Efficient Extraction of Ferulic Acid from Sugar Beet Pulp
Using the Culture Supernatant of Penicillium chrysogenum

(Received December 24, 2004; Accepted February 4, 2005)

Tatsuji Sakamoto, Sayuri Nishimura, Tomoki Kato, Yoichi Sunagawa, Moriyasu Tsuchiyama and Haruhiko Kawasaki

1Division of Applied Biochemistry, Graduate School of Agriculture and Biological Sciences, Osaka Prefecture University (I–I, Gakuen-cho, Osaka 599–8531, Japan)
2Department of Research and Development, Okumoto Flour Milling Co., Ltd. (5–7–24, Minamihorie, Osaka 550–0015, Japan)

Abstract: We found a microorganism, Penicillium chrysogenum 31B, that has high ability to release ferulic acid from sugar beet pulp. Approximately 85% of alkaline-extractable ferulic acid in sugar beet pulp could be released using the culture supernatant of P. chrysogenum 31B. However, the culture supernatant did not efficiently extract ferulic acid from wheat bran, peel of corn seed, or sugar-cane bagasse. A ferulic acid esterase (FAE-1) was purified from the culture filtrate of P. chrysogenum 31B. The molecular mass of the enzyme was determined to be 62 kDa by SDS-PAGE. Optimum conditions for enzyme activity were 50°C and pH 6–7. The enzyme showed activity towards methyl esters of hydroxybenzoinc acids including ferulic acid, p-coumaric acid, and caffeic acid, but was not active on methyl sinapinate or 3,4-dimethoxy cinnamate. The lack of activity of FAE-1 toward these substrates appears to be due to the presence of two methoxy groups on the benzene ring. The substrate specificity of FAE-1 seemed to be similar to that of ferulic acid esterase (CinnAE) of Aspergillus niger. However, there was a difference between FAE-1 and CinnAE in respect to activity towards methyl vanillate. It is remarkable that FAE-1 hydrolyzed methyl vanilllate, which, to our knowledge, is the first report of a ferulic acid esterase hydrolyzing a hydroxybenzoic acid methyl ester.

Key words: ferulic acid, ferulic acid esterase, Penicillium chrysogenum, sugar beet pulp

Ferulic acids have been found in the cell walls of many plant products including maize bran,1 sugar-cane bagasse,2 wheat bran,3 sugar beet pulp,4,5 and spinach.6 In the three former tissues, ferulic acid is esterified to the C-5 of arabinose in arabinoxylan, a β-1,4-D-xylan to which α-L-arabinofuranosyl residues are attached at position 2 or 3. In contrast, ferulic acids mainly attach to the C-2 position of α-1,5-linked arabinofuranose residues or the C-6 of β-1,4-linked galactopyranose residues in the hairy region of pectins of sugar beet and spinach.7,8 Recently, Levigne et al. demonstrated that ferulic acid is also linked to the C-5 position of arabinofuranose residues in sugar beet pectin by analysis of the structures of feruloylated oligosaccharides obtained by treating sugar beets with a commercial enzyme preparation (Driselase).9 Furthermore, some ferulic acids exist as dehydrodimers (e.g. 5,5-dehydrodiferulic acid) in the cell walls of several plant species, which are formed through an oxidation reaction catalyzed by peroxidases.10,11 Possible roles of ferulic acid in plant cell walls are to decrease the digestibility of the cell wall by microorganisms12 and to regulate cell growth13 by cross-linking cell wall polysaccharides.

