Journal of the Arkansas Academy of Science

Volume 48 Article 15

1994

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Abstract

Though enzymes responsible for rotting wood have been studied for some time, the enzymes and enzymatic systems responsible for breaking down lignin have only begun to be discovered. The lignin-degrading enzymes produced by soft-rot fungi, in particular, have not been sufficiently studied. The present study presents evidence that the enzyme called laccase, known to be associated with lignin biodegradation, is produced by the species *Chaetomium elatum*, a soft-rot fungus. *Cerrena unicolor*, a positive control, and *Chaetomium elatum* were grown in culture. These species were tested for the presence of laccase using syringaldazine as a chromogenic substrate. As expected, *Cerrena unicolor* showed laccase production after two weeks of growth indicating the experimental procedures were working. After three weeks, *Chaetomium elatum* showed laccase production.

Introduction

Lignin is produced in large quantities as a byproduct of pulp production and currently has very little economic value. An understanding of lignin biodegradation may have a positive effect on several industries.

Several studies have illustrated the benefit of using fungal decay in the processing of wood for pulp manufacturing. Paszcynski et al. (1988) demonstrated how wood chips and pulps can be delignified by natural methods. Eriksson and Vallander (1980) showed that pretreating wood chips with wood-rotting fungi saves 30% in the energy demand for processing these wood chips into pulp.

A better understanding of the enzymatic mechanisms required for lignin biodegradation could lead to the ability to use waste lignin for the production of useful chemicals. For instance, S.L. Rosenberg and C.R. Wilke (1980) investigated the possibility of cheaply producing ethyl alcohol from cellulose by removing lignin via wood-rotting fungi. In particular, soft-rot fungi such as *Chaetomium* sp. may offer suitable strains for utilization in industry (Kirk et al., 1980).

Understanding lignin biodegradation is also important environmentally. For over a century, large amounts of lignin-related compounds have entered the environment from pulp production (Salkinoja-Salonen and Sundman, 1980). Understanding the biodegradability of these compounds is essential to understanding the impact of these compounds on the environment and how to cure any problems that occur.

One way of understanding lignin biodegradation is by studying the enzymes involved. Investigations of the enzymes of fungi are helpful in determining what fungi have and what they lack in wood-rotting capacity. Studying the enzymology of fungi proves in some ways to be more helpful in understanding lignin-degrading capacities of fungi than by observing fungi in their wild habitat.

Observation of fungi in their wild habitat gives limited evidence of their ability to degrade lignin. A given species may degrade a part of wood so slowly or incompletely that its degradation may not readily be observed in nature. A more-capable species, rotting wood at a faster rate, will take the place of a less-capable one before the slow-acting activity of the less-capable species takes place. In this way the slower-acting wood-rotting capacities of a given species are almost undoubtedly hidden from the observer (Garrett, 1963).

One of the first enzymes associated with lignin-biodegradation to be isolated was called laccase. Numerous researchers have suggested that laccase is necessary for complete lignin breakdown (Harkin et al., 1974; Szlarz et al., 1989), but the role of laccase in the long process of lignin metabolism is still not completely understood. It seems that laccase plays an indirect but necessary role, as a phenol-oxidizing enzyme, but that it does not structurally change lignin to a large degree (Kirk 1983; Kirk, 1984).

Recently, the enzymatic functions of laccase produced by *Coriolus versicolor* on polymeric lignin were determined by Iimura et al. (1991). Using ^{13}C and ^{14}C labeled synthetic high-molecular-mass lignin and ^{13}C-NMR spectroscopy, they found that laccase can degrade the framework of lignin and make lignin water soluable.

Since laccase is necessary for lignin biodegradation, it has been looked for primarily in fungi known to be lignin degraders. These fungi are called white-rot fungi because they degrade the cellulose, hemicelluloses, and lignin of wood evenly and equally, leaving the wood a light color during decay. Most other wood-rotting fungi are referred to as "brown-rot fungi" because they only metabolize cel-

lulose and hemicelluloses. Lignin is left behind in brown bands resulting in an erratic decay pattern in wood. These fungi attack some cells extensively while leaving others unharmed. They seem to have the necessary chemistry for the rudimentary processes of lignin degradation (Kirk, 1983). Since the brown-rot fungi are blocked by lignin, the decay of wood from these fungi is generally not as great as the decay due to white-rots (Deacon 1984).

The third and last type of wood-rot fungi is called soft-rot fungi. They are most commonly seen on wood that is in contact with water (Deacon, 1984). Soft-rots have not been studied as extensively as the other two types, and the enzymes necessary for lignin biodegradation by soft-rot fungi are not known (Raven et al., 1992).

