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Short Communication

Detection and production condition of acetylxylan esterase from a wood-rotting fungus, *Coriolus versicolor*

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Acetyl esterase production was detected in a wood-rotting fungus, *Coriolus versicolor*, by the formation of a clear zone on a double layer agar plate containing glucose β-D-pentaacetate. Two polysaccharide acetates, carboxymethyl cellulose acetate and xylan acetate, also served as detectable substrates in place of glucose acetate to form clear zone. In an esterase assay, this fungal esterase showed a higher specificity to acetylxylan than did porcine liver esterase, indicating that it is an acetylxylan esterase.

Key Words——acetylxylan esterase; *Coriolus versicolor*; wood-rotting fungus.

Xylan is a component polysaccharide in the secondary cell wall of woody plants. Native xylan is a linear polysaccharide composed of β-1,4-linked D-xylose residues with various substituent side chains, such as 4-*O*-methyl glucuronide, arabinofuranoside and acetate (Timell, 1964, 1965). Biodegradation of xylan has been considered to depend on the hydrolysis of glycosidic linkages by endo-1,4-β-D-xylanase and β-D-xylosidase. Recently, attention has shifted to the role of debranching enzymes in removing the side chains.

Acetylxylan esterase is a debranching enzyme that can split acetyl groups linked to 2- or 3-positions of xylose residues, and is thought to contribute to xylan biodegradation in nature (Biely et al., 1985). Early reports demonstrated the production of acetylxylan esterase in some bacteria and fungi (Lee et al., 1987; Poutanen and Sundberg, 1988). Of the wood-rotting fungi, which can degrade wood components including xylan, however, information is available only on *Schizophyllum commune* Fr. (Biely et al., 1985, 1986). Here, we report on *Coriolus versicolor* (L.: Fr.) Quél. (IFO 30340), one of the most extensively investigated wood-rotting fungi.

Four detectable substrates were prepared to examine the production of acetyl esterase: glucose β-D-pentaacetate, glucose α-D-pentaacetate, CMC acetate and xylan acetate. Glucose β-D-pentaacetate was synthesized by acetylation of glucose with acetic anhydride and sodium acetate, and glucose α-D-pentaacetate was synthesized with acetic anhydride and pyridine (m.p.: 131.5 and 111.5°C, respectively). CMC acetate was prepared from CMC-Na (Nacalai Tesque, Inc.) with trifluoroacetic anhydride according to Bourne et al. (1949). Xylan acetate was prepared by acetylation of alkaline-extracted xylan of Japanese beech (*Fagus crenata* Bl.) with acetic anhydride and hydrogen sulfate.

Coriolus versicolor was cultivated at 28°C on a double layer agar plate containing Kirk's basal medium (Kirk et al., 1978) and 1% (w/v) glucose, and additionally 2% (w/v) glucose β-D-pentaacetate in the upper layer. After a few days, a clear zone formed around the colony, due to the deacetylation of glucose acetate to be soluble by the action of esterase. Thus, *C. versicolor* was determined to secrete acetyl esterase.

To examine the effect of carbon source in preculture, mycelium incubated in agar culture with glucose concentration of 0 or 1% (w/v) was inoculated on the above detection culture. Clear zone expansion rates were 0.72 and 0.20 mm/h for precultures with 0 and 1% glucose, respectively. Thus, glucose starvation led to enhanced esterase production, and subsequent experiments were conducted using mycelia precultured without glucose.

The optimal conditions for clear zone formation were examined by using various nutrient cultures: glucose concentrations of 0, 0.01, 0.1 and 1.0% (w/v); ammonium

Table 1. Clear zone formation on various acetate substrates.

Substrate	Relative expansion rates of clear zone formation[a)]
Glucose β-D-pentaacetate	100
Glucose α-D-pentaacetate	75.3
Xylan acetate	+[b)]
CMC acetate	+[b)]

Agar tests were performed in 5 replicate cultures.
a) Percent against the value on glucose β-D-pentaacetate.
b) Indication of clear zone formation.
Calculation of expansion rate was impossible (see text).

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Table 2. Esterase activities toward 4-nitrophenyl acetate and acetylxylan (unit) and their ratios.

Enzyme sample	Acetylxylan esterase (AX)	4-Nitrophenyl acetate esterase (4NA)	Ratio (AX/4NA)
C. versicolor (0% glucose in preculture)	0.23	0.13	1.77
C. versicolor (1% glucose in preculture)	0.14	0.075	1.91
Porcine liver esterase	0.094	14.4	0.0065

tartrate, ammonium phosphate, potassium nitrate and asparagine as nitrogen source at concentrations of 1, 10 and 100 mM; and pH values in the range of 2.5-8.0. The optimal conditions were as follows: 0% glucose, 10 mM ammonium tartrate as nitrogen source, and pH 3.5.

Three other acetates were tested as substrates in place of glucose β-D-pentaacetate, and the results are shown in Table 1. Glucose α-D-pentaacetate also gave a clear zone, but its expansion was slower than that with glucose β-D-pentaacetate. With two chemically acetylated polysaccharides, CMC acetate and beech xylan acetate, mycelium spread over the culture within 1 wk, and insoluble particles gradually dissolved in the whole culture after about 2 wk. The clear zone expansion rate could, therefore, not be calculated. Because acetylated polysaccharides are resistant to degradation by endopolysaccharases as a result of steric hindrance, hydrophobicity and so on, this finding indicates that acetyl esterase deacetylated these acetylated polymers to be water-soluble. It also suggests that CMC acetate would be an effective substrate for detection of acetyl esterase, because it cannnot be attacked by xylan-hydrolyzing enzymes.

Acetyl esterase activity was next measured using culture filtrate as an enzyme solution. *Coriolus versicolor* incubated in 20 ml of liquid medium prepared to give the above optimal culture conditions in 100-ml Erlenmeyer flasks at 28°C for 7 d. Acetyl esterase activity was determined using high-performance liquid chromatography (HPLC) by measuring the amount of acetic acid released from two substrates, acetylxylan extracted with dimethyl sulfoxide from Japanese beech and 4-nitrophenyl acetate. The reaction mixture contained 0.1 ml of substrate solution (10% (w/v) acetylxylan or saturated 4-nitrophenyl acetate (freshly prepared at the time of use) dissolved in 0.2 M phosphate buffer, pH 6.5) and 0.1 ml of enzyme solution, and was incubated at 30°C for 15 min. The conditions for HPLC were as follows: column, ChemcoPak NUCLEOSIL 120-10C18 (300 × 4.0 id mm); detection, UV detector (220 nm); solvent, 20 mM phosphate buffer, pH 2.8; flow rate, 1.0 ml/min; internal standard, propionic acid. One unit of enzyme activity was defined as the activity releasing one μmol of acetic acid per min per mil of enzyme solution.

The results of the esterase assay are shown in Table 2. While preincubation without glucose enhanced the esterase production, the ratios of esterase activities toward acetylxylan and 4-nitrophenyl acetate were similar, 1.77 and 1.91, respectively, for 0 and 1% glucose in preculture. This fungal esterase activity shows a higher specificity to acetylxylan than does porcine liver esterase, for which the activity ratio is 0.0065. Because xylan-degrading activities (xylanase and β-xylosidase) were not detected in the fungal enzyme samples, their influence was not considered in the measurement of esterase activity against acetylxylan. As specificity to carbohydrate acetate is one of the properties of acetylxylan esterase (Biely et al., 1986), the acetyl esterase of *C. versicolor* is considered to belong to acetylxylan esterase.

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