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Purification and characterization of an extracellular laccase from a *Pseudomonas* sp. LBC1 and its application for the removal of bisphenol A

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ABSTRACT

The *Pseudomonas* sp. LBC1 produced extracellular laccase when grown in the nutrient broth. The enzyme was purified using acetone precipitation and an anion-exchange chromatography. The molecular weight of the purified laccase was estimated as 70 kDa by sodium dodecyl sulfate polyacrylamide gel electrophoresis. An enzyme showed maximum substrate specificity towards *o*-tolidine than other substrates of laccase including 2,2′-azinobis, 3-ethylbenzothiazoline-6-sulfonic acid, hydroquinone, N,N′-dimethyl phenylene diamine, syringic acid and veratryl alcohol. The optimum pH and temperature for the laccase activity were 4.0 and 40 °C, respectively. Cyclic voltammogram revealed the redox potential of purified enzyme as 0.30 V. The laccase was stable up to 40 °C and within pH range 6.0–8.0. Sodium azide and EDTA strongly inhibited laccase activity. The purified laccase completely degraded the higher concentration of bisphenol A within 5 h. Biodegradation metabolites of bisphenol A were characterized by using FTIR, HPLC and GC–MS.

1. Introduction

Laccases (*p*-benzenediol: oxygen oxidoreductase EC 1.10.3.2) are the most numerous members of the multicopper protein family, which also includes tyrosinases, monoxygenases, and dioxygenases [1]. Laccases were widely distributed among plants, fungi and bacteria [2,3]. Laccases catalyze the oxidation of various substituted phenolic compounds by using molecular oxygen as an electron acceptor. The ability of laccases to act on a wide range of substrates makes them highly useful biocatalysts for various biotechnological applications [4]. The broad substrate specificities of this enzyme make them excellent candidates to catalyze the oxidation of the environmental pollutants including endocrine disruptors. Endocrine disruptors induce the adverse effects into wildlife and humans owing to their ability of interfering with an endocrine system. Bisphenol A (2,2-bis(4-hydroxyphenyl)propane) has been chosen as a model of the endocrine disruptors. Bisphenol A is an organic pollutant which affects the reproductive system of wildlife and humans by mimicking or interfere the action of endogenous gonadal steroid hormones. Bisphenol A is the major component in the production of various consumer products, including plastic packing materials, detergents, epoxy resins, phenol resins, polycarbonates, polyacrylates, polyesters and lacquer coatings on food cans [5]. The presence of these compounds in the effluents at low but environmentally relevant levels indicates that the traditional technologies are not sufficiently effective to remove these compounds [6]. Therefore, new technologies are under development to target such compounds for the treatment including enzymatic processes, which are characterized by high reaction rates and high stoichiometric efficiencies [7].

The practical applications of the laccase in biotechnology point out the need of isolating microorganisms with laccase activity with different physicochemical and catalytic properties [8]. However, a majority of the previous studies have been focused on relatively few laccase producing bacterial species, such as *Pseudomonas desmolyticum* NCIM 2112, *Azospirillum lipoferum*, *Marinomonas mediterranea*, *Sinorhizobium meliloti*, *Bacillus substillus* SF, *Escherichia coli*, *Pseudomonas putida*, *Pseudomonas syringae* and *Ralstonia metallidurans* [9–13]. In recent years, an enzyme-catalyzed polymerization and precipitation process have been explored as a new method for the treatment of aqueous phenols. This approach was used widely for the removal of bisphenol A. Lignin peroxidase was extensively used for the treatment of the endocrine disruptors [14]. Laccase does not require the harmful hydrogen peroxide for the oxidative reaction like lignin peroxidase. Thus, several researchers preferred laccases for the removal of phenolic contaminants from the wastewater than lignin peroxidase [15].

In this paper, we are reporting the presence of an extracellular laccase activity in *Pseudomonas* sp. LBC1. The paper deals with the purification, characterization and identification of metabolites formed after degradation of bisphenol A.

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2. Experimental

2.1. Chemicals and microbiological media

Bisphenol A was obtained from Sd fine Chemicals, India. ABTS (2,2′-azinobis, 3-ethylbenzothiazoline-6-sulfonic acid) was obtained from Sigma Chemicals Company (St. Louis, MO, USA). *o*-Tolidine, hydroquinone, pyrogallol, benzidine, guaiacol, veratrol, L-DOPA and coomassie brilliant blue R-250 were obtained from SRL Chemicals, India. Veratryl alcohol, syringic acid, catechol, N,N′-dimethylphenylene diamine, caffeic acid, *p*-cresol, peptone, yeast extract and agar powder were obtained from Hi-Media laboratory, India. The protein markers were obtained from Bangalore Genei, India. The textile dyes were obtained from local industry at Ichalkaranji, India. All chemicals were of highest purity and of an analytical grade.

