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Qualitative Evaluation of Ligninolytic Enzymes in Xylariaceous Fungi

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Abstract Sixty-one strains representing the main genera of wood-decaying xylariaceous fungi (mainly in *Daldinia*, *Hypoxylon*, *Kretzschmaria*, *Rosellinia*, *Penzigia*, and *Xylaria*) were tested for their ability to produce ligninolytic enzymes. The phenol oxidase activity and fungal growth of the xylariaceous fungi on gallic acid and tannic acid media showed a variation in their ability to degrade lignocellulose. A number of species showed equal or better ligninolytic enzyme activities than *Coriolus versicolor*, a known basidiomycete wood-degrader. A large variation of the enzyme activity was observed by individual strains as well as a substantial variation between the isolates of the same species. The most frequent ligninolytic enzymes were peroxidase and general oxidase. With 19% of the strains tested, peroxidase showed the strongest ligninolytic enzyme activity, while tyrosinase activity was detected only in 7% of the strains. All strains of *Kretzschmaria* and *Rosellinia* tested was positive for laccase. Xylariaceous fungi were able to degrade the macromolecule, lignin, using each specific ligninolytic enzyme in the specific lignin degradation pathway.

Key words: Wood decay, xylariaceous wood decay fungi, Bavendamm test, peroxidase, tyrosinase, laccase

Xylariales are a cosmopolitan group of fungi with some 800 species assigned to 92 genera. They are a clearly defined assemblage of taxa mostly occurring as saprophytes of wood and bark. Xylariaceous fungi are common wood inhabiting species in tropical forests where they play a significant role in the degradation of lignocellulose. Most are terrestrial fungi, however, a few occur on wood in mangrove habitats (e.g. *Halorosellinia oceanica*, *Nemania* sp.). Although most are wood inhabiting, some occur on animal feces, fruits, seeds, and leaves in the forest litter.

Three main groups of wood-decaying fungi have been recognized: white, brown, and soft rot fungi [6, 35]. A number of xylariaceous fungi have been reported to cause white rot of wood [25, 26, 33], yet the soft rot attack still predominates [10]. On the other hand, Abe [1] reported on a decay pattern that did not conform to any of these decay types. White rot and soft rot have been reported to be caused by lignin-degrading enzymes in the fungi, however, lignin degradation by xylariaceous fungi has not been well documented. The biological degradation of lignin is one of the most important events in the biospheric carbon and oxygen cycle, and various microorganisms (fungi and bacteria) are known to degrade lignin [4]. Fungal peroxidase, ligninase, manganese, and laccase have all been implicated in the biodegradation of the lignin complex [17, 22, 29, 31].

This study was initiated to obtain more detailed information on the wood decaying ability of xylariaceous fungi and constitutes a part of wider investigations on their role in the carbon cycle, mainly in tropical and temperate forests. Since xylariaceous fungi degrade lignocellulose, their potential application in the paper and pulp industry and in bioremediation is also of considerable interest.

Materials and Methods

Organisms and Culture Conditions

Fifty-two strains (in 43 species) of xylariaceous fungi and 3 basidiomycetes (for comparison) were selected for this investigation and are listed in Tables 1 and 2. Most specimens were collected from 1993 to 1995 from England, Hong Kong, Malaysia, Philippines, Thailand, Taiwan, and Korea [20]. Species cultures for inoculum were grown at 25°C on 25 ml of cornmeal agar media (CMA) in Petri dishes. Discs (diam. 5 mm) of mycelium were cut from the edges of the selected colonies with a sterile cork borer and used to inoculate experimental plates.

Phenol Oxidase (Bavendamm) Test

The ability of the isolates to produce polyphenol oxidases, which are involved in the degradation of lignin, was assessed using a modification of the technique of Gessner [11] and

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Davidson *et al.* [5]. This is a test for the presence/absence of extracellular oxidase. The fungus to be tested was grown on a malt agar to which gallic or tannic acid was added. A brown zone around the colony indicates the oxidation of tannic acid or gallic acid, and is considered to be a positive test for polyphenol oxidase activity [11]. The fungi were inoculated centrally onto a Petri dish with malt extract and added gallic (0.5%) or tannic (1.5%) acids.

