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ORIGINAL ARTICLE

Production, characterization and Congo red dye decolourizing efficiency of a laccase from *Pleurotus ostreatus* MTCC 142 cultivated on co-substrates of paddy straw and corn husk

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Abstract A laccase produced by *Pleurotus ostreatus* MTCC 142 under solid-state fermentation using co-substrates of paddy straw and corn husk (1.5:1.5, g w/w) showed an activity of 2.54 U gds^{-1}. Laccase activity was determined spectrophotometrically using 0.5 mM 2,2′- azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS). Supplementation with fructose and potassium nitrate resulted in maximum enzyme production at initial pH 5.8 ± 0.2 and initial moisture content of 70%. A carbon: nitrogen ratio of 0.5:0.1 yielded highest laccase activity in the presence of surfactant Tween 20 (0.05%, w/v). Incorporation of vanillin (5 mM) and copper sulphate (10 mM) facilitated enhanced synthesis of laccase. A 4.8-fold increase in enzyme activity was recorded after optimization of nutritional parameters. The apparent molecular mass of this enzyme was revealed as 43 kDa by sodium dodecyl sulphate–polyacrylamide gel electrophoresis. The laccase showed optimal activity at pH 3 and 35 °C with 82.8% residual activity after 1 h of incubation. The K_m and V_{max} values on ABTS were found to be 0.52 mM and 9.33 U gds^{-1}, respectively. The enzyme activity was enhanced by Cu^{2+} and remained unaffected with Ba^{2+}, Mn^{2+}, Pb^{2+}, Mg^{2+}, Ca^{2+} and Fe^{3+}. However, pre-incubation of the enzyme with reagents like sodium azide, sodium lauryl sulphate and 2-mercaptoethanol demonstrated an inhibition of its activity. Addition of crude laccase to Congo red dye solution resulted in 36.84% decolourization after 20 h of incubation at 35 ± 2 °C. This study discusses the production and characterization of a laccase from *P. ostreatus* strain with potential for azo dye decolourization.

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1. Introduction

Laccases (E.C. 1.10.3.2, *p*-diphenol: dioxygen oxidoreductases) are blue multicopper oxidases which catalyze the oxidation of various organic and inorganic compounds such as phenols, aromatic amines, polyphenols, benzenethiols and cyanide complexes of metals [33]. Laccases are abundantly produced by many white-rot basidiomycetous fungi which cause decomposition of litter by degradation of lignin [37]. However, the extent of lignin biodegradation depends on environmental conditions and the basidiomycete species involved. *Pleurotus ostreatus* is a basidiomycete that produces ligninolytic enzymes such as laccases, manganese peroxidases and veratryl alcohol oxidases but not lignin peroxidases [27].

Laccases have numerous biotechnological applications due to their ability to oxidize different phenolic and non-phenolic compounds. Some of the potential applications of laccase include treatment of effluents from paper, pulp and textile industries, bioremediation of polycyclic aromatic hydrocarbons and dye degradation [35].

Several factors such as the type of cultivation (submerged or solid-state), nutritional supplementation and concentration of microelements can influence the level of laccase production [14]. Solid-state fermentation facilitates better production of enzymes by filamentous fungi because it mimics the growth of the fungi in natural environment [28]. Various agro-industrial lignocellulosic residues have been used as growth substrates for laccase production from *P. ostreatus* [34].

The ability of laccase producing microorganisms or their purified laccases to bioremediate different pollutants is not only interesting but also a promising approach for environmental cleanup [32]. Several solid substrates have been utilized for laccase production from fungal systems but very few literatures are available which discuss the characterization of laccases from fungi cultivated on lignocellulosic co-substrates. Owing to numerous applications of fungal laccases in various industrial sectors, the present study was undertaken with the objectives of enhancing the production, characterization and determining an azo dye decolourizing potential of laccase from a strain of *P. ostreatus* cultivated on lignocellulosic co-substrates.

2. Materials and methods

2.1. Source of fungal strain, substrates and chemicals

The basidiomycete culture *P. ostreatus* MTCC 142 was obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India. Pure culture of the fungus was maintained on glucose yeast extract agar plates and stored at 4 °C until further use. Different agri-wastes (paddy straw, corn husk, sugarcane bagasse and saw dust) were procured from different regions of Bangalore. All the analytical grade chemicals and reagents were procured from HiMedia Laboratories Pvt. Ltd., Mumbai, India. ABTS was procured from Sigma–Aldrich (USA).

