Purification and Properties of Three Types of Xylanases Induced by Methyl β -Xyloside from *Streptomyces* sp.

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When mycelia of *Streptomyces* sp. No. 3137 were cultivated in a medium containing methyl β -xyloside, xylanases (EC 3.2.1.8) were inductively produced into the medium. Three types of enzyme from the culture filtrate have been purified by ultrafiltration with DIAFLO UM-10, chromatography on DEAE-Sephadex A-25, gel filtration on Bio Gel P-100, and isoelectric focusing with Servalyt 6~8 or 9~11. The three purified enzymes, tentatively named X-I, X-II-A, and X-II-B, were homogeneous by polyacrylamide gel electrophoresis at pH 4.3. The molecular weight of X-I was about 50,000 by SDS-polyacrylamide gel electrophoresis or gel filtration on Bio Gel P-100. The molecular weight of X-II-A and X-II-B were both approximately 25,000 by SDS-polyacrylamide gel electrophoresis and that of X-II-B was 25,680 by the sedimentation-equilibrium method. X-I had an isoelectric point at 7.10, and X-II-A and X-II-B had different isoelectric points, 10.06 and 10.26, respectively. The three enzymes were optimally active at 60~65°C and stable to 55°C. The optimal pH of X-I, X-II-A, and X-II-B were pH 5.5~6.5, 5.0~6.0, and 5.0~6.0, respectively. The ranges of two X-II's pH stability (pH 1.5~11.5) were wider than that of X-I's (pH 3.0~10.5). These purified preparations hydrolyzed xylotriase, xyloetraose, and xylan but not xylobiose, cellobiose, maltose, carboxymethyl cellulose, or soluble starch. Their actions were inhibited by Hg^{2+} and Fe^{3+} ions, sodium dodecyl sulfate, and *N*-bromosuccinimide.

There are many kinds of microorganisms which can produce xylanase (EC 3.2.1.8. β -1,4-xylan xylanohydrolase) in the presence of xylan as a carbon source.¹⁾ It has been reported that the enzyme system of *Streptomyces*^{2~6)} except *Streptomyces ostreogriseus*⁷⁾ consists of one xylanase. We found that the xylanases of *Streptomyces* sp. No. 3137 are produced in the presence of an inducer⁸⁾ and β -xylosides are effective and non-metabolizable inducers for the xylanase production of this microorganism.^{9~11)} The properties of xylanases induced by xylan have been investigated,¹²⁾ however, there has been no report about those xylanases that are produced by certain inducers, not by xylan. It is expected that the xylanase produced by induction is of such high purity that the purification should be more convenient. In this report the purification and

properties of xylanases induced by methyl β -xyloside have been detailed.

MATERIALS AND METHODS

Microorganisms. *Streptomyces* sp. No. 3137 isolated from soil⁸⁾ was used through this experiment. The microorganism was grown on Bennett agar¹³⁾ (0.1% glucose, 0.2% NZ Amine, 0.1% yeast extract, 0.1% beef extract, and 2.0% agar) and stored until use.

Materials. Xylan was "Sanpearl," a by-product from the mercerization process in pulp manufacturing, supplied by Sanyo Kokusaku Pulp Co., Ltd. Xylooligosaccharides were prepared from a xylan hydrolyzate by *Streptomyces* xylanase by the method of Kusakabe *et al.*^{14,15)} Methyl β -xyloside was synthesized from xylose and methanol using a cationic resin as catalyst by the method of Bollenback.¹⁶⁾ DEAE-Sephadex A-25 was a product of Pharmacia Fine Chemicals Inc., Uppsala, Sweden. Bio Gel P-100 was purchased from Bio-Rad Laboratory, California, U.S.A. Servalyt 6~8 and 9~11 as carrier ampholytes for isoelec-

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Abbreviations: SDS, sodium dodecyl sulfate; EDTA, ethylenediamine tetraacetic acid.

tric focusing were products of Serva Feinbiochemica GmbH and Co., F. R. Germany. Corn Steep Liquor (C.S.L.) was a gift from Nippon Shokuhinkako Co., Ltd. bovine serum albumin was a product of Wako Pure Chemical Industries, Ltd. The other chemicals used were of reagent grade and commercially available.