Ferulic acid esterases (FAEs) are enzymes that catalyze the hydrolysis of ester linkages between ferulic acids and sugars or alcohols. The number of studies concerning microbial FAEs has been increasing in the last ten years and many FAEs have been isolated and characterized. In the utilization of agro-industrial wastes such as sugar beet pulp, effective degradation of plant cell walls requires FAEs for the release of ferulic acid as well as polysaccharides such as xylanases14 and arabinanases.15 Moreover, ferulic acid has several potential applications: it may be useful as an anti-oxidizing agent,16,17 an anti-inflammatory drug,18,19 and a food preservative that inhibits microbial growth.20,21 Enzymatic extraction of ferulic acid from industrial wastes has been studied. Penicillium funiculosum FAEB releases 98% of esterified ferulic acid from wheat bran in the presence of xylanase and releases 35% of esterified ferulic acid from sugar beet pulp in the presence of a mixture of endo-arabinanase and α-L-arabinofuranosidase.22 To the best of our knowledge, that releases more ferulic acid from sugar beet pulp than any other enzyme.

In this paper we describe the screening of microorganisms that can achieve high yield extraction of free ferulic acid from sugar beet pulp. In addition, we describe the isolation and some of the characteristics of a novel FAE, termed FAE-1, produced by P. chrysogenum 31B.

MATERIALS AND METHODS

Chemicals and reagents. ResourceQ 6 mL, ResourceS 6 mL, MonoQ HR 5/5, MonoS HR 5/5 and HiLoad 16/60 Superdex 75 columns were purchased from Amersham Biosciences. All other chemicals were from Wako Pure Chemical Industries, Ltd. (Osaka) unless otherwise stated, and were of certified reagent grade.

Organism and cultivation conditions. The microorganisms were obtained from the Institute for Fermentation, Osaka (IFO) or isolated from rotten sugar beet, and main-
tained on agar slants containing 2% glucose, 0.5% yeast extract, and 0.5% peptone (pH 5.0) at 4°C. The medium used for the first screening of FAE-producing microorganisms was composed of 0.2% NH₄NO₃, 0.1% KH₂PO₄, 0.05% MgSO₄·7H₂O, 0.05% KCl, 0.001% FeSO₄, 0.1% peptone, 0.1% glucose and 2% sugar beet pulp (pH 5.0).

In the first screening, the microorganisms were cultivated in test tubes (i.d. 24 mm) containing 10 mL of the medium, at 30°C for 10 days under static conditions. For purification of the FAE-1 produced by the *P. chrysogenum* 31B strain, the microorganism was cultivated statically in 2.5 L of the above liquid medium in a 5-L Erlenmeyer flask at 30°C for 12 days.

**Substrates.** Sugar beet pulp, wheat bran, peel of corn seed, and sugar-cane bagasse were kindly provided by Nippon Beet Sugar Manufacturing Co., Ltd. (Tokyo), Okumoto Flour Milling Co., Ltd. (Tokyo), Sanwa Cornstarch Co., Ltd. (Nara, Japan), and Okinawa Prefectural Agricultural Experiment Station, respectively. The materials were powdered with a mill, dried at 40°C, and used for substrates in the enzyme reaction.

Sugar beet pectin (SBP) was extracted from sugar beet pulp with water. The hairy regions of SBP (HR) were isolated by digestion of sugar beet pulp with water. The hairy regions of SBP (HR) obtained on agar slants containing 2% glucose, 0.5% yeast extract, and 0.5% peptone (pH 5.0) at 4°C. The medium used for the first screening of FAE-producing microorganisms was composed of 0.2% NH₄NO₃, 0.1% KH₂PO₄, 0.05% MgSO₄·7H₂O, 0.05% KCl, 0.001% FeSO₄, 0.1% peptone, 0.1% glucose and 2% sugar beet pulp (pH 5.0).

In the first screening, the microorganisms were cultivated in test tubes (i.d. 24 mm) containing 10 mL of the medium, at 30°C for 10 days under static conditions. For purification of the FAE-1 produced by the *P. chrysogenum* 31B strain, the microorganism was cultivated statically in 2.5 L of the above liquid medium in a 5-L Erlenmeyer flask at 30°C for 12 days.

