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Micromorphological characteristics of bamboo (*Phyllostachys pubescens*) fibers degraded by a brown rot fungus (*Gloeophyllum trabeum*)

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Abstract The decay pattern in bamboo fibers caused by a brown rot fungus, *Gloeophyllum trabeum*, was examined by microscopy. The inner part of the polylaminate secondary wall was degraded, while the outer part of the secondary wall remained essentially intact. Degradation in bamboo fiber walls without direct contact with the fungal hyphae was similar to wood decay caused by brown rot fungi. Degradation in polylaminate walls was almost confined to the broad layers whereas the narrow layers appeared resistant. The *p*-hydroxylphenyl unit lignin in middle lamella, particularly in the cell corner regions, was also degraded. The degradation of lignin in bamboo fibers was evidenced by Fourier transform infrared spectra. The present work suggests that the decay of bamboo fiber walls by *G. trabeum* was influenced by lignin distribution in the fiber walls as well as the polylaminate structures.

Key words Brown rot fungus · *Gloeophyllum trabeum* · Bamboo fiber · Polylamellar layers · Decay pattern

Introduction

Brown rot in wood is characterized by rapid and extensive decomposition of cellulose in the initial stages of wood decay. It can be easily distinguished under microscopy from other fungal attacks from a combination of features, including loss of birefringence, absence of erosion troughs, and near-normal morphological appearance of the degraded wood cells.[1,2] Microscopy studies have shown that the S_2 layer of tracheids is preferentially degraded by the brown rot fungi whereas the S_3 layer and lignin-rich middle lamella remain intact.[3] The structural polysaccharides in the S_2 layer are degraded without direct contact of hyphae with the wood cell wall. However, the micromorphology of wood degraded by the brown rot fungi is variable depending upon the host and the wood cell wall types attacked. In some situations, cavities reminiscent of soft rot attack were observed.[4,5]

Compared with degradation of tracheids, little is known about the decay of bamboo fibers by brown rot fungi. The formation of a cell wall in graminaceous plants such as bamboo proceeds in a quite different manner to that in woody plants. No thickening growth of the culm occurs.[6,7] Bamboo fibers are characterized by a thick polylamellate secondary wall. Chemical composition and microfibril orientation are different depending upon the layers in the polylamellate fibers.[8,9] There are also marked differences in the process of lignification and the deposited lignin among various cells in graminaceous plants.[10,11] Ultraviolet (UV) microscopic work has shown that different lignin moieties are present in bamboo fibers and that the concentration of lignin in the bamboo fiber wall is also different.[12] Cho et al.[13] found that *p*-hydroxylphenyl units (H) were dominant in the compound middle lamella in bamboo. Consequently, the existing models of the lignified cell walls of wood xylem might not directly apply to graminaceous plant cell walls. Similarly, modes of fungal degradation of wood cell walls may not be the same for fungal degradation of bamboo fiber walls.

The present study was undertaken to understand the decay of bamboo fiber walls by the brown rot fungus *Gloeophyllum trabeum* using various microscopic techniques in comparison with the mode of brown rot of wood cell walls. We were particularly interested in the influence of lignin composition and distribution in bamboo fiber walls on the mode of degradation of these walls by *G. trabeum* and the effect of polylamellate structures on the restriction of brown rot.

Materials and methods

Brown rot fungus, *Gloeophyllum trabeum* 6737, was obtained from the Korea Research Institute of Bioscience

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and Biotechnolgy (KRIBB) in Daejeon, Korea. Blocks (10 × 10 × 8 mm) were prepared from 2-year-old *Phyllostachys pubescens* bamboo growing in Damyang Province, close to Gwangju, Korea. The fungus was cultivated in a petri dish on a potato–dextrose agar medium until the medium was completely covered by mycelium. Steam-sterilized bamboo blocks of *P. pubescens* were placed on a feather slip of birch wood. After 16 weeks of fungal incubation at 25°C, bamboo specimens were harvested and the mass loss calculated.

