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Ultrastructural Morphology of the Hyphal Sheath of Wood Decay Fungi Modified by Preparation for Scanning Electron Microscopy

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INTRODUCTION

Our previous attempts to elucidate the structure and extent of the hyphal sheath of wood-decay fungi by scanning electron microscopy (SEM) showed that different preparative methodologies yield differing and frequently conflicting results. Foisner et al. (1985a and b), Highley and Murmanis (1985, 1987), and Green et al. (1989) provided substantial ultrastructural evidence for the presence of extracellular membranous structures that assume a variety of forms, including sheets, tubules, vesicles, and fibers. Evans et al. (1981) reported a fibrillar sheath surrounded by a tripartite pellicle on rapidly growing Bipolaris maydis. Day et al. (1986a and b) also provided evidence for extracellular fungal structures, called "fungal fimbriae", which the authors described as primarily proteinaceous. Foisner et al. (1985a and b) analyzed isolated, extracellular membranous structures, which were reportedly composed of carbohydrate, protein, and lipid. These structures were better visualized by transmission electron microscopy (TEM) after treatment with osmium and/or ruthenium red.

Thus, the literature supports the hypothesis that the membranous structures comprising the hyphal sheath form a tripartite lipid bilayer, without phospholipids, associated with protein and carbohydrate. The carbohydrate component is probably fungal glucan in various forms. The

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presence of fungal glucan in a variety of filamentous fungi has been well documented; fungal glucan has been referred to variously as mucilage, extracellular slime, mucoid substance, and mucopolysaccharide. The glucan moiety is composed of 90% glucose residues (Foisner et al., 1985b; Micales and Highley, 1990). Bonfante-Fasolo et al. (1987) suggest that extracellular polysaccharides are rich in low molecular weight sugars, usually lost during conventional procedures for electron microscopy.

The extracellular materials of microorganisms are frequently viewed as contaminants, which should be removed prior to fixation by treatments as simple as washing in an isotonic buffer (Hayat, 1981; Wilcox and Brier, 1987). Often, the presence of adherent slime on the surface of tissues examined by SEM obscures underlying structures (Honegger, 1985; Waterman, 1982).

We consider the term 'hyphal sheath' to refer to a sheath that includes the various structural membranous elements as well as glucan. The hyphal sheath is not confined to only the immediate vicinity of the hyphae, but is ubiquitous and extensively distributed over the lumen surface of the wood cell wall, as is the decay process. Presence of glucans in cell wall and extrahyphal extensions are frequently overlooked (Fleet and Phaff, 1981).

In this SEM study, we utilized several conventional fixation techniques routinely used in TEM for visualizing wood-decay fungi. We conjecture that membrane-specific fixatives enhance preservation of the organized lipid layers in association with protein and glucan. Conventional fixation techniques were supplemented with additives reported to enhance membrane stabilization, e.g. $CaCl_2$, OsO_4, picric acid, saponin, and glutaraldehyde. Cryofixation was also used, as a means of excluding chemical fixation and solvent dehydration.

Central to any ultrastructural study is the acknowledgement of the possible generation of artifacts that are not identical to actual in vivo structures (Crang, 1988). However, the modification of biological structure by a variety of fixation protocols is important from the perspective that certain structures, especially membranes, will respond in a predictable manner, thus revealing their identity. The purpose of this study was to extend our previous observations (Green et al., 1989) of the ultrastructural morphology of the hyphal sheath of brown- and white-rot fungi by SEM using diverse preparative protocols. The results presented here illustrate a variety of morphological modifications of the hyphal sheath observed after fixation and dehydration for SEM. These modifications provide additional evidence for the hypothesis that the hyphal sheath is an extensive extramembranous structure.

MATERIALS AND METHODS

Fungi and Culture Conditions

Wood blocks (8 by 8 by 4mm) of various species were decayed by different fungi using the ASTM soil-block procedure (ASTM, 1971). Southern yellow pine (Pinus spp.) blocks were decayed by Postia placenta (Fr.) M.Lars et Lomb. (isolate # MAD-698). Maple (Acer spp.) blocks by Trametes versicolor (L. :Fr) Pilat (isolate # MAD-697), and poplar (Populus spp.) blocks by Phanerochaete chrysosporium Burds. in Burds. et Eslyn (isolate # MAD-461). Wood blocks were removed at selected intervals during the 12-week decay period for examination by SEM.

Fixation

Several fixation schedules were used for decayed wood blocks; (1) cryofixation by rapid quenching in precooled (30 lb/in^2, -21°C) liquid nitrogen (QLN) and lyophilization without chemical fixation; (2) aldehyde fixation--1.0% glutaraldehyde (G) and 4.0% paraformaldehyde (F) in 0.1 M cacodylate buffer (pH 7.2) for 1 to 12 h, double fixation in the same fixative plus 0.05% $CaCl_2$ and 2% OsO_4, the same fixative plus 0.05% $CaCl_2$ and 0.05% saponin (SAP) added (Hayat, 1981), or the same fixative postfixed in 1% OsO_4; (3) chemical fixation in saturated picric acid, 2% paraformaldehyde and 3% glutaraldehyde (PAFG) in phosphate buffer as described by Waterman (1982) for 2 h, or the same fixative postfixed in 2% OsO_4 overnight; and (4) chemical fixation in 1% aqueous OsO_4 vapors for 4 to 8 h in a chemical hood (Cole, 1986).