**Substrates.** Sugar beet pulp, wheat bran, peel of corn seed, and sugar-cane bagasse were kindly provided by Nippon Beet Sugar Manufacturing Co., Ltd. (Tokyo), Okumoto Flour Milling Co., Ltd. (Tokyo), Sanwa Cornstarch Co., Ltd. (Nara, Japan), and Okinawa Prefectural Agricultural Experiment Station, respectively. The materials were powdered with a mill, dried at 40°C, and used for substrates in the enzyme reaction.

Sugar beet pectin (SBP) was extracted from sugar beet pulp with water. The hairy regions of SBP (HR/SBP) were obtained as follows: SBP was digested with the cloned Bacillus subtilis pectin lyase in 20 mM Tris-Cl buffer (pH 8.0) at 50°C to remove the homogalacturonic acid regions. The reaction mixture was boiled for 5 min to inactivate the enzyme, dialyzed against water, precipitated by the addition of three volumes of ethanol, and finally dried at 40°C, giving HR/SBP.

Methyl derivatives of hydroxycinnamic acids and hydroxybenzoic acids were chemically prepared and the synthesis was confirmed by 1H-NMR. Chemical structures of the compounds are shown in Fig. 1. Ethyl and butyl ferulates were kindly donated from Tsuno Food Industrial Co., Ltd. (Wakayama, Japan).

**Enzyme assays.** A standard assay for FAE activity was performed with methyl ferulate (MFA) as the substrate. The reaction mixture contained 190 μL of 0.05% MFA in 100 mM acetate buffer (pH 5.0) and 10 μL enzyme solution and was incubated at 37°C for 20 min. The mixture was boiled for 5 min to inactivate the enzyme. The ferulic acid released was quantified by high-performance liquid chromatography (HPLC). One unit was defined as the amount of enzyme that releases 1 μmol of ferulic acid at 37°C in 1 min.

**HPLC conditions.** HPLC was performed with a Mighlysil RP-18 GP column (250×4.6 mm; Kanto Chemical Co., Inc., Tokyo) using 50% methanol containing 0.1% acetic acid as the solvent at a flow rate of 0.7 mL/min. The effluent was monitored by measurement of absorbance at 320 nm.

**Alkaline extraction of ferulic acid from natural products.** Reaction mixtures containing 100 mg of each sample and 6 mL of 0.5 N KOH solution were incubated at 50°C for 6 h. After incubation, 2 mL of 2 N HCl was added to the mixtures and the mixtures were centrifuged at 30,000×g for 20 min. The supernatants were extracted with an equal volume of ethyl acetate and the ethyl acetate extract was evaporated to dryness. The residues were dissolved in the same solvent used for HPLC analysis. Ferulic acid contents were determined by HPLC.

**Enzymatic extraction of ferulic acid from natural products.** Ferulic acid released from different natural products using the culture supernatant of *P. chrysogenum* 31B was assayed as follows. Extractions utilized a mixture containing 100 mg of each natural product and 10 mL of the crude enzyme solution, which was previously dialyzed against 5 L of 20 mM acetate buffer (pH 5.0), at 37°C for 24 h. The remaining insoluble materials were removed by centrifugation at 30,000×g for 20 min followed by filtration through a 0.45 μm-membrane filter. The solubilized ferulic acid liberated from natural products was measured by HPLC.

