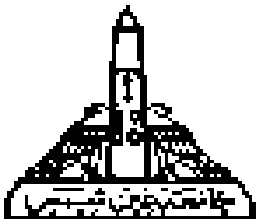

University College of Women
For Arts Science and Education
Botany Department

Biochemical Studies on the Production of Some Lignin-degrading Enzymes (LDEs) by Some Marine Fungal Isolates.

Thesis Submitted For
Fulfillment of the Degree of
Doctor of Philosophy in Science
(Microbiology)

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2011

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(ميكروبيولوجى)

قسم النبات
كليه البنات للآداب و العلوم و التربية
جامعه عين شمس

2011

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ABSTRACT

Marine-derived fungi are a potential for the search of new compounds with relevant features. Among these, the ligninolytic enzymes have potential applications in a large number of fields, including the environmental and industrial sectors. This work aimed to evaluate the enzymatic activities of eighty eight marine fungal isolates obtained from different algae, sea grasses and decayed wood samples collected from Abou-keer, Alexandria, Egypt. All fungal isolates screened for the presence of lignin-degrading enzymes by qualitative and quantitative assay methods. Results indicated that the marine fungal isolate *Trematosphaeria mangrovei* showed the highest laccase activity and the other two ligninolytic activities could not be detected. Among the different agricultural residues screened for laccase production, saw dust was the most suitable substrate for enzyme production (59.92±0.73 U/ml). Results also showed that the simple Boyd &Kohlmeyer medium gave laccase activity of 76.80±1.07 U/ml and supplementation of (2.5 mM) CuSO4.5H_2O on day 6 yielded high amounts of laccase (441.43±10.59 U/ml) at an incubation period of 14 days and incubation temperature of 28-30 ºC under static conditions. Different experiments were carried out to optimize the cultural and nutritional conditions for the production of active laccase such as concentration and time of addition of the inducer, concentration of carbon and nitrogen sources, percent of salinity, rate of agitation, optimum temperature and pH. General properties of the crude laccase enzyme were

determined. Partial purification of the crude enzyme was carried out by fractional precipitation with 60% acetone. The purification was achieved on sephadex G-100 column and SDS-PAGE of the purified laccase enzyme showed a single band at 48 K Dalton. The pure laccase reached its maximal activity at temperature 65 ºC, pH 4.0 with Km equal 1.4 mM and Vmax equal 184.84 U/mg protein. The substrate specificity of the purified laccase was greatly influenced by the nature and position of the substituted groups in the phenolic ring. The pure laccase was tested with some metal ions and inhibitors, $FeSO_4$ completely inhibited laccase enzyme and also highly affected by (NaN_3) at a concentration of 1mM. Amino acid composition of the pure enzyme was also determined. Carbohydrate content of purified laccase enzyme was 23% of the enzyme sample. The U.V absorption spectra of the purified laccase enzyme showed a single peak at 260-280 nm.

Key words: Marine fungi, lignin-degrading enzymes, lignin-modifying enzymes, ligninases, laccase, fermentation, agricultural wastes.

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