Dehydration and Drying

Three techniques were used for dehydration and drying: critical point drying, freeze drying, and air drying. For critical point drying (CPD), fixed decayed blocks were dehydrated in a series of increasing concentrations (30, 50, 70, 85, 90, 95, 100%) of ethanol/buffer or acetone/buffer and dried in 5 to 10 changes of liquid CO_2 (transition fluid) in a Balzer's[2] critical point dryer equipped with a high pressure filter (Union Carbide, NJ). For freeze drying (FD), cryofixed decayed blocks were transferred to a precooled cryovessel and lyophilized overnight at −55°C for 12 h.

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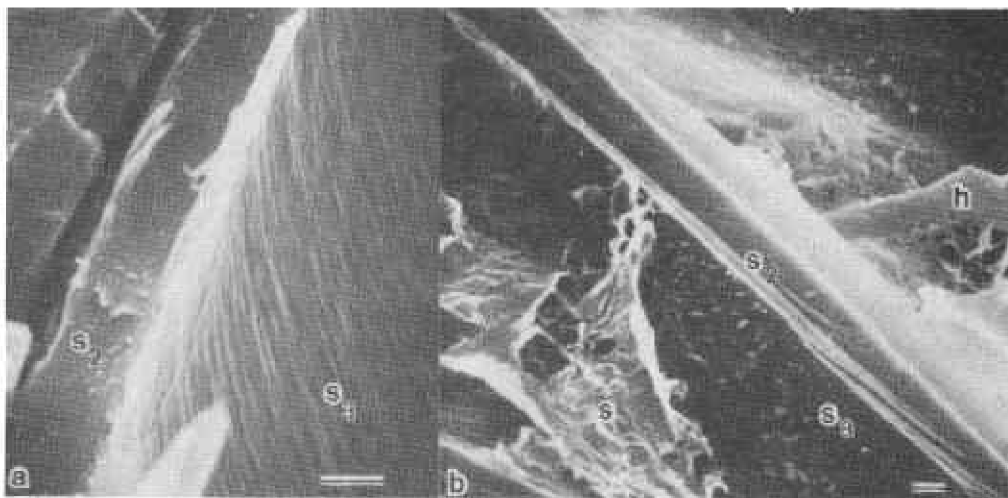

Figure 1. Scanning Electron Micrographs of Undecayed and Decayed Wood of Pinus spp. (QLN-FD). (a) Undecayed Control, (b) Wood Decayed by Postia placenta. Note Prominence of Fiber Angle in S3 Layer (a); (b) Fiber Angle Occluded by Hyphal Sheath (s) Covering S3 Layer and Hypha (h). Scale Bar in (a) = 10 µm; (b) = 5 µm.

Coating and SEM

Dried wood blocks were split longitudinally with a razor blade to expose the radial face and then coated with gold (Au) in a Polaron sputter-coater for approximately 22 s resulting in an Au-layer approximately 65 to 70 A thick. Specimens were examined with a Hitachi S-530 scanning electron microscope at an accelerating voltage of 25 Kv and working distance of approximately 5 to 10 mm.

RESULTS

The ultrastructural features of the hyphal sheath and associated extracellular hyphal structures of the brown-rot fungus P. placenta (Figures 1 to 3, and 4e-f), and the white-rot fungi T. versicolor (Figure 4a-b) and P. chrysosporium (Figure 4c-d) were illustrated by SEM. In general, the hyphal sheath of both brown- and white-rot fungi appeared extensive and similar. Different morphological variations in hyphal sheath structure were observed and were dependent upon the preparative methods used for fixation and dehydration,

The hyphal sheath covered the hyphae and extended out from them onto the lumen surfaces of the wood cell walls. The sheath often appeared disrupted at the hyphal-S3 interface, This was probably due to shrinkage during dehydration (Figure 2b, d, and g). The extensive nature of the

