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Purification and Properties of Xylan Degrading Enzymes Produced by *Penicillium expansum* O-385-10 Concerning Spoilage of Apple Fruit

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Extracellular xylan-degrading enzymes (xylanase I, xylanase II and β -xylosidase) were purified as electrophoretically and isoelectrophoretically homogeneous proteins from the culture broth of *Penicillium expansum* O-385-10 concerning the spoilage of apple fruit. The molecular weights of xylanase I, xylanase II and β -xylosidase were 21.0, 40.0 and 91.0 kDa, respectively. The pIs of xylanase I, xylanase II and β -xylosidase were 9.20, 4.30 and 4.20, respectively. Xylanase I produced xylose and several xylooligosaccharides from xylan. On the other hand, xylanase II mainly produced xylose and xylobiose from an early stage of the reaction, and oligosaccharides over xylotriose were almost absent. In the presence of K⁺ ion, the activity of xylanase I increased almost two-fold, while the activity of xylanase II decreased to about 80%. When xylanase I and II were simultaneously incubated with apple fruit, reducing sugar was hardly liberated from the fruit tissue, but the tissue was significantly affected by adding β -xylosidase to this incubation mixture, and a large amount of the sugar was liberated.

Key words: Xylanase, β -Xylosidase, *Penicillium expansum*, Apple fruit

Introduction

Penicillium expansum was found to be responsible for the post harvest spoilage of apples¹⁾. However, there is very little information on the relationship between the components of apple fruit and physiology of the fungus²⁻⁷⁾. *P. expansum* O-385-10 extracellularly produced the xylan degrading enzyme and pectin degrading enzyme in a xylan medium and pectin medium, respectively. It was clarified by electron microscopy that the xylan degrading enzyme played a role in the decomposition of cell walls and the pectin degrading enzyme in the dissociation of the

combination between cells²⁾. From these facts, it has been estimated that the xylan and pectin degrading enzymes play an important role in spoilage process of apple fruit by *P. expansum*. Only one of the pectin degrading enzymes (polygalacturonase) produced by the fungus has been purified until now, and the characteristics have been clarified⁸⁾. However, no reports on the purification and characterization of xylan degrading enzyme have appeared.

This paper deals with the purification and characterization of the xylan degrading enzyme produced by *P. expansum* growing in a medium containing washed wheat bran⁹⁾ as a xylan source. Two xylanases and β -xylosidase

were purified. Their properties were examined, and the action of these purified enzymes on apple fruit was also examined.

Materials and Methods

Microorganism and culture conditions

Penicillium expansum O-385-10 was obtained from the National Institute of Health Sciences (Tokyo, Japan). Stock cultures were maintained on potato dextrose agar (PDA, Difco) slants²⁾. The basal medium for enzyme production contained the following compounds (per liter): KH_2PO_4 , 2 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1 g; $(\text{NH}_4)_2\text{SO}_4$, 5 g; mineral solution, 2 ml; washed wheat bran⁹⁾ as a xylan source, 20 g. The mineral solution had the following composition (per liter): $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.05 g; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.10 g; $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 0.02 g; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.02 g. The pH was adjusted to 5.0 with 1 N NaOH, and the medium was sterilized by autoclaving at 121°C for 20 min. One loop of the stock culture was inoculated on a PDA slant and then incubated at 30°C for a week. After the cultivation, 10 ml of sterilized distilled water was added to the slant and shaken vigorously by a vortex mixer. This suspension (0.2 ml) was inoculated to a 100-ml Erlenmeyer flask containing 30 ml of the basal medium and incubated at 30°C on a rotary shaker (160 rpm) for a week. After the incubation, cells were removed by centrifugation. The resulting supernatant was concentrated to one-eighth volume by a flash evaporator. To the concentrated solution, solid ammonium sulfate was added to give 70% saturation. The precipitate was collected by centrifugation and stored below 4°C until use.

Enzyme assay

Xylanase activity was determined by measuring the amount of the reducing sugar liberated from xylan (Sigma, from oat spelts). The reaction mixture contained 1.0 ml of the purified enzyme or crude enzyme preparation and 3.0 ml of 0.5% xylan in 100 mM sodium acetate buffer (pH 5.0). After 20 min incubation at 30°C, the reducing sugar in the reaction mixture was measured by the Somogyi-Nelson method¹⁰⁾. One unit(U) of xylanase was defined as the amount of enzyme that liberated 1 μmol of xylose per min.

