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Production of acetyl esterase during wood biodegradation by *Coriolus versicolor*

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A role of acetyl esterase in wood biodegradation by *Coriolus versicolor* was examined by the assay of enzyme production and the chemical analysis of decayed wood meal of Japanese beech (*Fagus crenata*). Enzyme assay demonstrated that the degradation proceeded in two stages and acetyl esterase production was correlated with the cellulolytic and xylanolytic enzyme production in the second stage, not with the production of phenol-oxidizing enzymes. From the results of chemical analysis, acetyl and xylose contents in wood meal were observed to decrease simultaneously in the second stage. In contrast, rapid decrease of lignin was recognized during the initial three wk of incubation, and it was closely related with the production of phenol-oxidizing enzymes in the first stage. These results show that acetyl esterase of *C. versicolor* participates in the degradation of acetylxylan and acts with the cellulolytic and xylanolytic systems, not with the ligninolytic system.

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

Wood hemicellulose contains a large number of acetyl groups as side chain moieties, such as glucuronoxylan of angiosperms (hardwoods) and galactoglucomannan of gymnosperms (softwoods) (Timell, 1964,1965; Lindberg et al., 1973a, b). In wood biodegradation, deacetylation has been overlooked, although it is important for enzymic breakdown of hemicelluloses. Biely (1985) pointed out the importance of enzymic deacetylation to degrade the native acetylated xylan. Acetyl esterases which release acetic acid from acetylated xylan and glucomannan were reported to contribute to the biodegradation of these polysaccharides (Biely et al., 1985; Biely et al., 1986; Poutanen et al., 1990; Tenkanen et al., 1993). In practice, acetyl esterase like acetylxylan esterase is widely distributed in wood-rotting fungi (Tsujiyama and Nakano, 1996b). However, the role of esterases in wood-degrading systems has not been fully clarified. Furthermore, wood hemicellulose as a constituent of the secondary cell wall has been proposed to be complexed with lignin, resulting in the formation of a lignin-hemicellulose matrix (Kerr and Goring, 1975). Therefore, biological degradation of native woody hemicelluloses with acetyl groups would not only involve hydrolysis by hemicellulolytic enzymes and acetyl esterase but also be affected by the decomposition of the lignin portion. The role of acetyl esterase in the biodegradation of wood is assumed to be more complicated than that in the biodegradation of isolated hemicelluloses alone.

In this study, the role of acetyl esterase in the wood decaying system was investigated based on the enzyme assay and the chemical analysis of wood meal decayed by a white-rot fungus, *Coriolus versicolor* (L.: Fr.) Quélet.

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Materials and Methods

Microorganism and cultivation A white-rot fungus, *C. versicolor* IFO 30340, was obtained from the Institute for Fermentation, Osaka, Japan (IFO).

Basal medium used in this study was according to Kirk et al. (1978) with 10 mM ammonium tartrate added as N-source to enhance the production of acetyl esterase (Tsujiyama and Nakano, 1996a). Two grams of wood meal of Japanese beech (*Fagus crenata* Blume) (40–100 mesh) was added to 15 ml of the liquid medium in 100-ml Erlenmeyer flasks; the wood meal culture was incubated stationary at 28°C after inoculation with *C. versicolor*.

Enzyme assays Enzyme assay was performed using an extract obtained from the wood-meal culture with 20 mM succinate buffer (pH 4.5) according to Kofujita et al. (1992).

Acetyl esterase activity was measured spectrophotometrically using *p*-nitrophenyl acetate (Wako Pure Chem.) according to Biely et al. (1986). One unit of enzyme activity was defined as the amount of the enzyme that released 1 μmol of *p*-nitrophenol per s. Acetylxylan esterase activity was measured as reported previously (Tsujiyama and Nakano, 1996a). One unit of enzyme activity was defined as the amount of the enzyme that released 1 μmol of acetic acid from a substrate per s.

