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Enzymic Demethylation of Lignin Model Polymers

T. D. FRICK AND R. L. CRAWFORD

University of Minnesota, Gray Freshwater Biological Institute, Navarre, MN 55392, USA

ABSTRACT

We have studied the demethylation of [$O^{14}CH_3$]-polyguaiacol by *Phanerochaete chrysosporium* as a model for the fungal demethylation of lignin. Demethylating activity of whole-cell ligninolytic cultures was compared to demethylating activities of various oxygen-activating systems. Some of these systems demethylated polyguaiacol (e.g., Fenton's reagent, rose bengal sensitized photolysis, and horseradish peroxidase + H_2O_2). Other systems did not (e.g., xanthine/xanthine oxidase). Even where oxygen-activating systems did demethylate polyguaiacol, we found no convincing evidence that these systems are used by *Phanerochaete*.

We have detected in concentrated extracellular culture filtrates of ligninolytic *Phanerochaete* cultures an enzymatic activity that demethylates [$O^{14}CH_3$]-polyguaiacol. The activity was stabilized greatly by concentrating culture filtrates by pressure dialysis (20,000 MW cutoff membrane). Concentrated enzyme preparations could be filter sterilized and stored at 4°C for several days without extensive loss of activity. The methoxyl label released by our enzyme preparation was nongaseous (e.g., not $^{14}CO_2$, ^{14}CO, or $^{14}CH_4$), but volatile (e.g., CH_3OH or CH_2O). The amount of labeled methoxyl released by the enzyme preparation was about the same as that released by intact cultures. The enzyme preparation contained ~50 μg/mL of protein and had laccase activity against catechol or hydroquinone. Unsupplemented preparations lacked activity against *o*-dianisidine, a dye used to assay peroxidase. However, when H_2O_2 was provided (0.8 m*M*), *o*-dianisidine was oxidized rapidly. This indicates that the preparation contained peroxidase, but lacked substrate levels of H_2O_2. Demethylation of polyguaiacol by the enzyme preparation was not stimulated by NADH, NADPH, FAD, or FMN. Demethylation was stimulated by >50% upon addition of H_2O_2 (0.5 m*M*).

Concentrated culture filtrates of *Phanerochaete* produced ethylene from methional, a reaction that has been used as an indicator of hydroxyl radical generating systems. However, the ethylene-generating activity and the

demethylase activity in such preparations showed different purification and stability characteristics. Pure horseradish peroxidase and H_2O_2 demethylated polyguaiacol and produced ethylene from methional. *Phanerochaete* does produce H_2O_2, so our demethylase activity appears to be similar to a peroxidase, although we have not yet determined the identity of the methyl product of either enzyme preparation. We suspect that the demethylase operates by a free-radical mechanism, and that the methyl product released is likely to be methanol. Confirmation of these hypotheses provides the basis for our future work with this novel fungal enzyme system.