Assay of Laccase, Tyrosinase, and Peroxidase

The production of laccase, tyrosinase, and peroxidase was determined using a modified procedures of Egger [7], Gessner [11], Harkin and Obst [12], Harkin *et al.* [13], and Zare-Maivan and Shearer [34] (Fig. 1). The fungi were grown on both cornmeal agar (CMA) and malt extract agar (MA) in Petri dishes for 12 days and 7-mm diameter wells were cut with a flamed cork borer within the outermost 10 mm of the colony circumference at 4 radii 90° apart.

For laccase (indirect method) detection, one drop of a freshly prepared solution of 0.1% (w/v) pale yellow syringaldazine in 95% ethanol was added to one well; an appearance of a red to purple color, usually within 25 min, indicates the presence of laccase [13]. Laccase was determined by a modified method of Higuchi [14], Higuchi and Kitamura [15], and Lee [20]. For tyrosinase, one drop of 0.1 M cresol in 95 % ethanol was added to the second well and the production of an orange-brown color indicates tyrosinase activity [7]. For peroxidase, one drop each of a freshly prepared 1% (w/v) aqueous solution of pyrogallic acid and 0.4% hydrogen peroxidase was added to the third well and the presence of a golden yellow to brown color indicates peroxidase activity [7]. Ethanol was added to the fourth well as a control for the enzyme.

The appearance and relationship of the brown diffusion zone to the fungus mat as well as the time required for its development varied widely with different species.

Weight Loss of Wood Block

Three wood species were used for analyzing the wood decay capability of Xylariaceae. Blocks (1 cm×1 cm× 2 cm) of Scots pine (*Pinus sylvestris* L.), beech (*Fagus sylvatica* L.) and blocks (0.6 cm×0.6 cm×0.6 cm) of balsa (*Ochroma lagopus* Sw.) were cut and employed in this experiment. Test blocks were dried at 60°C for 12 h in an oven, cooled for 1 h in a desiccator, and weighed. Test blocks of Scots pine and balsa were autoclaved in dry condition and the beech blocks were autoclaved at 121°C for 15 min in both dry and wet conditions.

A disc (diam. 5 mm) of mycelium was cut from the edge of the selected colonies and placed 4.5 mm apart and inoculated on Pyrex glass jars, containing 80 ml of potato dextrose agar medium, with metal screw tops. The jars were incubated at 20°C for 2 weeks and the test blocks were then placed 50 mm apart on the inoculated mycelium in the Pyrex glass jars. After inoculation, the jars were incubated at 20°C in a growth room for 24 weeks to ensure total colonization of the test blocks. Test blocks were cleaned of aerial hyphae, dried at 60°C for 12 h in an oven, and cooled for 1 h in a desiccator. The dry weight of these blocks were determined.

Table 1. Production of general phenol oxidase, laccase, peroxidase, and tyrosinase by the selected xylariaceae fungi.

Species	Lac (dir.)	Lac (ind.)	Per	Gen	Tyr	Phe (gallic)	Phe (tannic)	No.
Asomycota								
Bixcogniauxia nummularia	-	++	-	-	-	+	+	3
Camarops lutea	-	-	+	-	-	+	-	2
Daldinia concentrica 2145	-	-	±	±	-	+++	++	2
D. concentrica 2162	-	-	-	-	-	+++	-	1
D. concentrica 2165	-	-	++	-	n	+	++	3
D. eschscholzii 2140	++	-	+	+	+++	++	+++	6
D. eschscholzii 2163	-	-	-	+	++	+	++	4
D. vernicosa 2152	-	-	+++	+	±	++++	+++++	4
D. vernicosa 2164	-	-	+	+	-	++	++++	4
Diatrype disciformis	-	-	++	-	+	+	-	3
Hypoxylon crocoplum	-	n	n	n	n	n	n	0
H. fuscum 2155	-	-	++	-	-	-	+	2
H. fuscum 2156	-	-	+	-	-	++	+	3
H. fuscum 2157	+	-	++	+	+	++	+++	6
H. fuscum 2158	-	-	-	+	-	-	+	2
H. fuscum 2159	-	-	+	-	-	-	-	1
H. fragiforme	+	++	+	++	+	+++	+++	7
H. mammatum	-	-	++	+	+	-	-	3

Table 1. Continued.

