO 1,000) to a volume of 5 ml. The ultrafiltrate was rechromatographed on a Sephacryl S-200 column under the same conditions as described above.

Protein concentration was determined by the method of Lowry with bovine serum albumin as a standard (10). In the case of column chromatography protein concentrations were determined by measuring absorbances at 280 nm.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis

Discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in 10% polyacrylamide gel containing 0.1% SDS at the 20 mA constant current condition (7). After the run, the gel was stained by a silver stain kit (Bio-Rad) according to the procedure described by the supplier. Size markers for

molecular weight determination were bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (29 kDa), trypsin inhibitor (20 kDa) and α-lactalbumin (14 kDa).

Affinities of the Xylanase to Xylan

Insoluble xylan was prepared by suspending 1 g of oat spelt xylan in 50 ml of distilled water, stirring for 24 h, precipitation by centrifugation (9,600 g, 1 min), washing with distilled water 3 times and suspending the pellet in 50 mM phosphate buffer (pH 6.5). About 0.6 g of insoluble xylan was obtained by this procedure. Affinities of the enzyme to xylan was measured with the prepared insoluble fraction of oat spelts xylan. Insoluble xylan suspension was mixed with the enzyme (2 U/ml) and incubated at 30°C for 30 min with shaking. After 30 minutes, enzyme activities of the supernatant were measured by the Cibacron Blue-xylan method as described above.

HPLC Analysis of Xylan Hydrolysates

The hydrolysis products of xylan by the xylanase were analyzed by high performance liquid chromatography (HPLC) using a carbohydrate analysis column (Waters) with 70% acetonitrile as a mobile phase at a flow rate of 1.5 ml/min. Oat spelts xylan (1%) was completely hydrolyzed by incubating with 0.5 U/ml of the enzyme for 8 h at 40°C and filtering through a cellulose nitrate membrane filter (pore size, 0.45 μm). Twenty μl of the sample was injected into the column and xylooligosaccharides were detected with a RI detector.

RESULTS AND DISCUSSION

Characteristics of the Isolated Bacterium

The bacterium isolated from compost showed a clear zone of hydrolysis on LB agar plates supplemented with Cibacron Blue-xylan indicating a high level of xylanase production. The isolated strain was an aerobic, gram positive, rod shaped (1.2×0.6 μm) motile and endospore forming bacterium. On LB agar this bacterium showed glistening, slimy and pale white colonies. The strain could utilize citrate, arabinose, gluconate, mannitol, N-acetyl glucosamine and malate and showed hydrolytic activities toward casein, gelatin, β-glucan, esculin and starch. The content of guanine and cytosine in the DNA (deoxyribonucleic acid) was 48.6 mol%. Taxonomic characteristics for the identification of the bacterium shown in Table 1 could place the isolate to a species in the genus *Bacillus* (12). However, the content of guanine and cytosine indicate that the bacterium could not be placed to any of the species in the genus *Bacillus*. Therefore, we refer to the bacterium as *Bacillus* sp. The *Bacillus* sp. showed a growth associated xylanase production during the course of incubation in LB medium at 30°C (Fig. 1). Upto 5 U/ml of xylanase was released into the culture medium during the course of incubation under the conditions mentioned above. A slight decrease in xylanase activity was observed after the cells entered stationary phase. For the optimization of xylanase production and application of the enzyme to industrial processes, further studies on the effect of nutrient and regulation of the enzyme production should be followed.

Table 1. Taxonomic characteristics of the bacterium isolated from compost.

Characteristics	Description
Cell Shape	Straight rod
Cell size	1.2×0.6 μm
Gram stain	+
Endospore	+
shape	Oval
Murein type	Meso-DAP[a]
G+C content	48.6 mol%
Reduce NO_3 to NO_2	+
Catalase	+
Oxidase	+
β-galactosidase	−
Urease	−
Acid from Glucose	+
Hydrolysis of Casein	+
Gelatin	+
Esculin	+
β-Glucan	+
Starch	+
Utilization of Citrate	+
Arabinose	+
Glucose	+
Xylose	+
Mannitol	+
N-acetyl-glucosamine	+
Maltose	±
Gluconate	±
Caprate	−
Adipate	±
Malate	+
Phenyl-acetate	±

[a]Diaminopimelic acid.

Purification of the Xylanase

Although molecular weight of the xylanase estimated by SDS-PAGE was 20,400, membranes with MWCO size smaller than 3,500 were used throughout the experiments. The xylanase was purified 647 fold and 1.5% of the culture supernatant activity was recovered. The results of purification of the xylanase from the culture supernatant are summarized in Table 2.

