Detecting Bacteria in Wood using a Fluorescent Lipid Probe

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Wood cells have strong autofluorescence in a wide wavelength band due to lignin in the cell walls. The detection of microorganisms in wood is very difficult when using fluorescent microscopy because of interferences. We have recently developed fluorescent staining techniques to differentiate fungal hyphae from wood cell walls (Singh, et al., 1997; Xiao, et al., 1997). This study was aimed at developing fluorescent techniques to visualize bacteria in wood using confocal laser scanning microscopy (CLSM). Nitrobenzoxadiazole glycerophosphoethanolamine (NBD-PE, Molecular Probes), a widely used membrane probe which accords strong fluorescence upon lipids, was compared with glutaraldehyde which had proved useful in our initial attempts to visualize fungal hyphae in wood because of the cell auto fluorescence it causes (Singh et al., 1997).

NBD-PE (excitation: 463 nm, emission: 536 nm) was dissolved in absolute ethanol at 1 mg/mL. 10 µl of this solution was diluted with 250 µl phosphate buffered saline (PBS, pH=7.4). 20 µm thick wood sections which had been infected by bacteria were stained in the above solution for 1 hour in the dark. The sections were then dehydrated in a series of 75%, 95%, 100% ethanol (each for 2 minutes), and then mounted in immersion oil on glass slides after air drying, covered with cover slips and the edges were sealed with nail vanish. Additional wood sections were also mounted and similarly treated - some after glutaraldehyde treatment and some without any treatment. The slides were then examined using Leica TCS NT confocal microscope equipped with an Argon-Krypton laser excitation source (488/568/647 nm). Images were captured in green channel through a band pass filter BP 530/30 nm and in red channel through a long pass filter LP 590 nm using a 63x oil lens with numerical aperture setting of 1.4. The digital image stacks obtained on CLSM were transferred to a SGI Indy workstation and processed using Image Space™ software (Molecular Dynamics). Dual channel projection was obtained by combining images collected at two channels, and image slices were composed together using Volume Workbench.

It was difficult to clearly visualize bacteria in the wood sections which had not been treated either with glutaraldehyde or with NBD-PE. Glutaraldehyde treatment produced enough cell autofluorescence to differentiate bacteria from wood cell walls or other wood structures such as pit membranes (Fig 1). However, superior contrast between wood and bacteria was obtained when sections were examined after treatment with NBD-PE, which produces much greater fluorescence to bacteria (Fig 2).

The technique described here provides a new approach in fluorescent microscopy study of wood-inhabiting bacteria by probing phospholipids in their membranes. It will be useful for studying gram-negative bacteria with a characteristic feature, a double membranous envelope which contains abundant lipopolysaccharides. Wood decay bacteria were reported to be gram-negative (Singh and Butcher, 1991), so this technique will be particularly valuable for the study of wood decay bacteria. It should also facilitate our ongoing work on a large project aimed to enhance the penetration of protective coating into wood through bacterial modification of wood pit membranes.

Some of the wood-inhabiting bacteria may be gram-positive, and we are presently trying a procedure using a combination of NBD-PE and a fluorescent stain specific for gram-positive bacteria to allow us to visualize both gram-negative and gram-positive bacteria in wood and distinguish these two different types.

The following hints may be useful to those interested in using the technique described in this article here in their own work. Ethanol should be used to wash

off the excess dye from wood; washing time will vary with different samples and should be kept to a minimum.

A number of phospholipids labeled with different fluorescent head groups are available in the market (Haugland, 1996). Each of them have different labeling location in the lipid bilayer as well as specific optical properties. We intend to compare some of these in our future work.

Haugland R. P., 1996. Handbook of Fluorescent Probes and Research Chemicals (Sixth Edition). Molecular Probes, Inc., Eugene.

Singh A. P. and J. A. Butcher, 1991. Bacterial degradation of wood cell walls: A review of degradation patterns. J. Inst. Wood Sci. 12 (3), 143-157.

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Xiao S.Y., A.P. Singh and R.N. Wakeling. 1997: Detection of fungal hyphae in wood using a chitin selective fluorescent probe. Microscopy in Focus Newsletter for Microscopy New Zealand Inc. No.3).

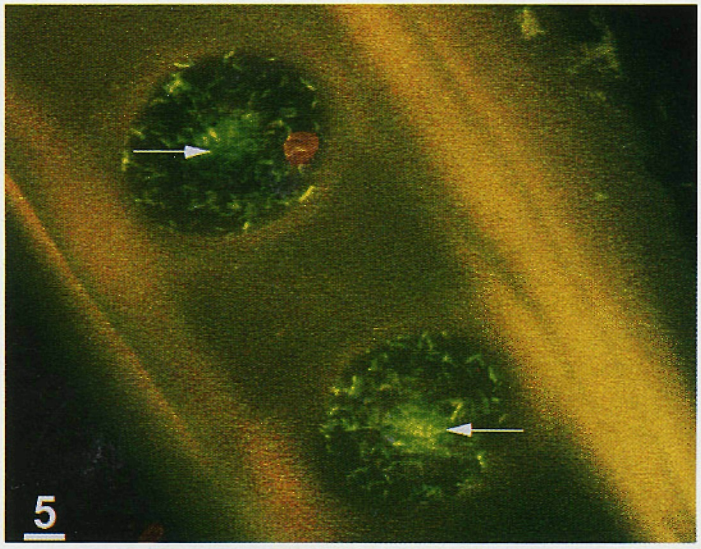

Fig 1: Glutaraldehyde treated sample showing bacteria colonising wood pit membranes. There are denser bacteria on torus (arrows). Bar = 5 µm.

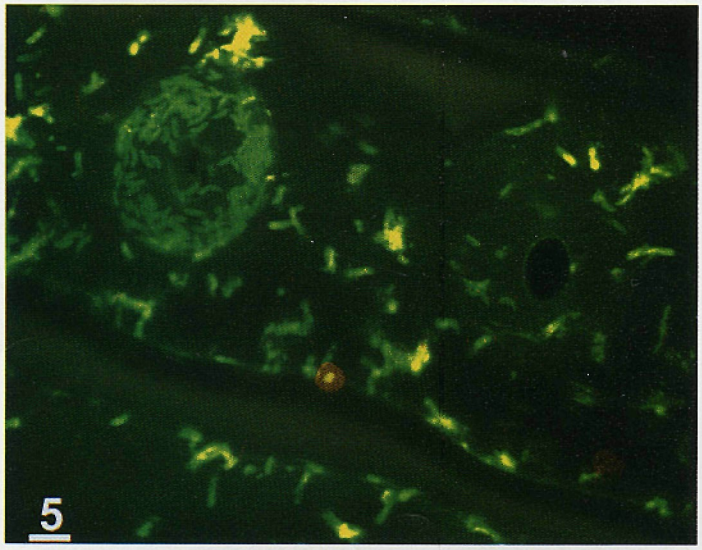

Fig 2: NBD-PE treated sample showing better differentiation between bacteria and wood than in Fig 1. Bar = 5 µm.