Endo-1,4-β-glucanase (CMCase), avicelase and endo-1,4-β-xylanase activities were assayed by using car-

boxymethyl cellulose sodium salt (CMC-Na) (Nakalai tesq.), microcrystalline cellulose FUNACEL-SF (Funakoshi) and alkali-extracted xylan from Japanese beech (*F. crenata*) as substrates, respectively. Beech xylan was prepared according to Browning (1967). Enzyme activities were measured by determining the amounts of reducing groups formed by the enzyme action by the Somogyi-Nelson method as reported previously (Tsujiyama and Nakano, 1996b). One unit of enzyme activity was defined as the amount of enzyme that released 1 μmol of reducing sugar (expressed as glucose) per min.

Glycosidase activities (β-D-glucopyranosidase and β-D-xylopyranosidase) were assayed by using *p*-nitrophenyl glycoside substrates as reported previously (Tsujiyama et al., 1992). One unit of enzyme activity was defined as the amount of enzyme that released 1 μmol of *p*-nitrophenol per min.

Three kinds of phenol-oxidizing enzyme activities (laccase, manganese-dependent peroxidase (Mn-peroxidase) and lignin peroxidase) were assayed. The activity of laccase was assayed by measuring the oxidation of syringaldazine (Aldrich) spectrophotometrically at 525 nm according to Leonowicz and Grzywhowicz (1981). The activity of Mn-peroxidase was measured in the same way as that of laccase except for the addition of H_2O_2 and $MnSO_4$ (final concentration: 0.4 mM and 0.3 mM, respectively) in the reaction mixture. The activity of lignin peroxidase was assayed by measuring the oxidation of veratryl alcohol (Tokyo Chemical Industry) spectrophotometrically at 310 nm according to Tien and Kirk (1988). One unit of these enzyme activities was defined as the amount of enzyme that oxidized 1 μmol of substrates per min.

Chemical analysis of wood components Lignin content was measured by using acetyl bromide according to Iiyama and Wallis (1988)

Neutral sugar components were analyzed as alditol acetates (Borchardt and Piper, 1970) by gas chromatography at 220°C on a column of OV-225 (2.6 mm × 2.1 m: GL Science) using inositol as an internal standard.

Acetyl content in the degraded wood meal was analyzed by a gas chromatography at 190–220°C (1°C/min) on a column of Gaskuropack 54 (3.0 mm × 2.1 m: GL Science) using propionic acid as an internal standard.

Mycelial weight in residual wood meal was estimated by the Kjeldahl-indophenol method (Bolleter et al., 1961).

The weight of residual wood was calculated by subtracting the mycelial weight from that of recovered insoluble materials. Glucose content of wood was evaluated by subtracting the glucose content of mycelium from total glucose amount analyzed by a gas chromatography. Acetyl content of residual wood meal was evaluated in the same was as glucose content.

Instrumentation Spectrophotometric analysis was carried out with a Shimadzu spectrophotometer UV-2200. Gas chromatographic analysis was carried out with a Shimadzu GC-12A equipped with a flame ionization detector.

Results

Enzyme production during wood degradation Enzyme activities during incubation are shown in Figs. 1–5.

Figure 1 shows that acetyl esterase activity was detected even in the early period of incubation and increased after 8 wk. On the other hand, acetylxylan esterase activity (Fig. 2) was remarkably observed at the beginning of incubation and after 6 wk. Probably, the

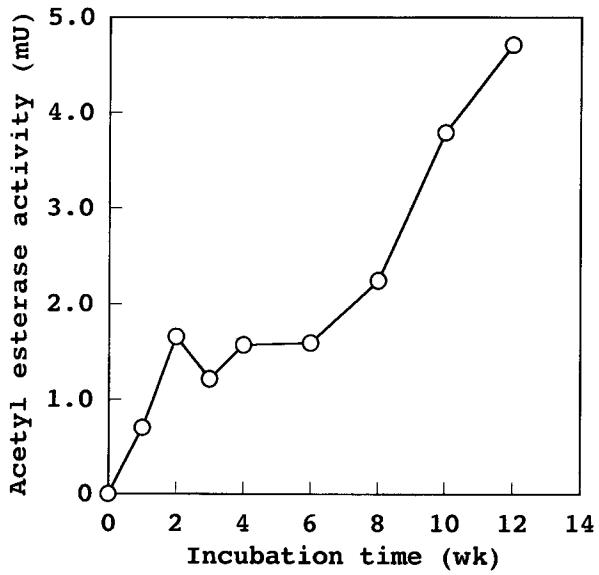

Fig. 1. Production of acetyl esterase by *Coriolus versicolor* on wood-meal culture.