Molecular Weight Determination

The purified xylanase showed a single band on SDS-PAGE gel by silver staining (Fig. 2). Molecular weight of the xylanase was determined to be 20,400. Wong *et al.* (17) has suggested that the xylanase produced by *Bacillus* sp. can be divided into two major groups, one with

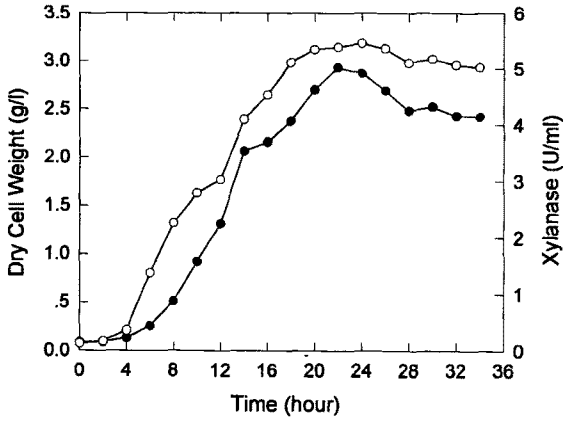

Fig. 1. Growth (○) and xylanase production (●) of the *Bacillus* sp.
The bacterium was grown in an Erlenmeyer flask (500 ml) containing 100 ml of LB medium at 30°C with shaking (180 rpm).

Table 2. Summary of purification of the xylanase.

	Volume (ml)	Protein (μg/ml)	Enzyme (U/ml)	Sp. act.[a] (U/mg)	Fold of purification	Recovery (%)
Culture supernatant	400	2740	4.09	1.49	1	100
Ammonium Sulfate[b]	6	7420	165	22.2	15	61
Sephacryl S-200 chromatography	50	35	3.74	107	71	11.4
Rechromatography on S-200	5	5	4.84	968	647	1.5

[a]Specific activity. [b]Ammonium sulfate precipitation and dialysis.

relatively low molecular weights (From 16,000 to 22,000) and the other with relatively high molecular weights (From 43,000 to 50,000). Some of the xylanase produced by *Clostridium*, *Streptomyces*, *Aspergillus* and *Trichoderma* species show similar pattern. In case of the xylanase studied in this work the xylanase could be assigned to the low molecular weight group. Gel filtration patterns show that the *Bacillus* sp. isolate produces a single xylanase during the course of culture in LB medium. This result is contrary to the report that xylanases expressed by most microorganisms are usually in multiples, and which are known to play role in hydrolysis of xylan in situ.

Substrate Specificity of the Xylanase

The purified enzyme was shown to be highly specific to xylan as a substrate (Table 3). About 69% of the reducing power produced from oat spelts xylan was produced with birchwood xylan at the same reaction conditions. Cross-specificity has been reported in many lignocellulolytic enzymes (9, 14) and some xylanases are known to possess relatively high side activities. In this

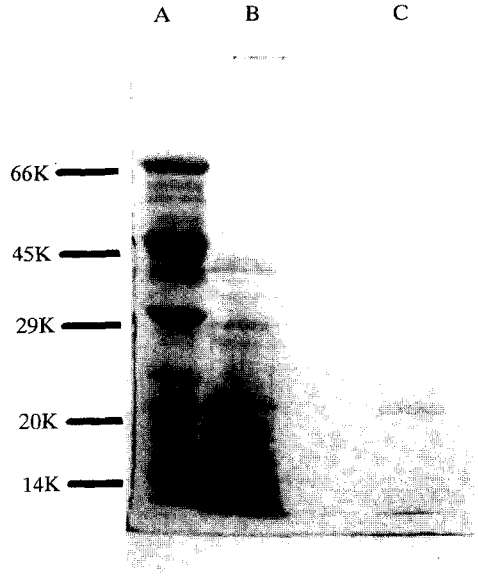

Fig. 2. Determination of the molecular weight of the xylanase by discontinuous SDS-PAGE.
Size markers for the estimation of the molecular weight were bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (29 kDa), trypsin inhibitor (20 kDa) and α-lactalbumin (14 kDa). Symbols: A, size marker; B, culture supernatant; C, purified xylanase.

Table 3. Substrate specificity of the xylanase.

