Biodegradative Oxidant Production by Fungi in Lignocellulose: In Situ Mapping and Gene Expression

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Project Goals: Naturally occurring, biological mechanisms for lignocellulose deconstruction are of interest because they may inspire the development of environmentally friendly technologies for conversion of lignocellulose into useful fuels and chemicals. The only organisms that degrade lignocellulose efficiently are certain filamentous lignin-degrading fungi, especially white rot basidiomycetes such as *Phanerochaete chrysosporium*, whose genome was recently sequenced. It is known that lignin degradation is oxidative, but the specific mechanisms remain obscure–numerous enzymes and cofactors have been proposed to have a ligninolytic role. To address this problem, it would be useful to visualize fungal ligninolytic oxidants on biodegrading lignocellulose, to map their position relative to the fungus, and to correlate their production with the expression of fungal genes.

Abstract Text: We have developed micrometer-scale silica beads that carry an oxidant-sensitive BODIPY dye, which fluoresces red in its native reduced state but green when oxidized. The beads were placed on thin sections of wood undergoing early decay by *P. chrysosporium* and the specimens were analyzed periodically by fluorescence microscopy. Concurrently, specimens were harvested for RNA extraction and transcript analysis by quantitative RT-PCR, using primers designed to amplify sequences encoded in the genome that have a proposed ligninolytic role. Three major findings have emerged from our study thus far: **(1)** In the first few days of biodegradation, there were marked gradients of oxidation around the fungal hyphae, i.e., beads close to the fungus were more oxidized than were more distant beads. This result is inconsistent with a ligninolytic mechanism that depends on small, freely diffusing oxidants. Instead, it indicates that enzymes associated with the fungal hyphae have a major oxidative role during the initial stage of biodegradation. **(2)** Bead oxidation was not continuous, but rather commenced abruptly several days after fungal inoculation onto the wood. This result indicates a rapid transition from nonligninolytic to ligninolytic metabolism by the fungus. **(3)** Concurrent with this oxidative burst, several fungal genes were highly up-regulated. Chief among these were several peroxidases that have been shown to cleave lignin in vitro. By contrast, a cellobiose dehydrogenase and two glycopeptides that have been proposed to produce ligninolytic hydroxyl radicals were not up-regulated. These results support a mechanism for incipient ligninolysis in which fungal peroxidases react directly with lignin to cleave it.

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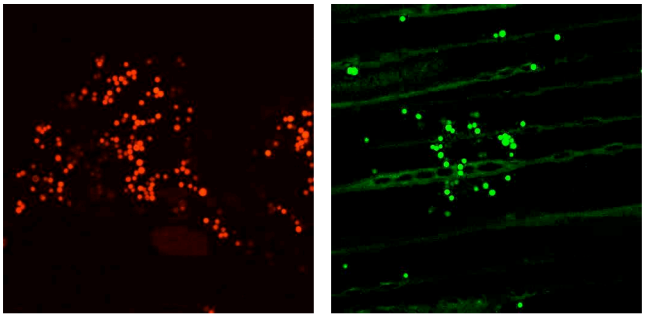

Fluorescence images of beads on spruce wood undergoing decay by *P. chrysosporium* at 40 h (left) and 96 h (right) after inoculation.