

A review of selected methods for the detection of degradative fungi

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Abstract

The primary decomposers of wood in non-aquatic environments are the brown and white rot fungi. These are basidiomycetous fungi which possess the appropriate enzymatic and non-enzymatic systems to allow them to degrade the wood lignocellulose. Many current methods for the detection of these organisms within the wood are unreliable or necessitate extensive destructive sampling. Our laboratory has focused on serological and molecular assays for the detection of degradative fungi. Polyclonal and monoclonal antibodies to specific fungal metabolites have been used with TEM immunolabelling and ELISA for the detection of decay agents. More recently we have developed a polymerase chain reaction assay which can allow us to reliably detect basidiomycete DNA in small wood samples. When used to examine fungi in wood, these primers are specific for basidiomycetes and do not react with non-degradative wood-inhabiting ascomycetes. Fungal colonization of wood can be detected early in the degradation process. Restriction digests can be used to confirm the identity of the amplified DNA. These assays are of potential utility for the detection and identification of wood degrading fungi. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Successful preservation of wood and wood artifacts may be limited by the attack and degradation of the wood by decay fungi. Decay is one of the most significant problems facing the wood products industry, limiting wood product utilization in adverse environments and costing millions annually for decay prevention and replacement of wooden structural elements (Zabel and Morrell, 1992). Detecting and monitoring decay in wood products ranging from bridges, to private homes and public buildings remains a problem, particularly where wood is in ground contact or exposed to moisture. Fungal decay is also a potential problem with works of art (Ciferri, 1999) and cultural and archeological artifacts made of wood (Blanchette et al., 1994). Optimal methods for the detection of decay fungi in wood have not, however, been developed. The development of a reliable assay for the early detection of decay in wood is needed. Techniques that have been used with varying degrees of success include culturing, chemical fluorescence staining, sonic analysis, NMR,

electrical resistance and serological methods (Goodell and Jellison, 1988). Ideally, methods for detecting incipient decay should be as non-destructive as possible. Our laboratory has been developing two such techniques to be briefly reviewed here, serological assays and DNA-based assays.

Immunodetection assays have been utilized since the mid 1980s for the identification of decay fungi in culture and the detection of decay fungi in wood (Goodell and Jellison, 1986, 1988; Jellison and Goodell, 1988, Clausen, 1997). Monoclonal antibodies to degradative fungi were first produced in 1986 (Jellison and Goodell, 1986) and polyclonal and monoclonal antibodies have subsequently been utilized in immunoTEM studies of fungal metabolites (Goodell et al., 1998; Daniel et al., 1991). Antibodies have also been used to examine the degree of homology among fungal cellobiohydrolases (Ishihara et al., 1993) and biochelators (Jellison et al., 1991). A review by Clausen (1997), of immunological detection of decay fungi summarizes the diversity of immunological techniques and their application to decay detection.

The development of the DNA-based polymerase chain reaction (PCR) (Mullis and Faloona, 1987) and taxon-specific primers (Gardes and Bruns, 1993; Taylor et al., 1993) is making it increasingly feasible to detect and

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study fungi in their natural substrates. The technique is used widely in medical and forensic work and allows very small amounts of DNA to be detected and identified. PCR of the internal transcribed spacer region (ITS1, 5.8s rDNA, ITS2) by the conserved universal primers ITS1 and ITS4, coupled with restriction digestion of the resulting product to generate restriction fragment length polymorphisms (RFLPs), has been used to detect and identify isolates of the dry rot fungus *Serpula* in the laboratory (Schmidt and Moreth, 1998) and to characterize tropical wood-decaying fungi (Zaremski et al., 1998) in a taxonomic study. Preliminary work in our laboratory (Jasalavich et al., 1998) has demonstrated the potential efficacy of using molecular techniques for the detection and identification of decay fungi.

2. Research results of serological assays

Serological methods have been used by our laboratory and others to allow decay fungi and/or fungal metabolites associated with the decay process to be detected and in some studies quantified and/or localized (Clausen, 1997; Jellison et al., 1991; Goodell and Jellison, 1988). Antibodies can be used in ELISA and other immunological assays and in immunoTEM studies (Daniel et al., 1991) both to detect and identify decay fungi in wood and to localize specific fungal metabolites within the degrading wood matrix (Goodell et al., 1997).

