Purification and Characterization of Xylanase II from *Trichoderma koningii* ATCC 26113

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A 1,4-β-D-xylanase, designated as xylanase II, was purified from the culture filtrate of *Trichoderma koningii* ATCC 26113 by column chromatography on Sephadex G-75, SP-Sephadex C-50, DEAE-Sephadex A-50 and Sephadex G-50 with an overall yield of 6.97%. It has a molecular weight of 21,000 and an isoelectric point of 9.4. The enzyme activity is optimal at pH 5.0 and at a temperature of 50°C. Xylanase II is stable up to 50°C, while 40 and 90% of its activity are lost after the incubation for 30 and 60 min at 60°C. The enzyme degrades xylan with relatively high activity, as well as carboxymethylcellulose and Avicel. Its K_m values for oat-spelt xylan, larchwood xylan and Avicel are 7.48, 1.98 and 13.33 mg/ml, respectively. The hydrolysis products of oat-spelt xylan by xylanase II are xylose, xylobiose, xylotriose and arabinoxylotriose, while the reaction products of larchwood xylan are xylose, xylobiose, xylotriose and small amount of higher oligomers. The action patterns of the enzyme demonstrate that xylanase II is endo-enzyme.

KEY WORDS □ ***Trichoderma koningii*, xylanase II, endo-xylanase**

Xylan is a heterogeneous polysaccharide found in the cell walls of higher plants and forms a major part of hemicelluloses. In a pure state, it is composed of β-1,4-linked D-xylose polymer, even though L-arabinose, D-glucose and uronic acid residues may be detected in different amounts depending on the plant species. The hydrolysis of its backbone involves endo-xylanase (1,4-β-D-xylan xylanohydrolase; EC 3.2.1.8) and β-xylosidase (1,4-β-D-xylan xylohydrolase; EC 3.2.1.37). Generally, endo-xylanase hydrolyzes polysaccharides in a random manner, yielding fragments of xylan of various chain length, and β-xylosidase release xylosyl residues by endwise attack of xylooligosaccharides.

Since xylan is abundant and renewable resource, it has recently received considerable attention in the exploitation of plant biomass. The use of microbial enzymes for the industrial hydrolysis of xylan is advantageous owing to the high specificity of enzyme reactions and the mildness of the reaction conditions. Hydrolysis products obtained from enzymatically treated xylan may be subsequently converted into single-cell protein, liquid fuel, solvents and other chemical products.

Many microbial xylanases have been described from bacteria, such as *Bacillus* (2), *Clostridium* (18) and *Streptomyces* (15, 28), yeast, such as *Cryptococcus* (3, 20), and fungi, such as *Aspergillus* (12, 23), *Trichoderma* (7, 17, 21), *Ceratocystis* (9) and *Schizophyllum* (21).

Trichoderma koningii is also one of the best producer of xylanase and has been shown to possess endoxylanase activity (30). To extend our comprehension of the xylanases produced by *T. koningii*, we have recently purified xylanase I from *T. koningii* and investigated its characteristics and action patterns (14). We now report the purification of a second enzyme, named as xylanase II, from *T. koningii* and describe its properties and hydrolysis patterns.

MATERIALS AND METHODS

Chemicals

Oat-spelt xylan, larchwood xylan, laminarin, polygalacturonic acid, *p*-nitrophenyl-β-D-xyloside (PNPX) and *p*-nitrophenyl-β-D-glucoside (PNPG) were purchased from Sigma (U.S.A.). CMC (carboxymethyl cellulose) was supplied from Wako (Japan) and Avicel from Fluka (Swiss). All the other chemicals used were of the highest quality, commercially available.

Fungal strain and culture conditions

A conidial suspension of *Trichoderma koningii* ATCC 26113 was inoculated in Mandels' medium (18) containing (g/l): Avicel, 5.0; CMC, 5.0; Bactopeptone, 1.0; $(NH_4)_2SO_4$, 1.4; KH_2PO_4, 2.0; urea,

0.3; $MgSO_4 \cdot H_2O$, 0.3; (mg/l): $FeSO_4 \cdot H_2O$, 5.0; $MnSO_4 \cdot H_2O$, 1.6; $ZnSO_4 \cdot H_2O$, 1.4; $CoCl_2$, 2.0; and (ml/l): Tween 80, 2.0; Antifoam A emulsion, 5.0. The final concentration of conidia was about 5×10^5 conidia/ml. They were grown in 1000 ml flasks containing 250 ml of medium and incubated at 28°C on a reciprocal shaker for 8 days.

Enzyme purification

The culture filtrate after 8 day-incubation was recovered by centrifugation and the solution was precipitated with 20 to 80% ammonium sulfate saturation. The precipitate was dissolved in 50 mM sodium acetate buffer (pH 5.0; buffer A) and dialyzed against buffer A at 4°C. The enzyme solution was loaded to a Sephadex G-75 column equilibrated with buffer A and eluted with the same buffer. The active fractions were pooled and applied to a SP-Sephadex C-50 column equilibrated with buffer A. The column was washed with buffer A and then eluted with a linear concentration gradient of 0~0.25 M NaCl. Fractions containing xylanase activity were collected and ultrafiltered through a YM 5 membrane (Amicon, U.S.A.). The enzyme solution was exchanged for 20 mM sodium phosphate buffer (pH 6.8; buffer B) by repeated ultrafiltration. The concentrated enzyme was placed on a DEAE-Sephadex A-50 column equilibrated with buffer B and eluted with the same buffer. Further fractionation was performed on a Sephadex G-50 column. The solution was applied to the column and eluted with buffer A. Final purification was accomplished by rechromatography on Sephadex G-50 column.