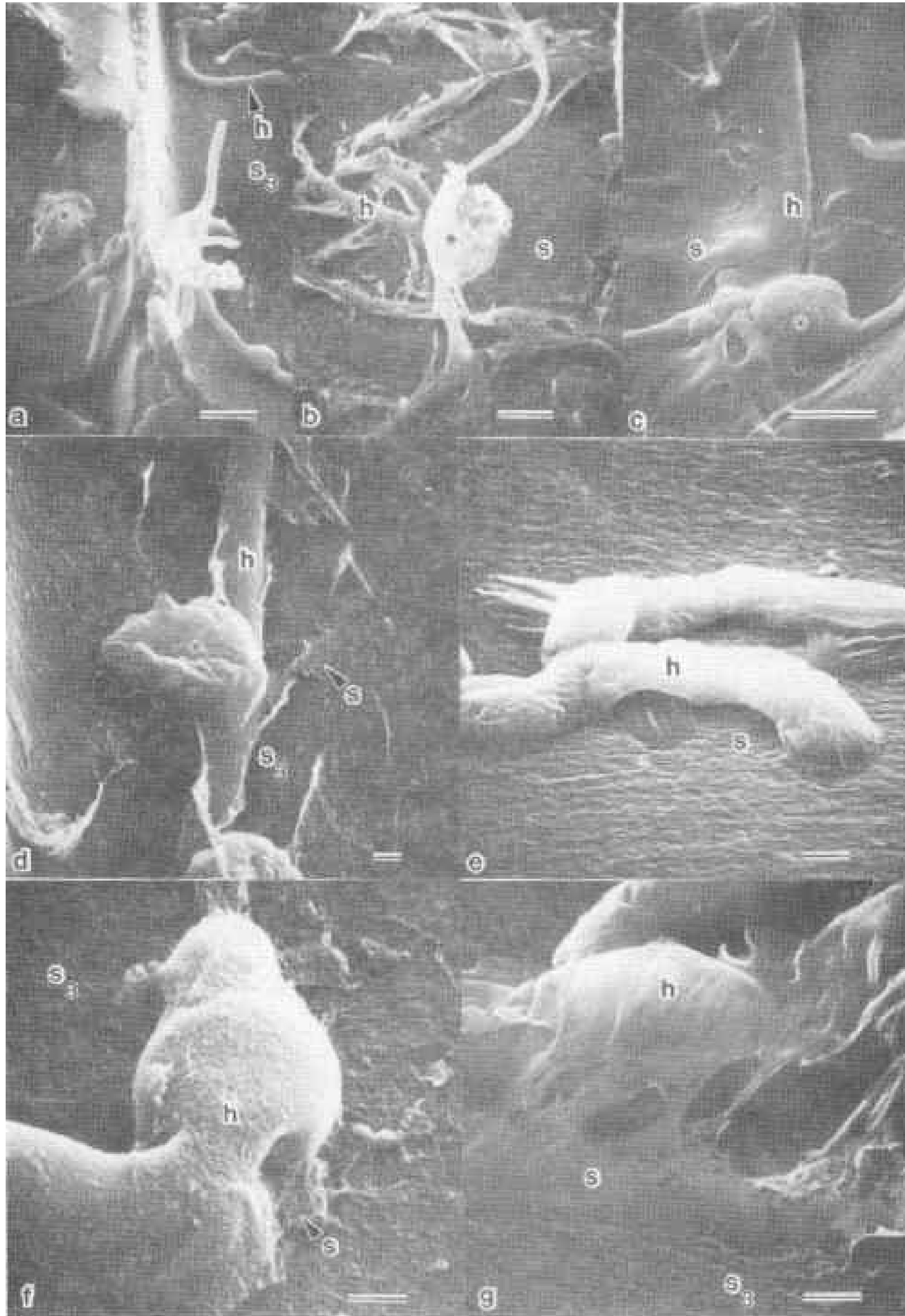

Figure 2. Sequential Modifications of Hyphal Sheath of <u>Postia placenta</u> on <u>Pinus spp.</u> by Various Preparative Methods. (a) GF 1 h–Etoh–CPD, (b) GF 2 h–Acctonc–CPD, (c) GF+$CaC1_2$ SAP 12 h–Etoh–CPD, (d) PAFG 2 h-OsO_4 12 h–Etoh–CPD, (c) OsO_4 Vap. 4 h–QLN–FD, (f) GF-Acetonc–CPD, (g) GF+$CaCl_2$ +SAP 1 h–Etoh–CPD. (*Note Extrahyphal Structures) Scale Bars = 1 µm.

