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Section 1: Biology

Towards Understanding the Biology of Wood Decay

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ABSTRACT

Our previous research has focused primarily on ways to identify the wood decay fungi and microbial community. We continue to explore this complex and dynamic community and its interactions through microbial community ecology studies, gene expression interactions and proteomics. However, in order to better understand the mechanisms of fungal decay, we have sequenced the genome of a copper tolerant brown rot fungus, *Antrodia radiculosa*. To advance our goals, we will be using structural and comparative genomics to identify novel genes and functional genomics and transcriptomics to systematically discover what genes are activated during wood decay under different environmental conditions.

Keywords: microbial community analysis, genomic sequencing, gene expression

INTRODUCTION

The decomposition of wood occurs every day under our feet, yet our understanding of the fungal community responsible for wood decay and the processes these organisms use to break down wood is incomplete. The structure and composition of wood cell walls provide a natural barrier to decomposition. The degradation of wood is a result of a complex interaction involving such variables as environmental conditions, the diversity of microorganisms that colonize wood, the types of wood decay fungi present, and the chemical composition of the wood itself. Wood decomposition is a critical step in carbon cycling within all global ecosystems. Wood and other plant biomass are currently the only worldwide sustainable sources of fuel and materials. Additionally, wood is the only renewable, recyclable, and sustainable building material, and understanding how to control or prevent the decay of wood products will reduce the tremendous costs associated with replacement and repair of damaged wood. The long term objective of our research is to unravel the complexities and better understand the process and regulation of fungal decay of wood.

TOWARD UNDERSTANDING MICROBIAL COMMUNITIES

Microbial Community Ecology Studies

Microbial community ecology studies can help unravel the contributions and interactions of the various bacteria and fungi that colonize wood, and how environmental changes alter this community and its functions. Our first community study assessed the effects of chlorothalonil (CTN) and/or butylated hydroxytoluene (BHT) and ACQ on the fungal and bacterial community on southern yellow pine (SYP) at two field sites using T-RFLP analysis. Terminal restriction fragment length polymorphism (T-RFLP) analysis uses fluorescently labeled primers, with the

terminal fragment of each species detected using a capillary detection system. The result is a DNA fingerprint of each species of a specified taxonomic group found within a sample. Results from this study indicated that the presence of wood preservatives: (1) increased initial colonization by bacteria that decreased over time (2) slowed the initial colonization of field stakes by fungi resulting in lower richness and diversity that increased over time, and (3) increased richness and diversity of basidiomycetes. Preservative treatment changed the community composition of bacteria, fungi, and basidiomycetes, which became more similar over time to untreated controls for fungi and basidiomycetes, but not bacteria (Kirker 2008).

A current study is evaluating the changes in bacteria, total fungi and basidiomycetes on 9 species of wood when exposed above ground at two sites over a 5-year period. The species being evaluated are the naturally durable or invasive species Honey Mesquite, Alaskan Yellow Cedar, Black Locust, *Pawlownia tomentosa*, Western Juniper, Catalpa, Eastern red cedar, Western Red Cedar, and Southern Yellow Pine. Another current study is comparing the basidiomycete populations on a variety of treated wood in ground contact over the course of a year. These studies are being done in conjunction with Carol Clausen, Rick Green and Grant Kirker of the US Forest Service Forest Products Laboratory in Madison, WI and Dr. Tor Schultz of the Department of Forest Products, Mississippi State University.

Effects of Microbial Interactions on Gene Expression during Wood Decay

qReal-time PCR was used to assess the effects of interspecific microbial interactions on the expression of genes associated with lignolytic enzymes. Lignin peroxidase, manganese peroxidase and alcohol oxidase were actively expressed in colonized wood under optimal conditions. Expression levels were quantitated in one-, two- and multiple-organism interaction tests with the basidiomycetes *Trametes elegans*, *Phanerochaete chrysosporium*, *Gloeophyllum sepiarium* and *Gloeophyllum trabeum.* Differential expression was detected in three genes in the two-organism interaction tests. Soil microflora strongly inhibited the growth the fungi and inhibited the expression of manganese peroxidase I in *T. elegans*. The *T. elegans* strain used in this study was isolated from the same site as the soil, potentially explaining why *T. elegans* alone survived to colonize the test wafers in the presence of unsterilized soil. The lack of manganese peroxidase production by this fungus in the presence of the soil microflora from which it was isolated illustrates the complexity of transcriptional patterns in environmental microbial communities (Mangum 2009). Another study was recently completed in which gene expression of *Phelbia radiata* was measured on three wood types over 15 months in soil contact. This work was done in collaboration with Young-Min Kang and Dr. Lynn Prewitt of the Department of Forest Products, Mississippi State University.