Species	Lac (dir.)	Lac (ind.)	Per	Gen	Tyr	Phe (gallic)	Phe (tannic)	No.
H. multiforme	-	++	++	++	-	++++	+++++	5
H. rubiginosum I	-	-	-	-	-	±	±	0
H. rubiginosum II	+++	-	-	+	-	±	±	2
H. stygium	++	n	n	n	n	++++	+++++	3
H. subgilvum	-	n	n	n	n	++	+	2
Kretzschmaria deusta	+	n	n	n	n	++++	++++	
K. holisus	+++	-	++	++	-	n	n	3
K. intracoloratum	++	++	++	+	-	+++++	+++++	6
Kretzschmaria sp.	+	-	+++	+	-	++	++	5
Leprieuria bacilla	-	-	++	+	-	+++	++++	4
Nemania albocinctum	+++	++	+	++	+	++++	++++	7
Penzigia sp. I	++	++	++	+++	+	+++++	+++++	7
Penzigia sp. II	++	-	+++	+	-	n	n	3
Poronia pileiformis	-	-	-	-	-	+	-	1
P. punctata	+	-	-	-	+	-	-	2
Rosellinia britanica	+	-	-	-	-	++	++	3
R. bunodes	+++	-	+++	++	-	++++	++++	5
R. necatirx	+++	++	+++	+	++	++	++++	7
Rosellina sp.	++++	-	-	++	-	-	-	2
Rosellina sp.	+	n	n	n	n	+++	++++	3
Xylaria allantoidea	+++	++	-	+	-	±	+++	4
X. allantoidea	n	n	n	n	n	+++	+++++	2
X. cf. myosurus	++++	n	n	n	n	++	+++	3
X. cubensis 2149	++	n	n	n	n	+++	+++	3
X. cubensis I	++	n	n	n	n	++	-	2
X. cubensis II	-	-	++	+	+++	++	+++	5
X. cubensis III	++	-	+	+	-	n	n	3
X. curta	+	n	n	n	n	n	n	1
X. aff. feejeensis	-	-	+	-	-	-	+	2
X. grammica I	+++	n	n	n	n	++	++	3
X. grammica II	+	n	n	n	n	+++	++++	3
X. longipes	-	-	+/±	-	++	-	-	2
X. maitlandii	-	-	-	++	-	++++	++++	3
X. obovata	+++	-	+++	++	-	++++	++++	5
X. oligotoma I	+++	-	+++	++	-	+	+	5
X. oligotoma II	++++	++	-	++	-	n	n	3
X. oligotoma III	+	n	n	n	n	n	n	1
X. polymorpha	++	-	++	+	++	++	++++	6
X. tanganyikaensis	+	-	++	+	+++	+++	+++	6
Xylaria taxonomic sp.3 I	++	-	-	+	-	+	-	3
Xylaria taxonomic sp.3 II	+	-	+++	++	-	+	-	4
Xylaria taxonomic sp.3 III	+	-	+++	++	-	n	n	3
Xyalria sp.	+++	++	+	++	-	++	++	6
Basidiomycota								
Coriolus versicolor	+++	+++	++	+++	+	+++	n	6
Lentinus sp.	-	n	n	n	n	-	n	0
Serpula lacrymans	-	n	n	n	n	+	n	1

Lac, laccase; per, peroxidase; gen general oxidase, tyr, tyrosinase; phe, phenol oxidase; No , number of reaction; +, positive reaction, -, negative reaction; n, not tested· ±, no clear positive reaction

For an evaluation of enzyme activity, the following system was used to record the reactions of the species on the gallic acid and tannic acid media:

-. Negative, no brown discoloration under or around the mat.

+ Diffusion zone light to dark brown, formed under most of the mat extending to the margin and only visible from underneath.

++, Diffusion zone, light to dark brown, extending to a short distance beyond the margin of the mat and visible from the upper side

+++, Diffusion zone, light to dark brown, extending to quite a distance beyond the margin of the mat and visible from the upper side.

++++, Diffusion zone. very intense dark brown and opaque, forming a wide corona around the mat.

Table 2. Xylariaceous fungi which produced higher individual enzyme activity.

General oxidase	Laccase	Peroxidase	Tyrosinase
Penzigia sp. I	*Rosellinia* sp. I	*D. vermicosa* 2152	*D. eschscholzii* 2140
C. versicolor	*X. cf. myosurus*	*Kretzschmaria* sp.	*X. cubensis* III
	X. oligotoma II	*Penzigia* sp. II	*X. tanganyikaensis*
		R. bimodes	
		R. necatrix	
		X. obovata	
		X. oligotoma	
		Xylaria sp. I	
		Xylaria sp. II	

RESULTS

Phenol Oxidase Activity in the Bavendamm Test

The data on the qualitative phenol oxidase activity determined after 2 weeks of growth at room temperature are presented in Table 1. The phenol oxidase activity in the gallic acid malt extract agar medium was categorized as follows: 1) good activity (shown by 7 species, e.g. *K. intracolorata*, *Penzigia* sp.); 2) weak to moderate activity (32 species), and 3) no activity (11 species). Of the basidiomycetes, *Lentinus* sp. showed no activity, and *C. versicolor* and *S. lacryman* showed only weak activity. On tannic acid, 17 species showed good activity, 32 showed moderate to weak activity, while 12 species showed no activity.

