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Criteria for Estimation of Laccase, Lignin Peroxidase, and Manganese Peroxidase Activity in Crude Fungal Broth Cultures

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The unequivocal detection of the enzyme laccase in the culture broths of medicinal mushrooms can be complicated by the presence of other oxidase enzymes that can act on similar substrates. Criteria have therefore been developed to determine the presence of lignin peroxidase, manganese peroxidase, and laccase activities in crude culture broths containing various combinations of these enzymes.

Broths were first concentrated 10-fold and examined for the presence or production of hydrogen peroxidase using a bicinchoninic acid assay. Lignin peroxidase activity was then determined from the oxidation of veratryl alcohol at pH 2.5. It was found that this substrate was not oxidized by the enzyme at pH 5. Manganese peroxidase was determined by monitoring the oxidation of 2,2′-azino-*bis*-(3-ethylbenzothiazoline)-6-sulfonate in the presence of manganous ion and hydrogen peroxide at pH 5 against a control that contained EDTA. EDTA can inhibit oxidation of this substrate by manganese peroxidase, but will not inhibit the laccase reaction. Laccase activity was then determined as the oxidation of 2,2′-azino-bis-(3-ethylbenzothiazolinone)-6-sulfonate in the presence of EDTA. In this case, any hydrogen peroxide present was removed by treatment of the reaction mixture with catalase from *Aspergillus niger* Tiegh.

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