Xylanase production. One liter of the 1st medium contained 30 g of glucose, 3 g of urea, 1 g of yeast extract, 10 g of KH_2PO_4 , 0.5 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 5 g of C.S.L. One loopful of spores from a Bennett agar slope was inoculated into a 500-ml shake flask containing 100 ml of the 1st medium, and the flask was incubated on a shaker at 120 strokes per minute at 36°C for 40 hr. Mycelia obtained from the 1st culture were put into a 500-ml shake flask with 100 ml of the 2nd medium containing 0.3 g of methyl β -xyloside, 0.2 g of KH_2PO_4 , and 0.01 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. The conditions of incubation were the same as that of the 1st culture except for the time, 24 hr.

Xylanase assay. Xylanase activity was measured by incubating 0.2 ml of diluted enzyme solution with 1.0 ml of 2% xylan solution and 0.8 ml of McIlvaine buffer (pH 5.5) for 10 min at 55°C, and measuring the released reducing sugar by the method of Somogyi.¹⁷⁾ Xylan used for the determination of enzyme activity was prepared as follows: Xylan was dissolved into 5% NaOH and precipitated with addition of an equal volume of ethanol. The precipitate was collected by centrifugation and suspended to water. The suspension was adjusted with acetic acid at pH 5 and washed with ethanol and acetone. After drying, the xylan was powdered by a mortar and pestle. One unit of enzyme activity was defined as the amount causing production of 1 μmol of reducing sugar as a xylose equivalent per minute, under optimal conditions of measurement.*¹

Protein estimation. Protein was measured by the method of Lowry *et al.*¹⁸⁾ using bovine serum albumin as the standard, or from the absorbance at 280 nm.

Carbohydrate estimation. The carbohydrate content of xylanases was measured by the phenol-sulfuric acid method¹⁹⁾ and the orcinol- Fe^{3+} method.²⁰⁾

Slab polyacrylamide gelelectrophoresis. A polyacrylamide gel was prepared by the method of Reisfeld *et al.*²¹⁾ The "pH 4.3 ~ 10%" gel was set in a slab (50 $\times$ 75 $\times$ 1 mm). The electrophoresis was done for 45 min at 15 mA and room temperature with a KS-8010MSE apparatus, Marysol Co., Ltd., Japan, and the gel was stained with a solution of 0.25% Coomassie Brilliant Blue R-250-acetic acid-methanol (5:1:5 by volume) to locate the protein bands.

Sedimentation analysis. The sedimentation-equilibrium was done with an ultracentrifugal machine (MSE

Centriscan 75II, MSE, England). The centrifugation was 13,000 rpm for 24 hr and the system came to an equilibrium state. By measuring the concentration of the protein at 280 nm as a function of the distance from the center of rotation, the molecular weight can be calculated.

Isoelectric focusing. The isoelectric focusing procedure followed that of Matsuo and Horio,²²⁾ with 110-ml columns (LKB Produkter AB, Bromma, Sweden). Servalyt 6~8 or 9~11 ampholyte were employed. The focusings were done for 48 hr at 900 V and 4°C, and fractions of 2.0 ml were removed from the bottom of the column in the usual manner.²²⁾

Hydrolysis of xylooligosaccharides. Ten units of enzyme in 1 ml of McIlvaine buffer (pH 5.5) containing 1% xylooligosaccharides was incubated at 35°C, and aliquots of the incubation mixtures were withdrawn at 0, 1, 3, 6, and 12 hr and were kept in a boiling water bath for 1 min to stop the enzyme reactions. The sugars were identified by thin layer chromatography. Samples of hydrolyzates were spotted on cellulose plates (TLC plastic sheets cellulose F254, Merck) and developed by the ascending technique in a solvent system, *n*-butanol-pyridine-water (6:4:3 by volume) at room temperature. Sugars were detected with silver nitrate-sodium hydroxide reagent.²³⁾

RESULTS

Xylanase purification

All operations were done below 5°C. The purification was summarized in Table I.

