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Microchemical Studies of Wood Degradation by Brown Rot and White Rot Fungi in Two Tropical Timbers

B. O. Ejechi,[a]* C. O. Obuekwe[b] & A. O. Ogbimi[b]

[a]*Department of Microbiology, Delta State University, Abraka, Nigeria*
[b]*Department of Microbiology, University of Benin, Benin City, Nigeria*

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An observation that the growth of the brown rot fungus *Gloeophyllum sepiarium* and another species of the same genus isolated from decaying timbers-in-buildings, 'bleached' mahogany (*Khaya ivorensis*) timber, prompted a laboratory investigation of their pattern of wood degradation in obeche (*Triplochiton scleroxylon*) and mahogany. The white rot fungus *Pleurotus ostreatus* was studied for comparison. Microchemical and quantitative analyses indicated that the *Gloeophyllum* species degraded cellulose, but not lignin. *P. ostreatus* attacked lignin after substantially degrading cellulose. Spot tests indicated laccase production by all three species studied. Lignin peroxidase was detected only with *P. ostreatus*. It is hypothesized that laccase may be partly associated with breakdown of the decay-retarding pigmented extractives of the timbers. 'Bleaching' may have been due to lightening of timber colour caused by removal of these pigmented compounds. Copyright © 1996 Published by Elsevier Science Limited.

INTRODUCTION

The decay of wood by fungi is due to the breakdown of lignin, cellulose, and hemicellulose components. The white rots attack all the polysaccharides and may modify the lignin. The brown rots attack the cellulose, but not the lignin. The microscopic and morphological changes associated with decay in various woody hosts by both groups of fungi have been reported by many workers e.g. Greaves & Levy (1965), Kirk (1971), Kaarik (1974) and Santra & Nandi (1976).

Gloeophyllum species are decay fungi known to cause brown rot (Cartwright & Findlay, 1958). The basidiocarp of *Gloeophyllum sepiarium* and another unidentified species of the same genus are a common sight in decaying timbers in buildings in Benin City, Nigeria. Moreover they were observed to bleach the wood of mahogany (*Khaya ivorensis*) (unpublished data). This is an attribute of white rot fungi suggesting an ability to attack lignin.

Therefore, we investigated the wood degrading pattern of the two *Gloeophyllum* species in two commonly used Nigerian timbers through quantitative and micro-chemical analyses. The white rot fungus *Pleurotus ostreatus* was used for comparison.

*To whom correspondence should be addressed.

MATERIALS AND METHODS

Wood degradation tests

Cubic blocks (20 mm in each dimension) were sawn from obeche (*Triplochiton scleroxylon*) and mahogany (*Khaya ivorensis*) obtained from the Federal Wood Utilization Centre, Benin City, Nigeria. On average, the blocks weighed 1.00 and 3.10 g per block for obeche and mahogany, respectively, after drying to a constant weight at 103°C.

Decay resistance was tested by the soil jar method (Henningsson, 1977). One wood block was placed in each soil jar, in replicates of six for each fungus and wood type, and inoculated with 5 ml potato dextrose broth mycelial suspension of

Gloeophyllum sepiarium, *Gloeophyllum* sp. and *Pleurotus ostreatus* (Ejechi & Obuekwe, 1993). Control soil jars contained wood blocks and were not inoculated. Control and inoculated jars were incubated at room temperature (30±2°C) for 12 weeks, after which the blocks were removed, cleared of mycelia, and dried to constant weight as before.

Wood blocks for microchemical qualitative estimation of the degradation of cellulose and lignin were softened in boiling water for 30 min and soaked in 1:1 mixture of glycerol and methanol for another 30 min (Santra & Nandi, 1976). Thereafter, free-hand wood sections from brown, bleached and incipient decay zones, were stained as described by Jenseen (1962). Lignin was detected using the Weisner test and cellulose was detected with iodine dissolved in potassium iodide solution. The intensity of the colours were visually compared for control and inoculated wood blocks.

