



## POLYAROMATIC OXIDATION BY A TRAMETES VERSICOLOR LACCASE WITH MEDIATORS

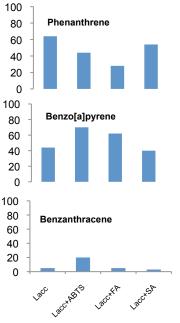
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Introduction. The ability of various enzymes from white rot fungi to degrade PAHs has been studied extensively. More recently, the polyphenoloxidase laccase has been added to this group of PAH-degrading enzymes. Laccases (EC1.10.3.2) are widely distributed in nature, especially in fungal species that degrade lignin. The action of laccase is commonly limited to these classes of substrates. However, it was shown recently that, in the presence of appropriate lowmolecular mass compounds called *mediators*, laccase is able to oxidize a much wider range of compounds, including PAHs. In this work, we have explored PAH degradation using the laccases from Trametes versicolor in the presence of mediators.

Methods: Laccase enzyme was obtained after 10 days of growth of T. versicolor with wheat bran as a substrate. The ultrafiltrated extract was used as source of enzyme for PAHs oxidation. Laccase mediator oxidation of PAHs was determined by incubating a mixture containing; PAH (20 µM) and ABTS (1mM), syringic acid (SA) (1mM) and ferulic acid (FA) (1mM), in 5% acetonitrile in 0.1 M sodium acetate buffer (pH 4.5) with 5U of laccase in a volume of 2 ml. The assay was initiated by the addition of the enzyme and terminated by the addition of ethyl acetate. The supernatant (20µl) was analyzed by HPLC using C<sub>18</sub> reverse-phase column. The isocratic conditions, 10% waterand 90% acetonitrile, were used for the analysis. The wavelengths were 250, 285 and 295 nm for determination of Phenanthrene, 1.2 Benzanthracene Benzo[a]pyrene, and respectively.

Results and discusion. The volumetric production activity of laccase was approximately 350 U/L in wheat bran. In the PAHs oxidation, the T. vesicolor laccase was able to degrade the three PAHs from 20 to 70 percent. We observed that laccase was able to oxidase phenanthrene without mediator at 64 % of oxidation, with mediators the oxidation levels were less. For benzo[a]pyrene, the oxidation level was increased in presence of ABTS and FA as mediators. In the 1,2 benzanthracene, we observed that the oxidation level was very low without mediators, however, we observed an increase of oxidation in presence of ABTS.



**Conclusions.** Laccase from *T. versicolor* was able to oxidase PAHs. ABTS as a mediator was able to increase the oxidation level of the PAHs.

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**References: 1.** Jing. D. et al. (2007). Optimization of laccase production from *T.versicolor* by solid fermentation.Can. J.Microbiol.53:245-251.

**2.** Liu, W. et al. (2004). Biodecolorization of azo, antraquinonic and triphenylmethane dyes by White-rot fungi and laccase-secreting engineered strain. J. Ind Biotechnol. 31: 127-132.

**3.** Collins, M. et al. (1996).Oxidation of anthracene and benzo[a]pyrene by laccases from *T. versicolor*. Appl. Environ. Microbiol 62; 4563–4567.