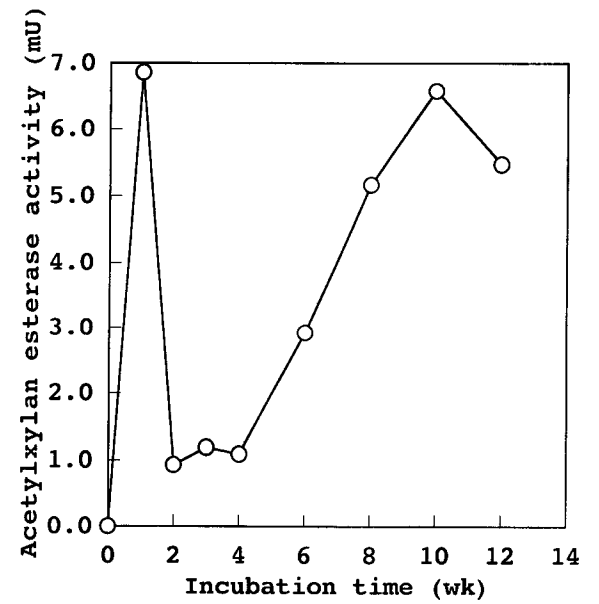

Fig. 2. Production of acetylxylan esterase by *Coriolus versicolor* on wood-meal culture.

value of acetyl esterase activity against acetylxylan would be affected by the cooperative action of the xylanolytic enzymes as shown below. Therefore, in the presence of high active xylanolytic enzymes, acetyl esterase activity using acetylxylan as a substrate indicates not the esterase activity against acetylxylan but the releasing activity of acetic acid from acetylxylan. These results reveal that acetyl esterase was produced mainly after 8 wk of incubation.

The glycosidic linkage-hydrolyzing enzymes assayed were polysaccharide-degrading enzymes (cellulases and xylanase) and glycosidases. Cellulases and xylanase had two production peaks, i.e., before 4 wk and after 8 wk, as shown in Fig. 3. The latter peak seems to coincide with the production of acetyl esterase. Two glycosidases (β-glucosidase and β-xylosidase) were produced actively in the latter part of the incubation period (Fig. 4).

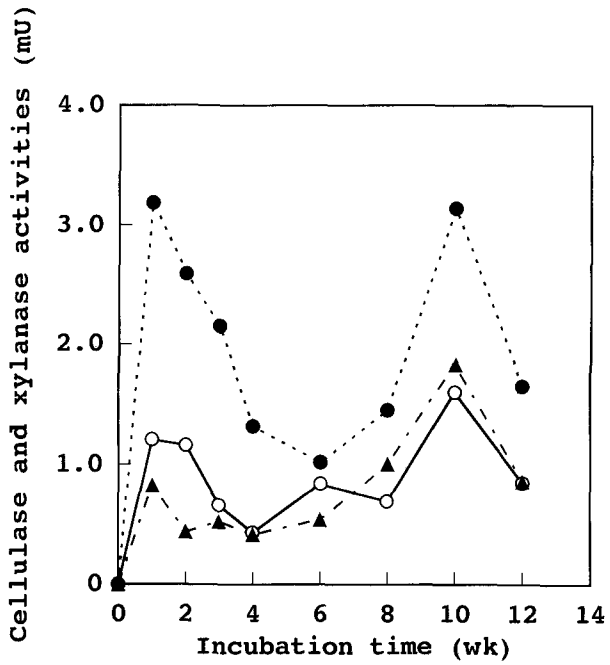

Fig. 3. Production of cellulases and xylanase by *Coriolus versicolor* in wood-meal culture.
Symbols: avicelase (○), CMCase (●), xylanase (▲).