Enzyme assay

Activities toward xylan (1% wt/vol), CMC (0.5% wt/vol), Avicel (0.5% wt/vol), laminarin (0.5% wt/vol) and polygalacturonic acid (1% wt/vol) were estimated by incubating a 0.5 ml reaction mixture containing each substrate and diluted enzyme in 50 mM sodium acetate buffer (pH 5.0) for 20 min (xylan, CMC, laminarin and polygalacturonic acid) or 1 h (Avicel) at 40°C. The amount of reducing sugars liberated was measured by the method of Somogyi-Nelson (24). One unit of enzyme activity was defined as the amount of enzyme that catalyzed the release of 1 mol of reducing sugars per min.

β-Xylosidase and β-glucosidase activity were determined by measuring the amount of *p*-nitrophenol (PNP) released from PNPX and PNPG, respectively. The reaction mixture was composed of 2 mM substrate, 50 mM sodium acetate buffer (pH 5.0) and an appropriate amount of enzyme in a final volume of 0.5 ml. After incubation at 40°C for 30 min, 1 ml of 1 M sodium carbonate solution was added to the mixture. The mixture was then diluted with 5 ml of distilled water and the absorbance at 420 nm was measured. One unit of enzyme activity was defined as the amount of the enzyme that catalyzed the release of 1 μmol of PNP per min.

Protein content was determined by the method of Bradford (4), with bovine serum albumin (Sigma, U.S.A.) as standard.

Determination of molecular weight

The molecular weight of the purified enzyme was determined by SDS-polyacrylamide gel electrophoresis as described by Laemmli (16). The reference proteins were supplied from Sigma (U.S.A.) and contained phosphorylase *b* (97,400), bovine serum albumin (66,200), hen egg white albumin (43,000), bovine carbonic anhydrase (31,000), soybean trypsin inhibitor (21,500) and lysozyme (14,000).

Determination of isoelectric point

Isoelectric focusing was carried out on a Pharmacia Phast gel (Pharmacia Fine Chemicals, Sweden) containing Ampholine of the pH range of 3~10 and calibration kit proteins were used. After electrofocusing, the gel was stained with Coomassie brilliant blue R-250.

Effects of various chemicals on enzyme activity

The enzyme was preincubated with various chemicals dissolved in 50 mM acetate buffer (pH 5.0) at 40°C for 10 min. The final concentration of chemicals was 10 mM. After the reaction, each enzyme activity was determined in comparison to the control which had no reagents treated.

Kinetic studies

For the determination of K_m and V_{max} values of the purified enzyme for several substrates, the enzyme activity was assayed at various concentrations of the substrates. The substrate concentrations were ranged over 0.3125~5 mg/ml for oat-spelt and larchwood xylan, and 1.25~20 mg/ml for Avicel. K_m and V_{max} values were determined from the Lineweaver-Burk plot.

Hydrolysis of xylan by the enzyme

To investigate the extent of hydrolysis of xylan and product distribution by the purified enzyme, 0.8 μg of xylanase II was incubated with 10 ml of oat-spelt xylan (1% wt/vol) or larchwood xylan (1% wt/vol), in 50 mM acetate buffer (pH 5.0) at 40°C. Samples were removed at time intervals and the reaction was stopped by heating for 10 min. Reducing sugars released were measured by the method of Somogyi-Nelson (24). The percent hydrolysis was calculated as (micromoles of reducing sugars released/micromoles of xylan as xylose).

Identification of reaction products by HPLC

The hydrolysis products were analyzed by HPLC. Hydrolyzates were centrifuged in a microcentrifuge (Eppendorf, Germany) at 12,000 rpm for 30 sec in order to remove any solids and the supernatants were filtered through 0.45 μm-filter (Waters, U.S.A.). These filtrates were analyzed on the μ-Bondapak NH_2 column (Waters, U.S.A.) using 76% acetonitrile as eluant

at a flow rate of 1.5 ml/min. The sugar products were detected with a Waters R 401 differential refractometer.

Amino acid composition

20 μg of the purified enzyme was dried *in vacuo* and hydrolyzed with 6 N HCl containing 1% phenol *in vacuo* and oxygen-free nitrogen condition at 105°C for 24 h. To the thoroughly dried hydrolyzates, 10 μl of redrying agent [ethanol: water:triethylamine (TEA)=2:2:1 (V/V/V)] was added and the sample was dried again. Then 20 μl of the reagent solution [ethanol:water:TEA: phenylisothiocyanate (PITC)=7:1:1:1 (V/V/V/V)] was added to the sample, reacted for 20 min at room temperature, and dried. To the dried sample, 250 μl of sample diluent (5% acetonitrile in sodium phosphate buffer, pH 7.4) was added and analyzed by HPLC system containing a model 6000 A pump and a Pico-Tag column (Waters, U.S.A.). Samples were eluted by a linear concentration gradient of solvent A (2% sodium acetate, 0.05% TEA, 6% acetonitrile) and solvent B (60% acetonitrile) at a flow rate of 1.0 ml/min.

