EXTRACELLULAR DEGRADATION OF POLYETHERS BY THE BROWN ROT FUNGUS GLOEOPHYLLUM *TRABEUM*

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ABSTRACT

Brown rot fungi are uniquely able, to decay wood by selectively degrading its cellulose. Their ability to depolymerize crystalline cellulose and to penetrate the recalcitrant lignin barrier in wood suggests the involvement of low molecular weight diffusible agents, for example iron-binding compounds that might carry out one-electron oxidation reactions. We found that cultures of the brown rot fungus *Gloeophyllum trabeum* efficiently degraded U-[^{14}C]cellulose, synthetic methoxyl-[^{14}C]lignin and l-[^{14}C]L-glucose to $^{14}CO_2$. The fungus also depolymerized and mineralized poly(ethyleneglycol) (PEG), a biologically recalcitrant polymer. The degradation of polyethers by *G. trabeum* probably reflects the operation of nonspecific extracellular oxidants. These oxidants could contribute to the depolymerization of crystalline cellulose in the fungal natural habitat.

INTRODUCTION

Biodegradation of lignocellulose by brown rot fungi is of interest both because of the need for decay prevention in wood and because of the potential utility of degradative processes in biotechnological applications. Brown rot fungi have the unusual ability to penetrate the recalcitrant lignin barrier in wood and remove the cellulose selectively (1). The initial steps of brown rot are probably not enzymatic because immunoelectron microscopic studies have shown that a wide variety of enzymes are incapable of penetrating the wood cell wall during early stages of decay (2-4). Some researchers have attempted to explain these findings by suggesting that cellulose degradation during brown rot is hydrolytic, and that it results either from the action of unusually small cellulases or from a gradual acidification of the wood during fungal colonization (5, 6). However, most researchers agree that brown rot is primarily oxidative. This proscess could occur via the action of diffusible oxyradicals or iron-oxo complexes that initiate free radical fragmentation reactions (5).

For example, brown rot fungi might use ferrous iron and hydrogen peroxide (Fenton's reagent) to produce hydroxyl radicals: $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^- + HO^{\cdot}$ (7). Hydroxyl radicals are known to degrade cellulose, and extensively brown-rotted wood resembles wood which has been treated with Fenton's reagent (8-10). However, the actual mechanism for brown rot remains unknown. Here we present new data on the biodegradation mechanisms of the brown rot fungus *Gloeophyllum trabeum.*

RESULTS AND DISCUSSION

Mineralization of Cellulose, Lignin and L-Glucose by G. trabeum

In preliminary work, we found that the brown rot fungus *G. trabeum* is capable of mineralizing both U-[^{14}C]cellulose and synthetic methoxyl-[^{14}C]lignin in 5 ml stationary liquid cultures (Fig. 1). These results agree with the biodegradative capabilities of *G. trabeum* on its natural substrate, and therefore show for the first time that brown rot can be studied in defined medium cultures of this fungus. The level of nutrient nitrogen was found to have no significiant effect on the mineralization of either polymer.

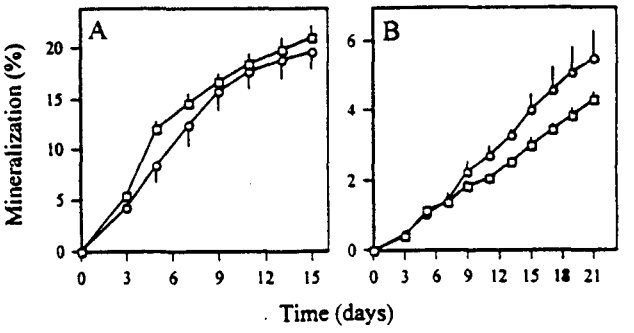

Fig. 1. Mineralization of U-[^{14}C]cellulose (A) and synthetic methoxyl-[^{14}C]lignin (B) by *G. trabeum in* 5-ml stationary liquid cultures, which were grown in medium (11) that contained 20 mM nitrogen(□) or 2 mM nitrogen (o). Error bars indicate one standard deviation.

The ability of *G. trabeum* to demethylate lignin suggested the existence of a nonspecific oxidative extracellular system in this fungus. To address this hypothesis further. the degradation of 1-[^{14}C]-L-glucose, which is not metabolized by most organisms, was studied. Mineralization of the L-glucose by *G. trabeum* reached 25-35% in 2 weeks. By contrast the white rot fungus *Phaneroehaete chrysosprium* mineralized only 4% of the initially added L-glucose in this time. These results support the idea that brown rot fungi produce nonspecific oxidants that are lacking (or produced at much lower levels) in white rot fungi.