Step 1. Ultrafiltration with DIAFLO UM-10. The second culture filtrate (2500 ml) was concentrated to one-tenth of the original volume (250 ml) by ultrafiltration with DIAFLO UM-10.

Step 2. Chromatography on DEAE-Sephadex A-25. The concentrated filtrate (250 ml) was put on a DEAE-Sephadex A-25 column (2 $\times$ 60 cm) previously buffered with McIlvaine buffer (pH 5.5). The effluent (300 ml) was decolorized by this column and then was concentrated to 30 ml by an evaporator under reduced pressure at 35°C.

Step 3. Gel filtration on Bio Gel P-100. The concentrated effluent (10 ml) was put on a Bio Gel P-100 column (2 $\times$ 90 cm) previously buffered with McIlvaine buffer (pH 5.5). Filtration was done with the same buffer at 4°C at

*¹ One unit by this assay method corresponds to 1.5 units by the method described in our previous paper.⁸⁾

TABLE I. PURIFICATION OF XYLANASES FROM *Streptomyces* sp. No. 3137

Step	Volume (ml)	Protein (mg)	Activity (units) $\times 10^3$	Specific activity (units/mg)	Yield (%)
Culture filtrate	2500	4900	200	41	100
UM-10	250	2700	180	67	90
DEAE-Sephadex A-25	300	2100	168	80	84
Bio Gel P-100					
X-I	150	340	46	135	23
X-II	130	460	66	143	33
(sum)					(56)
Isoelectric focusing					
X-I		260	42	161	21
X-II-A		132	14	106	7
X-II-B		290	45	155	23
(sum)					(51)

FIG. 1. Bio Gel P-100 Column Chromatography.

The concentrated effluent from DEAE-Sephadex A-25 column chromatography (10 ml) was put on a Bio Gel P-100 column (2 $\times$ 90 cm) equilibrated with ten times diluted McIlvaine buffer (pH 5.5). The flow rate was 10 ml/hr and fractions of 5 ml were collected. Symbols: $\bigcirc$ — $\bigcirc$, xylanase activity; $\bullet$ — $\bullet$, absorbance at 280 nm.

the rate of 7 ml/hr. Xylanase activities were recovered in two fractions, tubes 20~29 and 59~84 (5 ml per tube), which were named X-I and X-II, respectively (Fig. 1). The two fractions were separately dialyzed against distilled water and concentrated to 1 mg of protein/ml by an evaporator under reduced pressure at 35°C.

Step 4. Isoelectric focusing with Servalyt 6~8 or 9~11. The concentrated X-I or X-II (10 ml) was put on a column of 110-ml capacity for isoelectric focusing. The pattern of X-I using Servalyt 6~8 is shown in Fig. 2. Xylanase activity was recovered in one fraction, which

FIG. 2. Isoelectric Focusing of X-I with Servalyt 6~8.

The dialyzed and concentrated X-I fraction (about 10 ml) was put on a 110-ml column containing 1% carrier ampholyte pH 6~8 (Servalyt 6~8). The voltage was adjusted to 900 V. After this was focused for 48 hr at 4°C, fractions of 2 ml were collected. Symbols: $\bigcirc$ — $\bigcirc$, xylanase activity; $\bullet$ — $\bullet$, absorbance at 280 nm; — —, pH.

was named X-I, and the only peak of protein was in the same fraction. The isoelectric point of X-I was 7.10. The pattern of X-II using Servalyt 9~11 showed two fractions of xylanase activity (Fig. 3). Their isoelectric points were 10.06 and 10.26, which were named X-II-A and X-II-B, respectively. The activity of X-II-B was three times as great as that of X-II-A, and the specific activity of X-II-B was one

FIG. 3. Isoelectric Focusing of X-II with Servalyt 9~11. The dialyzed and concentrated X-II fraction (about 10 mg) was put on a 110-ml column containing 1% carrier ampholyte pH 9~11 (Servalyt 9~11). The voltage was adjusted to 900 V. After this was focused for 48 hr at 4°C, fractions of 2 ml were collected. Symbols: ○—○, xylanase activity; ●—●, absorbance at 280 nm; ———, pH.