β -Xylosidase activity was measured by a spectrophotometric method with *p*-nitrophenyl

β -D-xylopyranoside (Sigma) as a substrate. The reaction mixture contained 200 μl of the substrate solution (2 mM *p*-nitrophenyl β -D-xylopyranoside in 50 mM sodium phosphate buffer, pH 6.0), and 200 μl of diluted enzyme solution. After 10 min incubation at 30°C, the reaction was stopped by the addition of 1.6 ml of 1 M Na_2CO_3 . The absorbance at 405 nm due to the release of *p*-nitrophenol in the mixture was measured. One unit(U) of β -xylosidase was defined as the amount of enzyme that liberated 1 μmol of *p*-nitrophenol per min in the reaction mixture under these assay conditions.

Purification of xylan degrading enzymes

The 70% saturated ammonium sulfate precipitate as described above was dissolved in a small amount of 10 mM potassium phosphate buffer (pH 7.45). The solution was dialyzed against the same buffer at 4°C overnight. All of the following procedures were carried out at 4°C unless otherwise indicated. The dialysate was centrifuged to remove insoluble substances and then applied on a DEAE-cellulose DE-52 (Whatman) column ($\phi 1.5 \times 40$ cm) equilibrated with 10 mM phosphate buffer (pH 7.45). The column was washed with the phosphate buffer and eluted with a linear gradient of sodium chloride (0 to 500 mM) dissolved in the same buffer. The xylanase activity was divided into the fraction (xylanase I) washed out and fraction (xylanase II) eluted from the column with the gradient buffer. The washout fractions were pooled and lyophilized. The lyophilized preparation was dissolved in a small amount of 10 mM sodium acetate buffer (pH 5.0), and applied on a CM-cellulose 52 (Whatman) column ($\phi 1.5 \times 40$ cm) equilibrated with the same buffer. The column was washed with 400 ml of the above buffer and eluted with a 1,000 ml linear gradient of sodium chloride (0 to 500 mM) in the acetate buffer at a flow rate of 10 ml/hr. The active fractions were pooled and lyophilized. The lyophilized preparation was dissolved in a small amount of 10 mM acetate buffer (pH 5.0), and applied on a Sephadex G-100 (Pharmacia) column ($\phi 1.6 \times 70$ cm) previously equilibrated with the above buffer. The column was eluted with the same buffer at a flow rate of 7 ml/hr. The active fractions were collected and concentrated by a Centriprep 10 concentrator (Amicon). The resultant prepara-

tion was stored as the purified xylanase I at -20°C for later analysis. In addition, the active fractions (xylanase II) eluted from the first DEAE-cellulose DE-52 column were pooled and lyophilized. The lyophilized preparation was dissolved in a small amount of 10 mM acetate buffer (pH 5.0). To the dissolved solution, ammonium sulfate was added to give 30% saturation. The ammonium sulfate solution was applied to a Butyl-Toyopearl 650M (Tosoh) column ($\phi 1.5 \times 40$ cm) equilibrated with the acetate buffer containing 30% saturation of ammonium sulfate. The column was eluted with a linear decreasing gradient of ammonium sulfate (30 to 0%) in the acetate buffer at a flow rate of 10 ml/hr. The active fractions were combined and lyophilized. The lyophilized preparation was dissolved in a small amount of 10 mM acetate buffer (pH 5.0), and applied to a Sephadex G-150 (Pharmacia) column ($\phi 1.6 \times 70$ cm) equilibrated with the acetate buffer. The column was eluted with the same buffer at a flow rate of 7 ml/hr. The active fractions were combined and stored as a purified xylanase II at -20°C .

The above 70% saturated ammonium sulfate precipitate of the culture broth was dissolved in a minimal amount of 10 mM potassium phosphate buffer (pH 7.4). The resultant solution was dialyzed against the same buffer at 4°C overnight. The dialysate was centrifuged again to remove insoluble substances and then applied to a DEAE-cellulose DE-52 (Whatman) column ($\phi 1.5 \times 40$ cm) equilibrated with 10 mM phosphate buffer (pH 7.4) and washed with the same buffer. The column was eluted with a linear (0 to 500 mM) NaCl gradient in the buffer. The active fractions (β -xylosidase) were combined and lyophilized. The lyophilized preparation was dissolved in a small amount of 10 mM sodium acetate buffer (pH 5.0). To the dissolved solution, ammonium sulfate was added to give 30% saturation. The resulting solution was applied to a Butyl-Toyopearl 650M (Tosoh) column ($\phi 1.5 \times 40$ cm) equilibrated with the acetate buffer containing 30% saturation of ammonium sulfate. The column was eluted with a linear decreasing gradient of ammonium sulfate (30 to 0%) dissolved in the acetate buffer at a flow rate of 10 ml/hr. The active fractions were combined and lyophilized.