RESULTS AND DISCUSSION

Purification of xylanase II

The xylanase activity was slightly separated into three main peaks on Sephadex G-75 column, as shown in Fig. 1 of Kim *et al.* (14). Fractions containing the lowest xylanase activity among three peaks showed a higher activity for CMC than for xylan. Therefore, it is deduced that the enzyme corresponding to the first peak is cellulase. Fractions containing relatively high xylanase activity were pooled and fractionated by SP-Sephadex C-50 column. The main xylanase was eluted at about 0.13 M NaCl. On DEAE-Sephadex A-50 column, the enzyme failed to bind to the column and eluted as a single peak (Fig. 1). Further purification was carried out on a Sephadex G-50 column and the xylanase was partially resolved into two peaks on the column. Fractions corresponding to the second peak (X_2) were collected and concentrated. Homogeneous xylanase was obtained by rechromatography of Sephadex G-50 (Fig. 2). The enzyme, named as xylanase II, was purified 9-fold, with a yield of 6.97% over the culture filtrate.

Molecular properties

Analysis of the purified enzyme by SDS-polyacrylamide gel electrophoresis exhibited a single protein band, as shown in Fig. 3, with molecular weight of 21,000 calculated from a standard curve (Fig. 4). Analytical isoelectric focusing of the enzyme on a pH gradient of 3 to 10 revealed a single band with pI of 9.4, indicating a homogeneous protein (Fig. 5). These values are appreciably higher than those of endoxylanase II reported by Wood and MacCrae (30), which were estimated to be 18,000 (Mr) and 7.3 (pI). Our xylanase has relatively high isoelectric point. Isoelectric point above 9 has been found in several xylanases from *Trichoderma pseudokoningii* (1), *Cryptococcus flavus* (19), *Streptomyces roseiscleroticus* (11), *Trichoderma harzianum* (27) and

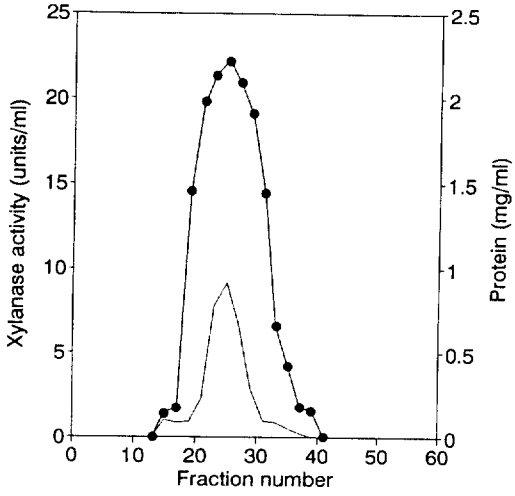

Fig. 1. *Ion exchange chromatography of xylanase II on DEAE-Sephadex A-50.*
The column (2.2×50 cm) was eluted with 20 mM phosphate buffer (pH 6.8) at a flow rate of 12 ml/h. Fractions of 6 ml were collected. ●, Xylanase; ----, protein concentration.

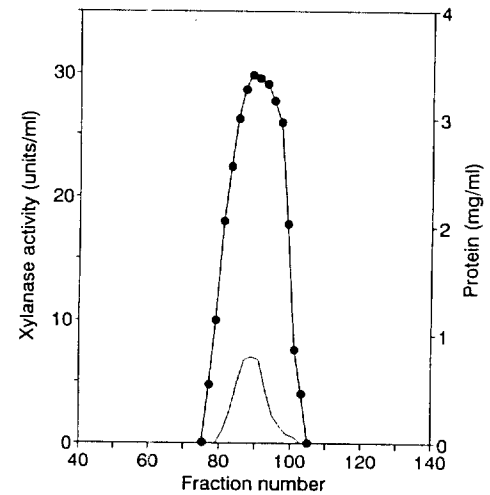

Fig. 2. *Gel filtration chromatography of xylanase II on Sephadex G-50.*
The column (2.3×100 cm) was eluted with 50 mM acetate buffer (pH 5.0) at a flow rate of 8.3 ml/h. Fractions of 2 ml were collected. ●, Xylanase; ----, protein concentration.

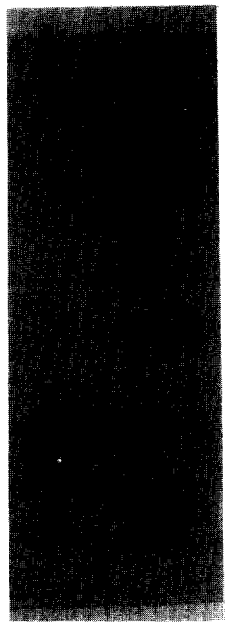

Fig. 3. *SDS-polyacrylamide gel electrophoresis of xylanase II.*
Samples were electrophoresed in 12.5% gel. Lane A, xylanase II. Lane B, molecular weight markers: 1, phosphorylase *b* (97,400); 2, bovine serum albumin (66,200); 3, hen egg white albumin (43,000); 4, bovine carbonic anhydrase (31,000); 5, soybean trypsin inhibitor (21,500); 6, lysozyme (14,000).

