RESEARCH in Progress

Coalition for Advanced Wood Structures
a university, industry, government partnership

Identification of Fungal Contributors to Decay of Southern Yellow Pine Using Terminal Restriction Fragment Length Polymorphism (T-RFLP)

Testing standards for evaluating wood preservatives in the laboratory are partly based on their performance against a few fungal isolates. With few exceptions, the industry has relied on *Gleoephyllum trabeum, Trametes versicolor,* and *Serpula lacrymans* for evaluation of wood preservative treatments. Although these fungi are relatively easy to culture and maintain, they are no doubt an integral part of the decay process, and do contribute greatly to the durability of wood in service, other groups of fungi may be present in wood and contribute to decay. Culture-independent molecular methods (such as terminal restriction fragment length polymorphism (T-RFLP), denaturing gradient gel electrophoresis (DGGE), and single-strand conformation polymorphism (SSCP)) can give accurate estimates of community diversity and richness in environmental samples and can also be used to identify fungi to a species level. An increased understanding of fungal communities that are present in decaying wood would provide more insight to decay process and also identify key events in the process that might be prevented in order to slow the breakdown process. The resultant community profiles will also be used to compare different preservative systems as well as above- and below-ground exposures. The ultimate goal is to provide a more holistic approach to characterizing fungal species causing wood decay. An added benefit is the simultaneous generation of data that can be used to characterize environmental impact of preservative systems on indigenous fungal communities.

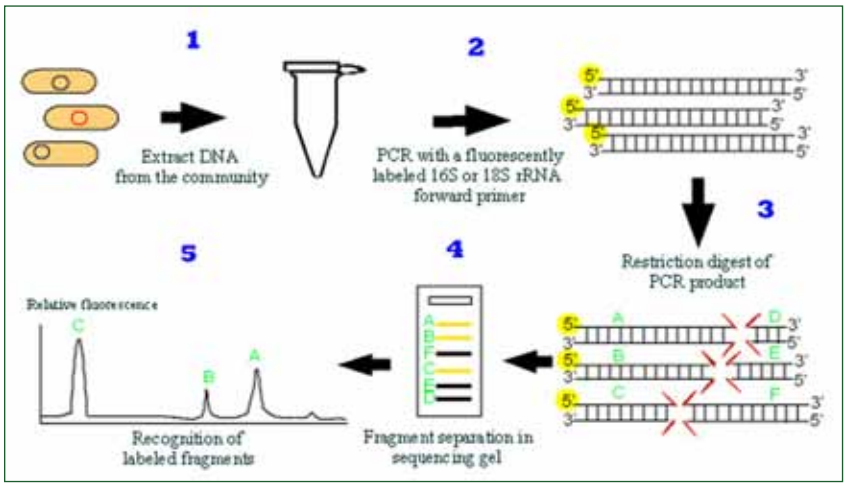

Figure 1. Stepwise explanation of the T-RFLP procedure. (V. Grüntzig et al 2002)

Background

T-RFLP analysis is a molecular tool that involves taking DNA from organisms contained in an environmental sample and amplifying targeted regions using fluorescently tagged primers (see Figure 1). The amplified DNA is digested using restriction enzymes that recognize specific nucleotide sequences and cut off the terminal ends that are flagged with the fluorescent tag. These fragments are separated on a capillary electrophoresis column. Each unique species contained in the sample is represented by a single peak in the T-RFLP output; the result is a community fingerprint that can be used to characterize changes in community composition (Figure 2) and to identify species using database matching against libraries of known fungi.

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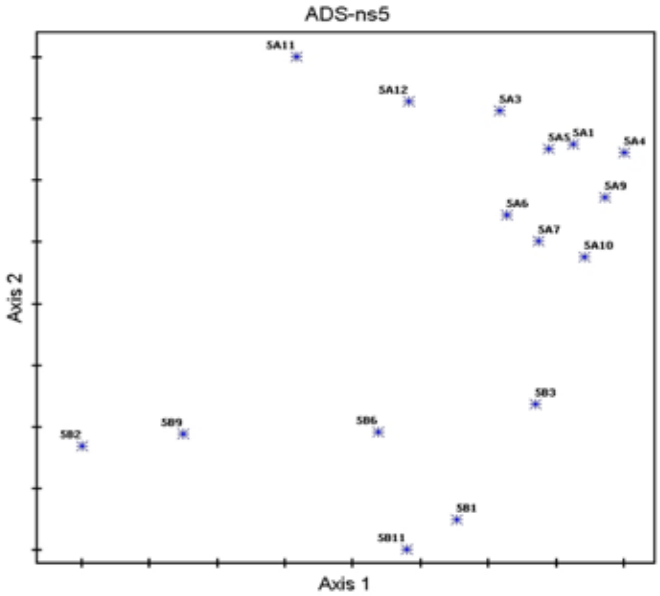

Figure 2. T-RFLP data can be used to determine differences in fungal species composition. Compared here are above- and below-ground fungal communities in an accelerated decay test (A denotes aboveground; B, below ground). The fungal DNA used in this experiment was extracted from southern yellow pine treated with 0.25% chlorothalonil. Points on this graph are spatially oriented based on their degree of species similarity.

Objectives

The objective of this study is to use T-RFLP to identify fungal species present in treated southern yellow pine during the decay process. Several industry standard treatments will be used and compared with untreated controls. Wood from both above- and below-ground exposure will be included in the analyses.

Approach

Existing terminal restriction fragment (TRF) matching software can take unknown samples and match them to TRF profiles of known fungal isolates. This approach is used routinely in analysis of vascular arbuscular mycorrhizal (VAM) fungi. This project will add wood decay fungi to those databases. The Forest Mycology Research Unit herbarium located at the Forest Products Laboratory and is one of the largest repositories for basidiomycetous fungi in the world. Their current inventory catalogues 178 isolates that occur on southern yellow pine. This group will serve as a starting point for our database. In the future, we may add additional fungal species and other wood species of interest, but for now we are focusing on southern yellow pine because of its dominance in the treated lumber market.

Expected Outcomes

Findings and method improvements will be published in the appropriate journals and presented at professional meetings (American Wood Protection Association (AWPA) and International Research Group on Wood Protection (IRG-WP)). The finished product will provide a faster way to confirm identification of fungal decay organisms at any stage of decay.

Timeline

Fungal profiles will be added to the library as they are created, with 40–50 of the most commonly occurring isolates catalogued by December 2011. At that point, we will begin to screen test blocks inoculated with known fungi to validate our procedure and conduct final tests to analyze unknowns from environmental samples. Final results of this project will be available by December 2013.

Cooperators

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