**Purification of the enzyme.** The culture filtrate (5 L) of *P. chrysogenum* 31B was concentrated by ultrafiltration (10-kDa cut off), dialyzed against 20 mM acetate buffer (pH 5.0) and used for enzyme purification. Proteins were precipitated from the filtrate by the addition of solid ammonium sulfate up to 80% saturation followed by centrifugation at 20,000×g for 10 min. The precipitate was dissolved in 20 mM acetate buffer (pH 5.0) and extensively dialyzed against the same buffer. The sample was loaded on a ResourceS column equilibrated with the acetate buffer. The column was washed with 60 mL of the same buffer and a 0 to 0.3 M NaCl linear gradient (120 mL) was applied at a flow rate of 3 mL/min. Three-mL fractions were collected and fractions containing FAE activity were pooled. FAE activities were found in both the bound and unbound fractions. The bound fraction was further purified and termed FAE-1. The enzyme solution was then dialyzed against the acetate buffer and loaded onto a ResourceQ column equilibrated with the dialysis buffer. The column was washed with 60 mL of the same buffer and bound proteins were eluted by a linear gradient of NaCl (120 mL, from 0 to 0.5 M) at a flow rate of 3 mL/min. Three-mL fractions were collected and the FAE-containing fractions were pooled. The enzyme solution was desalted and put on a MonoS column equilibrated with the acetate buffer. The adsorbed proteins were eluted.
by a linear gradient of NaCl (20 mL, from 0 to 0.3 M) at a flow rate of 1 mL/min. The active fractions were collected, dialyzed against the acetate buffer, and loaded onto a MonoQ column equilibrated with the dialysis buffer. The bound proteins were eluted by a linear gradient of NaCl (from 0 to 0.5 M) at a flow rate of 1 mL/min. The FAE-containing fractions were pooled, concentrated by centrifugal filtration at 5,000 × g with a 10-kDa cut-off filter (Ultrafree-MC; Nippon Millipore Ltd., Tokyo), and put on a size-exclusion column of Superdex 75 equilibrated with 100 mM NaCl in the acetate buffer. Proteins were eluted with the same buffer at a flow rate of 1 mL/min and 1-mL fractions were collected.

RESULTS

Selection of FAE-producing strains.

Our objective was to select microorganisms that had a high ability to release ferulic acid from sugar beet pulp. In the first screening, the concentration of ferulic acid released in the culture filtrates was measured by HPLC. Among 249 strains tested, 7 produced high concentrations of ferulic acid (data not shown). In the second screening, FAE activity in the above culture filtrates, which were dialyzed against 20 mM acetate buffer (pH 5.0), were measured using HR/SBP as the substrate (Table 1). HR/SBP seemed to be a more suitable substrate in this screening than synthetic substrates such as MFA. P. chrysogenum 31B showed the highest enzyme activity and seemed to be the best strain that released ferulic acid from sugar beet pulp.

Enzymatic extraction of ferulic acid using a crude enzyme solution of P. chrysogenum 31B.

The percentages of ferulic acid released from the different substrates using the culture filtrate of P. chrysogenum 31B in comparison with alkaline extraction are listed in Table 2. Peel of corn seed produced much more alkaline-extractable ferulic acid than did the other three substrates. The culture filtrate of P. chrysogenum 31B was very active on sugar beet pulp, releasing 87% of the alkaline-extractable ferulic acid. The higher specificity of the enzyme preparation for sugar beet pulp might be because ferulic acids in sugar beets are linked to arabinoxylan. As mentioned earlier, ferulic acid is esterified to the C-5 of arabinose in arabinoxylan. FAE in the culture filtrate might not be active on the ester linkage. Surprisingly, no ferulic acid in wheat bran was released by the enzyme solution.

Purification and characterizations of FAE-1.

FAE-1 was purified from 5 L fermentation broth of P. chrysogenum 31B. Throughout the purification steps, enzyme activity was measured using MFA as the substrate under the standard FAE assay conditions. The culture medium was filtered, precipitated with ammonium sulfate, resuspended and separated by cation-exchange chromatography using a ResourceS column. Although about 5% of the initial FAE activity did not bind to this column, this fraction is not described further here. Major FAE activity eluting at 0.1 M NaCl concentration was designated FAE-1 and purified further (Fig. 2). The purification procedure

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Alkaline-extractable ferulic acid (mg/100 mg of substrate)</th>
<th>Yield of enzymatic extraction (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar beet pulp</td>
<td>0.60</td>
<td>87</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>0.25</td>
<td>0</td>
</tr>
<tr>
<td>Peel of corn seed</td>
<td>2.43</td>
<td>6</td>
</tr>
<tr>
<td>Sugar-cane bagasse</td>
<td>0.43</td>
<td>26</td>
</tr>
</tbody>
</table>

The culture supernatant of P. chrysogenum 31B was used for enzymatic extraction of ferulic acid from the substrates. Experimental procedures are described in the text. *Yields of enzymatic extractions are expressed as a percentage of the amount of alkaline-extractable ferulic acid.