Laccase Activity

Only 22.9% of the strains tested positive for laccase (indirect method), the lowest figure for all the enzymes evaluated in this study. However, 60.6% tested positive for laccase when tested by the direct method. Most strains of *Kretzschmaria*, *Penzigia*, *Rosellinia*, and *Xylaria* gave positive reactions.

Table 3. Wood-degrading fungi and their ligninolytic enzymes.

Fungi	Ox	La	Per	Ty	Reference
Terrestrial Basidiomycota					
Trametes hirsuta	+	+	-	n	Nerud *et al.* (1991)
Corillopsis polyzona	-	+	-	n	
Pycnoporus cinnavarinus	-	+	+	n	
Stereum hirsutum	-	+	-	n	
Ganoderma valesiacum	-	+	-	n	
Cerrena unicolor	n	+	n	n	Bekker *et al.* (1990)
Lentinus lepideus Fr.	n	-	-	n	Harkin & Obst (1973)
Coriolus versicolor (L.) Quel.	n	+	+	n	
Poria subvermispora Pil.	n	+	+	+	
Serpula pinastri (Fr.) Bond.	n	-	-	-	
Poria pannocincta (Rom.) Lowe	n	-	-	-	
Tyromyces fumidiceps Atk.	n	+	+	n	
Terrestrial Ascomycota					
Monocillium indicum Saxena	n	+	n	n	Thakker *et al.* (1992)
Marine fungi					
Halosarpheia retorquens Shearer & Creane	n	+	+	+	Zare-Maivan & Shearer (1988)
Leptosphaeria sp.	n	+	+	+	
Nais inornata Kohlm.	n	+	+	+	
Nectria haematococca Berk. & Br.	n	+	+	+	
N. lucidum Hoehn.	n	+	+	-	
Clavariopsis aquatica	n	-	+	-	
Aigialus mangrovei Borse	n	-	-	-	Raghukumar *et al.* (1994)
Halosarpheia ratnagiriensis Patil et Borse	n	-	n	n	
Xylariaceous fungi					
Hypoxylon deustum (Hof. ex Fr.) Grev.	n	-	-	-	Harkin *et al.* (1974)
Halorosellinia oceanica (S. Schatz) Whalley, Jones, Hyde & Lassøe	n	+	n	n	Raghukumar *et al.* (1994)

Ox, Oxidase, Per, peroxidase; La, laccase; Ty, tyrosinase; +, positive reaction; -, negative reaction; n, not tested.

Peroxidase Activities

Over 72% of the fungi tested showed peroxidase activity with 100% positive reactions for the genus *Kretzschmaria*, while the two *Poronia* species tested negative. Nine of the 47 strains tested (19.1%) showed strong peroxidase activity, while 11 strains showed weak activities.

General Oxidase Activity

Some 72% of the test strains were positive for this enzyme. The *Penzigia sp.* showed an enzyme activity as high as *C. versicolor*, a known white rot basidiomycete, while the *Poronia* species tested negative (Tables 2 and 3). All *Kretzschmaria* strains (100%) and most *Xylaria* stains (86.7%) were positive, while the *Daldinia* and *Hypoxylon* species showed less activity (5.7% and 60%, respectively).

Only 4 fungi (*Hypoxylon fragiforme*, *Nemania albocinctum*, the *Penzigia* species, and *Rosellinia necatrix*) produced all five enzymes by the 7 methods, while a few strains (*Daldinia eschscholzii*, *H. fuscum* 2157, *Kretzschmaria intracolorata*, *Xylaria polymorpha*, *X. tanganyikaensis*, and *Xylaria* sp.) were positive for 6 enzymes, along with the basidiomycete *C. versicolor* (Table 1). Table 2 lists the fungi producing the highest ligninolytic enzyme activity.

