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DEGRADATION OF LIGNIN AND DECOLORIZATION OF PAPER MILL BLEACH PLANT EFFLUENT (BPE) BY MARINE FUNGI

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Very little is known about BPE decolorization or lignin degradation by marine fungi. In this study, we report on the ability of three marine fungi to produce the lignin modifying enzymes; laccase, manganese peroxidase (MNP) and lignin peroxidase (LIP), and to mineralize ^{14}C-ring-labeled synthetic lignin. We also demonstrate, for the first time, the ability of these marine fungi to decolorize paper mill BPE.

MATERIALS AND METHODS

Isolation of the marine fungi *Sordaria fimicola* (Roberge) Cesati et De Notaris (# 298), *Halosarpheia ratnagiriensis* Patil et Borse (# 321) and an unidentified basidiomycetous fungus (# 312) and their maintenance on malt extract (ME) agar was described earlier by Raghukumar *et al.* (1994). Agitated low nitrogen medium (LNM; pH 4.5) cultures, growth measurement and BPE decolorization procedures were as described by Michel *et al.* (1991). For testing decolorization of BPE at alkaline pH, the cultures were grown in LNM medium containing Glycine-NaOH buffer (pH 8.2). BPE added to heat-killed mycelial pellets, to plain medium, and to cultures of *Phanerochaete chrysosporium* served as controls. Culture supernatants were assayed for MNP and LIP activities as described by Michel *et al.* (1991). Laccase activity in culture supernatants was assayed as described by Niku-Paavola *et al.* (1988). Mineralization of ^{14}C-synthetic lignin was determined in LNM using methods described by Forney *et al.* (1982).

RESULTS

Laccase and MNP activities for the three marine fungi were as shown (Fig. 1 a, b). LIP activity was not detected in cultures of any of the three fungi studied. The white-rot fungus, *P. chrysosporium*, produced both MNP and LIP activities in LNM but no detectable laccase activity (Fig.1a, b). Mineralization of ^{14}C-ring-labeled synthetic lignin by strain 312 was comparable to that observed for *P. chrysosporium* (positive control; Fig. 1c) while *S. fimicola* and *H. ratnagiriensis,* showed a much lower extent of mineralization. Strain 312 showed 74% BPE decolorization at alkaline pH (8.2) and 98% decolorization at pH 4.5 which was comparable to that seen with *P. chrysosporium*

(Fig.1d). By comparison, *S. fimicola* and *H. ratnagiriensis* decolorized BPE by 55% and 85%, at pH 4.5 (Fig. 1d). None of the three organisms studied showed major differences in growth or the levels of lignin modifying enzymes in BPE amended LNM cultures as compared to unamended LNM cultures.

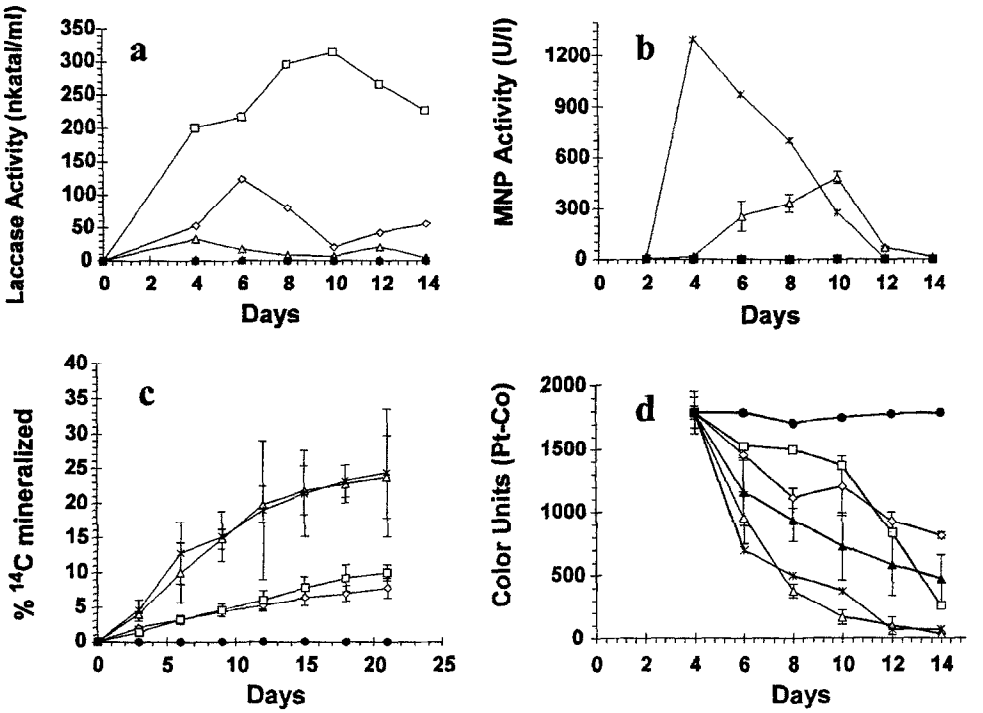

Fig. 1. Laccase activity (a), manganese peroxidase activity (b), U-ring-^{14}C-labeled synthetic lignin mineralization (c), and BPE decolorization (d) in cultures of the marine fungi *S. fimicola* (◇), Strain 312 (△ pH 4.5; ▲ pH 8.2) and *H. ratnagiriensis* (□). *P. chrysosporium* (✳), and an uninoculated culture (●) were used as positive and negative controls. Values represent the means ± one standard deviation.

In summary, the results of this study show, for the first time, MNP production by strain #312, a basidiomycetous marine fungus, and BPE decolorization by all the three lignin-degrading marine fungi. The results also show that marine fungi possessing laccase alone are able to effectively decolorize BPE and mineralize ^{14}C-ring labeled synthetic lignin to $^{14}CO_2$.

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