For confocal laser scanning microscopy (CLSM), sections obtained on a rotary microtome were examined with a CLSM (Olympus FV 500) for lignin fluorescence after staining with 0.0001% acriflavine for 10 min. Confocal images were acquired using an argon laser with an excitation wavelength of 490 nm and an emission wavelength of 520 nm. For transmission electron microscopy (TEM), small pieces of bamboo samples were fixed in a mixture of 2% paraformaldehyde and 2% glutaraldehyde in 0.05 M cacodylated buffer (pH 7.2). Thereafter samples were dehydrated using a graded ethanol series and embedded in Spurr's resin. The embedded samples were sectioned on an ultramicrotome using a diamond knife. The ultrathin sections (100 nm) were examined with a Jeol 1200 TEM after staining with 1% potassium permanganate (prepared in citrate buffer) to contrast the lignin component in bamboo cell walls. For characterization of chemical changes in bamboo after brown rot, bamboo blocks were freeze-dried, ground to a fine powder, and thoroughly dried at 60°C overnight. A KBr pellet mixed with the powder of bamboo (0.5% concentration) was used for Fourier transform infrared (FT-IR) spectroscopy (Nicolet 520P, Polaris/ICON).

For UV microspectrophotometry, small pieces of bamboo samples were prepared using the same procedures used for TEM. Semi-thin transverse sections (1 µm thick) were prepared using an ultramicrotome with a diamond knife. The sections were transferred to quartz slides, immersed in a drop of non-UV-absorbing glycerine and covered with quartz coverslips. UV absorbance spectra were recorded using a Zeiss UMSP 80 micro-spectrophotometer. The sections were investigated by point measurements with a spot size of 1 µm^2 taken at wavelengths from 240 to 400 nm in 2-nm steps using the program LAMWIN (Zeiss International).

Results and discussion

Detailed CLSM and TEM observations showed that the bamboo fiber secondary wall consisted of alternate thick and thin concentric layers (Figs. 1, 2). The narrow laminar layer showing strong autofluorescence under CLSM (Fig. 2) also stained strongly with $KMnO_4$ (Fig. 1) in ultrathin section, suggesting the presence of an elevated level of lignin in the narrow layers compared with the adjacent broad layers.

Parenchyma cells were severely attacked and deformed (not illustrated). Confocal microscopy (Fig. 2) showed that *Gloeophyllum trabeum* removed the mid-region of polylamellate structures, while the inner region remained essentially intact. Figure 3, which is the TEM view, confirms this and also shows that bamboo fiber walls are degraded at a considerable distance from fungal hyphae, with degradation occurring uniformly leading to the presence of a clear zone in the mid-region of the secondary wall. The outermost region of the secondary wall was not degraded. It is noteworthy that the innermost part of the wall, which is in direct contact with fungal hyphae, is swollen but not degraded.

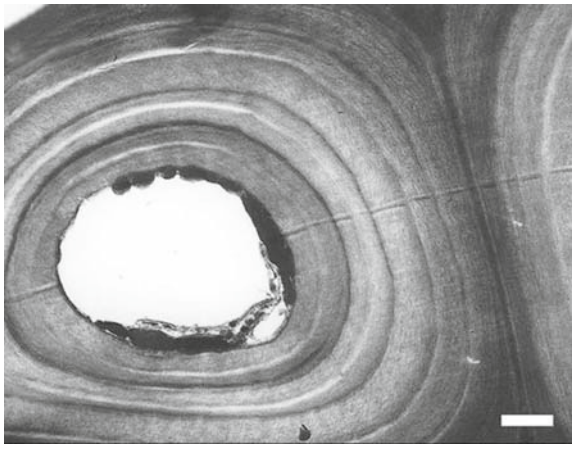

Fig. 1. Polylaminate layers in bamboo fibers viewed by transmission electron microscopy (TEM). $KMnO_4$ staining. *Bar* 1 µm