FIG. 4. Slab Electrophoretic Pattern of Xylanase Preparation in Each Purification Step.

Proteins (5~10 μ g) were put on a slab (50 $\times$ 75 $\times$ 1 mm) of 10% polyacrylamide gel and allowed to migrate in β -alanine-acetic acid buffer (pH 4.3) at 15 mA for 45 min. Proteins were stained with a solution of 0.25% Coomassie Brilliant Blue R-250-acetic acid-methanol (5:1:5 by volume). Symbols: A, Culture filtrate; B, Effluent of DEAE-Sephadex A-25 chromatography; C, X-I fraction of Bio Gel P-100 gel filtration; D, X-II fraction of Bio Gel P-100 gel filtration; E, X-I fraction of isoelectric focusing with Servalyt 6~8; F, X-II-A fraction of isoelectric focusing with Servalyt 9~11; G, X-II-B fraction of isoelectric focusing with Servalyt 9~11.

and a half times that of X-II-A. The contaminating carrier ampholytes in the three fractions were removed by salting out with am-

FIG. 5. Measurement of Molecular Weight of Xylanases.

Samples containing 0.01 M phosphate buffer (pH 7.2), 1% SDS, 5% 2-mercaptoethanol, 25% glycerol, and 0.1% protein were left for 5 min in boiling water. Then samples of 5~10 μ l were put on a slab (50 $\times$ 75 $\times$ 1 mm) of 10% polyacrylamide gel and allowed to migrate in 0.1 M phosphate buffer (pH 7.2) containing 0.1% SDS at 15 mA for 7 hr. Proteins were stained with a solution of 0.25% Coomassie Brilliant Blue R-250-acetic acid-methanol (5:1:5 by volume). Symbols and molecular weights of standard proteins: 1, RNA polymerase from *E. coli* β' -subunit (mol wt, 165,000); 2, RNA polymerase from *E. coli* β -subunit (155,000); 3, bovine serum albumin (68,000); 4, ovalbumin (45,000); 5, RNA polymerase from *E. coli* α -subunit (39,000); 6, chymotrypsinogen A (25,000); 7, trypsin inhibitor (21,500); 8, cytochrome *c* (12,500).

monium sulfate (70% saturation). After that the enzyme solutions were dialyzed against distilled water with cellulose tubes. Slab polyacrylamide gel electrophoresis of the purified xylanases gave a single protein band in the gel system "pH 4.3~10%" (Fig. 4).

Molecular weight

The molecular weights of X-I, X-II-A, and X-II-B were found by SDS-polyacrylamide gel electrophoresis (Fig. 5). They were estimated to be approximately 50,000, 25,000 and 25,000, respectively. The molecular weight of X-I estimated by gel filtration on Bio Gel P-100 column was also approximately 50,000. The molecular weight determination of X-II-A and X-II-B by gel filtration failed because of interaction between the enzymes and the gel. The molecular weight of X-II-B calculated from the sedimentation analysis was 25,680.

Carbohydrate content

The carbohydrate contents in X-I, X-II-A

FIG. 6. Effects of Temperature on Xylanase Activities. The reaction mixtures containing 1.0 ml of 2% xylan, 0.8 ml of McIlvaine buffer (pH 5.5), and 0.2 ml of enzyme solution (10 units) were incubated at various temperatures for 10 min. Symbols: $\triangle$ — $\triangle$, X-I; $\bullet$ — $\bullet$, X-II-A; $\circ$ — $\circ$, X-II-B.

FIG. 7. Thermal Stabilities of Xylanases.