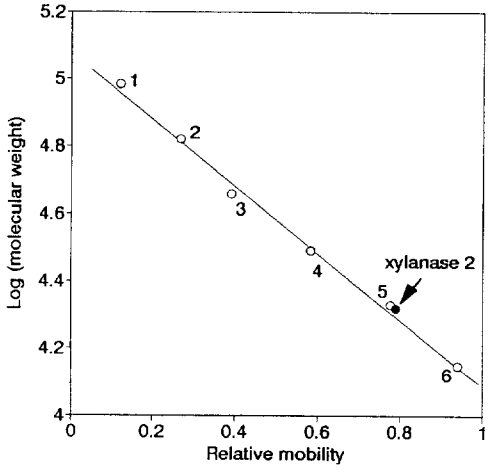

Fig. 4. *Molecular weight estimation of xylanase II by SDS-PAGE.*
Relative mobility was plotted against log molecular weight of standard proteins. The enzyme was indicated by the closed circle. Protein markers are the same as those of Fig. 3.

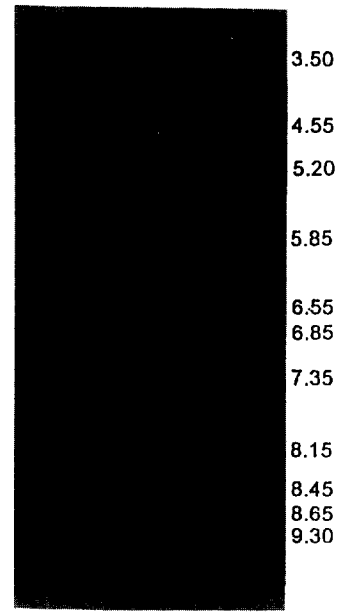

Fig. 5. *Analytical isoelectric focusing of xylanase II using a pH range of 3~10.*
Lane A and B contain xylanase II. Lane C contains Pharmacia protein mixture. pI values are at right.

Trichoderma viride (29). Xylanases of microbial origin are generally divided into two categories, i.e., basic xylanases with low molecular weights and acidic xylanases with high molecular weights (29). In *Trichoderma*, low M.W.-basic xylanases are common (29). Our enzyme is also basic protein having low molecular weight.

Effects of pH and temperature on xylanase activity

The xylanase exhibited the highest activity at pH 5.0 and more than 90% of maximal activity was observed in the broad range of 4.2~7.2 (Fig. 6). The optimum temperature for enzyme activity was 50°C. The Arrhenius plot of xylanase II showed an activation energy of 9.8 kcal/mol from 30 to 50°C (Fig. 7). There is an abrupt change to 19.29 kcal/mol below 30°C. This transition in activation energy appears to reflect a phase change in the substrate itself from a sol at temperature above 30°C to a gel at lower temperatures. In thermal stability, the enzyme was stable up to 50°C, while it lost 40 and 90% of its activity after the incubation for 30 and 60 min at 60°C (Fig. 8).

Effects of various chemicals on xylanase activity

The activity of the enzyme was inhibited by Hg^{2+}, Mg^{2+}, Mn^{+} and Fe^{2+}, whereas the activity was stimulated by Ag^{2+}, Ba^{2+} and DTT (Table 1). The enzyme, which was inactivated by Hg^{2+}, was fully reactivated by the addition of 10 mM

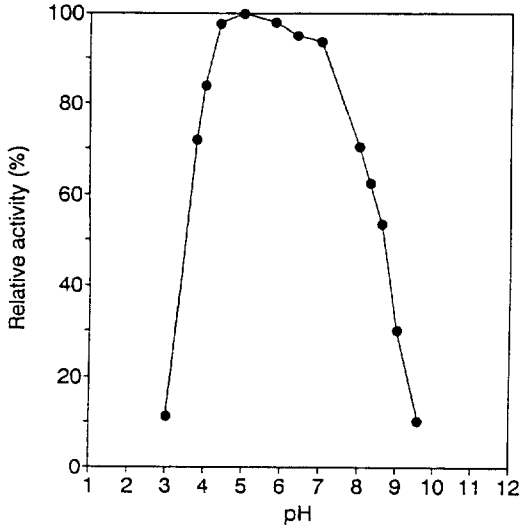

Fig. 6. *Effect of pH on the activity of xylanase II.*
The optimal pH of xylanase was measured at various pH values. The buffer solutions were the following: pH 3.0~3.8, citrate phosphate buffer; pH 4.0~5.8, sodium acetate buffer; pH 6.0~8.0, sodium phosphate buffer; pH 8.2~9.5, Tris-HCl buffer.