2.2. Production of laccase

The laccase production ability was monitored by observing a dark green colour around the mycelial plug on glucose yeast extract agar supplemented with ABTS (0.5 mM). The effect of different lignocellulosic substrates on laccase production was determined by adding separately 3 g of each substrate into 100 mL Erlenmeyer flasks and moistening them with 10 mL of mineral salt solution containing (g L^{-1}) NH_4Cl, 0.3; KH_2PO_4, 0.2; $MgSO_4$, 0.1; $CaCO_3$, 1; and distilled water, adjusted to an initial pH of 5.8 ± 0.2, initial moisture content of about 70% and autoclaved. The sterile substrates were inoculated with two mycelial plugs (6 mm diameter) from a seven day old culture plate. The flasks were incubated at 25 ± 2 °C for 14 days under static condition in the dark. Post incubation, 10 mL of sodium citrate buffer (pH 5.0) was added to each flask, the contents were shaken for 15 min and homogenized using a sterile mortar and pestle. The extract obtained was filtered using Whatman's No. 1 filter paper and centrifuged at 8000 rpm for 15 min to obtain a clear supernatant. This supernatant was subjected to laccase assay and further purification.

2.3. Laccase assay

Laccase activity was determined by the oxidation of ABTS. The assay protocol of More et al. [24] was followed with slight modifications. The reaction mixture contained 2.8 mL of 0.5 mM ABTS in 200 mM sodium acetate buffer (pH 5.5) and 0.2 mL of enzyme and incubated for 5 min at 35 °C. Oxidation of ABTS was monitored by determining the increase in absorbance (ε_{420}, 3.6×10^4 M^{-1} cm^{-1}) resulting in an intense blue-green colour spectrophotometrically read at 420 nm against a suitable blank. One unit of laccase activity was defined as the amount of enzyme that oxidized 1 μmol of ABTS substrate per min under the assay conditions and expressed as Units per g of dry substrate (U gds^{-1}).

2.4. Optimization of laccase production

The effect of co-substrate concentration on laccase production was determined by mixing the two selected substrates (paddy straw and corn husk) at different ratios such that the total content in each flask did not increase beyond 3 g. Various carbon supplements such as glucose, sucrose, maltose, lactose, starch (soluble), fructose, mannitol or molasses were added at 1% (w/v) concentration to the mineral salt solution. Different organic and inorganic nitrogen supplements such as peptone, beef extract, tryptone, yeast extract, corn steep liquor, KNO_3, $(NH_4)_2SO_4$ or NH_4Cl were added at 1% (w/v) concentration. The effect of C/N ratio was investigated by adding the selected carbon and nitrogen supplements at different concentrations. The effect of inducers (vanillin or ABTS) was analyzed by incorporating the membrane filter (0.2 μm) sterilized solutions into the flasks containing autoclaved co-substrates. Surfactants (Triton X-100, sodium lauryl sulphate (SLS), Tween 20 or Tween 80) were added at 0.05% (w/v) concentration to the mineral salt solution before autoclaving. Various metal salts ($ZnSO_4$, $CuSO_4$, $MnCl_2$ or $MgSO_4$) were added at 10 mM concentration to the mineral salt solution (devoid of any additional $MgSO_4$) before autoclaving. For each study, inoculated control was maintained with mineral salt solution devoid of any supplements. Solid-state cultivation of the basidiomycete was carried out under all the optimized cultural conditions and the crude laccase was harvested.

2.5. Purification of laccase

Purification of laccase was performed as per the protocol of Chefetz et al. [5] with a few modifications. The chilled clear supernatant containing crude laccase was subjected to ammonium sulphate precipitation till 80% saturation was attained at 4 °C. The resulting precipitate was centrifuged at 5000 rpm for 30 min at 4 °C and resuspended in 5 mL of 200 mM sodium acetate buffer (pH 5.5) at 4 °C. The enzyme solution was dialyzed using dialysis membrane 50 (Himedia, Mumbai, India) against 10 mM sodium acetate buffer (pH 5.5) at 4 °C overnight. The dialyzed enzyme was loaded onto DEAE cellulose column (2 × 10 cm) pre-equilibrated with 10 mM sodium acetate buffer (pH 5.5) and eluted with a linear gradient of (0–200 mM) NaCl in the same buffer at a flow rate of 0.5 mL min^{-1}. Eluted fractions showing laccase activity were pooled and stored at 4 °C.