TOWARD UNDERSTANDING WOOD DECAY

Whole genome sequencing is extremely valuable as it allows novel genes to be discovered in a rapid and systematic manner and provides insight into how gene products function and interact within a cell. After a genome is sequenced, thousands of genes can be identified and their functional roles predicted. Currently, wood decay basidiomycete genes often have only a 50-70% match to the same genes from non-basidiomycete fungi. Thus advances in wood decay research are seriously hindered by the lack of annotations that describe gene function, gene product localization in the cell, and gene product role in cellular processes.

Functional Genomics of Copper Tolerance

Copper-based preservatives remain the primary biocide used to protect wood from ground-contact exposure. Certain brown rot fungi are tolerant of copper-based preservatives, and can decay copper-treated wood. The mechanism of copper tolerance is still unknown. To advance

our current objective we (J. Tang) have sequenced the genome of *Antrodia radiculosa*, an aggressive copper tolerant brown rot decay fungus. We used Illumina paired-end short read sequencing (76 nt, 300 bp insert size) to produce a de novo assembly with an estimated genome size of 30-33 Mbp and predicted 8000 and 5700 genes from contigs >= 20 kb for two max N50 assemblies. The approximate genome size for wood decay fungi is 30-33 Mbp with 10,000 to 15,000 genes. Details of this assembly can be found in an IRG paper from this meeting (Tang *et al*., 2010). This work demonstrates that gene prediction from short read sequencing data of fungi is technically feasible and represents a significant step towards accelerating a genome-wide understanding of how brown rot fungi decay wood and tolerate high levels of copper. This work is in collaboration with Shane Burgess at the Life Sciences and Biotechnology Institute at MSU and Tad Sonstegard at the USDA ARS Bovine Functional Genomics Laboratory, Beltsville, MD.

Transcriptomics and Gene Expression of Copper Tolerance

Sequenced and annotated genomes are essential for studies in functional genomics, and proteomics. We (J. Tang) are currently working on a transcriptomics study to identify genes that are differentially expressed by *A. radiculosa* when grown on wood in the presence/absence of copper. Transcriptomics quantifies all the expressed genes in the tissue at the time of extraction and provides sequence information for the expressed genes. Transcriptomics sequencing should lead to a better understanding of the biochemical pathways used by the fungus to protect itself from the toxic effects of copper. It should also help determine the genetic mechanisms underlying enzymatic and non-enzymatic brown rot decay, most of which are unknown. This work is in collaboration with Shane Burgess at the Life Sciences and Biotechnology Institute at MSU and Tad Sonstegard at the USDA ARS Bovine Functional Genomics Laboratory, Beltsville, MD.

When a fungus encounters wood treated with a copper-preservative, there is a lag phase before it begins to degrade the wood. The tolerance of fungal strains to copper-based preservatives varies with preservative formulation as well as with fungal species. Copper tolerance has been linked to increased production and accumulation of oxalate for some wood decay species (Clausen and Green 2003; Green and Clausen 2005). Another study just beginning, will compare isolates of *A. radiculosa* that exhibit differing levels of copper tolerance for their differential expression of genes involved in the production of oxalic acid (K. Jenkins). This work is in collaboration with Carol Clausen and Rick Green of the US Forest Service Forest Products Laboratory in Madison, WI.

Transcriptomics of Selective Delignification

Although quite a bit is known about some of the decay enzymes in select wood decay fungal species, less is known about regulation of these enzymes during the process of decay and what other metabolic events are occurring that are integral to the decay process. Why does a fungus 'choose' selective delignification over simultaneous decay or why does selective delignification target only lignin? To begin to understand these questions, a new study has just begun using transcriptomics to compare gene expression when a white rot fungus is undergoing selective decay compared to simultaneous decay (L. Parker). This work is in collaboration with Shane Burgess at the Life Sciences and Biotechnology Institute at MSU and Tad Sonstegard at the USDA ARS Bovine Functional Genomics Laboratory, Beltsville, MD.

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