The lyophilized preparation was dissolved in a small amount of 10 mM acetate buffer (pH 5.0), and applied to a Sephadex G-150 (Pharmacia) column ($\phi 1.6 \times 70$ cm) equilibrated with the above buffer. The column was eluted with the same buffer at a flow rate of 7 ml/hr. The active fractions were combined and stored as a purified β -xylosidase at -20°C .

Protein assay

Protein was measured by the method of Lowry *et al.*¹¹⁾ with bovine serum albumin as a standard protein. Protein eluted from a column was monitored by measuring the absorbance at 280 nm.

Electrophoresis

The native protein sample was directly run on a 5% polyacrylamide gel in 50 mM Tris-glycine buffer (pH 8.3)¹²⁾. Electrophoresis of the denatured protein samples was done in 25 mM Tris-glycine buffer (pH 8.3) containing 0.1% SDS on a 13.5% gel by the method of Laemmli^{13, 14)}.

In both cases, protein was stained with 0.25% Coomassie brilliant blue R-250 in 50% methanol-10% acetic acid solution.

Molecular weight estimation

The molecular weight of the native enzyme was estimated by HPLC on a TSK G-3000 SW (Tosoh) column equilibrated with 50 mM Tris-HCl buffer (pH 7.5) containing 0.1 M NaCl. The column was eluted with the same buffer. In addition, the molecular weight of the denatured enzyme was estimated by SDS polyacrylamide gel electrophoresis as described above.

Measurement of isoelectric point

Isoelectric focusing was performed in a IEF-PAGE pH 3-10 Gel (4% gel, TEFCO). The isoelectric focusing calibration kit (pH 3-10) was purchased from Amersham Pharmacia Biotech. Protein was stained with 0.25% Coomassie brilliant blue R-250.

Paper chromatography

Reaction products of xylanases as described above were assayed by paper chromatography. Each sample was spotted on a filter paper (No. 50, Toyoroshi). The paper developed by the multiple ascending method using the following solvent system: *n*-butanol-pyridine-water (6:4:3, v/v/v). Saccharides were detected by an aniline hydrogen phthalic salt solution¹⁵⁾.

N-terminal amino acid sequences

The N-terminal amino acid sequences were identified using a protein sequencer PPSQ-10 (Shimadzu). About 100 pmol of pure proteins were used in each run.

Results

Purification of xylan degrading enzymes

Xylan degrading enzymes (xylanase I, xylanase II and β -xylosidase) were purified from the culture broth of *P. expansum* O-385-10 grown in a salt medium containing 2.0% (w/v) washed wheat bran⁹) as a xylan source. The purification procedures are described in Materials and Methods. These enzymes were easily

Fig. 1. Ion exchange chromatography of xylanases and β -xylosidase from *P. expansum* O-385-10 on DEAE cellulose DE-52. The column was eluted with a linear gradient of 0–0.5 M sodium chloride in phosphate buffer (pH 7.2) at a flow rate of 10.0 ml/h. Each fraction was collected in 10 ml. (—), absorbance at 280 nm; (●), xylanase activity; (○), β -xylosidase activity; (---), concentration of NaCl.

separated from each other by the DEAE-Cellulose column chromatography (Fig. 1). A typical purification of these three enzymes is summarized in Table 1. The purified enzyme preparations of xylanase I, xylanase II and β -xylosidase were obtained in a yield of 4.96, 4.34 and 11.1%, respectively, and had the specific activity of 846, 1,460 and 3.52 U/mg protein, respectively. The final purification factors were 46.7, 80.7 and 704-fold, respectively.

The purified enzymes were homogeneous as judged by the criterion of non-denaturing polyacrylamide gel electrophoresis and isoelectrophoresis. Moreover, no evidence of contamination was found on SDS-polyacrylamide gel electrophoresis [Fig. 2(A) and Fig. 2(B)].

Molecular weight of the enzymes

The molecular weights of xylanase I, xylanase II and β -xylosidase were estimated with HPLC on a TSK G-3000 SW (Tosoh) column and SDS-polyacrylamide gel electrophoresis method. The molecular weights of xylanase I, xylanase II and β -xylosidase were calculated to be about 21.0, 40.0 and 91.0 kDa, respectively [Fig. 2(A) and Fig. 2(B)].