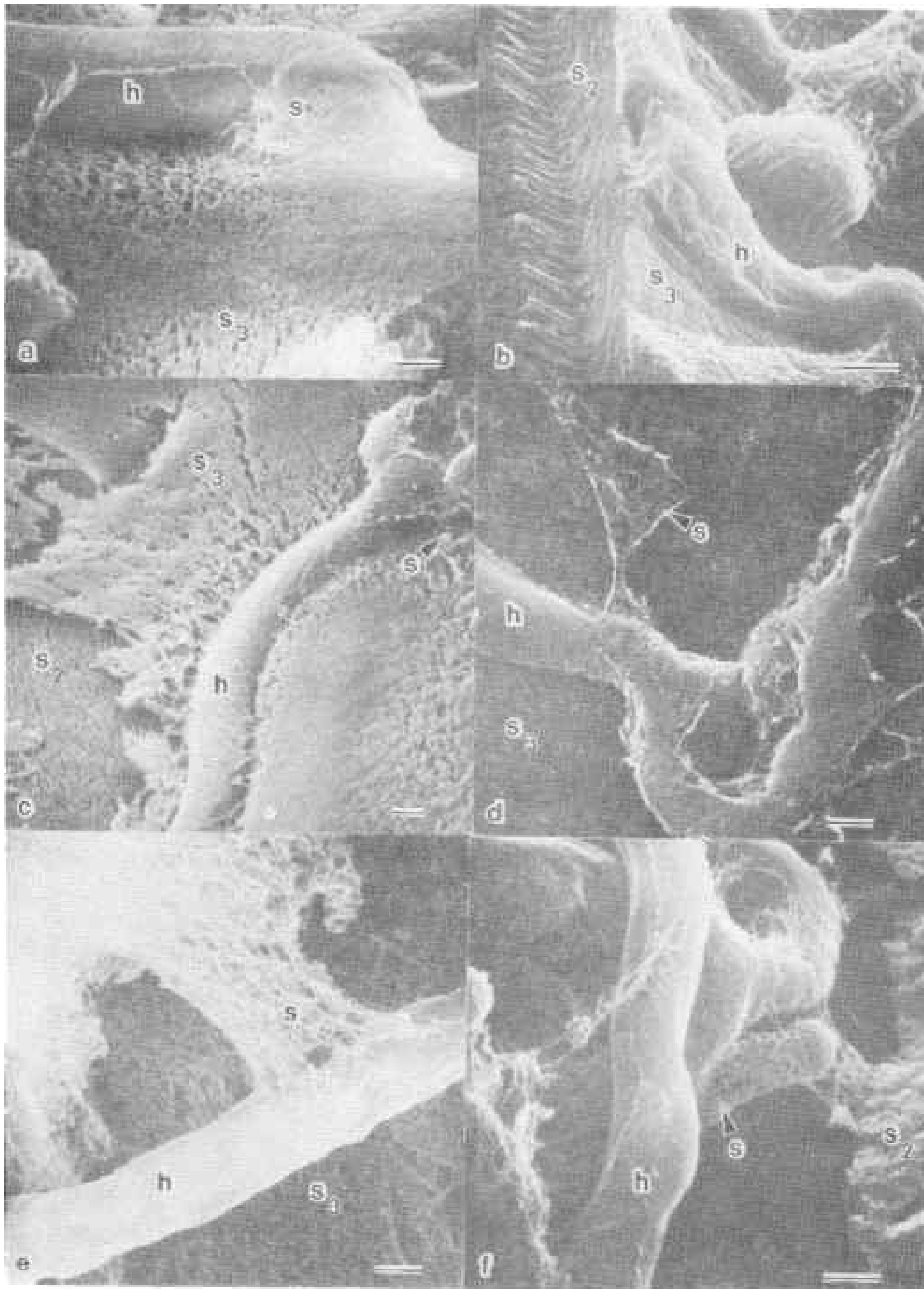

Figure 3. Fibrillar Modifications of Hyphal Sheath of *Postia placenta* on *Pinus spp.* by Various Fixation–Dehydration Protocols. (a) Fixed PAFG 12 h–Etoh–CPD, (b) OsO_4 Vap. 4 h–AD, (c) PAFG 2 h-OsO_4 12 h–CPD, (d) GF+$CaCl_2$ +OsO_4 8 h–Etoh–CPD, (e) GF–Acetone–CPD, (f) PAFG 2 h–Etoh–CPD. Scale Bars = 1 μm.