Table 1. FAE activity in culture filtrates of strains selected in the first screening.

<table>
<thead>
<tr>
<th>Strain</th>
<th>FAE activity (mU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. awamori IFO 4033</td>
<td>0.15</td>
</tr>
<tr>
<td>A. flavus IFO 5839</td>
<td>0.65</td>
</tr>
<tr>
<td>A. foetidus IFO 4122</td>
<td>0.24</td>
</tr>
<tr>
<td>A. niger IFO 4343</td>
<td>0.33</td>
</tr>
<tr>
<td>A. terreus IFO 6123</td>
<td>0.71</td>
</tr>
<tr>
<td>A. terreus IFO 6365</td>
<td>1.87</td>
</tr>
<tr>
<td>P. chrysogenum 31B</td>
<td>2.69</td>
</tr>
</tbody>
</table>

Culture supernatant was dialyzed against 20 mM acetate buffer (pH 5.0) and used for the enzyme assay. For determination of enzyme activity, 0.5% of HR/SBP was used as the substrate instead of 0.05% of MFA in the reaction mixture for the standard FAE assay. One unit is defined as the amount of enzyme that releases 1 µmol of ferulic acid at 37 °C in 1 min.

Table 2. Comparison of alkaline and enzymatic extraction of ferulic acid from various natural products.

Table 3. Purification of FAE-1 from P. chrysogenum 31B.

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Protein (mg)</th>
<th>Activity (U)</th>
<th>Sp act (mU/mg)</th>
<th>Purification (fold)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture filtrate</td>
<td>848</td>
<td>53.7</td>
<td>63.3</td>
<td>--</td>
<td>100</td>
</tr>
<tr>
<td>(NH₄)₂SO₄</td>
<td>596</td>
<td>57.4</td>
<td>96.3</td>
<td>1.52</td>
<td>107</td>
</tr>
<tr>
<td>Resource S</td>
<td>127</td>
<td>33.6</td>
<td>264</td>
<td>4.17</td>
<td>63</td>
</tr>
<tr>
<td>Resource Q</td>
<td>24.1</td>
<td>24.7</td>
<td>1020</td>
<td>16.1</td>
<td>46</td>
</tr>
<tr>
<td>Mono S</td>
<td>5.37</td>
<td>16.3</td>
<td>3040</td>
<td>48.0</td>
<td>30</td>
</tr>
<tr>
<td>Mono Q</td>
<td>4.50</td>
<td>11.9</td>
<td>2640</td>
<td>41.7</td>
<td>22</td>
</tr>
<tr>
<td>Superdex 75</td>
<td>3.20</td>
<td>10.1</td>
<td>3160</td>
<td>49.9</td>
<td>19</td>
</tr>
</tbody>
</table>
for FAE-1 is summarized in Table 3. SDS-PAGE analysis of the purified enzyme showed that there was a single protein band at a molecular mass of 62 kDa (Fig. 3). Size-exclusion chromatography of FAE-1 using Superdex 75 indicated a molecular mass of 63 kDa. These results confirmed the monomeric structure of the protein. The enzyme was glycosylated because the molecular mass of FAE-1 was decreased from 62 kDa to 60 kDa by treatment with endoglycosidase H (Fig. 3). The N-terminal amino acid sequence of FAE-1 was determined to be AFQSXXVEFGAQIDIPNVKVNFF, where X indicates an undetermined residue. The sequence was compared with those of A. niger FAEA,20 A. niger FAEB,20 P. funiculosum FAEB,22 Talaromyces stipitatus FAEC20 and Pseudomonas fluorescens XYLD.21 FAE-1 showed relatively high homologies to A. niger FAEB (50%) and T. stipitatus FAEC (41%) (Fig. 4). The amino acid sequences of the latter two proteins are similar to those of Acinetobacter sp. chlorogenate esterase and A. oryzae tannase.20