Serological work in our laboratory has included both polyclonal and monoclonal antibody studies. Most studies have focused on fungal enzymes or other metabolites produced by known degradative fungi such as the brown rot organisms *Postia placenta* and *Gloeophyllum trabeum*. Early work established the feasibility of using immunoassays such as the enzyme-linked immunosorbent assay (ELISA) to detect and quantify decay fungi in wood. Immunoassays such as ELISA were able to detect and quantify decay fungi in wood early in the degradative process. Assay sensitivity was, however, inhibited by wood extractives (Jellison and Goodell, 1989). Antibodies to degradative enzymes such as cellulase (Ishihara et al., 1993), Mn-peroxidase (Daniel et al., 1991) and laccase (Goodell et al., 1998) have also been used in basic studies aimed at enhancing our understanding of degradative mechanisms. For example, studies using an antibody made to purified cellobiohydrolase I from *Trichoderma viride* were used to confirm the presence of homologous sequences and structures in the enzymes of white rot fungi and their absence in enzymes of brown rot fungi (Ishihara et al., 1993). We have also used antibodies to study the role low molecular weight iron chelating compounds produced by the brown rot fungi may play in non-enzymatic degradation of wood. Antibodies prepared to TLC or HPLC purified biochelators from *G. trabeum* grown in culture have been shown to react with the

fungus in degrading wood in ELISA and have allowed us to visualize the chelators in situ using immuno TEM studies (Jellison et al., 1991; Goodell et al., 1997). Polyclonals to HPLC purified biochelators were visualized by immunolocalization around the fungal hyphae, penetrating into the cell wall of infected spruce wood early in the decay process. ELISA was used to detect degradation within the wood and to provide evidence that biochelators produced by the fungus in culture are also found in the more natural environment of the degrading wood (Goodell et al., 1997).

3. Research results of DNA-based assays

We have also developed a DNA-based assay to reliably detect brown rot and white rot fungi in wood at different stages of decay. Specific primers were selected to allow us to reliably amplify and analyze fungal DNA from culture. DNA extraction methods used were similar to those of Taylor et al. (1993) and Wilson (1987). The polymerase chain reaction (PCR) was used to selectively amplify DNA sequences of basidiomycetous fungi. DNA, isolated by a series of CTAB and organic extractions, was amplified by polymerase chain reaction using published universal primers (White et al., 1990) and basidiomycete-specific primers derived from ribosomal DNA sequences (Gardes and Bruns, 1993). Taxon-specific primers were developed for PCR detection of fungi by designing primers that correspond to sites where all strains within the fungal taxon have conserved DNA sequences and will amplify, while other organisms, including woody plants and other fungi, have differences that prevent amplification. Ribosomal DNA was used because it is particularly well suited to the development of taxon-specific primers due to interspersed regions of conserved and non-conserved sequence and a large copy number per genome. Primers used included both universal and basidiomycete-selective primers such as ITS4-B (Gardes and Bruns, 1993). This work differs from work previously done by using sets of primers which react only with fungal DNA and not with plant DNA. This gives us the potential to examine DNA both from fungal cultures and also directly extracted from the wood.

We have examined 7 species of brown rot fungi, 7 species of white rot fungi, 1 ectomycorrhizal basidiomycete, 1 non-wood decay basidiomycete, 25 species of wood-inhabiting ascomycetes (pathogens, endophytes, and saprophytes) and 2 species of non-wood-inhabiting ascomycetes. DNA was isolated from pure cultures of these fungi (Jasalavich et al., 2000). DNA was also isolated from spruce wood blocks colonized for 8 months by individual wood decay isolates or isolates of some of the wood-inhabiting ascomycetes (ASTM, 1994; Jasalavich et al., 2000). Wood blocks inoculated with species of brown rot fungi exhibited more decay as measured by percent

Table 1
PCR detection of decay fungi in spruce wood blocks sampled one and two weeks after inoculation^a

Fungus	Orientation	Week	%Wt. loss	PCR diagnosis
<i>P. placenta</i>	Radial	1	2.6	+++
<i>P. placenta</i>	Longitudinal	1	0.5	+-
<i>P. placenta</i>	Longitudinal	2	3.0	++-
<i>G. trabeum</i>	Radial	1	2.4	+++
<i>G. trabeum</i>	Longitudinal	1	0.4	+++
<i>G. trabeum</i>	Longitudinal	2	1.9	+++