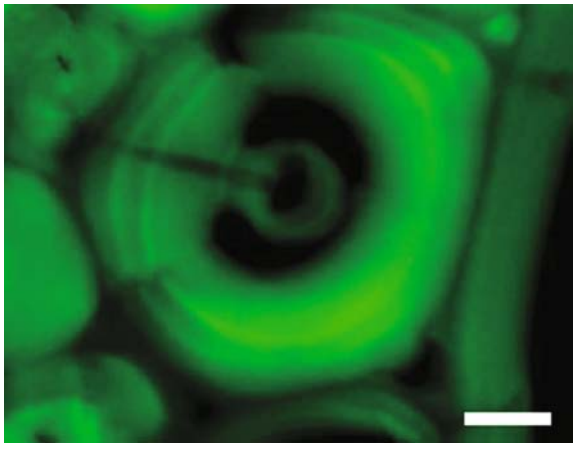

Fig. 2. Confocal laser scanning microscopy (CLSM) view of selective degradation in inner layer of bamboo fiber walls by the brown rot fungus *Gloeophyllum trabeum*. Note the polylaminate wall layers and degradation mainly in the broad layer. *Bar* 5 µm

The degradation pattern of bamboo fiber cell wall by *G. trabeum* was similar to other brown rot fungi in terms of preferential removal of the middle part of the secondary walls, and that the inner polylaminate layer remains intact. Micromorphological changes of wood cells due to attack by a brown rot fungus (*Poria placenta*) have been documented by light microscopy and TEM.[3] The S_2 layer of the wood cell wall is attacked first, whereas the S_3 layer and the middle lamella remain intact even in advanced stages of decay. It is not known, however, how the degrading agents released from fungal hyphae can penetrate deeply into the polylami-

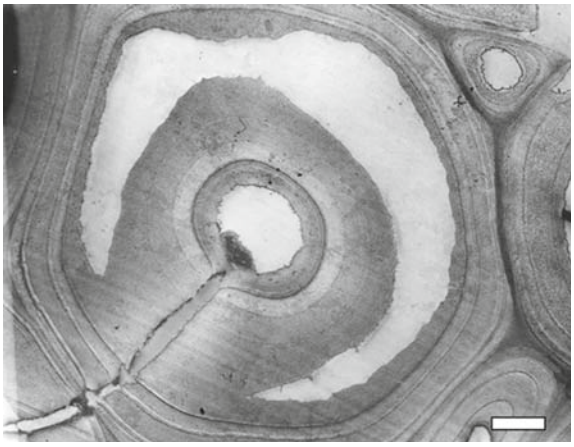

Fig. 3. TEM view of selective degradation in inner layer of bamboo fiber walls by *G. trabeum*. Note the occurrence of degradation in the broad layer. $KMnO_4$ staining. *Bar* 2 µm

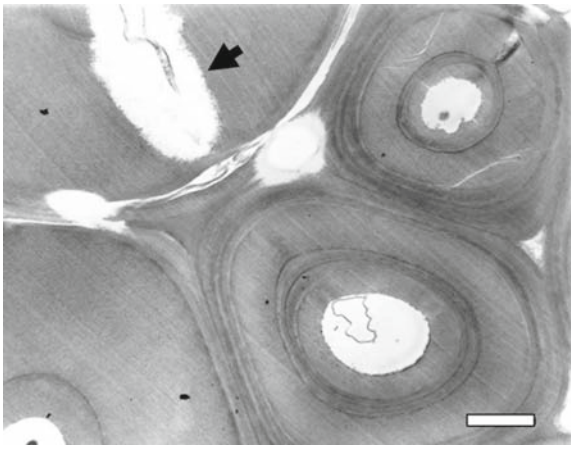

Fig. 5. TEM view of degradation of cell corner and middle lamella by *G. trabeum.* Note the enlargement along the pit in the fiber (*arrow*). $KMnO_4$ staining. *Bar* 2 µm