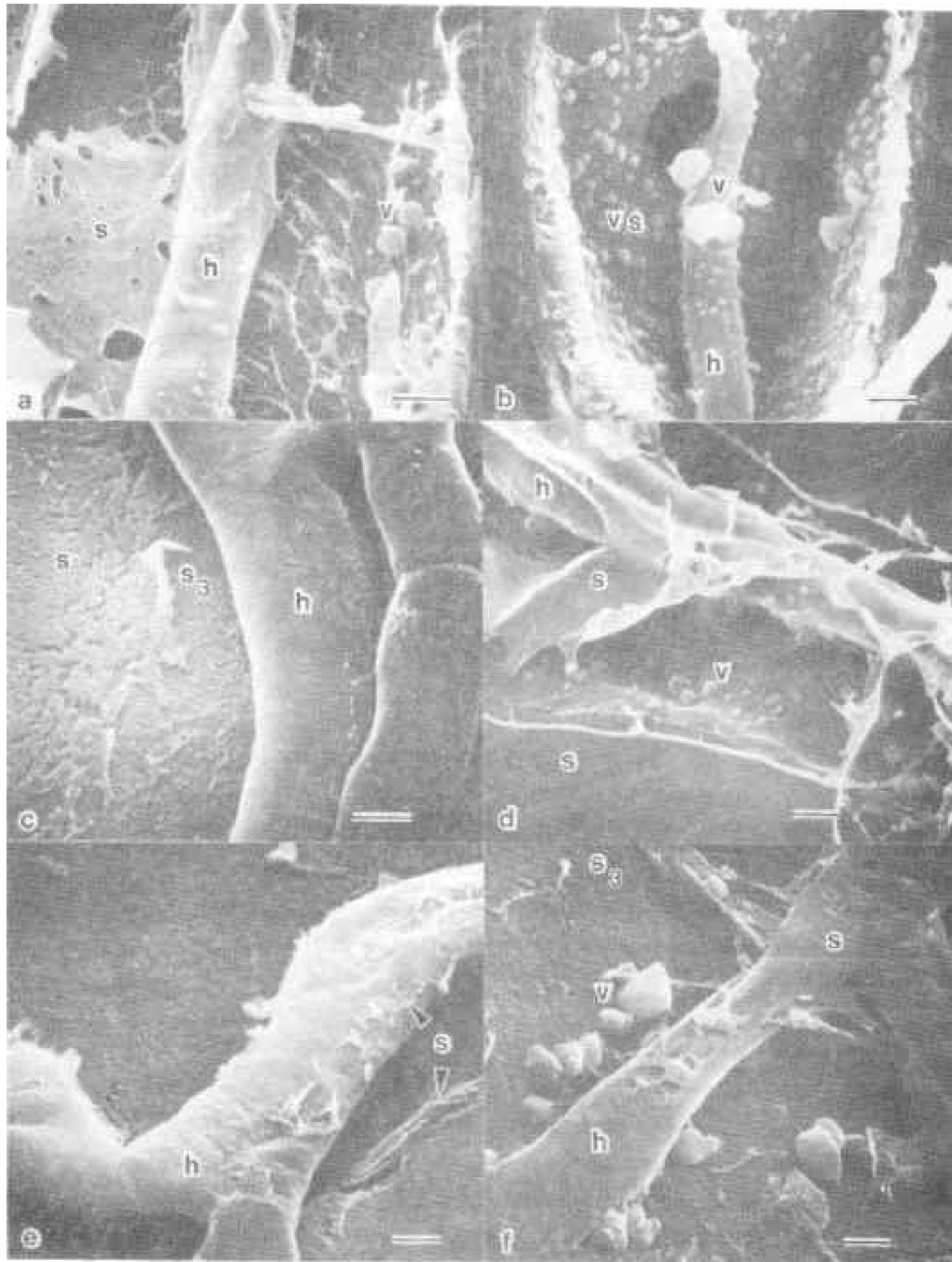

Figure 4. Vesiculated and NonVesiculated Hyphal Sheath of Brown- and White-Rot Fungi. (a–b) Trametes versiclor : QLN-FD; (c–d) Phanerochaetae chrysosporium : (c) GF–Etoh–CPD, (d) QLN–FD; (e-f) Postia placenta : (e) PAFG–Etoh–CPD, (f) QLN–FD. Scale Bars = 1 µm.

hyphal sheath and its ability to cover and occlude the linearity of the fiber angle of the S3 surface layer of the wood cell wall are evident in Figures 1b and 2c, as compared to the sheath of the undecayed control (Figure 1a).

Removal of the hyphal sheath from the outer surfaces of hyphae and the S3 lumen surface of the wood cell wall occured frequently after conventional chemical fixation and CPD (Figures 2a-f and 3e). Sequential removal of sheath components was commonly observed in different areas of the same wood block, most likely because partial penetration of fixatives and/or solvents during CPD. Underneath the hyphal sheath, other extrahyphal structures, which appeared convoluted and membranous, were visible (Figure 2a-c,*). Inclusion of "membrane-stabilizing agents" during fixation (that is, picric acid, $CaCl_2$, saponin, OsO_4), as well as increasing fixation times from 2 to 12 h, apparently improved retention of sheath components and structures during dehydration (Figures 2c-e and 3f). Figures 2e and 3b illustrate the apparetnt collapse of both hyphae and intact smooth sheath covering the S3 layer.

The increased porosity of the S3 layer of the wood cell wall during the decay process were revealed by dehydration in organic solvents and CPD (Figure 3a-f). Extensive fibrillar sheath structure was observed after aldehyde fixation followed by dehydration in either alcohol or acetone and CPD (Figure 3a, c, d, and f). The fibrillar elements of the hyphal sheath extending from the hyphae were similar to the residual wood structures (lignin ?) and associated porosity of the lumen surface of the wood cell wall (Figure 3a, c, and e).

Structural variation or modification artifacts of the hyphal sheath included lamellar sheets (Figures 1b; 2b, d, g; and 4a, d, f), fibrillar arrays extending between the hyphae and the S3 layer (Figure 3d, and e, and f), and vesicular structures (Figure 4a, b, d, and f). these modifications were often seen in one preparative method but were absent in others. For example, in both <u>T. versicolor</u> and <u>P. chrisosporium,</u> vesicles wsere observed after cyrofixation (QLN) Figure 4a, b and d), wereas both lamellar and vesicular structures were absent following DPD (Figure 4c). In <u>P. placenta,</u> however, angular vesiculation was observed only after primary fixation with PAFG, supplemented with $CaCl_2$ and saponin, and followed by CPD (Figure 4f), and never after QLN (Figure 4e) or exposure to OsO_4 vapors. Smooth, extracellular membranous sheets were most consistently observed and were associated wtih all three wood- decay fungi after QLN, even though these sheets were often disrupted with holes and cracks caused by shrinkage during dehydration (Fiures 1b; 2b and g; 3d; and 4a, d, and f).

DISCUSSION

This study was a preliminary survey of preparative methodology for fixation and dehydration of decayed wood blocks for examining the hyphal sheath of brown- and white-rot basidiomycetous fungi by SEM. Clearly, wide morphological variation in hyphal sheath structure was observed within and between different preparative methods. This variability was due in part to the biological variability inherent in the interaction of wood-decay fungi and substrate and in part to the partial or sequential modification of hyphal structures during specimen preparation (fixation and dehydration). No single preparative method was adequate for determining sheath structure. All methods produced artifacts. Nevertheless, several general principles emerge from this study with regard to the interpretation of sheath structures.