Although the lignin-degrading enzymes used by soft-rot fungi have not been studied adequately, the extent to which soft-rot fungi can degrade lignin has been studied. A comparison of the weight losses resulting from attack by each wood-rotting type shows that soft-rot fungi do not degrade lignin nearly as quickly as white-rto fungi, but faster and to a larger degree than brown-rto fungi (Kirk, 1983). As one can see in the succession of fungi on trees or soil, soft-rot fungi prefer carbohydrates to lignin, degrading them much faster (Ander and Eriksson, 1978).

Eslyn et al. (1974) determined that the ability to degrade various parts of the wood varies greatly with each species of soft-rot fungi. An examination of the wood-rotting activities of just six soft-rot fungi indicated a range of 2 to 20% lignin depletion. The amounts of cellulose and hemicellulose depleted varied even more. With such varying degrees of wood-rotting capabilities, it could be assumed that the enzymology varies considerably as well.

The enzyme laccase is found in all white-rot fungi, but it has not been found in all soft-rot fungi. The goal of this research is to find laccase producing fungi among soft-rot species. Species belonging to the genus *Chaetomium* were tested for the ability to produce laccase. *Chaetomium* is a rather large genus containing more than eighty species (Ames, 1963), all of which are considered to be true soft-rot fungi. Three species found to grow on wood were chosen for study.

Materials and Methods

Chaetomium and *Cerrena* species were obtained from The American Type Culture Collection (Rockville, MD). Cultures were kept on 5% malt agar slants at about 20°C.

To test for laccase, cultures were transferred to a liquid medium using the methods described by Lindeberg and Fahraeus (1952). The medium contained in one l of H_2O: 1.8 g $NaNO_3$, 5 g glucose, 0.5 g $MgSO_4*7H_2O$, 0.47 g KH_2PO_4, 0.48 g Na_2HPO_4, 0.05 g $Ca(NO_3)_2*4H_2O$, 8.5 mg $Mn(CH_3COOO)_2$, 3.2 mg $FeC1_3*6H_2O$, 2 mg $Zn(NO_3)_2$, 2.5 mg $CuSO_4$, 0.05 mg thiamine, pH 5.6.

The fungi were grown in 250 ml Erlenmeyer flasks containing 50 ml of medium at 23° C for two weeks in a static culture. Eight cultures were grown for each species. Each of the cultures were tested for laccase activity at one week intervals for two months. *Cerrena unicolor* was used as a positive control to check testing procedures. A study by Harkin et al., (1974) confirmed that *Cerrena unicolor* shows signs of laccase activity.

Laccase activity was detected using syringaldazine as a chromogenic substrate. The reaction mixture contained in 1 ml: 0.4 Mellvaine buffer (0.2 M phosphate-0.1 M citrate), pH 6.5, 0.1 ml 1 mM syringaldazine (in an ethanol solution), and the medium sample. After the broth and mycelia have been mixed with syringaldazine, a pink color appears in the broth and mycelia when laccase activity is present (Szlarz et al., 1989). The reaction mixture was watched for two minutes.

Results

Species	Result	Growth	Reaction Time
Cerrena unicolor	positive	2 weeks	instant
Chaetomium elatum	positive	3 weeks	15 s
Chaetomium olivaceum	negative		
Chaetomium succineum	negative		

"Growth" refers to the length of time the species grew in the medium before a positive result was detected. "Reaction time" is the length of time the broth took to turn pink after it was added to the syringaldazine mixture.

Discussion

Harkin et al. (1974) tested over one hundred wood-rotting species and found that syringaldazine can be used for an indicator of the production of laccase. Before Harkin's discovery, tincture of guaiac was used as a test for laccase (Nobles, 1958). All species known to produce laccase tested positive to the Harkin test, and those known to lack the ability to produce laccase tested negative. Since their discovery, syringaldazine has repeatedly been used for detection of all types of laccases, even those produced by plants (Bao et al., 1993). Syringaldazine is thought, therefore, to be an accurate test for the presence of laccase.

It was determined that *C. elatum* is a laccase producing fungus. It is not certain that *C. elatum* degrades lignin simply because it produces laccase, an enzyme associated with

lignin biodegradation. Further research must be done to make the conclusion that it has lignin degrading abilities. The fact that the species is a laccase producer does make it a good candidate species for lignin biodegradation.

If it is a lignin-degrading fungus, it should be tested for evidence of activity of other enzymes associated with lignin degradation so that lignin degradation among soft-rot fungi can be better understood.

It has been noted that soft-rot fungi such as *Chaetomium* sp., which degrade wood differently than brown-rot or white-rot fungi, may offer suitable strains for utilization in industry, but too little is known of these species to say with certainty how useful they may be (Kirk et al., 1980). To determine the usefulness of *Chaetomium elatum* the extent to which it degrades other parts of wood and the ease with which its degrading ability can be manipulated should be tested.

Acknowledgements

The first author acknowledges financial support from the UALR Donaghey Scholars Program, The UALR Department of Physics and Astronomy and from the U.S. Department of Energy.

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