Mycoscience 37: 289–294, 1996

Distribution of acetyl esterase in wood-rotting fungi

Sho-ichi Tsujiyama and Naoko Nakano[1)]

Faculty of Agriculture, Kyoto Prefectural University, Shimogamo-nakaragi-cho, Sakyo-ku, Kyoto 606, Japan

Accepted for publication 3 July 1996

The distribution of acetyl esterase was studied in 30 strains of wood-rotting fungi. A screening test on agar plates using glucose β-D-pentaacetate as a substrate indicated that all tested fungi produced acetyl esterase to form a clear zone on the culture. All fungi also showed positive responses in an agar test using carboxymethyl cellulose acetate. Enzyme assay showed that extracellular acetylxylan esterase activity was present in the filtrates of wood-meal culture of all these fungi. The ratio of fungal acetylxylan esterase activity to 4-nitrophenyl acetyl esterase activity were higher than that of porcine liver esterase, indicating that fungal esterases have high affinity for acetylated carbohydrates. Acetyl esterase is suggested to be distributed widely in wood-rotting fungi for degradation of native acetylated hemicelluloses.

Key Words——acetyl esterase; wood biodegradation; wood-rotting fungi.

Wood-rotting fungi degrade wood component materials: cellulose, hemicelluloses and lignin. Some of hemicelluloses that constitute wood cell walls are present as acetyl-substituted forms, such as acetylxylan in angiospermous wood and acetylglucomannan in gymnospermous wood (Timell, 1964, 1965). The contents of acetylated hemicelluloses can reach 20–30% in wood component materials.

Recently, acetyl esterase was reported to play an important role in biodegradation of such acetylated polysaccharides as acetylxylan (Biely, 1985; Poutanen and Sundberg, 1988) and acetylglucomannan (Tenkanen et al., 1993). As acetylated polysaccharides are resistant to glycosidic bond hydrolyzing enzymes because of low affinity, steric hindrance and other factors, deacetylation must occur prior to glycoside hydrolysis. In wood biodegradation, the action of acetyl esterase is assumed to be necessary for degradation of acetylated hemicelluloses. There are a few reports about acetyl esterase-producing wood-rotting Basidiomycetes (Biely et al., 1985, 1986; Tenkanen et al., 1993; Tsujiyama and Nakano, 1996). In this study, the distribution of acetyl esterase was examined in 30 wood-rotting basidiomycete fungi.

Materials and Methods

Organisms Thirty strains of wood-rotting fungi were used: 1. *Armillaria mellea* (Vahl: Fr.) Karst. (KPUF 9301), 2. *Auricularia auricula* (Hook.) Underw. (KPUF 7502), 3. *Auricularia polytricha* (Mont.) Sacc. (KPUF 2394), 4. *Coriolus hirsutus* (Wulf.: Fr.) Quél. (KPUF 2594), 5. *Coriolus versicolor* (L.: Fr.) Quél. (IFO 30340), 6. *Elfvingia applanata* (Pers.) Karst. (KPUF 0694), 7. *Fomes fomentarius* (L.: Fr.) Fr. (small type) (KPUF 1094), 8. *F. fomentarius* (large type) (KPUF 0894), 9. *Fomitopsis palustris* (Berk.: Curt.) Gilbn. et Ryv. (=*Tyromyces palustris* (Berk.: Curt.) Murr.) (IFO 30339), 10. *Fomitopsis pinicola* (Swartz.: Fr.) Karst. (KPUF 0394), 11. *Laetiporus sulphureus* (Fr.) Murr. var. *miniatus* (Jungh.) Imaz. (KPUF 1194), 12. *Laetiporus versisporus* (Lloyd) Imaz. (KPUF 0495), 13. *Lentinula edodes* (Berk.) Sing. (KPUF 0395), 14. *Lentinus lepideus* (Fr.: Fr.) Fr. (KPUF 0194), 15. *Lenzites betulina* (L.: Fr.) Fr. (KPUF 2194), 16. *Microporus* sp. (KPUF 0795), 17. *Oligoporus caesius* (Schrad.: Fr.) Gilbn. et Ryv. (KPUF 1695), 18. *Oligoporus tephroleucus* (Fr.) Gilbn. et Ryv. (KPUF 0994), 19. *Onnia vallata* (Berk.) Aoshima (KPUF 0695), 20. *Phaeolus schweinitzii* (Fr.) Pat. (KPUF 0995), 21. *Phanerochaete chrysosporium* Burd. (IFO 31249 (ATCC 34541)), 22. *Piptoporus betulinus* (Bull.: Fr.) Karst. (KPUF 1595), 23. *Pleurotus ostreatus* (Jacq.: Fr.) Kummer (KPUF 2494), 24. *Polyporus alveolarius* (DC. ex Fr.) Bond. et Sing. (KPUF 0794), 25. *Pycnoporus coccineus* (Fr.) Bond. et Sing. (KPUF 2094), 26. *Schizophyllum commune* Fr.: Fr. (KPUF 8805), 27. *Serpula lacrymans* (Wulf.: Fr.) Schroet. (KPUF 7802), 28. *Tremella foliacea* Pers.:Fr. (KPUF 2294), 29. *Truncospora ochroleuca* (Berk.) Pilat (KPUF 0494), 30. *Tyromyces incarnatus* Imaz. (KPUF 0594).