There are more morphological similarities than dissimilarities in the ultrastructural characteristics of the hyphal sheaths of the three wood-decay fungi. This is in concert with the conclusions of Highley (1987), Foisner et al. (1985), and Green et al. (1989) that the hyphal sheath plays a central and ubiquitous role in wood decay by both the brown- and white-rot fungi, and that it is operative at large distances from the hyphae.

Conventional aldehyde fixation (glutaraldehyde and paraformaldehyde), followed by solvent dehydration and CPD, removed and/or modified the hyphal sheath unless supplemented with membrane-stabilizing agents in the primary fixative or during postfixation. The dehydration steps for CPD included graded ethanol and acetone series to 100% concentration. Unless lipids or carbohydrates are adequately preserved, extensive removal during dehydration can be expected. Acetone is reported to remove 95% of lipids from membranes of mycoplasmas (Razin, 1969).

A universal feature of all cell membranes is the presence of some amphipathic (bimodal) structural molecule, usually phospholipids in animal cells and glycolipid in plant cells (Green and Tzagoloff, 1966). Lipid extraction procedures demonstrate that membrane-associated polar lipids require polar solvents, such as ethanol, to disrupt hydrogen bonds or electrostatic forces between lipid-protein complexes. In addition, alcohol dehydration coextracts sugars, amino acids, and salts (Kates, 1986). Neutral lipids are soluble in acetone, whereas polar lipids are insoluble in cold acetone (Kates, 1986). During lipid extraction, polar and nonpolar lipids are extracted by ethanol and acetone, respectively, and in addition, alcohol disrupts and extracts lipid-protein complexes (Kates, 1986). Foisner et al. (1985a) characterized the hyphal sheath as 90%

glucose residues and the remainder as protein and lipids. Phospolipids were not detected by Foisner et al. (1985a), but this does not exclude a role for structural glycolipids.

Ethanol precipitates extracellular glucan of wood-decay fungi during isolation procedures (Micales and Highley, 1990). Therefore, if lipids were removed by ethanol in CPD, associated glucan would precipitate and/or condense. Little evidence in the literature suggests that the sheath and related structures would undergo similar extraction during QLN, but freezing or freeze-thawing artifacts may be evident, Such modifications, however, are unlikely to represent a large-scale removal of proteins, lipids, or carbohydrates.

Glutaraldehyde is reported to fix biological membranes (Hayat, 1981), but glutaraldehyde or formaldehyde has only a slight reaction with lipids. Glutaraldehyde cross-links proteins without reducing the fluidity of the lipid bilayer; this can result in the formation of vesicles, blisters, and blebs (Crang, 1988). In addition, phospholipids, when present, may be removed and the lipid moiety of membranes destabilized by glutaraldehyde (Hayat, 1981).

In our study, the inclusion of OsO_4 in the primary fixative or during postfixation enhanced preservation of the hyphal sheath. Osmium is essential to prevent lipid loss, but it also hydrolyzes and extracts proteins (Todd, 1986). Osmium increases the visualization of lipids because of osmophillia (TEM) and stabilizes protein cross-links already formed (Cole, 1986). Postfixation with OsO_4 has even been shown to stabilize membranous artifacts produced during conventional fixation with glutaraldehyde (Hayat, 1981), When OsO_4 is used alone as a Primary fixative, it may produce more artifacts than it eliminates (Crang, 1988; Sleytr and Robard, 1982). Hyphal sheath structures have also been visualized in TEM with the aid of ruthenium red (Foisner et al., 1985); however, the specificity of this stain is in doubt because it also binds to acidic amino acids and Lipide (Todd, 1986). In Streptomyces salivarius, surface fibrils (probably carbohydrates) could not be seen after conventional aldehyde and OsO_4 fixation in TEM. Polymeric acidic polysaccharides and the neutral polysaccharide, dextran, may also be stained by ruthenium red (Handley et al., 1988).

In our study, addition of $CaCl_2$ and SAP to conventional aldehyde fixatives also resulted in better preservation of sheath structures. Divalent cations ($CaCl_2$) at very low concentrations stabilize mycoplasma membranes againgt osmotic lysis (Razin, 1969) and also minimize lipid loss during dehydration (Mayat, 1981), In addition, cell swelling during dehydration can be minimized by addition of divalent cations to the graded

ethonal/acetone series (Cole, 1986). To some degree, these effects are due to the increased osmolality of the fixative vehicle, eliminating the membranous blebs of hypotonic solutions. Saponin is a mild detergent that solubilizes membranes, but in concert with aldehyde fixation, SAP also stabilizes membrane structure after membrane permeability is altered (Hayat, 1981).

The hyphal sheath appears and behaves like an extracellular membranous structure covered by or embedded in glucan. We observed a variety of artifacts that are inherent in biological membranes, such as cracked and disrupted lamellar sheets and vesicles or blebs. The best SEM representation of the hyphal sheath of P. placenta (MAD-698) is shown in Figure 2c, where a smooth, uninterrupted matrix covers the entire S3 surface of the wood cell lumen. The sheath may be envisioned as a complex fluid structure of high water content.