2.6. Determination of molecular mass

The relative molecular mass of the partially purified laccase was determined by SDS–PAGE in a Mini Protean Tetra Cell vertical electrophoresis unit (Bio-Rad) using a 10% (w/v) polyacrylamide separating gel, following the method described by Laemmli [18]. Samples were analyzed after staining with Coomassie Brilliant Blue R-250 and molecular mass was estimated with reference to medium range molecular weight protein marker (Genei, Bangalore, India).

2.7. Zymography

In situ laccase activity was detected by zymography following a non-denaturing PAGE. After electrophoresis, the gel was placed in a petri dish containing 0.5 mM ABTS solution prepared in 200 mM sodium acetate buffer (pH 5.5). After incubation for 15 min at 35 °C, laccase activity was visualized as a green colour band [17].

2.8. Characterization of laccase

The optimum pH for laccase activity was measured using ABTS as the substrate, at 35 °C in buffers (200 mM) of different pH values such as citrate buffer (pH 3.0–5.0), phosphate buffer (pH 6.0–8.0) and glycine-NaOH buffer (pH 9.0–10.0). pH stability of laccase was determined after pre-incubating the enzyme with citrate buffer (pH 3) at 35 °C for 0.5, 1 and 24 h. The optimum temperature for laccase activity was determined in the temperature range of 4–85 °C using citrate buffer (200 mM, pH 3). The effect of substrate concentration on laccase activity was evaluated by incubating the enzyme (0.2 mL) with varying concentrations of ABTS (0.5–50 mM) at 35 °C. The K_m and V_{max} values were calculated from Lineweaver–Burk plot using Hyper32 software.

The effect of various metal salts and reagents on laccase activity was analyzed by pre-incubating the enzyme (0.2 mL) for 1 h at 35 °C in 200 mM citrate buffer (pH 3), containing 6 mM of each reagent ($CaCl_2$, $MgSO_4$, $MnCl_2$, $ZnCl_2$, $BaCl_2$, $HgCl_2$, $CuSO_4$, $FeCl_3$, $Ba(NO_3)_2$, EDTA, 2-mercaptoethanol, sodium azide (NaN_3) or SLS [2]. A control was maintained without the addition of any metal salt or reagent. Residual laccase activity was determined spectrophotometrically and expressed as percentage.

2.9. Protein estimation

The protein content was evaluated using the method of Lowry et al. [21] with 500 μg mL^{-1} of crystalline bovine serum albumin fraction V as the standard.

2.10. Determination of dye decolourizing efficiency

Crude laccase was tested for its ability to decolourize Congo red dye. The assay mixture contained 0.3 mL (0.15 U gds^{-1}) of crude laccase and 9.7 mL of citrate buffer (200 mM, pH 3) with the dye added to a final concentration of 100 mg L^{-1}. Suitable control was maintained without addition of the enzyme. The absorbance was spectrophotometrically monitored at the maximum wavelength for Congo red (497 nm).

The decolourization efficiency (%) was calculated as follows:

$$\text{Percentage of decolourization} = [(\text{Initial absorbance} - \text{final absorbance}) / (\text{initial absorbance})] \times 100$$

where initial absorbance indicated absorbance of the untreated dye at its characteristic wavelength and final absorbance indicated absorbance of the dye after treatment with laccase at the same wavelength after an incubation of 20 h at 35 ± 2 °C [9].

2.11. Statistical analysis

One-factor-at-a-time (OFAT) approach was followed during optimization of laccase production. Each experiment was conducted in triplicate and the data have been graphically presented as mean ± standard deviation ($n = 3$). All the data were analyzed by one-way Analysis of Variance (ANOVA) of repeated measures using GraphPad Prism version 7. p values < 0.05 were considered statistically significant.