Coriolus versicolor, *F. palustris* and *P. chrysosporium* were obtained from the Institute of Fermentation, Osaka (IFO), Japan. *Armillaria mellea*, *S. commune* and *S. lacrymans* were provided by Mr. Togashi, Hokkaido Forest Products Research Institute, Asahikawa. Other fungi were from Kyoto Prefectural University Forestry Collection. Two strains of *F. fomentarius* with morphologically different features (Imazeki, 1989) were included (Nos. 7 and 8). Decay type and host specificity are listed in Table 1.

Screening of acetyl esterase producing fungi Acetyl esterase-producing fungi were detected using a double-layer agar plate containing glucose β-D-pentaacetate or car-

[1)]Present address: Arborex Co. Ltd., Osaka 587, Japan.

Table 1. Detection of acetyl esterase on agar tests.

Species	Decay type[a]	Host specificity[b]	Clear zone expansion rate (mm/h)			
			Glucose acetate glucose conc.			CMC acetate[c]
			0%	1%		
Armillaria mellea	W	A, G	0.05	0.05		++
Auricularia auricula	W	A	0.12	0.09	*[d]	+
Auricularia polytricha	W	A	0.07	0.09		+
Coriolus hirsutus	W	A	0.33	0.29	*	++
Coriolus versicolor	W	A	0.48	0.33	*	+
Elfvingia applanata	W	A	0.23	0.21		+
Fomes fomentarius (small type)	W	A	0.24	0.23		++
Fomes fomentarius (large type)	W	A	0.14	0.14		+
Fomitopsis palustris	B	G	0.07	0.11	*	++
Fomitopsis pinicola	B	G	0.16	0.14	*	+
Laetiporus sulphureus var. *miniatus*	B	A	0.28	0.14	*	++
Laetiporus versisporus	B	A	0.35	0.28	*	+
Lentinula edodes	W	A	0.09	0.08		++
Lentinus lepideus	B	G	0.10	0.15	*	++
Lenzites betulina	W	A	0.34	0.35		+
Microporus sp.	W	A	0.21	0.21		+
Oligoporus caesius	B	G	0.42	0.10	*	+
Oligoporus tephroleucus	B	A	0.12	0.13		+
Onnia vallata	W	G	0.09	0.08		+
Phaeolus schweinitzii	B	G	0.10	0.09		+
Phanerochaete chrysosporium	W	A	0.92	0.07	*	++
Piptoporus betulinus	B	A	0.20	0.24	*	++
Pleurotus ostreatus	W	A	0.33	0.21	*	++
Polypolus alveolarius	W	A	0.12	0.20	*	+
Pycnoporus coccineus	W	A	0.47	0.21	*	+
Schizophyllum commune	W	A, G	0.32	0.29	*	++
Serpula lacrymans	B	G	0.05	0.04		+
Tremella foliacea	B	A	0.13	0.09	*	+
Truncospora ochroleuca	W	G	0.13	0.14		+
Tyromyces incarnatus	W	A	0.32	0.37	*	+

a) W: White-rot, B: Brown-rot.
b) A: Angiospermous wood, G: Gymnospermous wood.
c) As clear zone cannot be calculated (see text), degree of the clear zone formation was presented.
++: Clear zone was formed in whole culture, +: Clear zone was formed in parts of culture.
d) Significant difference between the values at two glucose concentrations is observed at 5% level.

