Current Research on Wood Decay in the USDA Forest Service[1]

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Abstract: The Forest Service's research on decay fungi and decay caused by fungi is done mainly in two research work units at the Forest Products Laboratory. One unit, the Center for Forest Mycology Research, performs biosystematic research on root-rot and products-rot fungi in the genera Armillaria, Phellinus, and Phlebia and maintains the culture collection supporting this research and other research on decay fungi. A second unit, Biodeterioration Research, concentrates on studying the basic mechanism of wood decay.

Most of the Forest Service's work on wood decay is being done in two work units in Forest Insect and Disease Research (FIDR) housed at the Forest Products Laboratory (FPL) in Madison, Wis. Work on wood preservation and biotechnological applications of wood decay fungi are also in progress at the FPL in Forest Products and Harvesting Research units, but the research on the decay fungi and biodeterioration is located in the FIDR units, the Center for Forest Mycology Research (CFMR) and Biodeterioration Research.

CENTER FOR FOREST MYCOLOGY RESEARCH

The investigations of the Center for Forest Mycology Research (CFMR) (Research Work Unit FPL 4501) mainly address the biosystematics of wood decay fungi and the maintenance of the Forest Service's culture collection of wood-rotting fungi. The problems under investigation by the scientists include the biosystematics and species delimitation in the genus Armillaria (the root- and butt-rot fungus), the taxonomy of the genus Phellinus (which causes substantial root and heart rots of forest trees), and the biosystematics of the genus Phlebia, (which, although it occurs mainly as a slash-rotting organism, is also found in products). In addition, several members of the genus Phlebia are of possible use in biopulping or other biotechnological endeavors. The other problem area under investigation in the CFMR involves the resolution of species complexes that are found within the culture collection.

Biosystematics and Species Delimitation in Armillaria

The work on the genus Armillaria has involved several approaches. One of the main approaches is the use of a rather unique serological technique to distinguish species of Armillaria. Instead of being inoculated into mice, goats or rabbits, the antigen (Almillaria fungal tissue) is inoculated into both sides of the breast and both legs of laying hens. After a second inoculation a week later, and a wait of 2 weeks for incubation within the chicken, the antibodies are at sufficient titer and deposited in the yolk of the eggs. The eggs are collected, the antibodies isolated, and the ELISA technique used to distinguish species on the basis of optical density of the color reaction. Our results to date indicate that we can distinguish several of the species readily. We will be putting more species into the system to see how widely applicable this technique might be, because now the system shows a great deal of promise. Using laying hens for antibody development is advantageous because of the ease of inoculation, the ease of obtaining the antibody material through the collection of eggs, the rapid increase of titer, and the apparent greater specificity (that is, less cross reactivity) of the antibodies developed in the egg yolk vs. the blood of the other animals.

Other investigations on Armillaria are also in progress, including attempts to elucidate the nuclear cycle within the life cycle of the fungus. Attempts are also being made to develop a method for consistent fruiting of the various Armillaria species so that genetic material will be regularly available for research on the Armillaria species. Refinement of the mating compatibility system and development of methods for cultural identifications are both under study.

Taxonomy of Phellinus

Research on the genus Phellinus has resulted in a published synopsis of the species of the genus Phellinus in the world. It is complete with keys and descriptions—many taken from the literature, but otherwise not available. Work is also progressing on the delimitation of Phellinus species especially as it concerns the variability within species. Phellinus tremulae (Bond.) Bond. et Boriss. has been collected and isolates made from all across North America, and the variability within this species is being studied. The genetic compatibility within the species and other variations, both morphological and biological, are being examined. Other species complexes in this group are being examined as well.

Biosystematics of Phlebia

Research on the genus Phlebia is taking a number of different routes: morphological, genetic, and molecular-biological. The genus fruits well in culture so that single-spore isolates for incompatibility studies are easily available. Morphological studies are combined with these incompatibility studies

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and cultural studies to aid in distinguishing the species. In addition, the utility of restriction fragment length polymorphisms and gene sequencing is being appraised. This should give an idea about the reliability of this methodology/technology to the wood-rotting basidiomycetes and give support to the other types of taxonomic investigations that are proceeding.

To date, the Phlebia rufa complex has been examined and found to consist of two different species. This information was supported by morphological and genetic data as well. Other species of this group are now under investigation. The results will not only give us a better idea of the taxonomy and identity of the various Phlebia species, but will also indicate the relationships among genera which are closely related to Phlebia.

Resolution of Species Complexes

We also have a program that is aimed at resolving species complexes in the culture collection. In cases in which we have numerous isolates of the same species and it is obvious that there are some misidentifications, these misidentifications are being resolved by finding the isolates that are similar in culture and then going to the fruiting body and finding the differences there as well. Two of the genera under investigation are Bjerkandera and Phellinus. A number of species that were previously put into genus Poria are also under investigation.