2.2. Isolation, screening and identification of microorganism

The microorganisms present in the soil samples from the effluent disposal site of a textile dyeing industry located in Solapur, India were enriched in the nutrient broth containing various textile dyes (100 mg of the dye l^{-1}) in static condition at 30 °C. After 48 h of incubation the 1 ml of cell suspension was transferred into fresh dye containing broth to screen the strain having color removing ability. The screening procedure in nutrient broth was continued until decolorization of the medium. A small amount of decolorized medium was transferred into nutrient agar plates containing various textile dyes (100 mg l^{-1}). Colonies surrounded by decolorized zones were selected and isolated by streak plate method. Isolates were then screened for their color removal ability in nutrient broth and the best isolate was selected. The 16S rRNA sequencing was done in genOmbio Pvt. Ltd. Pune, India. An isolated bacterium was analyzed on the basis of morphological and biochemical characteristics.

2.3. Microorganism, media and culture condition

Pseudomonas sp. LBC1 was grown at 30 °C under static condition and maintained on nutrient agar at 4 °C. The synthetic medium was used for an enzyme production. The composition of synthetic medium was NaCl 5 g l^{-1}, peptone 10 g l^{-1} and yeast extract 3 g l^{-1}.

2.4. Enzyme assays

Laccase activity was determined in a 2.5 ml reaction mixture containing 0.5 mM *o*-tolidine and 20 mM sodium acetate buffer (pH 4.0). The reaction was initiated by the addition of 0.2 ml of enzyme solution. The formation of the product was monitored at 366 nm [16]. One unit of enzyme activity was defined as the amount of enzyme required for an increase in 1.0 ABS unit min^{-1}.

Protein concentration was determined by Lowry method using bovine serum albumin as the standard [17]. The protein concentration was monitored based on the absorbance at 280 nm after an anion-exchange chromatography step.

2.5. Purification of laccase

The culture supernatant was collected by centrifugation of 24 h grown culture of *Pseudomonas* sp. LBC1 at 5000 rpm for 20 min. The chilled acetone was added slowly with constant stirring at 30% saturation into the culture supernatant and kept overnight at cold condition to precipitate the proteins. The precipitate formed after the treatment of acetone was dissolved in 50 mM potassium phosphate buffer (pH 8.0) and dialyzed against the same buffer for overnight. The dialyzed enzyme was applied on the DEAE-cellulose an anion exchange column (cylindrical glass column with 15 cm height and 1 cm diameter) equilibrated with same buffer at a flow rate of 1 ml min^{-1}. The retained proteins were eluted with a linear NaCl gradient from 0 to 0.4 M over 100 min.

2.6. Properties of purified laccase

The enzyme obtained after DEAE-cellulose an anion-exchange chromatography step was used for characterizations. Proteins in the enzyme preparation were analyzed by discontinuous sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and nondenaturing PAGE using vertical slab gel electrophoresis unit (the separating gel was 14% acrylamide, pH 8.3 and 5% stacking gel, pH 6.8). The activity staining was carried out using nondenaturing PAGE. The *o*-tolidine was used as a substrate for the activity staining [18]. The molecular mass of laccase was determined by SDS-PAGE using high molecular mass standards, such as phosphorylase b (98 kDa), bovine serum albumin (67.0 kDa), ovalbumin (44.0 kDa), GST (29.0 kDa) and lysozyme (16 kDa). Protein bands were visualized using coomassie brilliant blue R-250. Substrate specificity was studied using nonphenolic and phenolic compounds as substrate viz. veratryl alcohol, *o*-tolidine, ABTS, N,N′-dimethyl phenylenediamine, benzidine, pyrogallol, guaiacol, catechol, hydroquinone, tyrosine, *p*-cresol, and L-DOPA [18–23]. The oxidation of bisphenol A was determined in a reaction mixture containing the 0.1 mM bisphenol A and 20 mM sodium acetate buffer (pH 4.0). The reaction was initiated by the addition of 0.2 ml of enzyme solution. The decreased concentration of bisphenol A was monitored at 278 nm. One unit of enzyme activity was defined as amount of enzyme required to decrease 1.0 ABS unit min^{-1}. The H_2O_2 production was measured using the procedure reported earlier [24]. The effect of temperature and pH on laccase activity was studied by incubating the 0.100 U laccase at various temperatures viz. 20–50 °C, at pH 7.0 and various pH viz. 3–10, at 25 °C. The sodium citrate and sodium acetate buffers were used for maintaining the pH 3.0–6.0, potassium phosphate buffer for maintaining the pH 7.0–9.0 and sodium carbonate–sodium bicarbonate buffer for pH 9.0 and 10. Aliquots were transferred after specific time interval into the cuvette containing 0.5 mM *o*-tolidine and 20 mM sodium acetate buffer (pH 4.0) in order to determine the residual laccase activity. The purified laccase (30 μM) in 20 mM sodium acetate buffer (pH 4.0) was subjected for the wavelength scan (200–800 nm) on UV–vis spectrophotometer (Hitachi UV 2800). The redox potential of purified laccase was determined using cyclic voltammometer with 20 mV s^{-1} scan rate. The copper content was determined using atomic absorption spectrophotometer (PerkinElmer, model no. 4100).