boxymethyl cellulose (CMC) acetate as detectable substrates (Tsujiyama and Nakano, 1996). Tested fungus was cultivated at 28°C on a double layer agar plate containing Kirk's basal medium (Kirk et al., 1978), and additionally 2% (w/w) of above detectable substrates in the upper layer.

Enzyme assay Wood-meal culture was prepared as follows; 1% (w/v) wood meal was added in 20 ml of Kirk's basal culture (Kirk et al., 1978) and sterilized in an autoclave for 20 min at 121°C. Fungi were incubated at 28°C in above culture. Only *S. lacrymans* was incubated at 20°C. Wood-meal powders (<40 mesh) were prepared from Japanese beech (*Fagus crenata* Blume) and Japanese red pine (*Pinus densiflora* Sieb. et Zucc.) and extracted with hot water and subsequently with ethenol-benzene solution (1/2=v/v). To avoid the effect of host specificity on growth, wood-meal powder was mixed with both woods (1/1=w/w). After incubation for 1 or 2 wk, culture medium was filtered with a cloth filter, added distilled water to a final volume of 25 ml, and used as an enzyme solution.

Acetyl esterase activity was measured with 4-nitrophenyl acetate according to Biely et al. (1986). Acetylxylan was prepared by extraction with dimethyl sulfoxide from *F. crenata* holocellulose according to Bouverg and Lindberg (1965). Acetylxylan esterase activity

was measured with acetylxylan by using a commercial assay kit for detection of released acetic acid (Boehringer Mannheim Biochemicals) according to Lee et al. (1987).

Activities of xylan- and glucomannan-degrading enzymes were measured using alkali-extracted xylan and glucomannan from *F. crenata* and *P. densiflora*, respectively, as follows: reaction solution containing 0.2 ml of enzyme solution, 0.25 ml of 1% (w/v) substrate solution (xylan or glucomannan) and 0.1 ml of 0.5 M acetate buffer (pH 4.8), total volume 1 ml, was incubated at 36°C for 1 h. Reducing ends formed by enzyme action were estimated by the Somogyi-Nelson method (Somogyi, 1952) using glucose as a standard.

Two kinds of debranching glycosidase activity (α-L-arabinofuranosidase and α-D-galactopyranosidase) were measured using *p*-nitrophenyl glycosidic substrates according to Highley (1976).

Table 2. Effect of glucose on clear zone expansion rate.

Decay type	Host specificity			Total
	Angiosperm	Angiosperm and Gymnosperm	Gymnosperm	
White-rot	6	1	0	7
	1	0	1	2
Brown-rot	3	—	2	5
	1	—	2	3
Total	9	1	2	12
	2	0	3	5

Note: Upper and lower numbers are the numbers of fungi whose clear zone expansion rates were enhanced without or with glucose, respectively.

Table 3. Acetyl esterase activities of wood-rotting fungi.