3. Results and discussion

3.1. Laccase production by *P. ostreatus* MTCC 142

P. ostreatus is a white-rot basidiomycete which produces laccase as the major ligninolytic enzyme. The presence of utilizable carbohydrates in lignocellulosic wastes supports biomass formation and enzyme production by ligninolytic fungi [10]. Among the four different substrates used in the present study, paddy straw supported maximum laccase production (0.29 ± 0.006 U gds^{-1}) followed by corn husk (0.21 ± 0.006 U gds^{-1}) (data not shown). This may be due to the presence of utilizable lignin and cellulose and retention of moisture within the substrate fibres. This is in agreement with earlier reports which suggested that rice straw supported laccase production from *P. ostreatus* and *Trametes versicolor* [7,15].

3.2. Optimization of laccase production

3.2.1. Effect of nutritional parameters

Based on the results obtained, a combination of paddy straw and corn husk was used as co-substrates to study the effect

on laccase production by the basidiomycete strain. Improved laccase activity (0.35 ± 0.02 U gds^{-1}) was recorded when the ratio of co-substrates was 1.5:1.5 (g w/w). This result indicated that *P. ostreatus* MTCC 142 synthesized higher titres of laccase when cultivated on these co-substrates than when either substrate was used alone. Anike et al. [1] reported that cultivation of *P. ostreatus* on co-substrates of peanut shells and cornstalks enhanced lignin biodegradation when both the substrates were mixed at equal proportions.

All the carbon supplements enhanced laccase synthesis as compared to the unsupplemented control, though among these, any significant ($p = 0.1407$) difference in laccase production was not observed (Fig. 1a). However, the highest enzyme titre was noted in co-substrates supplemented with fructose (0.55 ± 0.09 U gds^{-1}). The results revealed the potential of this fungus to utilize any of the carbon supplements for laccase biosynthesis. Mansur et al. [22] reported that fructose as a carbon source resulted in 100-fold increase in specific activity of laccase obtained from a lignin degrading basidiomycete which belonged to Polyporaceae family.

Among the nitrogen supplements studied, KNO_3 demonstrated a significant ($p < 0.0001$) enhancement in laccase production (0.67 ± 0.02 U gds^{-1}), followed by beef extract (Fig. 1b). This may be due to faster assimilation of readily soluble inorganic nitrate which facilitated fungal growth and enzyme biosynthesis. Beef extract, on the other hand, might have provided the proteins, amino acids and accessory growth factors required for mycelial growth and metabolism. Dhakar et al. [8] reported that among the nutritional supplements, KNO_3 facilitated the highest production of laccase from *Penicillium pinophilum* (MCC 1049). A similar finding was reported by Ravikumar et al. [29] where KNO_3 as the nitrogen source supported maximum laccase production from the basidiomycete *Hypsizygus ulmarius*.

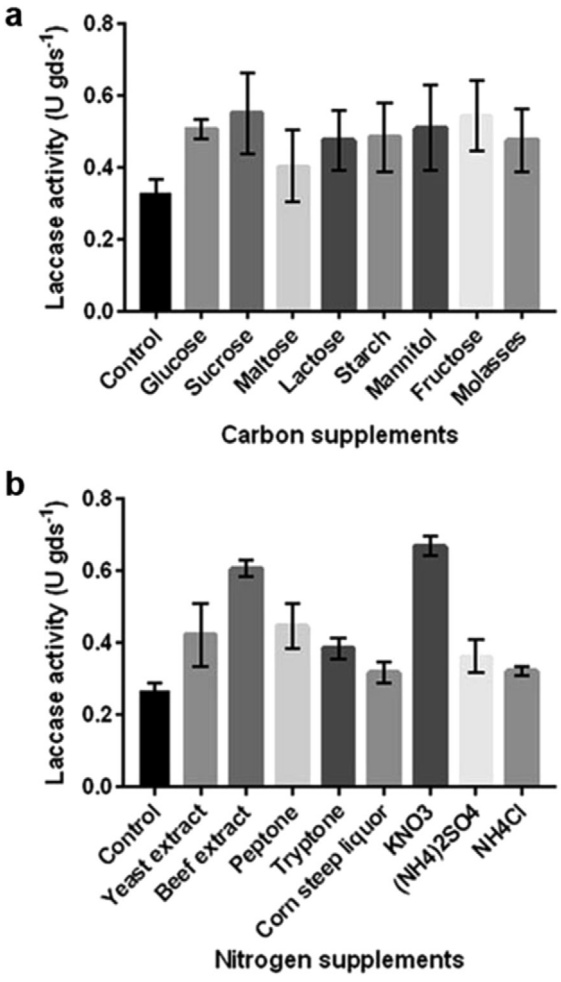

Figure 1 (a) Effect of carbon supplements on laccase production. (b) Effect of nitrogen supplements on laccase production. Data represent mean ± S.D. ($n = 3$); $p < 0.05$.