FAE-1 had the highest enzyme activity at 50°C. The thermostability of the enzyme was determined by incubating it at various temperatures at pH 5.0 and measuring residual activity. After incubation of the enzyme at pH 5.0 at 40°C for 1 h, the initial activity of the enzyme completely remained. FAE-1 was strongly inactivated by incubating the enzyme at 55°C; 75% of the initial activity was lost by treatment for 1 h at this temperature. Enzyme activity at 37°C was highest at pH 6 to 7. More than 90% of the initial FAE-activity remained after 16 h of incubation at pHs from 4.0 to 7.0 at 30°C.

The sensitivity of FAE-1 to metals was examined by adding metal chlorides at 3 mM to the reaction mixture of the standard assay. HgCl2 and FeCl3 caused a loss of 50 and 30% of the enzyme activity, respectively. No effect on activity was detected with the chloride salts of Ba++, Ca++, Cd++, Co++, Cu++, K+, Mg++, Mn++, Na+, Ni++, or Zn++.

Substrate specificity of FAE-1 towards various methyl esters of hydroxycinnamic acids and hydroxybenzoic acids is summarized in Table 4. FAE-1 showed the highest level of activity towards methyl p-coumarate (MpCA), moderate activity toward methyl ferulate (MFA) and caffeate (MCA), and no activity toward methyl sinapinate (MSA), methyl 3,4-dimethoxy cinnamate (MdMCA) or methyl syringate (MsyA). The lack of activity of FAE-1 toward MSA, MdMCA and MsyA appears to be due to the presence of two methoxy groups on the benzene ring. Surprisingly, FAE-1 was active towards methyl vanillate (MVA). MVA is a hydroxybenzoic acid methyl ester, and so this is the first report of the hydrolysis of this class of molecules by FAE. This data indicates that FAE-1 does not require a double bond between carbons on the aliphatic chain. The activities of FAE-1 toward methyl, ethyl, and butyl ferulate were in the ratio 1.00: 0.92: 0.90, indicating that the enzyme activity slightly decreased with increasing length of the aliphatic chain.

**DISCUSSION**

Side-chains of the rhamnogalacturonan region in sugar beet pectin are mainly composed of arabinose and galactose.25,26 Ralet et al. have reported that approximately 55 and 45% of total ferulic acid in sugar beet pectin is esterified to arabinose and galactose residues, respectively.27 Kroon and Williamson studied the effects of cell wall degrading enzymes on the liberation of feruloylated materials from sugar beet pulp28 and demonstrated that a relatively large amount of feruloylated oligosaccharides was released by treatment with a mixture of endo-arabinanase and α-1-arabinofuranosidase. However, endo-β-1,4-galactanase and β-galactosidase had little effect in releasing...
feruloylated oligosaccharides despite the presence of a large quantity of ferulic acid linked to galactose. They speculated that the accessibility of endo-galactanase and β-galactosidase to galactan side-chains in sugar beet pectins is limited due to the structural complexity of the rhamnogalacturonan regions. Galactan chains are thought to be placed between rhamnogalacturonan backbones and branched arabinans. Co-operation with several polysaccharidases may be necessary for galactanases to gain access to galactan chains in sugar beet pectins. *P. chrysogenum* 31B has been found to secrete at least five different arabinan-degrading enzymes including Abnx, Abne, AFQ1, AFSI1 and AbnSI (unpublished data) during culture in the presence of sugar beet pulp. Galactanase, rhamnogalacturonanase, polygalacturonase and cellulase activities were also detected in the culture filtrate. These enzymes appear to be involved in the effective release of ferulic acid from sugar beets.