Species	Substrate		Ratio of AX/4N
	Acetylxylan (AX)	4-Nitrophenyl acetate (4N)	
Armillaria mellea	0.044(U)[a]	0.006(U)	7.17
Auricularia auricula	0.460	0.267	1.72
Auricularia polytricha	0.089	0.015	5.90
Coriolus hirsutus	0.716	0.148	4.84
Coriolus versicolor	0.698	0.169	4.13
Elfvingia applanata	0.125	0.058	2.16
Fomes fomentarius (small type)	0.222	0.061	3.63
Fomes fomentarius (large type)	0.120	0.020	5.97
Fomitopsis palustris	0.275	0.071	3.87
Fomitopsis pinicola[b]	0.172	0.085	2.03
Laetiporus sulphureus var. *miniatus*[b]	0.009	0.017	0.55
Laetiporus versisporus	0.765	0.383	2.00
Lentinula edodes	0.470	0.206	2.28
Lentinus lepideus[b]	0.085	0.041	2.05
Lenzites betulina	0.701	0.035	19.91
Microporus sp.	0.414	0.084	4.94
Oligoporus caesius	0.326	0.311	1.05
Oligoporus tephroleucus	0.234	0.056	4.20
Onnia vallata	0.297	0.009	34.27
Phaeolus schweinitzii	0.193	0.132	1.47
Phanerochaete chrysosporium	0.666	0.448	1.49
Piptoporus betulinus	0.407	0.153	2.65
Pleurotus ostreatus	0.469	0.035	13.29
Polypolus alveolarius	0.167	0.034	4.96
Pycnoporus coccineus	0.639	0.217	2.94
Schizophyllum commune	0.330	0.302	1.09
Serpula lacrymans[b]	0.430	0.087	4.95
Tremella foliacea	0.265	0.517	0.51
Truncospora ochroleuca[b]	0.175	0.038	4.56
Tyromyces incarnatus	0.245	0.037	6.66
Porcine liver esterase	0.094	14.40	0.0065

a) 1 U = 1 mM released acetic acid/min/ml enzyme sol.
b) Incubation for 2 wk.

Results and Discussion

Screening of acetyl esterase producing fungi All 30 strains tested formed a clear zone around the colony on agar plates containing glucose β-D-pentaacetate, indicating that acetyl esterase was secreted causing deacetylation of glucose acetate. Lee et al. (1987) reported that only 13 of 350 strains of yeast showed positive responses in the same screening test. Though only 30 strains were tested here, they included various combinations of decay type and host specificity of esterase-producing fungi and the fact that all showed a positive response suggests that acetyl esterase is distributed widely in wood-rotting fungi.

The effect of glucose addition was investigated by measuring clear zone expansion rate. The measured expansion rates with and without glucose were shown in Table 1. Table 2 shows the numbers of fungi whose clear zone expansion rates were enhanced with or without glucose, classified according to decay type and host specificity. Seventeen of the 30 fungi were affected by glucose concentration. In 12 species, the expansion rate was enhanced by glucose starvation; and 5 species were more active in the presence of glucose. Glucose regulation to enhance esterase production was found more often in angiosperm-preferential fungi. White-rot fungi seem to have this property more frequently than brown-rot fungi. It has been reported that glucose regulation induced acetylxylan esterase production in an angiosperm-preferential white-rot fungus, *C. versicolor* (Tsujiyama and Nakano, 1996). This inductivity by glucose regulation appears to be relatively high in the same group.

In the screening test using CMC acetate, the clear zone expansion rate could not be calculated because insoluble CMC acetate particles gradually dissolved in the whole culture after mycelium had spread over the plate. After 1-2 mo, 30 strains all indicated positive responses

Table 4. Hemicellulose-degrading enzyme activities of wood-rotting fungi.

Species	Substrates		Ratio of GM/X
	Glucomannan (GM)	Xylan (X)	
Armillaria mellea	16.0(U)[a]	17.4(U)	0.92
Auricularia auricula	8.8	24.1	0.37
Auricularia polytricha	18.1	16.7	1.08
Coriolus hirsutus	10.2	13.9	0.73
Coriolus versicolor	18.8	25.6	0.73
Elfvingia applanata	14.5	39.0	0.37
Fomes fomentarius (small type)	103.4	118.1	0.88
Fomes fomentarius (large type)	58.9	56.0	1.05
Fomitopsis palustris	1201.4	985.6	1.22
Fomitopsis pinicola[b]	689.0	248.0	2.78
Laetiporus sulphureus var. *miniatus*[b]	33.3	160.0	0.21
Laetiporus versisporus	238.4	212.5	1.12
Lentinula edodes	103.3	146.3	0.71
Lentinus lepideus[b]	646.0	46.3	13.95
Lenzites betulina	20.8	27.1	0.77
Microporus sp.	126.9	107.9	1.18
Oligoporus caesius	367.1	275.9	1.33
Oligoporus tephroleucus	577.0	511.0	1.13
Onnia vallata	282.9	76.4	3.70
Phaeolus schweinitzii	254.6	238.0	1.07
Phanerochaete chrysosporium	640.7	619.4	1.03
Piptoporus betulinus	90.7	152.8	0.59
Pleurotus ostreatus	5.6	20.8	0.27
Polypolus alveolarius	37.5	14.8	2.53
Pycnoporus coccineus	153.7	250.9	0.61
Schizophyllum commune	304.0	399.0	0.76
Serpula lacrymans[b]	341.7	106.9	3.20
Tremella foliacea	47.7	136.1	0.35
Truncospora ochroleuca[b]	15.3	19.0	0.81
Tyromyces incarnatus	18.1	18.8	0.96