Enzyme solutions (100 units/ml), pH 5.5, were incubated for 30 min at various temperatures and then the remaining activities were measured by the standard assay method. Symbols: $\triangle$ — $\triangle$, X-I; $\bullet$ — $\bullet$, X-II-A; $\circ$ — $\circ$, X-II-B.

and X-II-B were less than 1% (carbohydrate/protein, w/w) as glucose by the phenol-sulfuric acid method¹⁹⁾ and under 0.5% (w/w) as xylose by the orcinol- Fe^{3+} method.²⁰⁾

Effects of Temperature

The activity of the enzymes was measured at various temperatures from 30 to 75°C for 10 min at pH 5.5. The optimal temperatures of X-I, X-II-A, and X-II-B were 60~65°C (Fig. 6). The enzymes were incubated at various temperatures for 30 min at pH 5.5, and after that the remaining activities were measured. The enzymes were stable up to 55°C (Fig. 7).

FIG. 8. Effects of pH on Xylanase Activities.

The reaction mixtures containing 1.0 ml of 2% xylan solution (10 units) were incubated for 10 min at 55°C. The buffers used were 0.1 M hydrochloric acid-sodium acetate buffer (pH 1.0~4.5), 0.1 M acetate buffer (pH 4.0~6.5), McIlvaine buffer (pH 4.0~8.0), 0.1 M phosphate buffer (pH 6.0~8.5), 0.1 M ammonium hydroxide-ammonium chloride buffer (pH 7.5~10.0) and 0.1 M glycine buffer (pH 9.5~12.5). Symbols: $\triangle$ — $\triangle$, X-I; $\bullet$ — $\bullet$, X-II-A; $\circ$ — $\circ$, X-II-B.

FIG. 9. pH-Stabilities of Xylanases.

Enzyme solutions (100 units/ml) of various pH values were incubated for 30 min at 35°C, and then adjusted to pH 5.5, and residual activities were measured by the standard assay method. The buffers used were 0.1 M hydrochloric acid-sodium acetate buffer (pH 1.0~4.5), 0.1 M acetate buffer (pH 4.0~6.5), McIlvaine buffer (pH 4.0~8.0), 0.1 M phosphate buffer (pH 6.0~8.5), 0.1 M ammonia water-ammonium chloride buffer (pH 7.5~10.0) and 0.1 M glycine buffer (pH 9.5~12.5). Symbols: $\triangle$ — $\triangle$, X-I; $\bullet$ — $\bullet$, X-II-A; $\circ$ — $\circ$, X-II-B.

Effects of pH

The enzyme activity was measured at various pH from 1.0 to 12.5 at 55°C with a pH meter (Horiba pH meter M-7). The results were shown in Fig. 8. The optimal pH of X-I was pH 5.5~6.5 and those of X-II-A and X-II-B were both pH 5.0~6.0. X-I had almost no

activity under pH 3.0 but X-II-A and X-II-B had 20~30% of their maximum activities at pH 2.0~3.0. In the range of pH 9.0~11.0 the activity of X-I was about twice as much as those of X-II-A and X-II-B. For examinations of pH stability, the enzymes were incubated at

various pH for 30 min at 35°C, and after that the remaining activities were assayed. X-I and two X-IIs were stable at pH 3.0~10.5 and 1.5~11.5, respectively (Fig. 9).

Effects of chemicals

The effects of metal ions, EDTA, SDS, *N*-bromosuccinimide, and *p*-chloromercuribenzoate on xylanases were shown in Table II. Hg^{2+} and Fe^{3+} ions, SDS and *N*-bromosuccinimide inhibited the activities of all the three xylanases. X-I was greatly inhibited by 5 mM Hg^{2+} ion or *N*-bromosuccinimide.

Substrate specificity

Substrates were incubated with the enzyme (6 units for xylan, xylo-tetraose, and xylo-triose, or 100 units for xylobiose, cellobiose, maltose, soluble starch, and CM-cellulose) and McIlvaine buffer (pH 5.5) for 1 hr at 40°C. Increased reducing sugar was measured by Somogyi's method. Activities were expressed as μg of reducing sugar per unit of enzyme per minute. As shown in Table III, xylobiose, cellobiose, maltose, soluble starch, and CM-cellulose were not attacked by the purified xylanase preparations. Xylan was hydrolyzed most easily and xylo-tetraose was done more easily than xylo-triose.