Isoelectric point of the enzymes

The pIs of xylanase I, xylanase II and β -xylosidase were 9.20, 4.30 and 4.20, respectively (Fig. 3).

Characteristics of xylanase I and II

Effects of pH on the enzymes

The optimum pHs of xylanase I and II were

Table 1. Summary of purification of xylanases and β -xylosidase produced by *P. expansum* O-385-10

Purification procedure	Total protein (mg)	Total enzyme activity (U)	Specific activity (U/mg)	Yield (%)	Purification (fold)
Xylanases					
Crude filtrate	1,430	25,800	18.1	100	1
(NH ₄) ₂ SO ₄ (70% sat.)	412	22,900	55.6	88.8	3.07
Xylanases I					
DEAE-cellulose DE-52	23.4	6,190	265	24.0	14.6
CM-cellulose	3.85	1,820	473	7.05	26.1
Sephadex G-100	1.51	1,280	846	4.96	46.7
Xylanases II					
DEAE-cellulose DE-52	25.8	5,110	198	19.8	10.9
Butyl-Toyopearl	1.92	2,130	611	8.26	33.8
Sephadex G-150	0.32	1,120	1,460	4.34	80.7
β-Xylanases					
Crude filtrate	1,430	7.65	0.005	100	1
(NH ₄) ₂ SO ₄ (70% sat.)	413	13.0	0.03	170	6
DEAE-cellulose DE-52	17.9	5.60	0.31	73.2	62
Butyl-Toyopearl	2.64	1.68	0.64	22.0	128
Sephadex G-150	0.24	0.85	3.52	11.1	704

Fig. 2. SDS-polyacrylamide gel electrophoresis of xylanases (xylanase I, xylanase II) and β -xylosidase from *P. expansum* O-385-10. Electrophoresis of denatured xylanase I, xylanase II and β -xylosidase was done on 13.5% SDS polyacrylamide gel. A: Lane 1, M_r standards: phosphorylase *b* (94,000), bovine serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), soybean trypsin inhibitor (20,100), α -lactalbumin (14,000). Lane 2, xylanase I. Lane 3, xylanase II. B: Lane 1, M_r standards: the same with A. Lane 2, β -xylosidase.

Fig. 3. Isoelectric focusing of xylanase I and xylanase II from *P. expansum* O-385-10. Electrophoresis was carried out with IEF-PAGE gel mini (pH 3–10, 4% gel). Lane 1, pI standards: amyloglucosidase (3.50), soybean trypsin inhibitor (4.55), β -lactoglobulin A (5.2), bovine carbonic anhydrase B (5.85), human carbonic anhydrase B (6.55), horse myoglobin (6.85), horse myoglobin (7.35), lentil lectin (8.15), lentil lectin (8.45), lentil lectin (8.65), trypsinogen (9.3). Lane 2, xylanase I. Lane 3, xylanase II.

about equal values of around 4.3. Xylanase I was stable in the wide pH range of 3 to 9, while the stability of xylanase II was in the narrower pH range of 5 to 8.

Effects of temperature on the enzymes

The optimum temperatures of xylanase I and II were about equal values of around 40°C

Table 2. Effect of metal ions on activity of xylanases of *P. expansum* O-385-10

Ion added (1 mM)	Relative enzyme activity (%)	
	Xylanase I	Xylanase II
None	100	100
K ⁺	207	81
Ca ²⁺	269	75
Fe ²⁺	229	41
Zn ²⁺	253	61
Ni ²⁺	228	75
Cu ²⁺	116	9
Mn ²⁺	21	32
Hg ²⁺	6	1
Ba ²⁺	216	96
Mg ²⁺	207	83

under the standard assay conditions. Both enzymes were rapidly inactivated above 30°C.

Effects of metal ions on the enzyme activities

The effects of metal ions on purified xylanase I and II were examined (Table 2). The activity of xylanase II was inhibited by Ca²⁺, Fe²⁺, Ni²⁺ and Zn²⁺, while the activity of xylanase I was strongly activated by these ions. In addition, xylanase I was also strongly activated by K⁺ ion. Both enzymes were inhibited by Mn²⁺, Hg²⁺.