Depolymerization and Mineralization of Poly(ethylegycol) (PEG) by *G. trabeum*

Both lignin and cellulose degradation must begin with steps that take place in the extracellular growth medium of *G. trabeum.* However, neither of these substrates lends itself to the detection of novel extracellular oxidants in fungal cultures, because lignin is structurally so complex that product analyses are difficult and cellulose is depolymerized by the cellulases that many fungi produce. Therefore, we decided to continue the study with an anthpogenic polymer, PEG ($HO\text{-}CH_2\text{-}CH_2\text{-}O\text{-}(CH_2\text{-}CH_2\text{-}O)_n\text{-}CH_2\text{-}CH_2\text{-}OH$). PEG has a simple repeating structure, cannot enter cell membranes, and resists attack by most organisms. The average molecular weight of the PEG used in these experiments was 4000, and the polymer was labeled with ^{14}C in its terminal hydroxyethyl moieties.

The results showed that *G. trabeum* mineralized PEG efficiently. In attempts to scale up to larger culture volumes, we found that the fungus was inordinately sensitive to agitation, but this problem was successfully addressed by supporting the fungus on a solid substrate, perlite (Fig. 2).

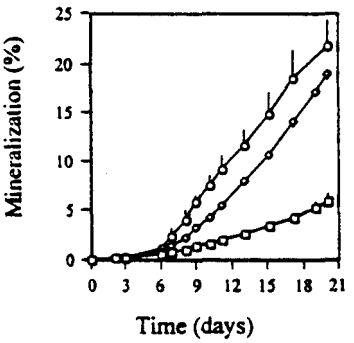

Fig. 2. Mineralization of [^{14}C]PEG (3 µg/ml) by *G. trabeum* in 5-ml stationary cultures (o), 15-ml stationary cultures (□), or 15-ml perlite-supported cultures (◇). Error bars indicate one standard deviation.

In mineralization experiments, the *G. trabeum* degradative system showed no sign of saturation over the concentration range 0.3-300 µg/ml (Fig. 3). The same results were obtained in gel permeation chromatography experiments, which showed that the fungus gradually shifted the molecular weight distribution of the PEG to lower values. Preliminary NMR spectrometric results suggest that *G. trabeum* cleaves PEG at random within the polymer chain and that end groups characteristic of hydroxyl radical attack are produced during cleavage.

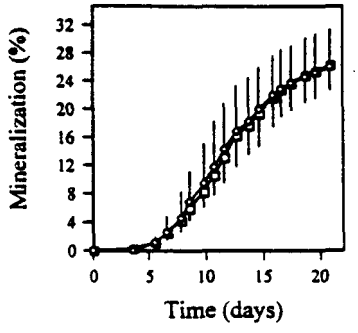

Fig. 3. Mineralization of [^{14}C]PEG by *G. trabeum* in 15-ml perlite-supported cultures. The initial PEG concentration was 0.3 µg/ml (◇) or 300 µg/ml (□). Error bars indicate one standard deviation.

SUMMARY

These reactions arc biologically unusual because other eukazyotes are unable to degrade PEG. The degdation appears to be oxidative rather than hydrolytic, because alkyl ethers are chemically very stable and can be hydrolyzed only under stringently acidic conditions that are unlikely to occur physiolosically. A few specialized bacteria are known to attack PEG, but they use enzymes to depolymerize it endwise and frequently display a high degree of selectivity for PEGs that fall within a limited molecular weight range (12).

The production of hydroxyl radicals by brown rot fungi has been suggested previously by other research groups (8, 13-15). Low molecular weight compounds with metal chelating capability have been found to be produced by several of these organisms, and it has been suggested that *G. trabeum* produces an iron chelator that might function to reduce Fe^{3+} to Fe^{2+}, a key reagent in Fenton chemistry (15).

A nonspecific, extracellular, oxidative system of this type could play a major role in biodegradation by *G. trabeum.* The free radical fragmentation reactions of alkyl ethers such as PEG and of acetals such as cellulose have many features in common (16), and nonspecific one-electron oxidants have long been known to degrade both of these polymers (5, 16, 17). We propose that the degradation of PEG by *G. trabeum* reflects constitutive operation of a brown-rot cellulolytic system that is oxidative rather than hydrolytic.

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