The patterns of thin layer chromatography

TABLE II. EFFECTS OF VARIOUS CHEMICALS ON THE ACTIVITY OF XYLANASES

The enzyme activities were measured by the standard assay method. The activity is expressed as a percentage of the activity level in the absence of chemicals.

Reagents (5×10^{-3} M)	X-I	X-II-A	X-II-B
None	100	100	100
FeCl_3	26	25	25
HgCl_2	5	40	42
CuCl_2	64	65	69
BaCl_2	96	100	104
CaCl_2	92	90	88
MgCl_2	93	107	106
MnCl_2	85	85	84
NiCl_2	83	90	91
SnCl_2	75	75	74
ZnCl_2	86	82	83
KCl	96	100	101
NaCl	85	101	103
LiCl	106	105	107
KMnO_4	85	81	82
EDTA	98	77	76
SDS	24	40	43
NBS	5	10	10
PCMB (1×10^{-4} M)	91	70	69

TABLE III. HYDROLYSIS OF SUBSTRATES BY XYLANASES

Substrate solutions (1 ml) were incubated with 0.2 ml of the xylanase (6 units for xylan, xylo-tetraose, and xylo-triose, or 100 units for xylobiose, cellobiose, maltose, soluble starch, and CM-cellulose) and 0.8 ml of McIlvaine buffer (pH 5.5) for 1 hr at 40°C. Activities were expressed as μg of reducing sugar per unit of enzyme per minute.

Substrates	Concentration	Activity (μg of reducing sugar $\cdot$ unit $^{-1}$ min $^{-1}$)		
		X-I	X-II-A	X-II-B
Xylan	1%	37.5	37.5	37.5
Xylo-tetraose	5 mM	11.3	9.0	9.8
Xylo-triose	5 mM	2.6	2.6	2.6
Xylobiose	5 mM	n.d.*	n.d.	n.d.
Cellobiose	5 mM			
Maltose	5 mM			
Soluble starch	1%			
CM-cellulose	1%			

* n.d. = not detected.

FIG. 10. Thin Layer Chromatography of Hydrolyzates of Xylooligosaccharides by Three Xylanases.

The reaction mixtures containing xylanase (10 units/ml) and 0.5% xylobiose, xylotriose, xylootetraose, xylopentaose, or xylan in McIlvaine buffer (pH 5.5) were incubated for 0~12 hr at 35°C. Samples of hydrolyzates were spotted on TLC plastic sheet cellulose, Merck. The solvent system was *n*-butanol-pyridine-water (6:4:3, by volume) at room temperature. Sugars were detected with silver nitrate-sodium hydroxide reagent.²⁵⁾ Symbols: X_1 , xylose; X_2 , xylobiose; X_3 , xylotriose; X_4 , xylootetraose; X_5 , xylopentaose; X_n , xylan; I, xylanase X-I; II-A, xylanase X-II-A; II-B, xylanase X-II-B; S, standard ($X_1 \sim X_5$); 0, incubation time 0 hr; 1, 1 hr; 12, 12 hr.

of xylooligosaccharide hydrolyzates are shown in Fig. 10. X-I, X-II-A, and X-II-B hydrolyzed xylotriose, xylotetraose, xylopentaose, and xylan to xylose and xylobiose, but were not able to attack xylobiose. At 1 hr of incubation time, X-II-A and X-II-B showed xylotriose spots in the hydrolysis of xylotetraose, xylopentaose, and xylan. Both xylose and xylobiose were products by X-I, X-II-A, or X-II-B at 12 hr of incubation time, except for xylobiose as a substrate.