In order to evaluate the combinatorial influence of carbon and nitrogen supplements, fructose and KNO_3 were incorporated at different concentrations. A C/N ratio of 0.5:0.1 (%, w/v) facilitated maximum laccase production (0.66 ± 0.006 U gds^{-1}) from *P. ostreatus* MTCC 142 (data not shown). In general, the requirement for C/N ratio differs with the species of basidiomycete used [32]. A C/N ratio of 5 showed the highest laccase activity from *P. ostreatus* cultivated on soybean hulls with added nitrogen sources [6]. This report also concluded that laccase production in some basidiomycetes reduces when C/N ratio increases.

Different compounds can act as inducers and mediators to stimulate laccase production. In the present study, both vanillin and ABTS enhanced laccase synthesis (0.64 ± 0.09 U gds^{-1} and 0.6 ± 0.02 U gds^{-1}, respectively), but no significant ($p = 0.1287$) difference was observed between each inducer. In a previous study conducted by Bhattacharya et al. [4], it was observed that both ABTS (artificial mediator) and vanillin (natural mediator) demonstrated positive effect on benzo[*a*] pyrene degradation involving laccase and other lignin-modifying enzymes from a strain of *P. ostreatus*.

Among the surfactants studied, Tween 20 facilitated highest laccase synthesis (1.05 ± 0.19 U gds^{-1}) (Fig. 2a). Saparrat et al. [30] reported 2.5-fold increase in laccase activity from a strain of *Minimidochium parvum* in the presence of Tween 20 (0.1%, v/v). This may be due to increased permeability of oxygen and transport of extracellular enzymes through the cell membranes of fungi [19]. However, maximum laccase production from a white-rot fungus *Stereum ostrea* was recorded with Tween 80 [36].

Among the metal salts studied, $CuSO_4$ facilitated maximum laccase production (1.14 ± 0.07 U gds^{-1}) (Fig. 2b). Copper is an important micronutrient for many microorganisms. Uptake of copper in fungi happens through a metabolism-independent surface binding followed by an energy-dependent metal influx [13]. Copper atoms are incorporated in the molecular structure of laccase. The results of the present study are in agreement with that of Palmieri et al. [26] who reported efficient production of laccase in copper-supplemented cultures of *P. ostreatus*. Shankar and Shikha [31] reported that Cu^{2+} (1.0 mM) induced maximum laccase production in a basidiomycete *Peniophora* sp.

In the present study, optimization of the nutritional parameters resulted in 4.8-fold increase in laccase activity as compared to the unoptimized control. In a recent study, Zhu et al. [39] optimized the production of laccase from *P. ostreatus* (ACCC 52857) and reported increased laccase activity upon the addition of yeast extract and copper to the production medium.

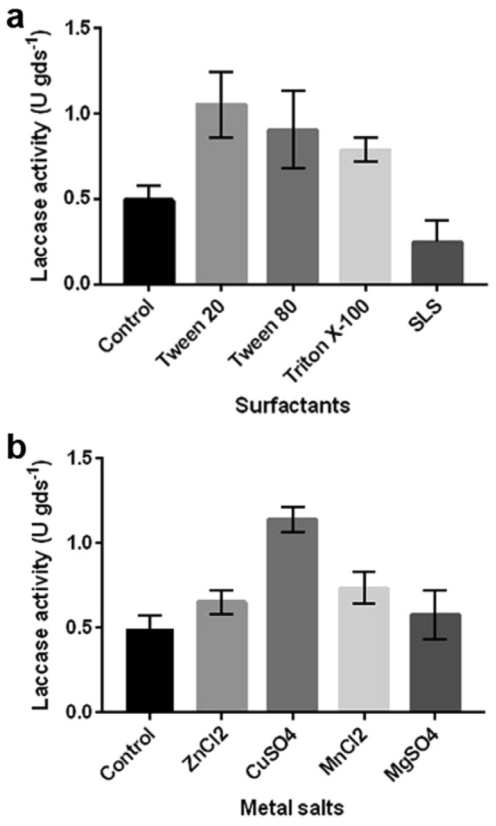

Figure 2 (a) Effect of surfactants on laccase production. (b) Effect of metal salts on laccase production. Data represent mean ± S.D. ($n = 3$); $p < 0.05$.

