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Synthetic Applications of Purified Laccase from *Pleurotus sajor caju* MTCC-141[1]

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Abstract—The study is devoted to the role of laccase, purified from the liquid culture medium of the indigenous fungal strain *Pleurotus sajor caju* MTCC-141, in selective bioconversion of toluene derivatives into substituted benzaldehydes with high yield. Coupling reactions of 3-(3,4-dihydroxyphenyl)propionic acid with 4-aminobenzoic, 4-aminoacetophenone and 1-hexylamine proceeded at room temperature under the action of laccase to give the corresponding products with excellent yields.

Keywords: laccase, *Pleurotus sajor caju,* 3-(3,4-dihydroxyphenyl)propionic acid, ABTS

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INTRODUCTION

Laccase (*E. C. 1.10.3.2*) belongs to a group of multicopper containing oxidases [1, 2] that catalyze [3–5] four electrons reduction of molecular oxygen to water. Laccase was extracted for the first time from Japanese lacquer tree *Rhus vernicifera* [6]. Little is known about higher plant laccases, probably due to their presence in cell walls. Laccases are the lignolytic enzymes and abundantly occur in the fungal systems [7]. Laccase is also reported in bacteria *Azospirrullum lipoferum* [8] which was the first laccase producing bacteria. It was also found in *Streptomyces spec.* [9, 10] and *Anabaena azollae* [11]. In addition to fungi, plants and bacteria the presence of laccase was detected in wasp venom [12] and in insects [13].

Catalytic activity of laccases depends on Cu atoms that are distributed among the three centres viz. type-1 or blue copper centre, type-2, or normal copper centre and type-3 or coupled binuclear copper center characterized by different electronic paramagnetic resonance signals [14, 15]. The organic substrate is oxidized by one electron at the active site of the laccase generating a radical which reacts further non-enzymatically. The electron is received at type-1 Cu center and is shuttled to the trinuclear cluster where oxygen is reduced to water. Fungal laccases are stable and have high biocatalytic efficiency.

The objective of this study was purification of laccase from the liquid culture growth medium containing natural lignin substrate bagasse particles of *Pleurotus sajor caju* MTCC-141 [16] and its introduction in selective oxidation of substituted toluene derivatives to corresponding benzaldehydes in presence of ABTS as a mediator and coupling of amines with 3-(3,4-dihydroxyphenyl)propionic acid at room temperature. In this communication the new active biocatalyst suitable for the syntheses is presented.

RESULTS AND DISCUSSION

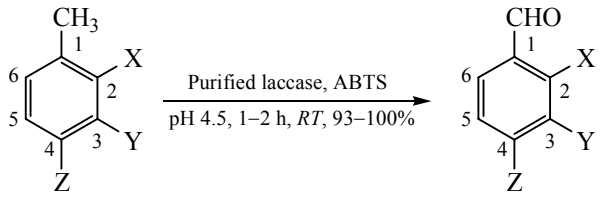
Purity of the enzyme samples designed for the synthesis was tested by SDS-PAGE. Selective bio-oxidation of the toluene methyl group to the aldehyde group is one of the most efficient reactions of laccases in organic syntheses. Generally such conversion requires tough conditions and is complicated by uncontrollable formation of carboxylic acids along with environmentally hazardous byproducts. In the current study oxidation carried out with laccase proceeded under mild conditions and high yield and was ecologically friendly (Scheme 1).

Syntheses of aryl substituted 3-(3,4-dihydroxyphenyl)propionic acid derivatives **Va–Vc** was carried out by the corresponding coupling processes catalyzed by purified laccase of *Pleurotus sajor caju* MTCC-141 (Scheme 2).

Progress of the reaction and purity of products were monitored and tested by HPLC. Formation of the

[1] The text was submitted by the authors in English.

Scheme 1. Oxidation of toluene and its derivatives by purified laccase of *Pleurotus sajor caju* MTCC-141 in the presence of ABTS as a mediator at room temperature



Ia–Id **IIa–IId**

I: X = H, Y = H, Z = H (**a**), X = Cl, Y = H, Z = H (**b**), X = H, Y = NO_2, Z = H (**c**), X = H, Y = H, Z = Cl (**d**). **II:** X = H, Y = H, Z = H (**a**), X = Cl, Y = H, Z = H (**b**), X = H, Y = NO_2, Z = H (**c**), X = H, Y = H, Z = Cl (**d**).

enzymatically synthesized products was justified by HPLC, IR, and ^{1}H NMR data.

EXPERIMENTAL

Materials. 4-Chlorotoluene and diethyl amino ethyl cellulose were purchased from Sigma Chemical Company (USA) and all other reagents from Fluka (Switzerland). The chemicals used in gel electrophoresis of protein samples were obtained from Bangalore Geni Pvt. Ltd. (India).