a) 1 U = 1 μM glucose/h/ml enzyme sol.
b) Incubation for 2 wk.

(Table 1). This result indicates that these fungi produced esterases which can deacetylate even chemically acetylated polysaccharides.

Acetyl esterase activity Acetyl esterase and acetylxylan esterase activities produced in wood-meal culture are shown in Table 3. Enzyme samples of all fungi showed both esterase activities, indicating that they secreted the extracellular esterase in wood-meal culture. The ratio of the esterase activity toward acetylxylan to that toward 4-nitrophenyl acetate is shown in Table 3. These ratios of the tested fungi were higher than that of porcine liver esterase purchased from Sigma Co. Ltd., indicating that these fungal esterases have higher activity toward acetylxylan than arylesterase. As a property of acetylxylan esterase is its high affinity for carbohydrate acetate (Biely et al., 1985), so these fungal esterases were deduced to be of a type that can attack the acetylated hemicelluloses, like acetylxylan, in wood.

Table 5. Debranching glycosidase activities of wood-rotting fungi.

Species	Substrates	
	Galactosidase	Arabinosidase
Armillaria mellea	0.39	0.74(U)[a]
Auricularia auricula	0.11	0.08
Auricularia polytricha	0.06	0.03
Coriolus hirsutus	0.07	0.31
Coriolus versicolor	0.50	2.56
Elfvingia applanata	1.02	1.49
Fomes fomentarius (small type)	2.20	0.23
Fomes fomentarius (large type)	0.28	0.50
Fomitopsis palustris	0.74	3.27
Fomitopsis pinicola[b]	5.29	11.30
Laetiporus sulphureus var. *miniatus*[b]	0.04	0.03
Laetiporus versisporus	0.19	0.31
Lentinula edodes	0.04	0.31
Lentinus lepideus[b]	2.63	0.81
Lenzites betulina	0.10	0.62
Microporus sp.	0.71	0.26
Oligoporus caesius	0.17	1.61
Oligoporus tephroleucus	0.07	0.08
Onnia vallata	0.06	0.19
Phaeolus schweinitzii	0.39	0.65
Phanerochaete chrysosporium	0.49	0.07
Piptoporus betulinus	0.09	3.99
Pleurotus ostreatus	0.11	0.25
Polypolus alveolarius	0.09	0.26
Pycnoporus coccineus	2.39	3.31
Schizophyllum commune	0.15	0.37
Serpula lacrymans[b]	0.04	0.88
Tremella foliacea	1.62	0.15
Truncospora ochroleuca[b]	0.00	0.10
Tyromyces incarnatus	0.38	0.10

a) 1 U = 1 mM/min/ml enzyme sol.

Hemicellulose degrading enzyme activity Table 4 shows the levels of hemicellulose-degrading enzyme activities, xylan- and glucomannan-degrading activities, which were present in all fungi under the culture conditions employed. From the ratio of the two enzyme activities, no remarkable host specificity was observable in the production of the hemicellulose-degrading enzymes. This is probably because the mixing of wood-meals regulated the occurrence of host specificity. Therefore, acetyl esterase produced by the tested fungi would participate in degradation of both acetylxylan and acetylglucomannan in wood. Tenkanen et al. (1993) reported that acetylglucomannan esterase activity was detected in all culture filtrates of six kinds of fungi, including a wood-rotting basidiomycete, *S. commune*. Together with our results, this suggests that acetyl esterase for deacetylation of acetylglucomannan might be widely distributed among hemicellulose-metabolizing fungi, such as wood-rotting fungi.