3.3. Purification of laccase

The crude fungal laccase showed a specific activity of 0.15 U mg^{-1} (Table 1). The specific activity of laccase was enhanced by 1.7-fold following ammonium sulphate precipitation and dialysis. The dialyzed enzyme was purified by DEAE cellulose ion exchange chromatography. The specific activity of the partially purified laccase increased by 2.1-fold in comparison to that of the crude enzyme.

3.4. Molecular mass of laccase and zymography

SDS–PAGE profile of partially purified laccase demonstrated a prominent band with an apparent molecular mass of approximately 43 kDa (Fig. 3). *In situ* laccase activity was detected by the formation of a green band on the non-denaturing gel stained with ABTS solution. This result is in close proximity with that of Marques and Peralta [23] who reported that a laccase from the white-rot fungus *Pleurotus pulmonarius* had an apparent molecular mass of 46 kDa.

3.5. Characterization of laccase

3.5.1. Effect of pH

Enzymes exhibit maximum activity at their optimum pH as their active sites have maximum interaction with the substrate. Any drastic alteration in the pH of a medium leads to denaturation of the enzyme resulting in loss of its activity. Maximum laccase activity was recorded at pH 3 (0.94 ± 0.01 U gds^{-1}), although the activity of the enzyme was retained till pH 10 (Fig. 4a). Beyond pH 5, the enzyme activity showed a steep decline indicating that the fungal laccase is highly active at low pH range. The results are in accordance with that of Neto et al. [25] who reported that for a tropical basidiomycete *Lentinus crinitus*, the highest laccase activity was obtained at pH 3.5.

The fungal laccase was stable at pH 3 for 0.5 h, whereas, 82.8% of its activity was retained after 1 h (Fig. 4b). However, after 24 h of incubation, 22% of its activity was retained.

3.5.2. Effect of temperature

Temperature plays a vital role in determining the activity of enzymes. 35 °C was found to be the optimum temperature for laccase activity (0.99 ± 0.007 U gds^{-1}) (Fig. 5). The laccase activity remained unchanged from 35 to 55 °C and thereafter showed a gradual decline. The enzyme retained 30% of its activity at 85 °C. This decrease in activity could be due to disruption of the tertiary structure of the enzyme and conformational change of the active site. El-Batal et al. [9] observed that a laccase produced from a local isolate of *P. ostreatus* had optimum temperature between 30 and 50 °C and rapidly lost activity at temperatures above 60 °C probably due to break down of the structural integrity of laccase protein.

3.5.3. Effect of substrate concentration

An enzymatic reaction involves the interaction of the active site of an enzyme with its specific substrate. With an increase in the substrate concentration, the active sites of enzyme molecules get saturated. The effect of substrate concentration on laccase activity was determined through Michaelis–Menten equation and Lineweaver–Burk plot using ABTS as the substrate. The K_m and V_{max} values of the laccase from *P. ostreatus* MTCC 142 were found to be 0.52 mM and 9.33 U gds^{-1}, respectively. The low K_m value indicated high specificity of the laccase towards the substrate ABTS. Marques and Peralta [23] stated that the K_m of a laccase from *P. pulmonarius* was 0.21 mM for ABTS substrate.

3.5.4. Effect of metal salts and reagents

Following 1 h of pre-incubation with the respective chemicals, a significant ($p < 0.0001$) increase (35%) in residual activity was detected in the presence of $CuSO_4$ (6 mM) (Fig. 6). It may be interpreted that this fungal laccase essentially requires Cu^{2+} for its catalytic action and therefore it is a copper-

Table 1 Purification profile of laccase from *P. ostreatus* MTCC 142.