Variation in the Enzyme Activity of Different Stains

More than one strain for 8 species were evaluated for enzyme activity and significant differences were observed. For example, all the *D. concentrica* strains showed poor enzyme activity (positive for only 1–3 of the tests); *H. fuscum* strain 2157, showed positive results for 6 enzymes,

Table 4. Xylariaceous fungi grouped based on their ligninolytic enzymes activity and wood-degrading ability.

Group	Species	Weight loss (%)			
		pine	beech (in dry)	beech (in wet)	balsa
Group 1 Strong activities of all enzymes	*H. fragiforme*	3.7	29.8	19.9	27.4
	Nemania albocinctum	29.5	31.4	28.6	49.4
	Penzigia sp. I	31.1	34.0	25.7	66.9
	R. necatirx	11.7	14.6	20.5	24.0
	Coriolus versicolor	62.5	80.7	34.9	90.8
Group 2 Strong activities of laccase and peroxidase	*R. bunodes*	6.0	22.7	13.5	19.5
	X. obovata		Not tested		
	X. oligotma I		Not tested		
Group 3 Strong activity of laccase	*H. rubiginosum*	4.8	9.0	6.7	11.6
	Rosellina sp.	28.6	27.3	24.2	53.0
	K. heliscus	22.3	29.4	23.3	61 0
	X. allantoidea	27.4	44.6	29.1	61.1
	X. gramica	10.0	-	12.4	26.9
	X. cf. myosurus	5.0	5.9	8.3	20 8
	X. oligotma II		Not tested		
	Xylaria sp.		Not tested		
Group 4 Strong activity of peroxidase	*D. vernicosa* 2152	1.5	13.5	10.7	27.2
	Kretzschmaria sp.	28.6	33.1	28.8	64.0
	Penzigia species II	31.2	29.8	25.7	60.7
	Xylaria taxonomic sp. 3	29.1	29.5	25.1	56.8
Group 5 Strong activity of phenol oxidase	*D. eschscholzii* 2140	2.4	68.8	17.9	53.8
	H. multiforme	4.6	4.2	34.2	14.5
	H. stygium	13.4	50.8	27.7	69.8
	Kretzschmaria deusta		Not tested		
	K. intracoloratum	22 3	29.4	23.3	61.0
	Leprieuria bacilla	25.5	41.5	31.6	62.8
	X. cubensis 2149	4.6	59.1	47.9	84.5
	X. maitlandii	13.9	15.4	22.0	41 1
	X. tanganyikaensis	39.2	49.5	46.7	82.1

while strain 2159 showed only very weak peroxidase activity. Of the four *C. cubensis* isolates tested, strain II was positive for 5 tests, while strain III was only positive for 3 enzymes. There was a good correlation between the two *D. vernicosa* strains tested.

Wood Decay Capabilities

The decay capability of 28 fungi, including one basidiomycete (as a control), was assessed using a weight loss method, and the results are presented in Table 4. On pine, none of the xylariaceous fungi caused weight losses comparable to the white rot basidiomycete *C. versicolor* (62.5%). Of the xylariaceous strains, three species caused weight losses in excess of 30% (*Penzigia* spp. and *Xylaria tanganyikaenis*). Eleven species caused weight losses greater than 20%, and these were mainly *Nemania*, *Penzigia*, and *Kretzschmaria* species. Surprisingly, *Hypoxylon* and *Daldinia* species produced little decay of Scots pine wood.

Weight losses of dry beech were grouped into 5 categories (Table 4). Species causing high weight losses included *D. eschscholzii* 2140 with weight losses greater than 60%. Eight species caused weight loss in excess of 30%, while only five species could be ranked to have high weight losses under wet conditions, such as *X. polymorpha* (73.6%) (Table 4). A number of species caused weak weight loss under dry conditions (e.g., *H. rubiginosum, X,* cf. *myosurus* and *H. multiforme*). Balsa wood was more heavily attacked than Scots pine or beech. Ten species caused weight loss in excess of 60%. The highest weight loss was caused by *Xylaria cubensis* 2149 and *X. tanganyikaensis*. Five species caused 30–60% weight losses. The remaining species caused greater than 10% weight losses.

DISCUSSION

Xylariaceous fungi, which cause both soft rot and white rot (mainly soft rot), have not been studied for lignin degradation in comparison with white rot basidiomycetes. Thakker *et al.* [31] first examined the characteristies of a laccase, showing peroxidase activity from the ascomycete, *Monocillium indicum* Saxena. For xylariaceous fungi, Abe [1] reported that all 13 strains studied tested positive to phenol oxidase in Bavendamm and gum guiac tests, and most were also postitive in the α-naphthol tests.