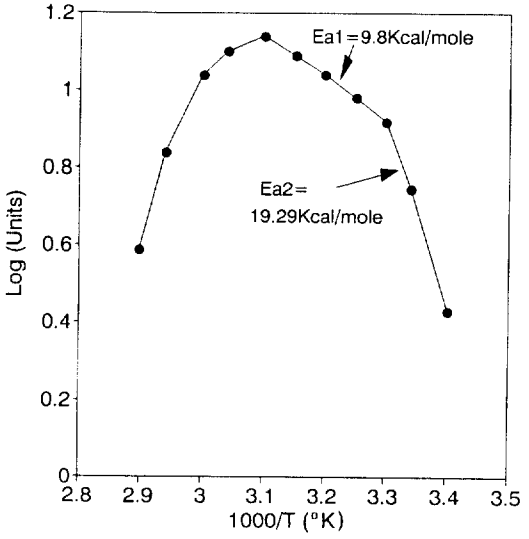

Fig. 7. *Arrhenius plot of xylanase II.*
The optimal temperature of xylanase was measured by incubating the enzyme at the defined temperatures.

cysteine. EDTA did not result in any significant inhibition on enzyme activity. Generally, thiols form complexes of varying stability with a wide variety of metal ions such as Cu^{2+}, Fe^{2+}, Zn^{2+},

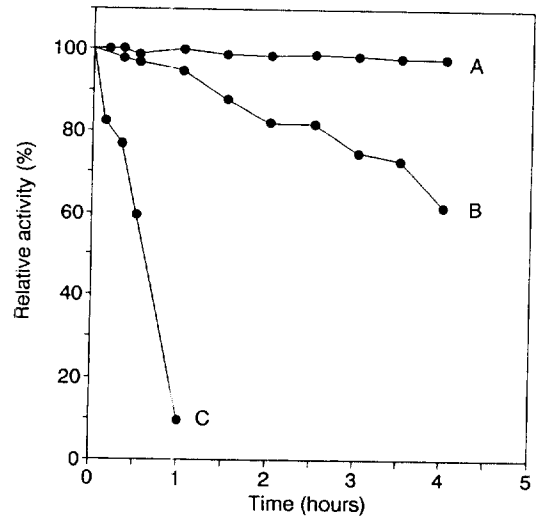

Fig. 8. *Thermal stability of xylanase II.*
The enzyme was preincubated in acetate buffer (pH 5.0) at 50°C (A), 55°C (B) and 60°C (C) in the absence of substrate. The enzyme activity was measured at intervals as described in Materials and Methods section.

Table 1. *Effects of various chemicals on the activity of xylanase II*

Chemicals[a]	Relative activity (%)
None	100
Dithiothreithol	177
$BaCl_2$	132
$AgNO_3$	130
$HgCl_2$ + cysteine[b]	102
$CuSO_4 \cdot 5H_2O$	100
$ZnSO_4 \cdot 7H_2O$	100
Ethylene diamine tetraacetate	98
$CaCl_2$	97
$CoCl_2$	95
$FeSO_4 \cdot 7H_2O$	77
$MnCl_2 \cdot 4H_2O$	77
$MgCl_2 \cdot 6H_2O$	65
$HgCl_2$	33
Sodium dodecylsulfate	24

[a]Each chemical concentration was 10 mM.
[b]The concentration of cysteine was 10 mM.

Mo^{2+}, Mg^{2+}, Hg^{2+} and Ag^{+}. The most stable thiols are inactivated with divalent mercury, Hg^{2+}. However, Ag^{+} and Zn^{2+} did not show inhibitory effect on xylanase II. The inhibition of enzyme activity by Hg^{2+} was similar to that observed for other xylanases (20, 25, 26). The inactivation by Hg^{2+} and reactivation by cysteine reveals that thiol-containing amino acids may be involved in

Table 2. *Activity of xylanase II against various substrates.*

Substrate	Relative activity (%)
Oat-spelt xylan	100
Carboxymethyl cellulose	29.18
Avicel	35.13
Laminarin	2.73
Polygalacturonic acid	8.52
Soluble starch	18.40
p-Nitrophenyl-β-D-xyloside	0
p-Nitrophenyl-β-D-glucoside	0

the active site of the enzyme.

Substrate specificity

The ability of the xylanase to hydrolyze various substrates was examined (Table 2). The enzyme, in addition to degrading xylan with relatively high activity, hydrolyzed CMC and Avicel. Xylanase II also showed slight activity toward soluble starch, polygalacturonic acid and laminarin, but no activity toward PNPX and PNPG. Thus they had no xylosidase and glucosidase activities. Since the chemical structure of the xylosyl residues in xylan differs only slightly from that of the glucosyl residues in cellulose, the enzyme would attack both substrates. Such enzymes have been described from *Trichoderma reesei* (17), *Sporotrichum dimorphosporum* (5, 6), *Aspergillus niger* (10), *Irpex lacteus* (13) and *Clostridium acetobutylicum* (18).