Purification step	Total activity (U)	Total protein (mg)	Specific activity (U mg^{-1})	Recovery (%)	Purification fold
Crude enzyme	17.8	117.5	0.15	100	1
Dialysis	8.8	35.4	0.25	49.4	1.7
DEAE cellulose	5.2	16.3	0.32	29.2	2.1

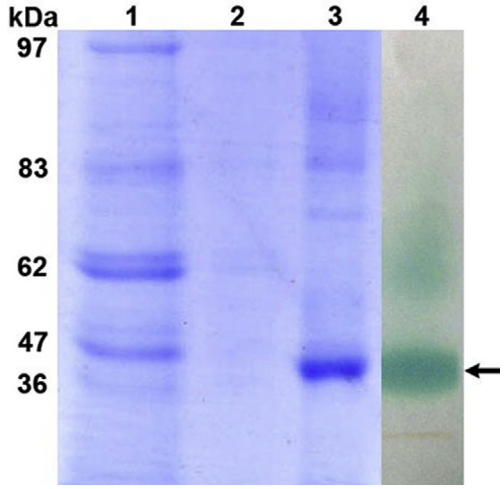

Figure 3 SDS–PAGE profile of laccase from *P. ostreatus* MTCC 142. 1 Protein marker. **2** Extract of uninoculated co-substrates. **3** Partially purified laccase. **4** Zymography of partially purified laccase showing a green band (indicated by arrow) on non-denaturing gel stained with ABTS solution. The molecular sizes of the marker proteins are shown on the left.

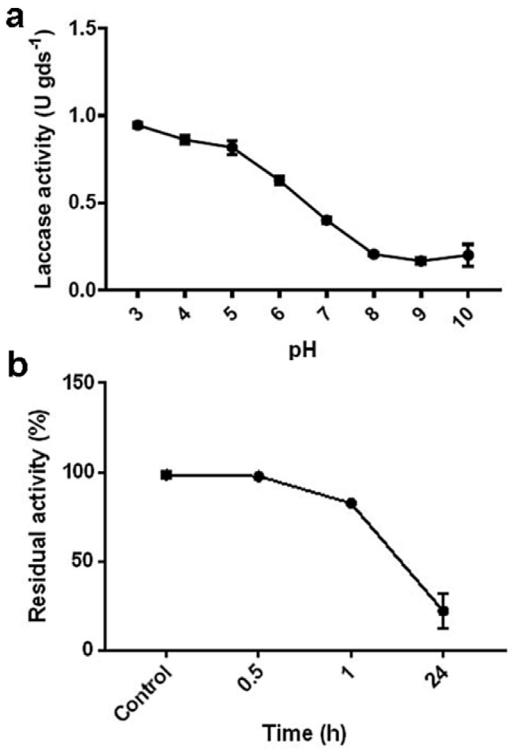

Figure 4 (a) Effect of pH on laccase activity. (b) Stability of laccase at pH 3, when incubated at 35 °C for different durations of time. Data represent mean ± S.D. ($n = 3$); $p < 0.05$.

dependent laccase. The stimulation of its activity may be due to the filling of type 1 Copper binding site by Cu^{2+} [32]. Baldrian and Gabriel [3] reported that the addition of $CuSO_4$ (0.05–50 mM) increased the laccase activity in *P. ostreatus*.

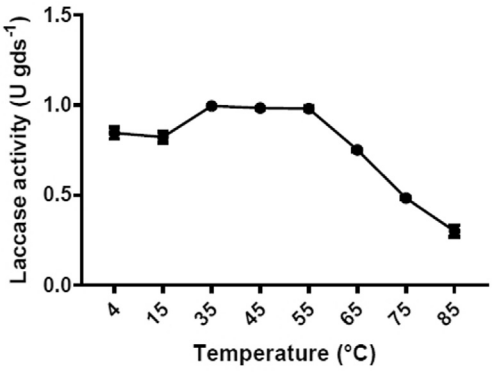

Figure 5 Effect of temperature on laccase activity. Data represent mean ± S.D. ($n = 3$); $p < 0.05$.

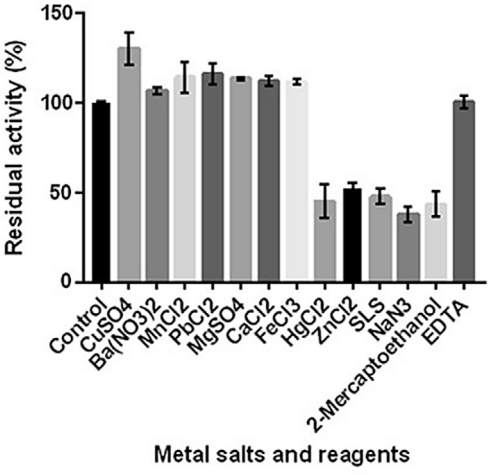

Figure 6 Effect of metal salts and reagents on laccase activity. Data represent mean ± S.D. ($n = 3$); $p < 0.05$.

