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Amination of *p*-Hydroquinone by Laccase of *Xylaria polymorpha* MTCC-1100[1]

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Abstract—The communication is devoted to the study of activity of crude laccase obtained from the liquid culture growth media of fungal strain *Xylaria polymorpha* MTCC-1100 in coupling processes of aromatic amines with *p*-hydroquinone. Products of monoamination were obtained under mild condition with high yields.

Keywords: laccase, *Xylaria polymorpha, p*-hydroquinone, monoamination

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INTRODUCTION

Laccase [E. C. 1.10.3.2] is a multicopper polyphenol oxidase [1–5] which has tendency to oxidize phenolic and non-phenolic substrates. Laccase reacts with substrates either in presence of a mediator or without mediator molecules [6-8]. Mediators can be readily oxidized and, hence, promote the laccases activity [9]. Laccases have very wide spectrum of applications in food, pulp, paper, textile, and cosmetic industries and in synthetic organic chemistry [10–17].

Laccases for the above purposes can be extracted from various sources, mostly from fungal systems [18] such as Ascomycetes, Deuteromycetes and Basidiomycetes. Almost all white rot fungi are laccase sources [18, 19] except *Phanerochaete chrysosporium*. Laccases are also found in bacteria *Azospirrullum lipoferum* [20], which was the first laccase containing bacteria. Laccases have also been detected in wasp venom [21] and insects [22].

RESULTS AND DISCUSSION

Coupling of amines with hydroquinones is the important type of laccases involving processes. Though such type of reactions had been performed previously [17], the authors' major objective was to find the new efficient biocatalyst for the synthesis of products of monamination. We have used several laccase proucing fungal sources different from the reported ones. Laccase from *X. polymorpha* MTCC-1100 demonstrated high activity in the synthesis of coupling products. In the present study the coupling reaction (Scheme 1) was promoted by crude Laccase (activity 1.96 IU/mL), produced from the liquid culture growth medium of *X. polymorpha* MTCC-1100, in presence of oxygen [17]. No aminohydroquinones were detected among products of the reaction [13]. Methyl hydroquinone was inefficient in the process with aromatic amines.

Secretion of laccase of mycelia of *X. polymorpha* was studied in different lignin substrates. It was determined that the growth medium containing wheat straw particles [23] had the highest secretion ability in the liquid culture medium (500 mg of wheat straw particles per 25 mL of the culture medium).

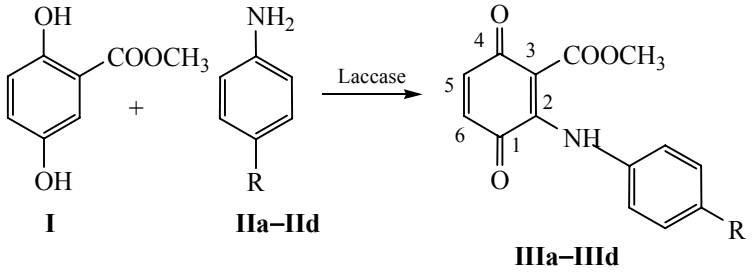
Progress of the reaction was monitored by UV-Vis spectrophotometry. All synthesized products were identified by HPLC and characterized by UV, IR and NMR spectroscopy.

EXPERIMENTAL

Materials. 4-Aminobenzoic acid and 4-amino-acetophenone were obtained from Fluka, Chemi Ulm (Switzerland). All other chemicals were obtained from Himedia laboratory Ltd. Mumbai (India) or E. Merck Ltd. Mumbai (India) and used without further purifications.

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

Scheme 1. Synthesis of monoaminated coupling products



I **IIa–IId** **IIIa–IIId**

R = COOH (**IIa, IIIa**); R = $COCH_3$ (**IIb, IIIb**); R = CH_2COOH (**IIc, IIIc**), R = $COOCH_3$ (**IId, IIId**).

The Fungal strain, its growth and enzyme assay. The fungal strain was obtained from the Microbial Type Culture Collection Center and Gene Bank, Institute of Microbial Technology, Chandigarh, (India) [24]. The growth medium for the fungal strain *Xylaria polymorpha* MTCC-1100, as reported in MTCC Catalogue of strains-2000, consisted of malt extract 20.0 g and agar 20.0 g in 1.0 L Milli-Q water.

Different natural lignin substrates were used in the liquid culture growth media that were composed of glucose 10.0 g, asparagine 1.0 g, yeast extract 0.5 g, $MgSO_4.7H_2O$ and $FeSO_4.7H_2O$, 0.01 g in 1.0 L of Milli-Q water [23, 25]. The above liquid culture growth media containing natural lignin substrates like coir dust, corn cob, wheat straw, saw dust and bagasse particles were prepared separately by adding 0.5 g of one of the natural lignin substrates to 25 mL of growth medium in 100 mL sterilized culture flasks that had been inoculated with small pieces of mycelia (0.5 × 0.5 cm) under aseptic condition. Culture flasks were stored under stationary conditions at 30°C in a biological oxygen demand (BOD) incubator. Accumulation of laccase in the liquid culture media was analyzed at regular intervals of 24 h using DMP as the substrate [23, 26, 27]. The plot of the enzyme concentration (unit/mL of the growth medium) against time (days) after inoculation of the fungal mycelia indicated the extracellular secretion of laccase. Concentration of the inducer wheat straw varied from 100 to 1000 mg per 25 mL of the growth media. The amount which gave maximum height of the enzyme activity peak was considered as the optimal amount of the inducer. Enzyme assay was done according to the method used by Chaurasia P.K. et al. [23].

Synthesis of 2-phenylamino-3-methylcarboxy-1,4-benzoquinones (IIIa–IIId). Equimolar solutions of *p*-hydroquinone **I** and an aromatic amine **IIa–IId** [17] were incubated with laccase (activity 1.96 IU/mL) in 20 mM sodium acetate buffer (pH 4.5) under atomsphere of oxygen at room temperature with constant stirring. Products were isolated after 24 h of the process as coloured solids and recrytallised from methanol. 20 μL of the methanol solutions of the recrystallized products were analyzed by Waters HPLC Model 600E using spherisorb C_{18} 5 UV, 4.5 × 250 mm column, methanol eluent at the flow rate of 0.5 mL/min, and Waters UV detector model 2487 at 254 nm.

Yields of 2-phenylamino-3-methylcarboxy-1,4-benzoquinones were following: 86% (**IIIa**), 79% (**IIIb**), 83% (**IIIc**), and 55 % (**IIId**).

IR spectra (KBr) of the products contained the characteristic bands at ca: 1710, 1695, 1725, 3026, 2915, 1270, 2770, 3432 cm^{-1}.

^1H NMR spectra (DMSO, 300 MHz) complied with the proposed structures of products **IIIa–IIId**.

CONCLUSIONS

Crude laccase of *X. polymorpha* has been efficiently used in syntheses of various monoaminated coupling products by its incubation with *p*-hydroquinone and amines in presence of oxygen. There were used the eqiuimolar concentrations of the reagents that minimizes the possibility of side reactions. The new strong biocatalyst for this type of enzymatic reactions has been studied. The reaction can be considered as an example of green chemistry processes.

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