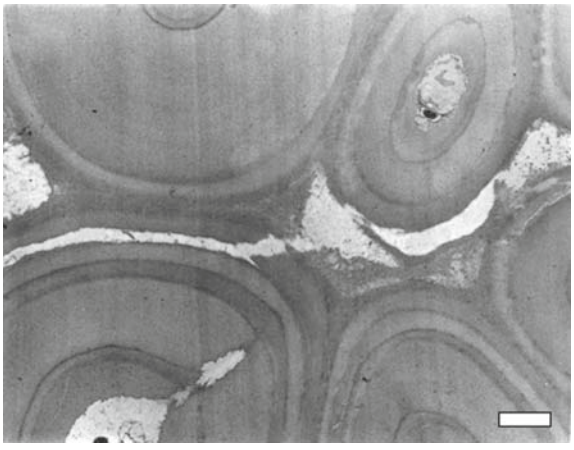

Fig. 4. TEM view of degradation of cell corner and middle lamella by *G. trabeum*. $KMnO_4$ staining. *Bar* 1 µm

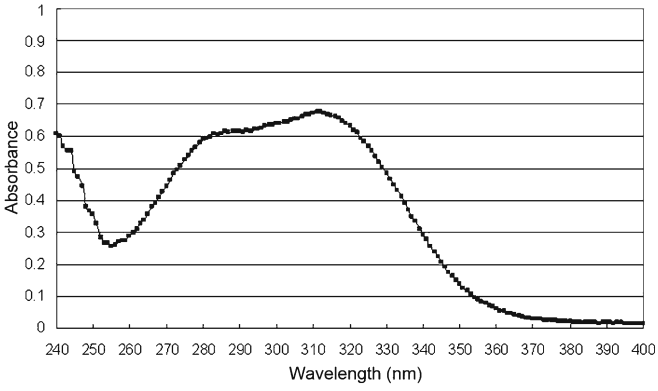

Fig. 6. Representative ultraviolet absorbance spectrum of compound middle lamella in bamboo fibers

nate wall of bamboo. Cellulolytic enzymes from brown rot fungi are too large to penetrate intact cell walls.[14,15] Hydroxyl radical or hydrogen peroxide is reported to be involved in the degradation of cellulose in conjunction with Fenton reagent.[16] Recently, Kim et al.[17] provided cytochemical evidence that hydrogen peroxide from the brown rot fungus *Coniophora puteana* diffused into the intact secondary cell walls. Further studies are necessary to confirm the diffusion of hydrogen peroxide into the polylaminated bamboo fiber cell walls.

TEM work showed that compound middle lamellae (CML) in bamboo fibers were degraded by the brown rot fungus *G. trabeum* (Figs. 4 and 5) at an early stage of decay. UV microscopy showed that absorbance maxima of CML including the cell corner were formed at 312–315 nm (Fig. 6), indicating the presence of H-unit lignin.[10,12] UV microscopy showed clearly that the compound middle lamella in bamboo was mainly composed of H-unit lignin. This means that the brown rot fungus was able to degrade the compound middle lamella rich in H-units. Graminaceous-plant lignins are more susceptible to degradation under chemical and microbiological conditions as compared with woody plant lignins.[11] FT-IR spectroscopy showed that absorbance bands assigned to lignin (840, 1270, 1330, 1430, and 1510 cm^{-1})[18,19] were slightly decreased by *G. trabeum* (Fig. 7). In particular, the band at 1646 cm^{-1}, which was assigned to conjugated carbonyl groups originating from lignin, significantly decreased. UV and FT-IR spectroscopy suggest that not only H-unit lignin but guaiacyl-type or syringyl-type lignin in bamboo were also degraded by the brown rot fungus.