The fungal strain, its growth, and purification of laccase The fungal strain was obtained from the Microbial Type Culture Collection Center and Gene Bank, Institute of Microbial Technology (India) and was maintained on agar slant as reported in MTCC Catalogue of strains-2000 [17]. For purification of the laccase *Pleurotus sajor caju* MTCC-141was grown in ten 100 mL culture flasks each containing 25 mL of sterilized growth medium reported by Coll and co-authors [18] and processed as presented in the publication [16].

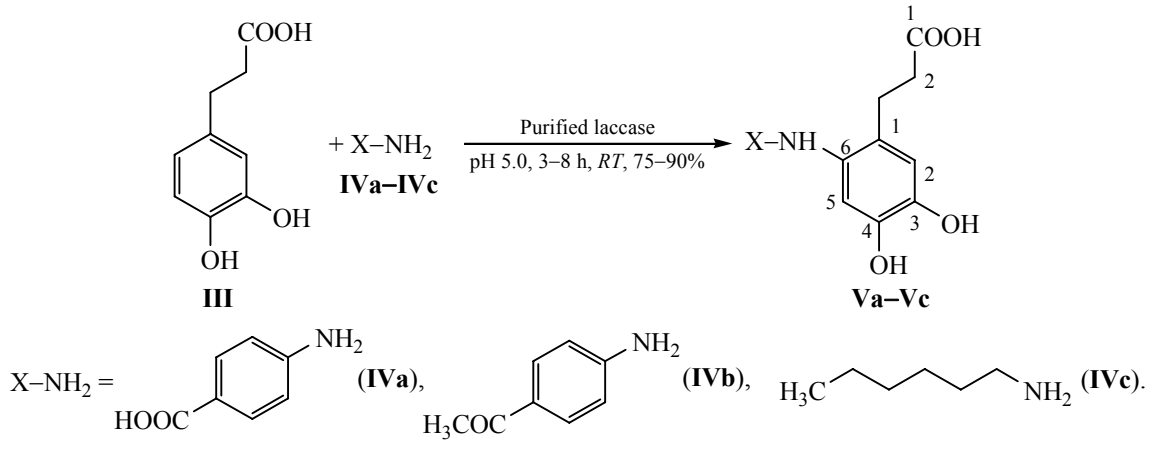
Purity of the prepared enzyme was tested by sodium dodecyl sulphate-polyacrylamide gel electrophoresis [19]. Polyacrylamide gel electrophoresis was run at the constant current 20 mA [20].

Enzyme assay. The assay solution 1.0 mL for DMP as the substrate [18] contained 1.0 mM DMP in 50 mM sodium malonate buffer, pH 5.0 (37°C). The process was monitored by absorbance change at λ 468 nm and $\varepsilon = 49.6 \times 10^3$ M^{-1} cm^{-1}. The UV–Vis spectrophotometer Hitachi (Japan) model U-2900 fitted with electronic temperature control unit was used in the study. One enzyme unit produced 1 μmol of the product per minute under the specified assay conditions.

Bioconversion of toluenes to benzaldehydes under the action of ABTS. The process was carried out in 100 mM sodium acetate buffer, pH 4.5, medium containing 20 mM of toluene in 20 mL of dioxane, 0.1 mM of ABTS [21–24] and 500 μL of two times diluted purified laccase (activity of concentrated laccase 1.12 IU/mL) upon vigorous stirring for 60 min. Completion of the reaction was monitored by UV–Vis spectrophotometry. The reaction solution was extracted by *n*-hexane (40 × 3 mL). 20 μL of *n*-hexane extract was injected in Waters HPLC Model 600E using spherisorb C_{18} 5 UV, 4.5 × 250 mm column, eluent methanol (flow rate 0.5 mL/min). Monitoring was performed by Waters UV detector model 2487 at λ 254 nm.

Biooxidation of 3-nitrotoluene, 2-chlorotoluene and 4-chlorotoluene were carried out according to the method presented above. The reaction mixtures was

Scheme 2. Syntheses of 3-(3,4-dihydroxyphenyl)propionic acid derivatives



stirred for 75 or 90 min. The compounds isolated required no further purification. In the course of oxidation no side reactions occured due to high specificity of laccase. Thus, extraction of products by ethyl acetate gave almost pure benzaldehyde and substituted benzaldehydes (yields >93%).

Synthesis of 3-[6-(4-carboxyphenyl)amino-3,4-dihydroxyphenyl] propanoic acid (Va), 3-[6-(4-acetophenyl)amino-3,4-dihydroxyphenyl]propanoic acid (Vb), and 3-(6-hexylamino-3,4-dihydroxyphenyl) propanoic acid (Vc) [25]. The pure enzyme (1.12 IU/mL) was diluted twice with 20 mM sodium acetate buffer, pH 5.0. 3-(3,4-Dihydroxyphenyl)propionic acid (1 mM) and 4-aminobenzoic acid (1 mM) were added to 2 mL of the solution. The reaction mixture was incubated for 4.15 h at room temperature upon vigorous stirring. The reaction was monitored by UV–Vis spectrophotometry. The reaction solution was extracted thrice with ethyl acetate. The ethyl acetate (20 μL) extract was injected in 4.5 × 250 mm column (Waters HPLC Model 600E, spherisorb C_{18} 5 UV), eluent methanol, flow rate 0.5 mL/min. The similar method was used for coupling of 3-(3,4-dihydroxyphenyl)propionic acid with 4-aminoacetophenone *n*-hexylamine, stirring time 4.30 h (**IVb**) and 6 h (**IVc**). Yields: 90% (**Va**), 86% (**Va**), and 75% (**Vc**).