2.7. Effect of metal salts, salinity and inhibitor on laccase activity

We have studied the effect of different metal salts (1 mM; $MgCl_2$, $CaCl_2$, $CoCl_2$, $MnCl_2$, $ZnSO_4$, $NiSO_4$, $CdCl_2$, KCl, and $CuSO_4$), salinity (1–100 mM) and inhibitors (5 and 15 mM; EDTA and sodium azide) on the laccase activity. The 0.100 U enzyme was incubated at various concentrations of metal salts, NaCl and inhibitors for 15 min. The laccase activity was determined using *o*-tolidine as substrate.

2.8. Bisphenol A degradation by using purified laccase

The reaction mixture for the degradation of bisphenol A contains 20 mM sodium acetate buffer (pH 4.0), 160 μM bisphenol A and 0.500 U purified laccase. The reaction mixture was incubated at optimum condition. Heat inactivated enzyme was used in the control experiment. Degradation of bisphenol A was measured using HPLC.

Table 1
Summary of purification of an extracellular laccase from cultural supernatant of *Pseudomonas* sp. LBC1.

Purification steps	Total activity (U)	Total protein (mg)	Specific activity (U mg^{-1})	Purification fold	Yield (%)
Crude culture	74	700	0.105	–	100
Acetone precipitation	17	90	0.185	1.76	22.65
DEAE-anion exchanger	1.0	1.54	0.649	6.1	1.35

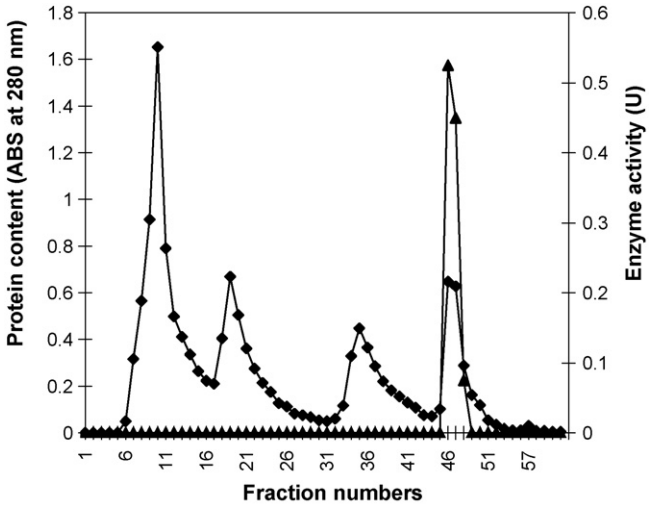


Fig. 1. DEAE-cellulose an anion exchange-chromatographic elution profile of laccase. The protein content in fractions (3 ml each) expressed in mg (■). The laccase activity in U (▲) as indicated by $A_{366\,nm}$, was assayed in each fraction with *o*-tolidine as substrate. The samples (200 μl) from each fraction were added in reaction mixture (see Section 2) and formation of oxidized product was measured at 366 nm.