Reaction specificities of the enzymes and reaction products

The reactions of purified xylanases with oat xylan and xylotri-ose were examined (Fig. 4). The reaction profile of xylanase I was clearly different from that of xylanase II. Xylanase I mainly produced oligomers of xylose such as xylobiose, xylotri-ose, and probably xylo-tetra-ose, from xylan. In contrast, xylanase II mainly produced xylose and xylobiose from the early stage of the reaction, and higher oligosaccharides were hardly detected in the incubation mixture through the reaction. When xylanase I was incubated with xylotri-ose, xylobiose was mainly produced, and small amounts of xylose and xylo-tetra-ose were detected in the incubation mixture. In the case of xylanase II, the enzyme almost completely decomposed xylotri-ose to xylose and xylobiose. Neither xylanase I nor xylanase II could hydrolyze xylobiose.

N-terminal amino acid sequences

The N-terminal amino acid sequences of the purified xylanase I and II were compared to those of enzymes from some fungi and bacteria whose complete sequences were already

Fig. 4. Paper chromatogram of hydrolyzates produced from xylan by xylanase I and II from *P. expansum* O-385-10 A-1: standards. Lane 1, xylose; Lane 2, xylobiose; Lane 3, xylotriose. A-2: xylan hydrolysis by xylanase I after various incubation time (1–12 hr). A-3: Lane 1, xylobiose hydrolysis by xylanase I; Lane 2, xylotriose hydrolysis by xylanase I. B-1: standards: the same with A-1. B-2: xylan hydrolysis by xylanase II after various incubation time. B-3: Lane 1, xylobiose hydrolysis by xylanase II; Lane 2, xylotriose hydrolysis by xylanase II.

known. The enzymes of these organisms belong to family 11 xylanase^{16, 17}. Figure 5 shows that xylanase I of *P. expansum* can probably be assigned to family 11 enzyme. On the other hand, it was not possible to read the N-terminal amino acid of xylanase II, indicating that the N-terminus of the enzyme was blocked. **Action of xylanase I, xylanase II and β -xylosidase on apple fruit**

The effects of purified enzymes on apple fruit tissue were examined by measuring the reducing sugar liberated (Fig. 6). When xylanase I and II were incubated with a piece of apple fruit, respectively, almost no increase of reducing sugar could be observed in the incuba-

Xylanase I (this study)	:	1	AITSNEQGTNNGYFYFSFWTNGGG
P.s XynA	:	33	TITSSQTGTNNGYYYFSFWTNGGG
A.nd XlnB	:	33	RSTPSSTGTSGGYYSFWTDGGG
S.livi. XlnC	:	3	TITTNTQGY.DGMYYSFWTDGGG
S.sp.S38 XylI	:	3	VITTNETGTNNGYYYFSFWTDGGG

Fig. 5. N-terminal amino acid sequences of xylanase I from *P. expansum* O-385-10 and comparison with family 11 xylanase from fungi and bacteria. P.s XynA, xylanase A from *Penicillium* sp.40; A.nd XlnB, xylanase B from *Aspergillus nidulans*; S.livi. XlnC, xylanase C from *Streptomyces lividans*; S.sp. S38 XylI, xylanase I from *Streptomyces* sp. S38.

Fig. 6. Liberation of reducing sugar from apple fruit. Discs (5 mm in diameter, 2 mm in thickness) obtained from apple fruit were incubated with each enzyme (about 5U) at 30°C. (●), apple; (○), apple+xylanase I; (△), apple+xylanase II; (□), apple+ β -xylosidase; (■), apple+xylanase I+xylanase II+ β -xylosidase.

tion mixture. A similar result was obtained when xylanase I and II were simultaneously incubated with the fruit. A clear increase of reducing sugar liberated from the fruit was observed when β -xylosidase was added to the incubation mixture of xylanases.

Discussion

In cultures of several *Penicillium* species, xylanolytic activities have been found^{18–20}. There have been a few reports on the enzyme purified as a homogeneous protein from *Penicillium capsulatum* and *Penicillium purpurogenum*^{18, 20}. However, little is known about relationship between the physiology of xylanolytic enzyme from phytopathogenic organisms and the components of host plant tissue. *Penicillium expansum* O-385-10 extracellularly produced a large amount of the xylan-degrading enzyme that played a role in the decomposition of apple cell wall². In this work, the xylan-degrading enzyme was purified, and xylanase I, xylanase II and β -xylosidase were obtained as electrophoretically homogeneous proteins,

respectively.