^aThe percent weight loss is given as an average of three test blocks. The PCR results shown represent the three blocks assayed separately. Colonization of radial cuts generally proceeded more rapidly than that of longitudinal. PCR results were positive for all radial blocks incubated with either fungus after both 1 and 2 weeks. PCR results for longitudinal blocks were positive for all *G. trabeum* inoculated blocks after both 1 and 2 weeks and for one out of three of the *P. placenta* blocks at 1 week and two out of three blocks at 2 weeks.

weight loss (up to 69.6%) after 8 months than those inoculated with white rot fungi (up to 40.1%) or wood-inhabiting ascomycetes (up to 2.7%). The primer pair ITS1-F (specific for higher fungi) and ITS4 (universal primer) amplified the internal transcribed spacer region from both ascomycetes and basidiomycetes from both pure culture and inoculated spruce wood, as expected. The primer pair ITS1-F (specific for higher fungi) and ITS4-B (specific for basidiomycetes) were shown to reliably detect the presence of wood decay basidiomycetes in both pure culture and wood; however, ascomycetes were not detected by this primer pair. In a time series of experiments we were often able to detect the presence of decay fungi in wood by PCR before measurable weight loss had occurred to the wood. *Gloeophyllum trabeum* was detected in spruce blocks in all of six blocks sampled 1 week after inoculation (weight losses in the blocks ranged between 0 and 2.1%). *Postia placenta* was detected in four out of six blocks at 1 week after inoculation (weight losses in the blocks ranged from 0 to 3.2%) and in five out of six blocks samples at 2 weeks (all weight losses exceeded 2.2%) (Table 1).

Basidiomycete identification can be achieved by restriction digestion of the PCR product. The RFLP profile so generated was visualized by electrophoresis in agarose or Sepharide gel matrix (gibco BRL) gels. The internal transcribed spacer region, the product amplified by primers ITS1-F and ITS4-B, ranged in size from 850 to 1460 bp, depending on the basidiomycete species. RFLP profiles were generated from this amplified DNA by restriction digestion with AluI, HaeIII, or TaqI, and the double digest TaqI/HaeIII. The majority of RFLP profiles generated for any given enzyme were unique at the species level, with a few exceptions. Different isolates of a given basidiomycete species usually had identical RFLP profiles for a particular restriction endonuclease, but in a few cases, isolates of a species could be distinguished from each other at the subspecies level. TaqI RFLP profiles clearly separated the following basidiomycetous species from each

other at the species level: the brown rot fungi, *Coniophora puteana*, *Fomitopsis pinicola*, *Gloeophyllum trabeum*, *G. sepiarum*, *Leucogyrophana pinastri*, *Postia placenta*, *Serpula lacrimans*; the white rot fungi, *Irpex lacteus*, *Lentinula edodes*, *Resinicium bicolor*, *Trametes versicolor*, *Trichaptum abietinum*, the ectomycorrhizal fungus, *Pisolithus tinctorius*; and the soil-borne plant pathogen, *Rhizoctonia solani*. The white rot fungi *Phanerochaete chrysosporium* and *Scytinostroma galactinum* were separated at the subspecies level by their TaqI RFLP profiles, in that each isolate of each of these two species had a unique TaqI RFLP profile that was also different as compared to all of the other species tested. We could confirm that different isolates of *S. galactinum* were the same species or that different isolates of *P. chrysosporium* were the same species, because their AluI RFLP profiles and HaeIII profiles were species specific, i.e. different isolates of a species had the same profile for a particular restriction enzyme. Different isolates of *T. versicolor* could be distinguished at the subspecies level by their HaeIII RFLP profiles and at the species level by their AluI RFLP profile and TaqI RFLP profiles (Jasalavich et al., 2000). The combined data from the different restriction digestions made it possible to identify each basidiomycete species tested.

4. Conclusions

Immunological and basidiomycete-specific primer systems offer considerable potential for detecting fungi in wood before they cause substantial effects on wood properties. The ability of these techniques to detect incipient colonization provides the opportunity to arrest attack before damage occurs. These techniques would use only small samples of wood, thus allowing for limited destruction of the sample. Antibodies can be used for identifying, quantifying and localizing fungi or fungal metabolites during the decay process. In more recent work, a basidiomycete-specific primer system has been developed that detects both white and brown rot fungi in wood. The use of basidiomycete-specific primer systems to selectively and specifically detect white and brown rot fungi has great potential as a tool for studying the fungal colonization and degradation of wood and when used in conjunction with RFLP analysis of the amplified product is a potentially useful tool to aid in the identification of degradative fungal species directly from the colonized wood.

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