

Title: Production of laccases by white rot fungi in static, submerged and semi-solid fermentation conditions

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Abstract:

Laccases are widely distributed enzymes among fungi and plants. These enzymes are multi-copper oxidases that metabolize several different substrates and present multiple functions in nature. Laccases also present potential commercial and environmental applications, such as in the pre-treatment of lignocelluloses for ethanol production and in bioremediation processes. White rot fungi are a group of wood-rotting organisms commonly known as great laccase producers. In this work, the production of enzyme extracts rich in laccase was studied with three white rot fungi: *Pleurotus ostreatus*, *Ganoderma lucidum* and *Trametes* sp., in static, submerged and semi solid cultivation conditions. The basic production medium contained glucose, monobasic potassium phosphate, magnesium sulfate and peptone. The effect of potential inducers of laccase was evaluated by the addition of rice husks, milled eucalyptus and copper (II) sulfate in the media. The cultivations were carried out in 7 days, at 28 °C and 120 rpm (submerged and semi solid). The enzymatic extracts were filtered through filter paper and the laccase activity was detected spectrophotometrically at 420 nm with 2,2'-Azino-bis (3-ethylbenzotiazolino-6-sulfonic acid) as substrate. The addition of rice hulls, milled eucalyptus and copper sulphate, individually or in combination, improved significantly the production of the enzyme by the fungi. The best results for each fungus were obtained in static conditions of cultivation. Laccase activities of 7182.81 ± 383.03 U/L, 2679.89 ± 369.58 U/L and 217.95 ± 7.77 U/L were detected for *Pleurotus ostreatus* with copper sulfate and eucalyptus, for *Trametes* sp. with copper sulfate and rice hulls and for *Ganoderma lucidum* with copper sulfate and eucalyptus, respectively. The addition of lignocellulosic residues and mineral copper induced laccase production, which corroborates data observed in literature. *Pleurotus ostreatus* was the best laccase producer and proved to be a potential fungus to obtain enzyme extracts rich in laccases (other enzymes such as manganese peroxidase and lignin peroxidase were not detected in culture filtrates). Enzyme extracts rich in laccases could be applied in processes such as the biobleaching of paper, degradation of soil contaminants, delignification of agricultural wastes for ethanol production, treatment of dyes, among others applications.

Key-words: Laccase; white rot fungi; eucalyptus; rice hulls; *Pleurotus ostreatus*

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