BIODETERIORATION AND PRESERVATION OF WOOD

Research Work Unit FPL 4551, Biodeterioration of Wood, is determining basic mechanisms involved in the decay process. The purpose of this work is to find the "Achilles heel" in the decay process that could guide the development of new or improved treatment. One approach is to use serological techniques including monoclonal antibody methodology to determine how the enzymes get into the wood. These studies use monoclonal antibodies to locate and characterize the enzymes responsible for wood decay. The presence of the enzymes in the sheath surrounding the hyphae and the delivery system transporting the enzymes into the wood are being studied. In addition, a technique for visualizing the delivery system of the enzymes into the wood substrates has been developed using the Scanning Electron Microscope. Results of this work indicate that the fungus does develop a sheath as has been suggested in other work. This sheath forms microfibrils and microfibrillar materials that penetrate the woody tissues and deliver the enzymes deep into the wood. The concept of the enzymes merely being distributed at random by diffusion is now regarded as improbable. This work is still in progress but may well provide information that will explain exactly how the enzymes are delivered to the woody substrate.

Considerable progress has been made in understanding the nature of the cellulose-degrading agent produced by brown-rot fungi. The physical and chemical properties of brown-rotted cellulose have been elucidated, and electron spin resonance (ESR) has been used to identify formation of hydroxyl radical and paramagnetic metals. These results demonstrate that brown-rot fungi depolymerize cellulose by oxidative cleavage. Because of the rapidity of the degradation, oxidative cleavage most likely takes place by formation of cellulose-metal ion complexes and reaction with oxygen radicals. Understanding the biochemistry of this system should provide tremendous opportunities to thwart decomposition of wood.

Other work is aimed at the use of innocuous fungi as biocontrol agents against mold, stain and decay fungi. Species of Trichoderma and Scytalidium are the most promising. Methods of applying the control agent and the most effective species and strains to use are still being evaluated. Field tests to date have provided mixed results.

Determining in a nondestructive manner that decay is present at an early stage involves the use of electron spin resonance. This technique is based on the idea that as decay progresses the element magnesium changes states. By using ESR the change in valence of the magnesium can be sensed, and the amount of decay can be determined at early stages. This work is still in progress.

From the standpoint of control, the regulation of key enzymes in digestion and growth is of interest. Important digestive enzymes are those decomposing cellulose, hemicellulose, and lignin. For growth, enzymes of importance are those involved in nitrogen metabolism, such as glutamine synthetase and polyamine enzymes, and those involved in cell wall formation, such as chitin synthetase.

Almost nothing is known about nitrogen metabolism in wood decay fungi, but it plays the most important role of the nonstructural nutrients in wood. The very low amounts of nitrogen in wood indicate that wood decay fungi have a very efficient mechanism for metabolizing and reusing nitrogen.

Polyamines are nitrogen compounds found in fungi that are essential for growth and development. The biosynthesis of polyamines in most organisms can proceed through one of two metabolic pathways. However, only one pathway has been reported to be the route of synthesis in fungi. This feature makes the pathway an ideal target for specific regulation of polyamine production in wood decay fungi. The result would be the specific control of decay by affecting only fungal growth and development. We are using "suicide" inhibitors such as difluoromethylornithine (DFMO) in the study of polyamine transport systems and determining reaction steps in metabolic pathways. The inhibitors are enzyme specific and irreversible, acting at the catalytic site of the enzyme, which results in "suicide." These inhibitors are ideal candidates for studies to target the "Achilles heel" of the decay fungi.

Another approach that might be utilized for specific inhibition of fungal growth and development is the use of chemicals that prevent synthesis of chitin, which is an essential component of fungal cell walls. Vertebrates do not possess chitinous tissue. This basic biological difference has stimulated interest in the development of pesticides that specifically inhibit chitin synthesis and have little or no effect on non-target organisms. We are presently studying inhibition of chitin synthetase from various fungi with chitin synthetase inhibitors.

To digest the cell wall constituents of wood, enzymes must be released external to the fungus. Thus, one method of preventing fungal attack of wood would be to inactivate the extracellular degrading enzymes in situ or prevent their release from fungal hyphae. Extracellular enzymes, however, appear quite stable—higher concentrations of chemical are often required to inactivate the enzyme than to prevent growth. A more promising approach, therefore, would be to find means to interfere with processes involved in enzyme secretion.

Tunicamyacin and calmodulin antagonists are compounds that we found to inhibit secretion of carbohydrate-degrading enzymes by wood decay fungi. Tunicamyacin interferes with glycosylation or sugar attachment to enzymes, and calmodulin antagonists interfere with calcium transport, which appears to be a part of the secretory process. We do not propose to use these materials as wood preservatives, but they may serve as model systems in the continuing search for more environmentally compatible fungicides.

Monitoring of the decay tests in both the deep South and other areas of the country is continuing, to learn about the effectiveness of various preservative treatments in preventing decay and stopping internal decay.

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