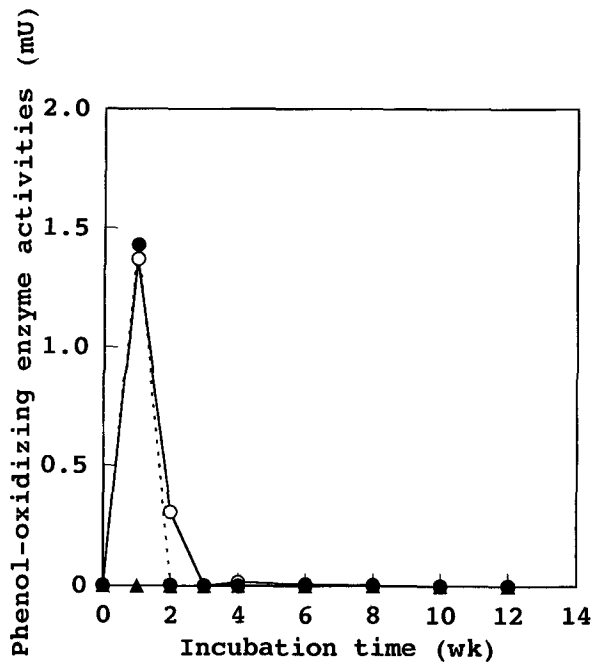

Fig. 5. Production of phenol-oxidizing enzymes by *Coriolus versicolor* in wood-meal culture.
Symbols: laccase (○), Mn-dependent peroxidase (●), lignin peroxidase (▲).

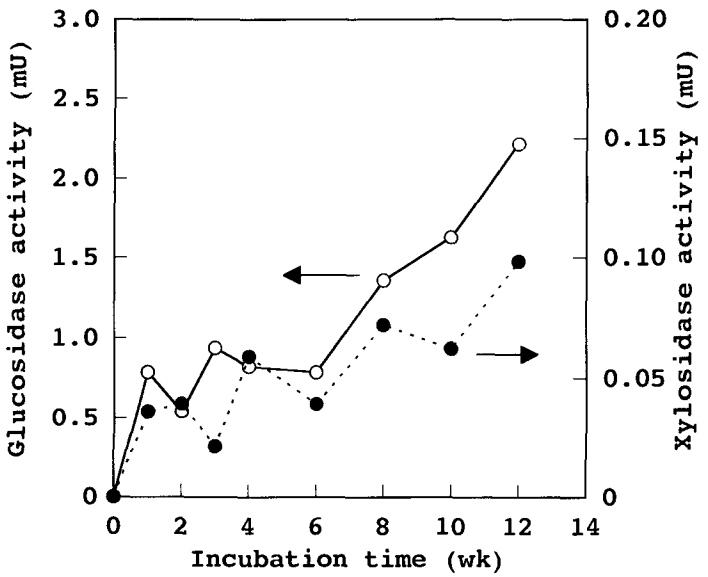

Fig. 4. Production of glycosidases by *Coriolus versicolor* in wood-meal culture.
Symbols: β-glucosidase (○), β-xylosidase (●).

Of the three phenol-oxidizing enzyme activities assayed in this study, *C. versicolor* produced high activities laccase and Mn-peroxidase in the early period of incubation, but very low activity of lignin peroxidase (Fig. 5). All three enzymes were scarcely detected after 4 wk.

Chemical composition changes in degraded wood meal

Change of chemical composition of wood meal is shown in Table 1. Weight of residual wood meal reached to 81.5% of the original weight after 12 wk.

Lignin decreased markedly only in the early period, as expected from the patterns of phenol-oxidizing enzyme production. In contrast, the amounts of sugars decreased little in the same period. Remarkable decreases in the amounts of glucose and xylose were observed after 8 wk. This indicates that cellulose and xylan in wood were decomposed mainly in the latter part of the incubation.

Acetyl content gradually decreased throughout the incubation, especially after 8 wk, when the production of acetyl esterase increased. However, in the beginning of incubation when high acetylxylan esterase activity was detected, decrease of acetyl content was not observed. This reveals that the esterase activity against acetylxylan does not reflect the deacetylation reaction of wood meal. One possible reason is that deacetylation of wood meal might need the modification of lignin because acetylxylan in wood cell wall forms the complex with lignin. Based on the enzyme assays, acetyl esterase is deduced to act cooperatively with the cellulolytic and xylanolytic enzymes in the latter part of the incubation period.