DISCUSSION

A replacement culture method which consisted of a proliferating culture with glucose and an inducing culture with methyl β -xyloside increased the efficiency of xylanase production in *Streptomyces* sp. No. 3137. Since there was only a small amount of unnecessary proteins in the culture filtrate, after the 3 to 4-fold purification of the culture filtrate, each purified enzyme, X-I, X-II-A, and X-II-B, showed a single protein band in polyacrylamide gel electrophoresis. For *Streptomyces xylophagus*,³⁾ *Streptomyces* sp.,^{24,25)} *Aspergillus niger*,²⁶⁾ *Schizophyllum commune*,²⁷⁾ *Termitomyces clypeatus*,²⁸⁾ *Bacillus* sp.,²⁹⁾ *Gliocladium virens*,³⁰⁾ and *Talaromyces byssochlamydoides*,³¹⁾ it needed 7 to 276-fold purifications of culture filtrate to get electrophoretically homogeneous preparations.

Enzyme systems of reported *Streptomyces* mostly were shown to consist of one xylanase,^{3,24,25)} however, there was one report that the enzyme system of *Streptomyces ostreogriseus* consisted of many xylanases.⁷⁾ The xylanase from *Streptomyces ostreogriseus* was absorbed to DEAE-cellulose and a single peak of xylanase activity was obtained from Sephadex G-100 gel filtration. The five xylanase fractions separated by gel electrophoresis were able to hydrolyze xylan to xylooligosaccharides,⁷⁾ however, no other properties were described in the report.⁷⁾

Three xylanases were induced by methyl β -xyloside from *Streptomyces* sp. No. 3137. The isoelectric point of X-I was much like those of

known xylanases (pI 5.7 ± 1.9),^{24,26,31-34)} and the isoelectric points of X-II-A and X-II-B were high values in comparison with those of X-I and the known xylanases. X-I, X-II-A, and X-II-B had wider ranges of pH stability than those of known xylanases (pH $3.9 \pm 1.2 \sim 8.0 \pm 1.5$).^{3,24,26-32)}

There was a strong similarity between X-II-A and X-II-B in chemical, physico-chemical, and enzymatic characters with a single exception of the isoelectric point. X-I was different from X-II-A and X-II-B in many respects, molecular weight, isoelectric point, and pH stability. X-I was twice as large as X-II-A or X-II-B in molecular weight, but it did not seem to be a dimer of X-IIs. The results of molecular weight estimation demonstrated that the xylanases from *Streptomyces* sp. No. 3137 are monomeric enzymes.

The three xylanases had the same ability to hydrolyze xylotriose, xylotetraose, and xylan (Table III). As shown in Fig. 9, they were able to convert xylotriose, xylotetraose, xylopentaose, and xylan to xylose and xylobiose, but not to hydrolyze xylobiose. They were like *Streptomyces* sp. E-86 xylanase,³⁵⁾ and were partially different from *Streptomyces* sp. KT-23 xylanase.²⁵⁾ Transxylosidation reactions were not observed in the three xylanases as well as the E-86 and KT-23 enzymes.

Our studies show that X-II-A and X-II-B were very similar and X-I was obviously different from them in enzymatic properties and protein structure.

It is interesting to compare them with enzyme systems induced by xylan and by methyl β -xyloside, and this will be reported elsewhere.

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REFERENCES

- 1) G. F. H. Dekker and G. N. Richard, *Adv. Carbohydr. Chem.*, **32**, 317 (1976).
- 2) I. Kusakabe, T. Yasui and T. Kobayashi, *Nippon*