Substrate[a] (major linkages)	Relative degree of hydrolysis (%)
Xylan (β-1,4)	
from oat spelt	100
from birchwood	69
Cellulose (β-1,4)	0
Carboxymethylcellulose (β-1,4)	0
β-glucan (β-1,4; β-1,3)	0
Lichenan (β-1,3-linked cellotriose)	0.15
Amylose (α-1,4)	0
Amylopectin (α-1,4; α-1,6)	0
pNP-β-Glu[b]	0
pNP-β-Xyl[c]	0

[a]Concentrations of the substrates were 10 mg/ml for polysaccharides and 5 mM for pNP derivatives. [b]*p*-Nitrophenyl-β-D-glucopyranoside. [c]*p*-Nitrophenyl-β-D-xylopyranoside.

case, the purified xylanase did not show much side activities. Little activity was detected on lichenan which was mainly composed of β-1,3-linked cellotriose units.

Effect of Temperature and pH on the Activity and Stability of the Xylanase

The optimal temperature for the xylanase activity was estimated to be 60°C (Fig. 3). The xylanase was stable at 30°C for 20 h. At 40°C, 45% of the initial activity was retained after incubation for 12 h, while at 50°C, 39% of the activity remained after 30 min. Although the maximum activity was shown at 60°C, only 24% of the initial activity

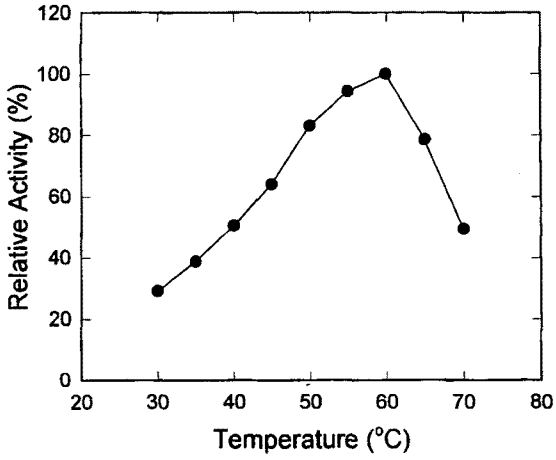

Fig. 3. Effect of temperature on the activity of the xylanase. Enzyme reactions were carried out in 50 mM phosphate buffer.

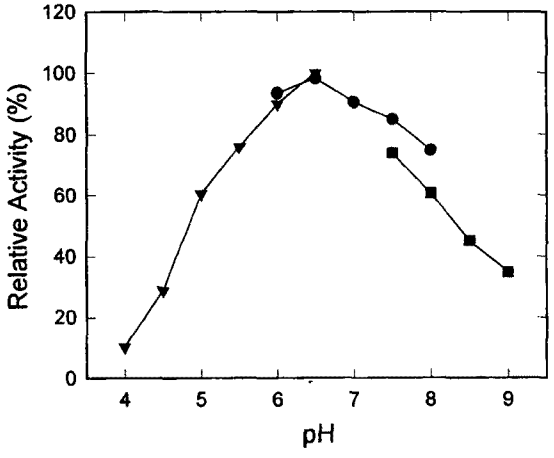

Fig. 4. Effect of pH on the activity of the xylanase. Enzyme reactions were carried out in 50 mM each of citrate-phosphate buffer (▼), phosphate buffer (●) and Tris-HCl buffer (■).

was detected at 60°C after 5 min.

The optimum pH for the activity was estimated to be 6.5 (Fig. 4). The xylanase was quite stable at 30°C at pH values ranging from 4 to 10. More than 70% of the initial activity was retained after 20 h. However, at pH 3.15, only 38% of the initial activity was remained after 24 h. It was slightly more stable in phosphate buffer than in citrate or Tris-HCl buffers.

Effect of Metal Ions and Some Chemicals

Almost all of the activity was lost by the addition of 2 mM mercury chloride (Table 4). Inhibition of the xylanase was observed with copper, ferrous and aluminum ions. Magnesium ion did not interfere with xylanase activity. A slight increase in activity was observed with calcium, cobalt and manganese ions. Addition of ethylenediaminetetraacetic acid (EDTA) decreased activity of

Table 4. Effect of metal ions and some chemicals on the activity of the xylanase.