Kinetic properties

K_m and V_{max} values of the enzyme for several substrates were determined using Lineweaver-Burk plot. K_m and V_{max} values of xylanase II for oat-spelt xylan, larchwood xylan and Avicel were 7.48, 1.98 and 13.33 mg/ml; 44.2, 35.9 and 12.2 units/mg protein. K_m values of the enzyme for oat-spelt xylan and larchwood xylan were 1.8 and 6.7 times higher than that for Avicel, respectively.

Identification of reaction products

The time course of hydrolysis of oat-spelt and larchwood xylan by the enzyme, and the hydrolysis products are shown in Fig. 9 and 10. Xylanase II degraded 27.5 and 30% of oat-spelt and larchwood xylan, respectively. The end products of oat-spelt xylan after 96 h-incubation were xylose (X_1), xylobiose (X_2), xylotriose (X_3) and arabinoxylotriose (AX_3), while the end products of larchwood xylan were xylose, xylobiose, xylotriose and small amount of higher oligomers. Any oligosaccharide product containing more than one arabinose residue (e.g., A_2X_3) is not present. The result shows that there are no instances where L-arabinose substituents occur in contiguous D-xylose residues of the D-xylan backbone. The enzyme did not release arabinose from the arabinoxylan. According to Dekker's

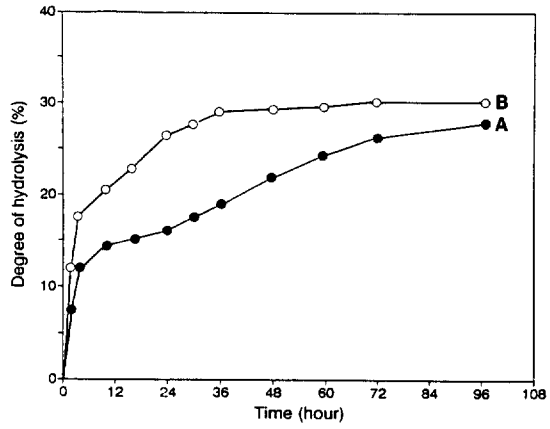

Fig. 9. *Time course of hydrolysis of oat-spelt (A) and larchwood xylan (B) by xylanase II.*
The reaction mixture contained 10 ml of each xylan (1% wt/v), 50 mM sodium acetate buffer (pH 5.0), and 0.8 μg of enzyme protein. At the times indicated, aliquots were taken and the degree of hydrolysis was estimated from the liberation of reducing sugars.

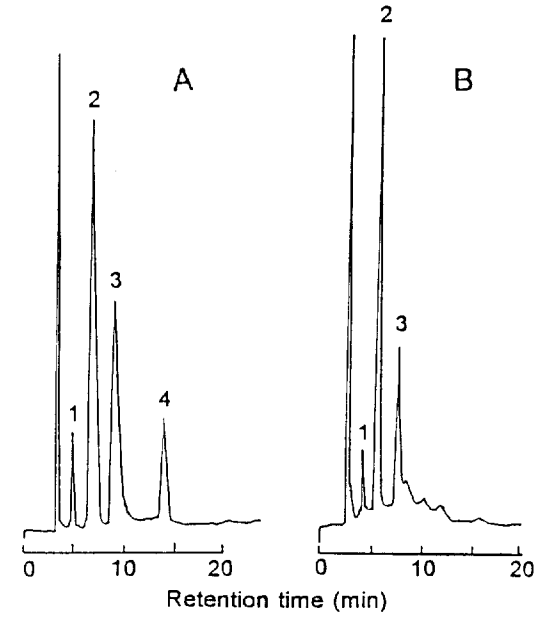

Fig. 10. *HPLC of hydrolysis products of oat-spelt (A) and larchwood xylan (B) by xylanase II.*
The reaction mixture was sampled after 96 h hydrolysis in the experiment shown in Fig. 9. 1, Xylose; 2, xylobiose; 3, xylotriose; 4, arabinoxylotriose.

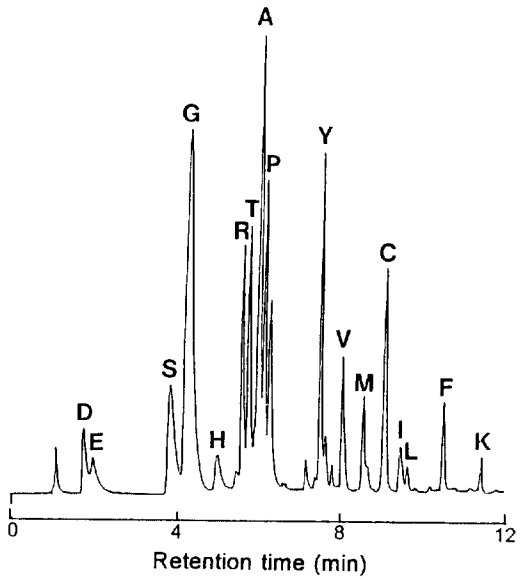

Fig. 11. *HPLC analysis of amino acid composition of xylanase II.*
Abb.: D, Asp; E, Glu; S, Ser; G, Gly; H, His; R, Arg; T, Thr; A, Ala; P, Pro; Y, Tyr; V, Val; M, Met; C, Cys; I, Ile; L, Leu; F, Phe; K, Lys.

classification of xylanases (8), this enzyme belongs to the group of "non-arabinose-liberating-xylanases". The nondebranching group of xylanases are by far the most common and degrade heteroxylans randomly (8). Such enzymes have been reported for fungal xylanases from *Aspergillus niger* (10), *Sporotrichum dimorphosporum* (5) and *Ceratocystis paradoxa* (9). The results on the hydrolysis of oat-spelt and larchwood xylan certainly indicate that the enzyme is endo-xylanase.