- Nôgeikagaku Kaishi*, **43**, 145 (1969).
- 3) H. Iizuka and T. Kawaminami, *Agric. Biol. Chem.*, **29**, 520 (1965).
 - 4) H. Iizuka and T. Kawaminami, *Agric. Biol. Chem.*, **33**, 1257 (1969).
 - 5) T. Kawaminami and H. Iizuka, *Agric. Biol. Chem.*, **33**, 1787 (1969).
 - 6) H. R. Goldschmid and A. S. Perlin, *Can. J. Chem.*, **41**, 2272 (1963).
 - 7) Y. K. Park and M. Toma, *Korean. J. Biochem.*, **14**, 37 (1982).
 - 8) K. Nakanishi, T. Yasui and T. Kobayashi, *J. Ferment. Technol.*, **54**, 813 (1976).
 - 9) K. Nakanishi, T. Yasui and T. Kobayashi, *J. Ferment. Technol.*, **54**, 801 (1976).
 - 10) T. Yasui, K. Nakanishi and T. Kobayashi, *Hakkokogaku*, **58**, 79 (1980).
 - 11) K. Nakanishi and T. Yasui, *Agric. Biol. Chem.*, **44**, 1885 (1980).
 - 12) K. Nakanishi, Doctoral thesis (1980).
 - 13) Society for the study of microbiology, "Experimental Methods in Microbiology," Kodansha, 1975, p. 423.
 - 14) I. Kusakabe, T. Yasui and T. Kobayashi, *Agric. Biol. Chem.*, **39**, 1355 (1975).
 - 15) I. Kusakabe, T. Yasui and T. Kobayashi, *Nippon Nôgeikagaku Kaishi*, **49**, 383 (1975).
 - 16) G. N. Bollenback, "Methods in Carbohydrate Chemistry," Vol. II, ed. by R. L. Whistler and M. L. Wolfrom, Academic Press Inc., New York, 1962, p. 326.
 - 17) M. Somogyi, *J. Biol. Chem.*, **169**, 61 (1945).
 - 18) O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
 - 19) M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith, *Analyt. Chem.*, **28**, 350 (1956).
 - 20) W. R. Fernell and H. K. King, *Analyst*, **78**, 80 (1953).
 - 21) K. A. Reisfeld, U. J. Lewis and D. E. Willians, *Nature*, **195**, 281 (1962).
 - 22) O. Vesterberg, "Methods in Enzymology," Vol. XXII, ed. by W. B. Jakoby, Academic Press Inc., New York, 1971, p. 389.
 - 23) W. E. Trevelyan, D. P. Procter and J. S. Harrison, *Nature*, **166**, 444 (1950).
 - 24) I. Kusakabe, M. Kawaguchi, T. Yasui and T. Kobayashi, *Nippon Nôgeikagaku Kaishi*, **51**, 429 (1977).
 - 25) T. Nakajima, K. Tsukamoto, T. Watanabe, K. Kainuma and K. Matsuda, *J. Ferment. Technol.*, **62**, 269 (1984).
 - 26) I. V. Gorbacheva and N. A. Rodionova, *Biochim. Biophys. Acta*, **484**, 79 (1977).
 - 27) M. G. Praise, L. Jurasek, M. R. Carpenter and L. B. Smillie, *Appl. Environ. Microbiol.*, **36**, 802 (1978).
 - 28) A. K. Ghosh, P. C. Banerjee and S. Sengupta, *Biochim. Biophys. Acta*, **612**, 143 (1980).
 - 29) F. Uchino and T. Nakane, *Agric. Biol. Chem.*, **45**, 1121 (1981).
 - 30) M. Takahashi and S. Kutsumi, *J. Ferment. Technol.*, **57**, 434 (1979).
 - 31) H. Yoshioka, N. Nagato, S. Chavanich, N. Nilubol and S. Hayashida, *Agric. Biol. Chem.*, **45**, 2425 (1981).
 - 32) M. M. Frederick, J. R. Frederick, A. R. Fratzke and P. J. Reilly, *Carbohydr. Res.*, **97**, 87 (1981).
 - 33) R. Esteban, J. R. Villanueva and T. G. Villa, *Can. J. Microbiol.*, **28**, 733 (1982).
 - 34) M. Vrsanska, I. V. Gorbacheva, Z. Kratky and P. Biely, *Biochim. Biophys. Acta*, **704**, 114 (1982).
 - 35) I. Kusakabe, T. Yasui and T. Kobayashi, *Nippon Nôgeikagaku Kaishi*, **51**, 439 (1977).
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