Wood Degradation and Host Specificity

In this study, 61 strains were tested for ligninolytic enzyme production and all, except *Hypoxylon crocoplum and X. rubiginosum* I, produced one or more enzymes. Some of them can be characterized as weakly ligninolytic because of the low activity: e.g., *Camarops lutea* on *Ulex* wood: *Daldinia concentrica* 2162 on all kinds of hardwood; *Hypoxylon fuscum* 2159 on *Buxus* wood; *Poronia pileiformis* and *P. punctata* on dung; *Xylaria curta* on tropical hardwood; *X. cf. myosorus* on tropical hardwood; *X. olgotoma* on dead decorticated wood; and *Xylaria* sp. 3 on dead decorticated wood. Some of these species grew on substrate other than wood (e.g., *P. punctata* on feces), in which cellulose may be the major component and lignin is in insignificant amount. In this survey, *H. fuscum* 2159 and *D. concentrica* 2162 showed weak ligninolytic activity in comparison to other strains of the same species. However, a significant variation among the strains of the same species was not found. In particular, all strains of *D. concentrica* and *H. fuscum* showed poor ligninolytic activity in comparision to other xylariaceous species, e.g., *Daldinia eschscholzii, Kretzschmaria intracoloratum, Nemania albocinctum*, and *Xylaria obovata*.

Hypoxylon fuscum has a significant host specificity for developing teleomorph stromata, even though it has been collected from five different hardwoods in the British Isles (A.J.S. Whalley, personal observation). Lee [20] reported that xylariaceous fungi show a substantial difference in their hyphal growth on different media, depending on the carbon source. Whalley [33] stated that, in relation to the distribution of some species, the nutritional status of the host is probably not significant. However, some species such as *H. udum* Pers.: Fr., a native species of the British Isles and Europe, is found onlyon *Quercus* and also only on decorticated wood that is highly rotten and usually water-sodden. Yet, many xylariaceous fungi can colonize a wide range of wooden substrata and degrade the lignin complex of the wood [20, 26, 33].

Comparison of Enzyme Activity with the Basidiomycete *Coriolus versicolor*

Coriolus versicolor is a well-known white rot basidiomycete [8], capable of producing a significant weight loss of wood (81.7% on beech) [16]. In biochemical tests carried out in this study, all the tests were positive. Only 3 xylariaceous fungi produced an enzyme profile similar to that of *C. versicolor*; namely, *Penzigia* sp. I, *Rosellinia necatirx*, and *X. obovata*, all wood-inhabiting xylariaceous fungi. When comparing the phenol oxidase activity of the xylariaceous fungi with *C. versicolor*, a number of species exhibited an equal activity (*D. eschscholzii*; *Hypoxylon fragiforme*; *Rosellinia* sp.; *X. obovata*; *X. tanganyikaensis*) or better activity (*Nemania albocinctum*; *D. vernicosa* 2152; *Rosellinia bunodes*: *Kretzschmaria deusta*; *Xylaria granmica*; *K. intracoloratum*; *X. maitlandii*; *Leprieuria bacilla*; *H. stygium*; *Penzigia* sp.). *Lentinus* sp. and *Serpula lacrymans*, two brown rot basidiomycetes, showed a weak to no ligninolytic activity in the present study and were included for comparison. This is in agreement with the observation of Kirk and Highley [19] in that the degradation of cellulose and hemicelluloses occurred rapidly during brown

rot decay, yet the lignin depletion was slow. When Davidson *et al.* [5] tested for phenol oxidase, using the Bavendamm's gallic and tannic acids methods, 80% of the brown rot fungi tested gave no reactions, while 96% of the white rot fungi gave positive reactions.

Thirty percent of the xylariaceous fungi tested produced an equal or better ligninolytic activity to that of *C. versicolor*. This clearly demonstrates their success in the colonization of fallen timber in forests in most geograpical locations, particularly in the tropics. Harkin *et al.* [13] found that *Polyporus dichrous* and *Stereum frustulatum* could produce phenol oxidases, despite their negative Bavendamm test reaction. The present results also showed a similar trend with no phenol oxidase in a few strains (*H. fuscum* 2159, *H. mammatum*, *P. punctata*, *Rosellinia* sp. I, and *X. longipes*), although they produced a certain amount of other polyphenol oxidases.