Amino acid composition

The amino acid composition of xylanase II was analyzed by HPLC (Fig. 11). The number of amino acid residues per molecule of the enzyme was calculated based on a molecular weight of 21,000 and from the average contents of amino acids (Table 3). Amino acid composition of a xylanase from *T. viride* (29) was compared with the present enzyme. Xylanase II has some resemblance to the xylanase from *T. viride*, in that both enzymes have similar molecular weight and high isoelectric point. Their amino acid composition shows a high percentage of serine and glycine, although xylanase II has lower aspartic acid and tyrosine, and higher alanine and cysteine than that of *T. viride*. The enzyme showed similarity to xylanases from *A. niger* (10), *Schizophyllum commune* (21) and *Bacillus subtilis*

Table 3. *Amino acid composition of xylanases from T. koningii and T. viride.*

Amino acid residue	*T. koningii* xylanase II Residue per 21,000 (%)	*T. viride* xylanase Residue per 22,000
Asp+Asn	11 (6.3)	23
Glu+Gln	7 (4.0)	10
Ser	19 (16.7)	24
Gly	35 (20.1)	28
His	2 (1.2)	4
Arg	5 (2.9)	6
Thr	13 (7.5)	17
Ala	14 (8.0)	7
Pro	7 (4.0)	6
Tyr	8 (4.6)	18
Val	10 (5.7)	13
Met	4 (2.3)	1
Cys	14 (8.0)	0
Ile	5 (2.9)	10
Leu	1 (0.6)	5
Phe	4 (2.3)	7
Lys	5 (2.9)	4
Total	174 (100)	189

(2) in having a high content of serine and glycine.

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REFERENCES

1. **Baker, C.J., C.H. Whalen, and D.F. Bateman,** 1977. Xylanase from *Trichoderma pseudokoningii*: Purification, characterization, and effects on isolated plant cell walls. *Phytopathology* **67**, 1250-1258.
2. **Bernier, R., M. Desrochers, L. Jurasek, and M.G. Paice,** 1983. Isolation and characterization of a xylanase from *Bacillus subtilis*. *Appl. Environ. Microbiol.* **46**, 511-514.
3. **Biely, P., Z. Kratky, M. Vrsanska, and D. Urmanicova,** 1980. Induction and inducers of endo-1,4-β-xylanase in the yeast *Cryptococcus albidus*. *Eur. J. Biochem.* **108**, 323-329.
4. **Bradford, M.M.,** 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the priciples of protein dye binding. *Anal. Chem.* **72**, 248-254.
5. **Comtat, J.,** 1983. Isolation, properities and postulated role of some of the xylanases from the basidiomycete *Sporotrichum dimorphosporum*.