For the quantitative estimation of changes in lignin and cellulose composition, wood blocks (control and inoculated) previously dried to constant weight were hammer-milled, extracted with ethanol:benzene (1:1, v/v) and delignified by the acidified chlorite method (Adams, 1965) after drying to constant weight. The difference in weight between extractive free un-delignified (untreated) wood blocks and delignified (treated) residue represent the weight of lignin. The delignified residue contains cellulose and hemicellulose. To determine the quantity of cellulose in the residue, hemicellulose was removed by the procedure of Whistler & Feather (1965) with modification. Boric acid (4%) was added to the NaOH before use to enhance the removal of mannan-containing cellulose, if present, and the de-oxygenation step was skipped since recovery of hemicellulose was not the objective. The hemicellulose-free residue dried to constant weight was the cellulose. All results represented means of determinations from six replicate wood blocks and were approximated as much as possible, to two decimal places.

Detection of laccase and lignin peroxidase

Laccase and lignin peroxidase were detected by the spot test of Stalpers (1978). A drop of a 1:1 mixture of 0.4% hydrogen peroxide and 1.0% pyrogallol (1, 2, 3 trihydroxy-benzene) in water, was placed on the actively growing marginal hyphae on malt extract agar (Oxoid) for the detection of lignin peroxidase. A yellowish-brown colour read after 3, 24 or 72 h was considered positive for lignin peroxidase. Detection of laccase was by a similar drop test using 0.1 M 1-naphthol in 96% ethanol, with a purplish discoloration considered as positive for laccase.

RESULTS AND DISCUSSION

Table 1 shows that *P. ostreatus* alone indicated the ability to attack lignin as judged by the reduction in the intensity of Weisner colour reaction, which was greater in incipient decay than in bleached or control wood. Bright red colour indicated the presence of lignin while a reduction in its brightness or intensity to red or dull/light red when compared to control indicated reduction in amount of lignin. The blue–black colour reaction indicative of cellulose was not detected in the zone

Table 1. Qualitative (Visual) Estimation of Decomposition of Lignin and Cellulose in Wood Exposed to Decay Fungi for 12 Weeks

Decay fungi	Zones decay	Intensity Weisner* colour reaction (lignin)		Intensity Iodine + test (Cellulose)	
		Obeche	Mahogany	Obeche	Mahogany
P. ostreatus	Bleached	+ +	+ +	+	+
	Brown incipient decay	+	+	—	—
G. sepiarium	Bleached	+ + +	+ + +	+ +	+ +
	Brown	+ + +	+ + +	+	+
Gloeophyllum sp.	Bleached	+ + +	+ + +	+ +	+
	Brown	+ + +	+ + +	+	+
Uninoculated (Control)	No discoloration	+ + +	+ + +	+ + +	+ +

*: + + +, bright red; + +, red: +, dull/light red.
+: + + +, blue–black; + +, blue; + light blue;
—, No colour reaction.

of incipient decay. Bleached portions, tested for cellulose, produced a light blue colour, indicative of a reduction in cellulose content. This reduction was indicated by lightening of the blue colour when compared with that of control. Also, as shown in Table 1, the colour reactions indicated the ability of the *Gloeophyllum* species to degrade only cellulose and this was more prominent in the brown zones.

When compared with controls, there were reductions in the quantities of lignin in wood blocks exposed to *P. ostreatus* while no changes in lignin content were observed in blocks exposed to the *Gloeophyllum* species (Table 2). On the other hand, the cellulose content was reduced in all cases with wood blocks exposed to *Gloeophyllum* sp. showing the highest reductions (Table 2). The pattern of changes in lignin and cellulose proportions due to decay, were identical in both timbers. The sum of the weight losses of lignin and cellulose after degradation, did not equal the loss in weight of the untreated wood when compared to control, thereby indicating the consumption of another substrate—probably hemicellulose.

The spot test for the detection of laccase was positive for the three fungi although the purplish discoloration was instantaneous and more intense in *P. ostreatus* culture. The reaction was slower in the two *Gloeophyllum* species cultures, with the colour appearing about 1 h after the application of the test. Lignin peroxidase was detected only in *P. ostreatus* culture although the positive colour reaction appeared after 24 h.