The profile of the xylan hydrolysis by xylanase I and II showed that these were the end type enzyme. However, the profiles of the hydrolysis reaction are remarkably different between both enzymes. In the reaction mixture with xylanase I, various xylooligosaccharides and a small amount of xylose were detected through the reaction. The reaction of xylanase I was similar to that of usual endo-xylanase²¹⁾. On the other hand, xylanase II formed mainly xylose and xylobiose from the initial time of the incubation, and production of oligosaccharides was hardly observed through the reaction. Little is known about how endoxylanase splits to the monomer of xylose from the early stage of the reaction with xylan. In the study of Tseng *et al.*²²⁾, co-incubation of the two endoxylanases from *Bacillus firmus* with xylan resulted in the release of xylose and the production of xylose was also shown to be increased with time. However, only a trace amount of xylose was detected at the time of adding solely xylanase. This suggested that different patterns of products released from the substrate correspond to different affinities of xylanase I and II for the substrate. There were also remarkable differences in the effects of metal ions on the enzyme activity between xylanase I and xylanase II. The activity of xylanase I was strongly enhanced by K^+ , Ca^{2+} , Fe^{2+} , Ni^{2+} and Zn^{2+} ions, while the activity of xylanase II was inhibited by all of these ions except K^+ . There has been no report about the effects of K^+ ion on xylanase activity. It is interesting that xylanase I was strongly activated by K^+ ion which is plentiful in apple fruit. K^+ ions of plant cells are usually localized in the protoplasm. The intracellular K^+ ions leak out when pathogenic organisms degrade the cells of host plant^{6, 23)}. Therefore, the effect of K^+ ions leaking out from host plant cells due to degrading enzymes of a pathogen is closely related to pathogenic activity.

It has been proposed that xylanase was classified into two groups of acidic high-molecular-mass enzyme (family 10) and basic low-molecular-mass enzyme (family 11)²⁴⁻²⁶⁾. Xylanase I (*pI*, 9.20; *M_r*, 21.0 kDa) of strain O-385-10 belongs to the basic low-molecular enzyme and xylanase II (*pI*, 4.30; *M_r*, 40.0 kDa)

belongs to the acidic high-molecular enzyme. The N-terminal amino acid arrangement of xylanase I shows a strong homology to that of several family 11 xylanases. As for xylanase II, it was not possible to read the N-terminal amino acids. From these facts, xylanase I seems to be a family 11 enzyme. At present time, the grouping of xylanase II is undecided.

There have been a few reports on the relationship between the components of apple fruit tissue and a purified carbohydrate degrading enzyme produced by fungi. Sasaki *et al.*⁴⁻⁷⁾, reported that β -glucosidase played an important role on the degradation of the pulp of apple by a phytopathogenic fungus, *Botrytis cinerea*. In the culture of *P. expansum* O-385-10, β -glucosidase has not been found²⁾. Xylanase I, II and β -xylosidase purified independently from strain O-385-10 did not affect apple fruit. When these enzymes were simultaneously incubated with apple fruit, a large amount of reducing sugar was liberated into the incubation mixture. This suggested that the cooperative and synergistic attack of xylanases and β -xylosidase against the apple tissue liberates a large amount of reducing sugar. From these facts, it seems that *P. expansum* O-385-10 spreads using three xylan degrading enzymes in apple fruit.

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リンゴ果実の腐敗に関与する *Penicillium expansum* O-385-10 の生産するキシラン分解酵素の精製と諸性質

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リンゴ果実の腐敗に関与する *Penicillium expansum* O-385-10 の液体培養上清液から、キシラン分解酵素 (キシラナーゼ I, キシラナーゼ II, β -キシロシダーゼ) を電気泳動ならびに等電点電気泳動的に単一なまでに精製した。キシラナーゼ I, キシラナーゼ II および β -キシロシダーゼの分子量はそれぞれ, 21.0, 40.0, 91.0 kDa, 等電点はそれぞれ, 9.20, 4.30, 4.20 と算出した。キシラナーゼ I は, キシランから少量のキシロースおよび多量の数種のキシロオリゴ糖を生成した。これに対して, キシラナーゼ II は反応初期から主にキシロースやキシロビオスを生成し, キシロトリ

オース以上のオリゴ糖はほとんど生成しないことがわかった。K⁺ イオンによりキシラナーゼ I の活性は約 2 倍に賦活化された。一方のキシラナーゼ II は約 80% まで阻害された。これらの精製酵素をリンゴ果実に作用させた場合、キシラナーゼ I や II だけではリンゴからの還元糖生成はほとんど見られなかったのに対し、 β -キシロシダーゼを同時に作用させることにより果実からの遊離還元糖が著しく増加してきた。