The exact mechanism underlying the decay patterns observed in bamboo fiber walls is unclear. It is possible that the degradation modes in bamboo resulting from attack by *G. trabeum* are related to both the enzyme systems of the brown rot fungus and different lignin composition of bamboo fibers. It has been generally believed that many of the brown rot fungi modify the chemical structure of lignin by demethylation and hydroxylation of aromatic nuclei, but the fungi probably cannot depolymerize lignin intensively.[20] However, the degradation of all wood components including lignin by *G. trabeum* was described by some inves-

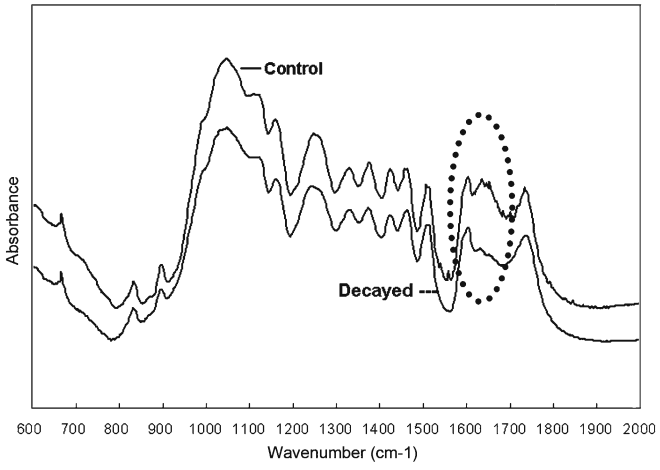

Fig. 7. Fourier transform infrared spectrum of bamboo degraded by the brown rot fungus *G. trabeum*, shown against a control spectrum. Note the diminished intensities of absorbance bands assigned to lignin and the significant decrease of the absorption band at 1646 cm^{-1} (*dotted circle*)

tigators.[21,22] Consequently, the degradation of bamboo is probably related with the degradation ability of *G. trabeum*.

Bamboo fibers consist of relatively thick secondary walls with high and low concentrations of lignin in alternate wide and narrow lamellae.[23] The narrow layers showed resistance against brown rot attack. CLSM showed that the main degradation occurred in the broad layers but not in the narrow layers (Fig. 2). While the results of TEM were consistent with those of CLSM, it also showed greater detail of cell wall organization and cell wall degradation. Narrow lamellae stained strongly with $KMnO_4$ (Fig. 1), indicating high concentration of lignin in them. The present work suggests that the narrow layers in bamboo fiber with higher lignin concentrations than in the broad layers have greater resistance to brown rot fungal attack, which is attributable to their high lignin concentration. Daniel and Nilsson[24] also found that polylaminate layering of the tropical hardwood species *Homalium foetidum* had a significant effect on cavity formation by soft rot fungi. The present work suggests that not only the degradation ability of *G. trabeum*, but also the lignin distribution plays an important role in producing different decay patterns by brown rot fungus in bamboo cell walls. Further studies such as the use of nitrobenzene oxidation are needed to elucidate the characteristics of lignin degradation by the brown rot fungus *G. trabeum.* Moreover, the effects of the physicochemical properties of bamboo, such as lignin distribution, microfibril angles, and bamboo age, on cell wall degradation micromorphologies produced by decay fungi should be examined.

Conclusions

Microscopy studies showed that *Gloeophyllum trabeum* removed the mid part of secondary walls in bamboo fibers, while the outer and inner parts remained essentially intact. Bamboo fiber walls were degraded at a considerable distance from the fungal hyphae. Compound middle lamellae in bamboo fibers were also degraded at an early stage of decay. The distribution of H-unit lignin in the middle lamella was confirmed by UV microscopy. The absorbance bands assigned to lignin were decreased in the FT-TR spectra. The present work suggests that the decay of bamboo fiber walls by *G. trabeum* was influenced by lignin distribution in the fiber walls. Polylaminate layers in bamboo fibers had an influence on cell wall degradation, with the narrow layers showing greater resistance than the broad layers.

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