Microchemical tests corroborated by quantitative analysis where there was no change in lignin composition indicated the inability of *Gloeophyllum* spp. to degrade lignin. Although laccase, an enzyme associated with lignin degradation (Lundell *et al.*, 1990) was detected, slower colour reaction in the spot test suggested lower activity than that of *P. ostreatus*—a white rot. Demethylation and demethoxylation have been shown to be processes in lignin modification of which laccase among other enzymes, plays a role (Lundell *et al.*, 1990). Laccase alone cannot effect complete lignin degradation. Hence, demethylation caused by growth of *Gloeophyllum sepiarium* on wood (Kirk, 1971) may, therefore, have served other purposes. Laccase is capable of removing methoxyl groups from phenolic compounds and may act as a detoxifying enzyme leading to the removal of toxic monomers from growth medium (Ander & Eriksson, 1976; Ander *et al.*, 1983; Kirk & Farrell, 1987; Leonowicz *et al.*, 1984). Some tropical timbers possess pigmented phenolic compounds known as extractives (Scheffer & Cowling, 1966; Hillis, 1971) which are toxic to decay fungi. The 'bleaching' of the timbers, may be due to the removal of the pigmented phenolic extractives by laccase and perhaps other enzymes, as part of a detoxification strategy to facilitate degradation of wood. The wood colour was, thus, lightened and not delignified *per se*. The lightening of wood colour observed in the early stages of attack by *Polysticus versicolor* was reported to be due to the destruction of pigmented materials (Cartwright & Findlay, 1958).

It was observed that 'bleaching' was more prominent in the more pigmented mahogony wood. During rinsing to remove sand, water from inoculated blocks became deeply coloured, implying greater solubility.

Table 2. Quantitative Estimation of Changes (Dry Weight) in Composition of Lignin and Cellulose in Wood Exposed to Decay Fungi for 12 Weeks.

Wood type	Decay Fungi	Mean weight (g) ± SD			
		Untreated* wood	Delignified residue	Lignin^{+}	Cellulose^{++}
Obeche	Uninoculated (control)	0.98±0.06	0.91±0.05	0.07±0.003	0.48±0.01
Obeche	*P. ostreatus*	0.70±0.05	0.65±0.02	0.05±0.002	0.30±0.02
Obeche	*G. sepiarium*	0.69±0.06	0.63±0.02	0.07±0.002	0.25±0.01
Obeche	*Gloeophyllum* sp	0.66±0.04	0.59±0.02	0.07±0.003	0.15±0.004
Mahogany	Uninoculated (control)	3.00±0.08	2.67±0.07	0.33±0.01	1.48±0.06
Mahogany	*P. ostreatus*	2.16±0.07	1.86±0.05	0.30±0.01	0.87±0.04
Mahogany	*G. sepiarium*	2.13±0.09	1.81±0.05	0.33±0.01	0.67±0.04
Mahogany	*Gloeophyllum* sp.	2.08±0.08	1.75±0.04	0.33±0.01	0.58±0.03

*, Extractive free wood not subjected to delignification or removal of hemicellulose.
+, Difference in weight between untreated wood and delignified residue.
++, Dry weight values of hemicellulose free residue; (See materials and methods).

Indication of lignolytic ability of *P. ostreatus* from microchemical tests, was corroborated by the detection of lignin peroxidase. Microchemical tests showed that the zone of incipient decay had no cellulose when lignin was still present, thereby suggesting that the fungus attacked only the cellulose for initial growth before attacking the lignin. The zone of incipient decay was the point of emergence of the basidiocarp suggesting intense metabolic activity. The lower loss of lignin when compared to that of cellulose also suggests that attack on lignin may have been delayed, and began after the cellulose was well under attack. Some white rot fungi e.g. *Phanerochaete chrysosporium* (Kirk *et al.*, 1976) do not degrade lignin without a growth substrate such as glucose or cellulose.

In conclusion, the two *Gloeophyllum* spp. attack mainly the cellulose and hemicellulose components of obeche and mahogany timbers, with concomitant destruction of extractives. On the other hand, *P. ostreatus* attacked all the structural components beginning with cellulose and hemicellulose. Quantitative assay of the laccase activity of the two brown rot *Gloeophyllum* species would be of interest.

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