Carbohydr. Res. **118**, 215-231.
6. **Comtat, J. and J.P. Joseleau,** 1981. Mode of action of a xylanase and its significance for the structural investigation of the branched L-arabino-D-glucurono-D-xylan from redwood (*Sequoia sempervirens*). *Carbohydr. Res.* **95**, 101-112.
7. **Dekker, R.F.H.,** 1983. Bioconversion of hemicellulose: aspects of hemicellulase production by *Trichoderma reesei* QM 9414 and enzymic saccharification of hemicellulose. *Biotechnol. Bioeng.* **25**, 1127-1146.
8. **Dekker, R.F.H.,** 1985. Biodegradation of the hemicelluloses, p. 505-533. *In*: T. Higuchi (ed), Biosynthesis and biodegradation of wood components. Academic Press, Tokyo.
9. **Dekker, R.F.H. and G.N. Richards,** 1975. Purification, properties, and mode of action of hemicellulase II produced by *Ceratocystis paradoxa. Carbohydr. Res.* **42**, 107-123.
10. **Fournier, A.R., M.M. Frederick, J.R. Frederick, and P.J. Reilly,** 1985. Purification and characterization of endo-xylanases from *Aspergillus niger*, III. An enzyme of pI 3.65. *Biotechnol. Bioeng.* **27**, 539-546.
11. **Grabski, A.C. and T.W. Jeffries,** 1991. Production, purification and characterization of β-(1-4)-endoxylanase of *Streptomyces roseiscleroticus. Appl. Environ. Microbiol.* **57**, 987-992.
12. **John, M., B. Schmidt, and J. Schmidt,** 1979. Purification and some properties of five endo-1,4-β-D-xylanases and a β-xylosidase produced by a strain of *Aspergillus niger. Can. J. Biochem.* **57**, 125-134.
13. **Kanda, T., Y. Amano, and K. Nisizawa,** 1985. Purification and properties of two endo-1,4-β-xylanases from *Irpex lacteus* (*Polyporus tulipiferae*). *J. Biochem.* **98**, 1545-1554.
14. **Kim, H.J., S.-O. Kang, and Y.C. Hah,** 1993. Purification and characterization of xylanase I from *Trichoderma koningii* ATCC 26113. *Kor. Jour. Microbiol.* **31**(1), 63-71.
15. **Kluepfel, D., S. Mehta, F. Aumont, F. Shareck, and R. Morosoli,** 1990. Purification and characterization of a new xylanase (xylanase B) produced by *Streptomyces lividans* 66. *Biochem. J.* **267**, 45-50.
16. **Laemmli, U.K.,** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* (London) **227**, 680-685.
17. **Lappalainen, A.,** 1986. Purification and characterization of xylanolytic enzymes from *Trichoderma reesei. Biotechnol. Appl. Biochem.* **8**, 437-448.
18. **Lee, S.F., C.W. Forsberg, and J.B. Rattray,** 1987. Purification and characterization of two endo-xylanases from *Clostridium acetobutylicum* ATCC 824. *Appl. Environ. Microbiol.* **53**, 644-650.
19. **Mandels, M. and D. Sternberg,** 1976. Recent advances in cellulase technology. *J. Ferment. Technol.* **54**, 267-286.
20. **Nakanishi, K., H. Arai, and T. Yasui,** 1984. Purification and some properties of xylanase from *Cryptococcus flavus. J. Ferment. Technol.* **62**, 361-369.
21. **Paice, M.G., L. Jurasek, M.R. Carpenter, and L.B. Smillie,** 1978. Production, characterization, and partial amino acid sequence of xylanase A from *Schizophyllum commune. Appl. Environ. Microbiol.* **36**, 802-808.
22. **Royer, J.C. and J.P. Nakas,** 1989. Xylanase production by *Trichoderma longibrachiatum. Enzyme Microb. Technol.* **11**, 405-410.
23. **Shei, J.C., A.R. Fratzke, M.M. Frederick, J.R. Frederick, and P.J. Reilly,** 1985. Purification and characterization of endo-xylanases from *Aspergillus niger*, II. An enzyme of pI 4.5. *Biotechnol. Bioeng.* **27**, 533-538.
24. **Somogyi, M.,** 1952. Note on sugar determination. *J. Biol. Chem.* **195**, 19-23.
25. **Takahashi, M. and S. Kutsumi,** 1979. Purification and properties of xylanase from *Gliocladium virens. J. Ferment Technol.* **57**, 434-439.
26. **Tan, L.U.L., P. Mayers, and J.N. Saddler,** 1987. Purification and characterization of a thermostable xylanase from a thermophilic fungus *Thermoascus aurantiacus. Can. J. Microbiol.* **33**, 689-692.
27. **Tan, L.U.L., K.K.Y. Wong, and J.N. Saddler,** 1985. Functional characteristics of two D-xylanases purified from *Trichoderma harzianum. Enzyme Microb. Technol.* **7**, 431-436.
28. **Tsujibo, H., K. Miyamoto, T. Kuda, K. Minami, T. Sukamoto, T. Hasegawa, and Y. Inamori,** 1992. Purification, properties and partial amino acid sequences of thermostable xylanases from *Streptomyces thermoviolaceus* OPC-520. *Appl. Environ. Microbiol.* **58**, 371-375.
29. **Ujiie, M., C. Roy, and M. Yaguchi,** 1991. Low-molecular weight xylanase from *Trichoderma viride. Appl. Environ. Microbiol.* **57**, 1860-1862.
30. **Wood, T.M. and S.I. McCrae,** 1986. Studies of two low-molecular weight endo-(1-4)-β-D-xylanases constitutively synthesised by the cellulolytic fungus *Trichoderma koningii. Carbohydr. Res.* **148**, 321-330.

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초 록: *Trichoderma koningii* ATCC 26113으로부터 Xylanase II의 순수분리 및 특성

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Trichoderma koningii ATCC 26113의 배양액으로부터 1,4-β-D-xylanase(xylanase II)를 순수분리하였다. 본 효소의 분자량은 21,000, 등전점은 9.4이었다. 효소의 반응에 있어 최적 pH는 5.0이었으며 최적 온도는 50°C이었다. 열안정성 조사에서 50°C까지의 온도에서 안정하였으나 60°C에서 30분과 60분 반응 후 각각 그 활성도의 40%와 90%를 손실하였다. 본 효소는 xylan 뿐 아니라 carboxy methyl cellulose와 Avicel에 대한 활성도 보여주었다. Oat-spelt xylan, larchwood xylan 및 Avicel에 대한 효소의 K_m값은 7.48, 1.98, 13.33 mg/ml이었다. Xylanase II에 의한 oat-spelt xylan의 분해 산물은 xylose, xylobiose, xylotriose 및 arabinoxylotriose이었으며 larchwood xylan의 분해 산물은 xylose, xylobiose, xylotriose 및 소량의 다당류이었다. 기질에 대한 효소의 작용 양상은 xylanase II가 endo 효소임을 시사해준다.