Two kinds of debranching glycosidases, α-L-arabinofuranosidase and α-D-galactopyranosidase, were also assayed and their activities are shown in Table 5. These two glycosidases are needed for degradation of hemicelluloses, acting to split side-chains of arabinofuranoside and galactopyranoside linkages substituted on xylan of *F. crenata* and glucomannan of *P. densiflora*, respectively. All fungi produced one or both of these enzymes in this culture. In view of the simultaneous productions of hemicellulose-degrading enzymes and acetyl esterase, these enzymes are thought to degrade hemicelluloses cooperatively as proposed by Biely (1985).

In conclusion, acetyl esterase was produced by all the tested wood-rotting fungi in wood-meal culture, indicating that this enzyme is widely distributed in wood-rotting fungi, acting to metabolize acetyl-substituted hemicelluloses, acetylxylan of angiospermous wood and acetylglucomannan of gymnospermous wood.

Acknowledgement——The authors wish to express their thanks to Mr. I. Togashi, Hokkaido Forest Products Institute, Asahikawa, for providing test fungi.

Literature cited

Biely, P. 1985. Microbial xylanolytic systems. Trends in biotechnol. **3**: 286–290.

Biely, P., Puls, J. and Schneider, H. 1985. Acetyl xylan esterase in fungal cellulolytic systems. FEBS Lett. **186**: 80–84.

Biely, P., Mackenzie, C. R., Puls, J. and Schneider, H. 1986. Cooperativity of esterases and xylanases in the enzymic degradation of acetyl xylan. Bio/Biotechnol. (USA) **4**: 731–733.

Bouveng, H. O. and Lindberg, B. 1965. Native acetylated wood polysaccharides. Methods in carbohydrate chemistry, vol. 5. (ed. by Whistler, R. L.), pp. 147–150. Academic Press, London.

Highley, T. L. 1976. Hemicellulases of white- and brown-rot fungi in relation to host preferences. Mater. Org. **11**: 33–46.

Imazeki, R. 1989. Polyporaceae. In: Colored illustrations of mushrooms of Japan, vol. II, (ed. by Imazeki, R. and Hongo,

T.), p. 172. Hoikusha Pub., Osaka. (In Japanese.)
Kirk, T. K., Schulz, E., Conners, W. J., Lorenz, L. F. and Zeikus, G. 1978. Influence of culture parameters on lignin metabolism by *Phanerochaete chrysosporium*. Arch. Microbiol. **117**: 277-285.
Lee, H., To, R. J. B., Latta, R. K., Biely, P. and Schneider, H. 1987. Some properties of extracellular acetylxylan esterase produced by the yeast *Rhodotorula mucilaginosa*. Appl. Environ. Microbiol. **53**: 2831-2834.
Poutanen, K. and Sundberg, M. 1988. An acetyl esterase of *Trichoderma reesei* and its role in the hydrolysis of acetyl xylans. Appl. Microbiol. Biotechnol. **28**: 419-424.
Somogyi, M. 1952. Notes on sugar determination. J. Biol. Chem. **195**: 19-23.
Tenkanen, M., Puls, J., Rättö, M. and Viikari, L. 1993. Enzymatic deacetylation of galactoglucomannans. Appl. Microbiol. Biotechnol. **39**: 159-165.
Timell, T. E. 1964. Wood hemicelluloses: Part I. Adv. Carbohydr. Chem. **19**: 247-299.
Timell, T. E. 1965. Wood hemicelluloses: Part II. Adv. Carbohydr. Chem. **20**: 409-482.
Tsujiyama, S. and Nakano, N. 1996. Detection and production condition of acetylxylan esterase of a wood-rotting fungus, *Coriolus versicolor*. Mycoscience **37**: 103-104.