Comparison of Ligninolytic Enzyme Activity with Other Fungi

Wood decay fungi produce specific extracellular enzymes that degrade the cell wall components of wood [2, 12, 13, 17, 21, 24, 29, 30]. Lignin can be removed selectively or slightly in advance of cellulose degradation, however, an extensive loss of cellulose and hemicellulose usually accompanies or immediately follows [3]. These findings would appear to suggest that wood-decaying fungi with different enzyme activities may include a specific reaction for the degradation of the lignin polymer complex. However, it is difficult to compare the production of extracellular enzymes with other groups which may produce different types of enzyme complexes due to a number of limiting factors, e.g., environmental, physical limitations, and the proper methodology for the detection of enzyme activity. For example, Evans [8] found that, using Western blotting techniques, all white rot fungi proved positive for peroxidase, yet Nerud *et al.* [21] reported that laccase was the most dominant enzyme. Using two different methods, similar results were obtained with the laccase test in the current study (Table 1). However, Zare-Maivan and Shearer [35] reported that most marine fungi tested produced laccase and peroxidase, and most fungi required different enzymes to degrade the wood cell walls and a considerable variation was observed in the enzymes produced by the individual strains.

Variation in the Enzymes Produced

A variety of enzymes are important in lignin breakdown [17, 24, 27], including lignin peroxidase and laccases which are capable of degrading non-phenolic and phenolic lignin-model compounds, respectively [8, 17, 28, 29]. A great variation was noted in the enzymes produced by individual species. *Nemania albocinctum*, *Penzigia* sp., and *R. necatrix* produced positive results for all the enzymes screened, while *D. eschschscholzii*, *H. fuscum* (2157), *K. intracoloratum*, *X. polymorpha*, and *Xylaria* sp. were positive for 6 enzymes, which were the same as for *C. versicolor* (Table 1). Similar observations have been made in the studies with diverse taxonomic groups. For example, Harkin and Obst [12] reported positive peroxidase and laccase activities for *C. versicolor*, negative for lignin oxidase, peroxidase, manganase peroxidase, and tyrosinase, while *Poria subermispora* was positive for 4 out of 6 reactions. Harkin *et al.* [13] and Raghukumar *et al.* [24] tested negative for all 6 enzymes screened for 2 xylariaceous fungi (Table 1). However, Zare-Maivan and Shearer [35] reported 4 positive reactions out of 6 for aquatic fungi, yet negative reactions for 3 wood-inhabiting mangrove ascomycetes (Table 3). Recently, Pointing *et al.* [23] investigated 5 lignicolous marine fungi, including *H. oceanicum* S. Schatz, which produced strong cellulolytic enzymes. They found that agitation of the cultures and an increased salinity reduced the enzyme production. However, *Julella avicenniae* (Borse) K.D. Hyde was unaffected by salinity within a range of 0–3%.

Relationship between Lignin Degradation and Ligninolytic Enzyme Production

Fungi vary considerably in their ability to degrade lignin, and, although some soft rot fungi can degrade it, their ligninolytic enzyme activity is very weak. This also applies to xylariaceous fungi and even white rot Basidiomycetes. In Table 4, the strains tested in this study were grouped into 5 groups, based on the strength of their enzyme activity together with data on their ability to degrade wood. None of the strains tested showed a greater weight loss than *C. versicolor*, that were strong for all 6 ligninlolytic enzymes activities. In pine, which consists mainly of syringyl lignin, *Xylaria tanganyikaensis* and *Penzigia* sp. II showed the highest weight losses with a strong enzyme activity; strong peroxidase-producing strains caused higher weight losses than laccase-producing strains. However, in beech and balsa, there was no significant difference in the laccase and peroxidase activities relative to the weight loss. However, the strains that produced all types of ligninolytic enzymes caused a lower weight loss than the individual enzyme-producing strains in balsa. A similar observation, although not significant, occurred on pine and beech. Thus, the present study confirmed that xylariaceous fungi have the ability to degrade the structure and substructure of the macromolecule, lignin, employing ligninolytic enzymes specific in each lignin degradation pathway. However, the detailed mechanism of lignin degradation by soft rot xylariaceous fungi with/without polysaccharide in wood elements is still unclear. Further characterization of the individual ligninlolytic enzyme pathways and the cooperative pathways in wood degradation will be the subject of future studies.

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