Correlative TEM evidence in the literature supports the hypothesis of the hyphal sheath as an extracellular membranous structure (Foisner et al., 1985a and b; Highley and Murmanis, 1985; Palmer et al., 1983). Foisner et al. (1985a and b) demonstrated tripartite, extracellular membranous structures using TEM. Recent studies showed that gold-labeled antibodies to ligninase label the extracellular membranes, suggesting that the hyphal sheath plays a role in decay by white-rot fungi (Daniel et. al., 1989; Blanchette et al., 1989).

The removal and modification of the hyphal sheath reveal an extensive fibrillar structure that is likely to represent condensation and precipitation of extracellular fungal protein components of the sheath. Our observations are similar to those of Tsuneda et al. (1987) for Lentinus edodes (Berk.) Sing.; however, these authors identified the structures as cellulose microfibrils. Fibrillar extracellular sheath structures were also observed by TEM in wood-decay fungi (Bracker, 1967; Nilsson et al. 1989), plant pathogenic fungi (Evans et al., 1981), and ericoid mycorrhizal fungi (Bonfante-Fasolo et al., 1987).

Vesicular modifications of the sheath were viewed in all three fungi studied. Vesicles, blebs, and blisters represent some of the most common artifacts visualized in membranes fixed in glutaraldehyde (Hayat, 1981). It is interesting to note that vesicles of the two white-rot fungi studied were seen only after QLN and that vesicles of P. placenta were seen only after CPD. We found little TEM evidence to support vesiculation of the sheath in P. chrysosporium or P. placenta and view these structures as artifacts. However, the numerous regular vesicles of T. versicolor have also been seen in TEM (unpublished results), and we reserve judgement as to

the possible role that these small vesicles play in enzyme delivery and membrane extension in this species.

This paper in no way exhausts the preparative methodology of fixation, dehydration, and preservation of labile biological structures for examination by SEM. Methods designed specifically to fix or remove the glucan component of the hyphal sheath have not yet been developed. Greatly improved preservation of fungal ultrastructure has been obtained by freeze-substitution (Hoch, 19861. Frozen-hydrated specimens better represent the in vivo condition of specimens than specimens prepared by conventional chemical fixation (Read et al., 1982; Beckett et al., 1982). We hope to confirm our observations of the hyphal sheath of wood-decay fungi by these and other methods in the future.

SUMMARY

A preliminary evaluation of different SEM preparative metod was made with specific emphasis on structural modifications of the extracellular sheath of brown-rot and white-rot wood-decay fungi. The variety of complex sheath structures (sheets, filaments, and vesicles) observed often depended on the preprative method. The hyphal sheath of the three basidiomycetes studied -- Postia placenta, Trametes versicolor, and Phanerochaete chrysosporium -- was similar and extensive, and it usually covered the entire S3 surface of the wood cell wall. Conventional aldehyde fixation, followed by organic solvent dehydration for CPD, apparently resulted in the largest morpholocial variation, unless supplemented by membrane-stabilizing agents. Specimens quenched in liquid nitrogen most consistenly showed smooth, lamellar hyphal sheath surfaces. These results provide additonal evidence for a "structural membrane element" of the hyphal sheath of wood-decay fungi, which can be sequentially removed and modified by SEM preparative methodology. A more precise understanding of the interaction of fixatives, stains, and solvents with the hyphal sheath components will be required to better approximate the in vivo morphology of the hyphal sheath.

REFERENCES

ASTM. (1971). Standard method for accelerated laboratory test of natural decay resistance of woods. American Society for Testing and Material Desig. D 2017, Philadelphia, PA.

Beckett, A., Porter, R., and Read, N.D. (1982). Low temperature scanning electron microscopy of fungal material. J. Microbiol., 125, 193-199.

Blanchette, R.A., Abad, A.R., Farrel, R.L., and Leathers, T.D. (1989). Detection of lignin peroxidase and xylanase by immunocytochemical labeling in wood decayed by Basidiomycetes. Appl. Environ. Microbiol., 55, 1457-1465.

Bonfante-Fasolo, P., Perotto, S., Testa, B., and Faccio, A. (1987). Ultrastructural localization of cell surface sugar residues in ericoid mycorrhizal fungi by gold-labeled lectins. Protoplasma, 139, 25-35.

Bracker, C.E. (1967). Ultrastructure of fungi. Ann. Rev. Phytopath., 5, 343-374.

Cole, G.T. (1986). Preparation of microfungi for scanning electron microscopy. In: Ultrastructure Techniques for Microorganisms, pp. 1-86, (Aldrich, H.C., and W.J. Todd, Eds.), Plenum Press, NY.

Crang, R.F.E. (1988). Artifacts in specimen preparation for scanning electron microscopy, In: Artifacts in Biological Electron Microscopy, pp. 107-129, (Crang, R.F.E., and K.L. Klomparens, Eds.), Plenum Press, NY.

Daniel, G., Nilsson, T., and Pettersson, B. (1989). Intra- and extracellular localization of lignin peroxidase during the degradation of solid wood and wood fragments by Phanerochaete chrysosporium by using transmission electron microscopy and immuno-gold labeling. Appl. Environ. Microbiol., 55, 871-881.

Day, A.W., Gardiner, B., Smith, R., Svircev, A.M., and McKeen, W.E. (1986a). Surface proteinaceous fibrils (fimbriae) on filamentous fungi. Can. J. Bot., 66, 2474-2484.