Metal ion or chemical[a]	Relative activity (%)
None	100
$CaCl_2$	115
$CoCl_2$	107
$MgCl_2$	96
$MnCl_2$	108
$CuCl_2$	60
$BaCl_2$	92
$FeCl_3$	87
$AlCl_3$	85
$HgCl_2$	0
EDTA[b]	93
DTT[c]	86
NBS[d]	0

[a]Concentration of metal ions and chemicals was 2 mM each. [b]Ethylenediaminetetraaceticacid. [c]Dithiotreitol. [d]N-Bromosuccinimide.

the enzyme slightly. The decrease in activity with the addition of EDTA and the increase with some metal ions indicates a possible involvement of divalent cations as an effector for the enzyme. Enzyme activity was slightly decreased by dithiotreitol and totally inactivated by N-bromosuccinimide which was known as a specific modifier of tryptophan residues in proteins. Complete inactivation by mercury ion and N-bromosuccinimide has been reported for many xylanases of different origins (1, 5). This result may indicate the presence of a tryptophan residue in the active site of this xylanase like some other xylanases reported (4).

Effect of Substrate Concentration

Initial velocities in the hydrolysis of oat spelts xylan and birchwood xylan were measured at their various concentrations. Kinetic parameters, K_m and V_{max}, were estimated by Lineweaver-Burk plot of the data (Fig. 5). Apparent K_m and V_{max} values for oat spelts xylan was 9.2 mg/ml and 1954 U/mg protein, respectively. For birchwood xylan, the values were 6.3 mg/ml and 1009 U/mg protein, respectively. The enzyme was more specific to oat spelts xylan as a substrate than birchwood xylan. However, it had higher K_m values for oat spelts xylan than for birchwood xylan.

Affinities of the Xylanase to Insoluble Xylan

The cibacron Blue-xylan method was used for the assay of the xylanase activities in the insoluble xylan-xylanase suspension because reducing groups released from the insoluble xylan by the enzyme action could interfere with the DNS method. Only 15% of the initial activity (0.3 U/ml) remained in the supernatant solution after incubation in 50 mg/ml insoluble xylan suspension (Fig. 6). Bound xylanase could be released from the insoluble xylan into the solution by adding the soluble fraction of the oat spelts xylan and incubating with shaking at 30°C

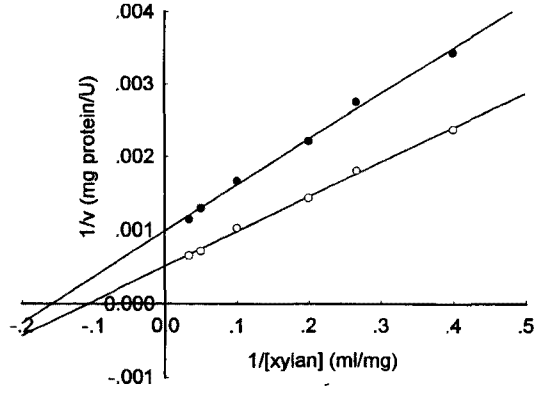

Fig. 5. Effect of substrate concentration on the activity of the xylanase.
Enzyme reactions were carried out in 50 mM phosphate buffer with varied concentration of oat spelt xylan (○) and birchwood xylan (●).

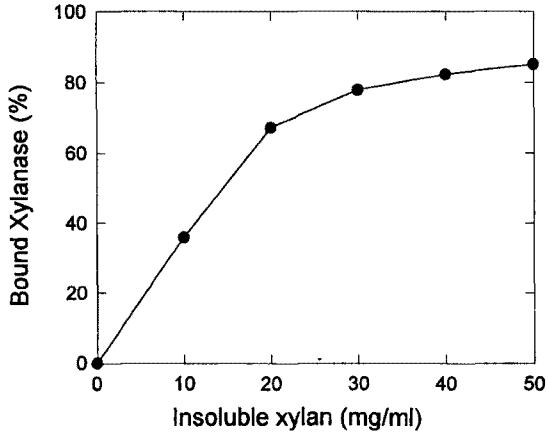

Fig. 6. Affinities of the xylanase to insoluble fraction of oat spelts xylan.
Enzyme solutions were mixed with the insoluble xylan suspension, incubated at 30°C for 30 min, centrifuged and supernatant solutions were measured for xylanase activity by the Cibacron Blue-xylan method.

(data not shown). Further studies on the affinities of the xylanase to the substrate could establish a method of purification of the xylanase.

HPLC Analysis of the Xylan Hydrolysate

The major products of the hydrolysis were xylobiose, xylotriose and xylotetraose as assayed by HPLC (Fig. 7). This result indicates that the enzyme is an endoxylanase (β-1,4-D-xylan xylanohydrolase; EC 3.2.1.8) which cleaves the internal backbone of xylan molecules. The products of hydrolysis are known to be selective growth stimulants to intestinal bifidobacteria, and the bacteria are regarded as beneficial for the maintenance of healthy intestinal microflora (11). In conclusion, the xylanase of the isolated strain could be effectively applied to the production of xylooligosaccharides that can be used as high quality food materials.