Discussion

Because of their high ligninolytic ability, white-rot fungi have been investigated for various applications, such as biological pulping, biological bleaching and lignocellulose fermentation. However, there are few examinations about acetyl esterase of white-rot fungi in the degradation of native acetylated hemicelluloses, especially in the degradation of native lignocellulosics containing acetylated polysaccharides, for which biological deacetylation is indispensable.

The present study has revealed two main stages with the different roles in the degradation of wood meal by *C. versicolor*. Acetyl esterase production correlated with the second stage, though it was detected throughout the incubation for wood degradation.

The first stage contributed to lignin degradation, because high activities of phenol-oxidizing enzymes other than lignin peroxidase were detected only in this period. Chemical analysis demonstrated a remarkable decrease of lignin content was determined in this period, supporting the suggestion derived from the enzyme analysis. In the same period, high activities of cellulases and xylanase were also detected, but the amounts of glucose and xylose did not decrease so drastically. Although lignin is complexed with hemicellulose to form the lignin-hemicellulose matrix (Kerr and Goring, 1975), hemicellulose decomposition did not occur simultaneously with lignin degradation. The action of cellulases and xylanase appears rather to cause the depolymerization of cellulose and xylan, thereby enhancing the accessibility of phenol-oxidizing enzymes to lignin in the matrix of wood cell walls.

In the second stage, i.e., after 8 wk, cellulose and xylan degradations were confirmed by chemical analysis. The enhanced productions of cellulolytic and hemicellulolytic enzymes (polysaccharases and glycosidases) and acetyl esterase in this stage also point to the decomposition of polysaccharides. In earlier reports, the simultaneous productions of both acetyl esterase and xylanolytic enzymes during acetylxylan degradation were observed in *Trichoderma reesei* Simmons, *Schizophyllum commune* Fr.: Fr., *Streptomyces* spp. and *Aureobasidium pullulans* (de Bary) Arnaud (MacKenzie et al., 1987; Biely et al., 1988; MacKenzie and Bilous, 1988; Myburgh et al., 1991). These enzymes are considered to act in the cooperative degradation of the isolated acetylated xylan (Biely et al., 1986). In this study, the wood-rotting basidiomycetes *C. versicolor* simultaneously secreted acetyl esterase and xylanolytic enzymes in the second stage to degrade acetylxylan in wood cell wall. It is noteworthy that active cellulases and β-glucosidase were also detected at this stage, and cellulose degradation was confirmed by the chemical analysis of the residual wood. Acetyl esterase may be a constituent enzyme

Table 1. Composition changes of wood meal degraded by *Coriolus versicolor*.

Incubation time (wk)	Lignin	Glucose	Xylose	Acetyl group	Residual wood meal (%)[a]
0	29.5	49.4	15.4	2.72	100.0
1	28.6	46.7	15.1	2.73	95.7
2	26.7	46.3	15.0	2.64	94.4
3	24.6	46.8	15.2	2.64	93.7
4	24.9	46.6	15.0	2.58	91.9
6	24.9	43.7	14.5	2.51	88.1
8	24.4	39.9	13.2	2.33	82.2
10	24.8	39.6	12.9	2.24	81.9
12	24.5	39.7	12.7	2.28	81.5

a) Percents against the weight of original wood.

not only of the xylanolytic system but also of the cellulolytic system in this fungus, as reported for *T. reesei* and *S. commune* (Biely et al., 1988). The production of acetyl esterase was observed to increase after the marked decreases of xylan, cellulose and acetyl contents. While acetyl esterase production is assumed to be enhanced to attack the released fragments containing acetylxylan, it may be induced by the degradation products in the same way as other cellulolytic and xylanolytic enzymes. Further study is needed to examine the inductivity of acetyl esterase by various substrates and its isozyme patterns in the cellulolytic and xylanolytic systems.

In conclusion, acetyl esterase of a wood-rotting fungus, *C. versicolor*, was produced together with the cellulolytic and hemicellulolytic enzymes in the period of the polysaccharide degradation that followed the lignin degradation.

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