Day, A.W., Gardiner, B., Smith, R., Svircev, A.M., and McKeen, W.E. (1986b). Detection of fungal fimbriae by protein A-gold immunocytochemical labelling in host plants infected with Ustilago heufleri or Peronospora hyoscyami f. sp. tabacina. Can. J. Microbiol., 32, 577-584.

Evans, R.C., Stempen, H., and Stewart, S.J. (1981). Development of hyphal sheaths in Bipolaris maydis race T. Can. J. Bot., 59, 453-459.

Fleet, G.H., and Phaff, H.J. (1981). Fungal glucans-structure and metabolism, In: Plant Carbohydrates II. Extracellular Carbohydrates, pp. 416-440, (W. Tanner and F.A. Loewus, Eds.), Springer-Verlag, Berlin.

Foisner, R., Messner, K., and Roehr, M. (1985a). Wood decay by basidiomycetes: Extracellular tripartite membranous structures. Trans. Brit. Mycol. Soc., 85, 257-266.

Foisner, R., Messner, K., Stachelberger, H., and Roehr, M. (1985b). Isolation and characterization of extracellular three-lamellar

structures of Sporotrichum pulverulentum. J. Ultrastruct. Res., 92, 36-46.

Green, F., Larsen, M.J., Murmanis, L.L., and Highley, T.L. (1989). Proposed model for the penetration and decay of wood by the hyphal sheath of the brown-rot fungus Postia Placenta. Intern. Res. Group Wood Preserv., IRG Document. No. 1391. Stockholm.

Green, D.E. and Tzagoloff, A. (1966). Role of lipids in the structure and function of biological membranes. J. Lipid Res., 7, 587-602.

Handley, P.S. Hargreaves, J., and Harty, D.W.S. (1988). Ruthenium red staining reveals surface fibrils and a layer external to the cell wall in Streptococcus salivarius HB and adhesion deficient mutants. J. Gen. Microbiol., 134, 3165-3172.

Hayat, M.A. (1981). Fixation for Electron Microscopy, 501 pp., Academic Press NY.

Highley, T.L. (1987). Biochemical aspects of white-rot and brown-rot decay. Intern. Res. Group Wood Preserv., IRG Document. No. 1391.

Highley, T.L., and Murmanis, L.L. (1985). Micromorphology of degradation in western hemlock and sweetgum by the brown-rot fungus Poria placenta. Holzforschung, 39, 73-78.

Highley, T.L., and Murmanis, L.L. (1987). Micromorphology of degradation in western hemlock and sweetgum by the white-rot fungus Coriolus versicolor. Holzforschung, 41, 67-71.

Hoch, H.C. (1986). Freeze-substitution of fungi. In: Ultrastructural Techniques for Microorganisms. pp. 183-212, (Aldrich, H.C., and W.J. Todd, Eds.), Plenum Press, NY.

Honegger, R. (1985). Scanning electron microscopy of the fungus-plant cell interface: A simple preparative technique. Trans. Brit. Mycol. Soc., 84, 530-533.

Kates, K. (1986). Techniques of lipidology-isolation, analysis and identification of lipids. Chapter 3-Lipid extraction procedures, In: Laboratory Techniques in Biochemistry and Molecular Biology v. 3, part 2, pp. 1-467. (R.H. Burden and P.H. Van Knippenberg, Eds.), Elsevier, Amsterdam.

Micales, J.A., and Highley, T.L. (1990). Factors associated with decay capacity of the brown-rot fungus Postia placenta. Biodeterioration Research 3. Plenum Publishing Corp., NY.

Nilsson, T., Daniel, G., Kirk, T.K., and Obst, J.R. (1989). Chemistry and microscopy of wood decay by some higher Ascomycetes. Holzforschung, 43, 11-18.

Palmer, J.G., Murmanis, L.L., and Highley, T.L. (1983). Visualization of hyphal sheath in wood-decay hymenomycetes. II. White-rotters. Mycologia, 75, 1005-1010.

Razin, S. (1969). The mycoplasma membrane, In: The Mycoplasmatales and the L-phase of bacteria, pp. 317-348. (L. Hayflick, Ed.), Appleton-Century-Crofts, N.Y.

Read, N.D., Porter, R., and Beckett, A. (1982). A comparison of preparative techniques for the examination of the external morphology of fungal material with the scanning electron microscope. Can. J. Bot., 61, 2059-2078.

Sleytr, V.B., and Robard, A.W. (1982). Understanding the artifact problem in freeze fracture replication : A review. J. Microscopy, 126, 101-122.

Todd, W.J. (1986). Effects of specimen preparation on the apparent ultrastructure of microorganisms, In: Ultrastructure Techniques for Microorganisms, pp. 87-100.

Tsuneda, A., Koshitani, H., and Furukawa, I. (1987). Micromorphological patterns of incipient wood decay by Lentinus edodes. Rept. Tottori Mycol. Inst., 25, 36-48.

Waterman, R.E. (1982). Preparation of embryonic tissues for SEM. Scann. Elect. Micr., 2, 21-44.

Wilcox, W.W., and Brier, Allison. (1987). Fixation improves image of fungal hyphae in SEM. IAWA Bull., (n.s.), 8, 78-79.