In the present study, Ba^{2+}, Mn^{2+}, Pb^{2+}, Mg^{2+}, Ca^{2+} and Fe^{3+} did not show any marked influence on laccase activity as compared to the control. These findings suggest the ability of this laccase to tolerate some heavy metals such as Ba^{2+}, Mn^{2+}, Pb^{2+} and Fe^{3+}. Therefore, it may be suitable for biotechnological applications involving the treatment of sites contaminated with different heavy metals [32]. Additionally, due to its tolerance to Mg^{2+} and Ca^{2+}, this enzyme may remain functionally active in hard water (often containing salts of Mg^{2+} and Ca^{2+}), thereby reducing the cost related to the usage of treated water in industries.

However, almost 50–55% reduction in the enzyme activity was noted in the presence of $HgCl_2$ and $ZnCl_2$. The inhibitory effect of Hg^{2+} may be attributed to its binding to the sulfhydryl (–SH) groups of the enzyme molecule, thereby causing distortion of the active site [3]. It was also observed that SLS, 2-mercaptoethanol and sodium azide inhibited the enzyme activity. SLS might have caused denaturation of laccase molecules. The inhibitory effect of 2-mercaptoethanol could be due to reduction of the oxidized substrate (ABTS) by this reducing agent. Inhibition of laccase activity by sodium azide may be

attributed to the binding of azide ion to the copper containing core of the enzyme, thus affecting the internal electron transfer and laccase catalyzed oxidation reactions [33]. A thermostable laccase obtained from an unidentified basidiomycete was also inhibited by sodium azide [16]. Interestingly, laccase activity remained unaffected in the presence of EDTA, which indicated its stability even in the presence of a chelating agent. This is in accordance with Lin et al. [20] who observed that EDTA did not inhibit the activity of a novel laccase from the basidiomycete *Coprinus cinereus*.

3.6. Dye decolourizing efficiency of crude laccase

Synthetic dyes are being extensively used in various industries. Among these, azo dyes are quite popular owing to their affordable synthesis and stability. However, they have been identified as environmental pollutants capable of causing toxicity and carcinogenicity. Laccases are employed either alone or in assistance with mediators for bioremediation of various industrial pollutants [12]. In the present study, the ability of crude fungal laccase to decolourize a synthetic diazo dye Congo red was evaluated. Addition of crude laccase to the dye solution resulted in 36.84% decolourization after 20 h of incubation at 35 ± 2 °C. The result indicated that the laccase from *P. ostreatus* MTCC 142 can moderately decolourize azo dye without the assistance of any costly mediator. This is in agreement with Zheng et al. [38] who reported that a laccase from the white-rot fungus *Lenzites gibbosa* could efficiently decolourize Congo red without the addition of redox mediators. However, in a study on synthetic dye decolourization, Forootanfar et al. [11] reported that the laccase from *Paraconiothyrium variabile* showed 18.5% decolourization of Congo red after 3 h of incubation in the presence of hydroxybenzotriazole as the laccase mediator. In the current context, it may also be suggested that since crude laccase extract was used for the decolourization study, occurrence of other extracellular ligninolytic enzymes in the crude preparation would have synergistically led to the dye decolourization.

4. Conclusions

An extracellular laccase was produced by *P. ostreatus* MTCC 142 on co-substrates of paddy straw and corn husk under solid-state fermentation. Optimization of various nutritional parameters resulted in 4.8-fold enhancement in laccase activity. The laccase showed optimum activity at pH 3 and 35 °C. The enzyme activity was enhanced by Cu^{2+} and remained relatively stable in the presence of various metal ions. Addition of crude laccase to the dye solution resulted in 36.84% decolourization of Congo red dye after 20 h of incubation at 35 ± 2 °C. Further studies are needed to scale up the fermentation process and evaluate the dye decolourizing ability of this fungal laccase in the presence of different mediators.

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