Crepin et al. proposed to classify FAEs into four groups depending on either their substrate specificities toward four synthetic methyl esters of hydroxycinnamic acids (ferulic acid, p-coumaric acid, sinapinic acid and caffeic acid) or their primary protein structure. Type A enzymes such as *A. niger* FAE-II and FAE-III are inactive towards MCA. Type B enzymes such as *A. niger* FAE-I and CinnAE are not able to hydrolyze MSA. Type C and type D enzymes are active towards all four substrates. However, type C enzymes such as *T. stipitatus* FAE can’t release ferulic acid dimer from synthetic diethyl differulates or natural substrates, such as wheat bran and sugar beet pulp, whereas type D FAEs, which include *P. fluorescens* XYL and *Neurospora crassa* FAED can release a 5-5 ferulic dehydrodimer from wheat cell walls in the presence of microbial xylanases. The substrate specificities of four FAEs (*A. niger* FAE-III, *A. niger* CinnAE, *T. stipitatus* FAEC and *N. crassa* FAED-3,544), which are examples of each of these respective groups, towards synthetic methyl esters are compared with that of *P. chrysogenum* FAE-1 in Table 4. Based on the substrate specificity of FAE-1 towards methyl esters of hydroxycinnamic acids, FAE-1 appears to belong to type B. However, *A. niger* FAEB and *T. stipitatus* FAEC, whose amino acid sequences have similarity to the N-terminal sequence of *P. chrysogenum* FAE-1, belong to the type C group based on a phylogenetic analysis. In fact, *A. niger* FAEB also has no activity on methyl sinapinate. Classification of FAEs is still unclear and will require more data on substrate specificity and the primary protein structure of FAEs. In any case, *P. chrysogenum* FAE-1, unlike type-B enzymes, was active on methyl vanillate. To the best of our knowledge, no FAEs that can hydrolyze synthetic methyl esters of hydroxybenzoic acids (e.g. vanillic acid) have been found previously. The FAE-1 described here may be the first enzyme with this mode of action.

The authors thank Professor K. Nishimura for his help in the preparation of methyl derivatives of phenolic acids. This research was financially supported in part by a grant from the Japanese Ministry of Education, Culture, Sports, Science and Technology (No. 14760057).

REFERENCES

Penicillium chrysogenum の培養上澄を用いたシュガービートパルプからの効率的なフェルラ酸抽出

シュガービートパルプからの酵素法による効率的フェルラ酸抽出を目的として、フェルラ酸遊離能の高い微生物のスクリーニングを行い、Penicillium chrysogenum 31B 株を選抜した。シュガービートパルプを主な炭素源とする培地にて本菌を培養し、その上澄をシュガービートパルプに作用させたところ、含有フェルラ酸の約 85 % を遊離させることができた。植物細胞壁からのフェルラ酸の遊離においてはフェルラ酸エステラーゼ以外に多糖分解酵素の作用が関与していると考えられている。効率的なフェルラ酸遊離のメカニズムを解明する第一段階として、本研究では Penicillium chrysogenum 31B 株の生産する主要フェルラ酸エステラーゼ (FAE-1) の単離と性質を決定した。

本酵素は分子量 62 kDa の糖タンパク質で、反応至適 pH と温度はそれぞれ 6.7 および 50°C であった。柑皮酸類および安息香酸類のメチルエステルに対する基質異性を検討した結果より、本酵素の活性はシンビ酸のようにベンゼン環に二つのメトキシ基が結合していると阻害されることが明らかとなった。これまでに安息香酸類のメチルエステルを分解するフェルラ酸エステラーゼの報告例はないが、FAE-1 はパラリン酸メチルエステルに対しても活性を有する新規酵素であることが判明した。また、この結果より本酵素の活性発現には脂肪酸中の炭素間 2 重結合が必要因子ではないことが示唆された。