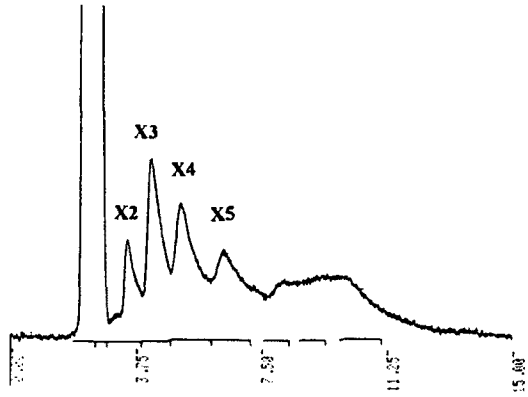

Fig. 7. HPLC analysis of the xylanase reaction product.
Symbols: X2, xylobiose; X3, xylotriose; X4, xylotetraose; X5, xylopentaose.

REFERENCES

1. Bastawde, K. B. 1992. Xylan structure, microbial xylanases and their mode of action. *World J. Microbiol. Biotechnol.* **9**: 221-224.
2. Biely, P. 1985. Microbial xylanolytic systems. *Trends Biotechnol.* **3**: 286-290.
3. Chaplin, M. F. 1986. Dinitrosalicylic acid assay. p. 3. *In* M. F. Chaplin, and J. F. Kennedy (ed.), *Carbohydrate analysis; a practical approach.* IRL press, Washington DC.
4. Deshpande, V., J. Hinge, and M. Rao. 1990. Chemical modification of xylanases: evidence for essential tryptophan and cysteine residues at the active site. *Biochem. Biophys. Acta* **1041**: 171-177.
5. Elegir, G., S. George, and W. J. Thomas. 1994. Purification, characterization and substrate specificities of multiple xylanases from *Streptomyces* sp. strain B-12-2. *Appl. Environ. Microbiol.* **60**: 2609-2615.
6. Holt, J. G., N. R. Krieg, P. H. A. Sneath, J. T. Staley, and S. T. Williams. 1986. *Bergey's Manual of Determinative Bacteriology*, Williams and Wilkins Co., Baltimore.
7. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**: 680-685.
8. Lee, J. J., S. K. Rhee, E. R. Park, and S. T. Lee. New insoluble dyed substrate for detection and assay of xylan degrading enzymes. 1994 Spring Meeting. Korean Society for Applied Microbiology.
9. Lee, S. F., C. W. Forsberg, and J. B. Rattray. 1987. Purification and characterization of two endoxylanases from *Clostridium acetobutylicum* ATCC 824. *Appl. Environ. Microbiol.* **53**: 644-650.
10. Lowry, O. H., N. J. Rosebrough, A. L. Far, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**: 265-275.
11. Okajaki, M., S. Fujikawa, and N. Matsumoto. 1990. Effect of xylooligosaccharides on growth of bifidobacteria. *J. Jpn. Soc. Nutr. Food Sci.* **43**: 395-401.

12. Sneath, P. H. A, N. S. Mair, M. E. Sharpe, and J. G. Holt. 1986. *Bergey's manual of systematic bacteriology*. vol. 2, Williams and Wilkins Co., Baltimore.
13. Thomson, J. A. 1993. Molecular Biology of Xylan Degradation. FEMS *Microbiol. Review* **104**: 65-82.
14. Toda, S., H. Suzuki, and K. Nishizawa. 1971. Some enzymatic properties and the substrate specificities of *Tricoderma* cellulases with special reference to their activity toward xylan. *J. Ferment. Technol.* **49**: 499-521.
15. Tomaoka and Komagata. 1984. Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol. Lett.* **25**: 125-128.
16. Wilkie, K. C. B. 1983. Hemicellulose. *Chem. Technol.* **13**: 306-319.
17. Wong, K. K. Y., U. L. T. Larry, and J. N. Saddler. 1988. Multiplicity of β-1,4-xylanase in microorganisms: Functions and applications. *Microbiol. Rev. 52*, **3**: 305-317.
18. Woodward, J. 1984. Xylanases: functions, properties and applications. Top. *Enzyme Ferment. Biotechnol.* **8**: 9-30.

(Received October 17, 1996)