CONCLUSIONS

The methyl group of toluene and its substituted derivatives were biooxidized into the aldehyde group under the action of ABTS as a mediator. The reaction of 3-(3,4-dihydroxyphenyl)propionic acid with amines leading to substitution in the aromatic ring of the acid **III** was carried out under the action of purified laccase of *sajor caju* MTCC-141 at room temperature and atmospheric pressure with high yield.

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REFERENCES

1. Hoegger, P.J., Kilaru, S., Jomes, T.Y., Thacker, J.R., and Kuees, U., *FEBS J.,* 2006, vol. 273, p. 2308.
2. Messerschmidt, A., *Multi-Copper Oxidases,* Singapore: World Scientific, 1997.
3. Riva, S., *Trends in Biotechnol.*, 2006, vol. 24, no. 5, p. 219.
4. Baldrian, P., *FEMS Microbiol. Rev.*, 2006, vol. 30, p. 215.
5. (a) Chaurasia, P.K., Bharati, S.L., and Singh, S.K., *Res. Plant Sci.*, 2013, vol. 1, no. 2, p. 32. (b) Chaurasia, P.K., Yadav, R.S.S., and Yadava, S., *Res. Rev. Biosci*., 2013, vol. 7, no. 2, p. 66.
6. Yoshida, H.J., *Chem. Soc.,* 1883, vol. 43, p. 472.
7. Messerschmidt, A. and Huber, R., *Europ. J. Biochem.*, 1990, vol. 187, p. 341.
8. Givaudon, A., Effose, A., Faure, D.P., Bouillant, M.-L.R., and Bally, R., *FEMS Microbiol. Lett.,* 1993, vol. 108, p. 205.
9. Arias, M.E., Arenas, M., Rodroguez, J., Soliveri, J., Ball, A.S., and Hernandez, M., *Appl. Envioron. Microbiol.*, 2003, vol. 69, p. 1953.
10. Suzuki, T., Endo, Y., Ito, M., Tsujibo, Y., Miamato, K., and Inamori, Y., Japan Patent 2003230392A2, 2003.
11. Malliga, P., Uma, L., and Subramanian, G., *Microbios*., 1996, vol. 86, p. 175.
12. Perkinson, N., Smith, I., Weave, R., and Edwards, J.P., *Insect Biochem. Mol. Biol.*, 2001, vol. 31, p. 57.
13. Thomas, B.R., Yonkura, M., Morgan, T.D., Czapla, T.H., Hopkins, T.L., an d Kramer, K.J., *Insect Biochem*., 1989, vol. 19, p. 611.
14. Solomon, E.I., Baldwin, M.J., and Lowery, M.D., *Chem. Rev.*, 1992, vol. 92, p. 521.
15. Bento, I., Armenia, M., Corrondo, P.F., and Lindloy, J., *Biol. Inorg. Chem*., 2006, vol. 11, p. 919.
16. Sahay, R., Yadav, R.S.S., and Yadav, K.D.S., *Chinese J. Biotechnol.,* 2008, vol. 24, no. 12, p. 2068.
17. *Catalogue of Strains-2000*, Microbial *Type Culture Collection and Gene Bank Institute of Microbial Technology*, Chandigarh, 5 ed., p.60.
18. Coll, M.P., Fernandez-Abalos, J.M., Villomueva, J.R., Somtamaria, R., and Perez, P., *Appl. Env. Microbiol*., 1993, vol. 59, p. 2607.
19. Laemmli, U.K. and Favre, V., *J. Mol. Biol*., 1973, vol. 80, p. 575.
20. *Polyacrylamide Gel Electrophoresis: Laboratory Techniques, Pharmacia, Laboratory Separation Division*, Uppsala Sweden, 1984.
21. Potthast, A., Rosenanu, T., Chen, C.-L., and Gratzl, J.S., *J. Org. Chem.* 1995, vol. 60, p. 4320.
22. Chaurasia, P.K., Yadava, S., Bharati, S.L., and Singh S.K., *Synth. Comm*., 2014, vol. 44, no. 17, 2535.
23. Chaurasia, P.K., Yadav, A., Yadav, R.S.S., and Yadava, S., *J. Chem. Sci.,* 2013, vol. 125, no. 6, pp. 1395–1403.
24. Chaurasia, P.K., Yadav, R.S.S., and Yadava, S., *Proc. Biochem*., 2014, vol. 49, p. 1647.
25. Mikolasch, A., Hammer, E., Jonas, U., Popowski, K., Stielow, A., and Schaner, F., *Tetrahedron*, 2002, vol. 58, p. 7589.