

# Structural and functional characterization of a laccase from *Pleurotus sajor caju*

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A study on production, purification, and characterization of an inducible fungal laccase was carried out. Laccases (LC, benzenediol:oxygen oxidoreductase, EC 1.10.3.2) are blue-copper proteins widespread among higher plants, bacteria, and ligninolytic fungi. Fungal laccases are extracellular glycoproteins; they are typically inducible enzymes, and a number of inducers have been found. Fungal laccases are deeply involved in lignin biodegradation, and with time new substrates, not necessarily chemically related to lignin, have been discovered. So, fungal laccases are of potential technological interest not only in the pulp and paper industry, but also in the field of bioremediation of many effluents, coming from agroindustries, textile and dyeing plants, and so on.

In the present study the edible white rot fungus *Pleurotus sajor caju* has been chosen as a laccase source, mainly because of its harmless nature and ease of cultivation. A wide variety of aromatic molecules, mainly phenolic in chemical nature, but also nonphenolics, and aromatic amines, have been tested and compared as possible laccase inducers. This allowed for stating a production protocol to obtain large amount of enzyme, which was then purified and characterized for its main biochemical and operational properties and features. Substrate specificity, catalytic performances under different pH and temperature conditions, resistance against organic solvents, and ability to decolorize some industrial dyes have been studied.

Among the various putative inducers, *p*-aminobenzoic acid and especially ferulic acid (*p*-hydroxy-*m*-methoxycinnamic acid, FA) showed the best performances, and therefore FA was pointed out as the inducer of choice to achieve LC overproduction. Unfortunately, FA led to large amounts of acidic blackish pigments, that required to be removed by calcium phosphate gel and by fibrous DEAE cellulose. Further chromatographic steps (DEAE cellulose, DEAE sepharose, HiLoad 16/60 Superdex 200) allowed a complete purification. The purified, monomeric enzyme showed a  $MW_r$  of 60 kDa (SDS-PAGE). Molecular mass

value of intact laccase was obtained by RP-HPLC-ESI-MS analysis; the deconvolution of the corresponding spectrum provided a theoretical mass value of  $55339 \pm 3$  Da, in agreement with other described fungal laccases. Its *pI* was 3.2. The purified protein was also spectrophotometrically investigated showing typical UV-vis peaks related to presence of type III and I copper metal ion. The enzyme was reasonably stable (only in the purified state, when a protease was eliminated) at room temperature for several hours and for three hours until 50°C. It maintained a satisfactory catalytic activity in the presence of polar organic solvents up to 20% v/v concentration, which allowed its potential use with sparingly water-soluble substrates. *P. sajor caju* LC shows a very wide substrate specificity, being able to oxidize many phenolics (quinols and catechols, but also *o*- and *p*-methoxyphenols such as FA, guaiacol, vanillic acid, 2,6-dimethoxyphenol). The enzyme is also active towards *o*- and *p*-phenylenediamine and some *o*- and *p*-aminophenols. The best substrate is syringaldazine, which has the additional advantage of producing and intensely ( $\epsilon = 65,000 \text{ mol}^{-1} \text{ cm}^{-1}$  at  $\lambda = 525 \text{ nm}$ ) pink quinone upon oxidation. Also some industrial dyes belonging to the class of triphenylmethanes and of hydroxyanthraquinones [1], proved to be LC substrates being efficiently bleached by the enzyme. This could open the way to some promising technological applications.

## References

- [1] Zucca P., Vinci C., Sollai F., Rescigno A., Sanjust E. 2008. Degradation of Alizarin Red S under mild experimental conditions by immobilized 5,10,15,20-tetrakis (4-sulfonatophenyl) porphine-Mn (III) as a biomimetic peroxidase-like catalyst. *J. Mol. Catal A: Chemical*. 288: 97-102.