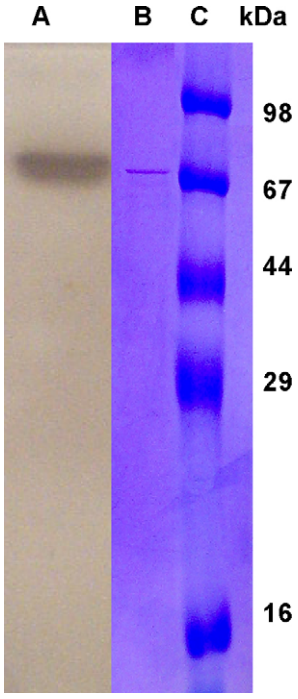


Fig. 2. Nondenaturing PAGE and SDS-PAGE of proteins obtained after purification of laccase from *Pseudomonas* sp. LBC1. Activity staining was carried out by using *o*-tolidine as substrate and protein staining by using coomassie brilliant blue as staining dye. The lane (A) represents the activity staining band of purified laccase on nondenaturing PAGE, lane (B and C) represents the protein staining band of purified laccase and molecular markers on SDS-PAGE, respectively.

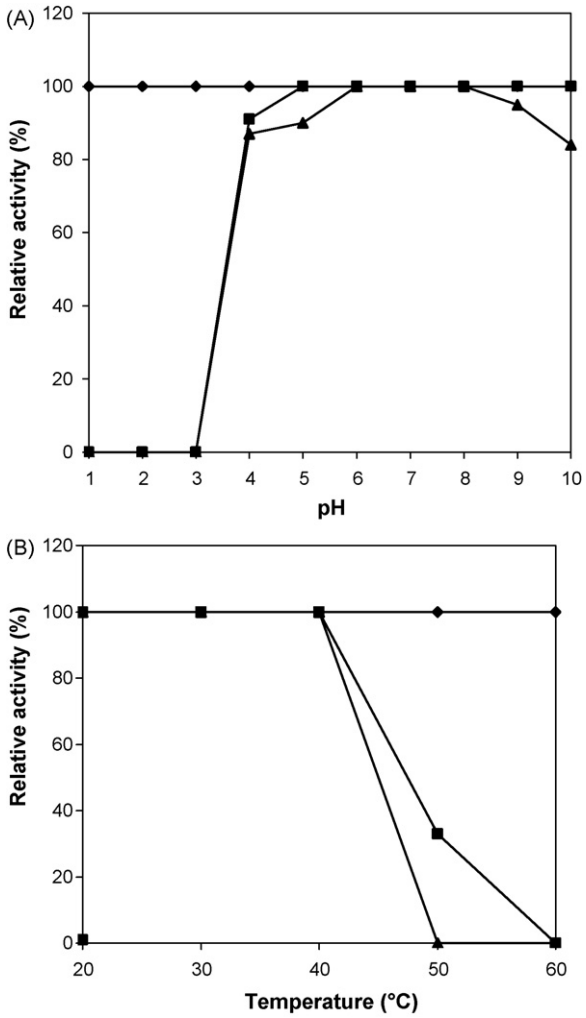


Fig. 3. The pH (A) and temperature (B) stability of purified laccase. Enzyme activity after 0 h (♦), after 2 h (■) and enzyme activity after 5 h (▲).

2.9. Extraction and analysis of biodegraded product

The reaction mixture was acidified to pH 2–3 by adding few drops of H_2SO_4. The mixture was extracted three times with 10 ml of ethyl acetate, with vigorous shaking. The combined organic phase was filtered over Na_2SO_4 on filter paper and concentrated in a rotary vacuum evaporator. The biodegradation of bisphenol A was analyzed using HPLC, FTIR and GC–MS. HPLC analysis was carried out (Waters model no. 2690) on C_{18} column (symmetry, 4.6 mm × 250 mm) using isocratic method with 10 min run time. The mobile phase was methanol with a flow rate 0.75 ml min^{-1} and UV detector at 280 nm. FTIR analysis was done in the mid-IR region of 400–4000 cm^{-1} with 16 scan speed. The pellets were prepared using spectroscopic pure KBr (5:95) and fixed in a sample holder. GC–MS analysis was carried out using a QP 5000 mass spectrophotometer (Shimadzu model no. U-2800). The ionization voltage was 70 eV. Gas chromatography was conducted in the temperature pro-

Table 2
The oxidation of phenolic and nonphenolic substrates by using *Pseudomonas* sp. LBC1 laccase.

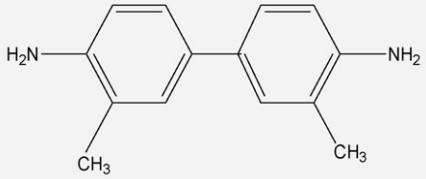
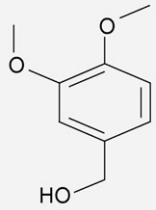
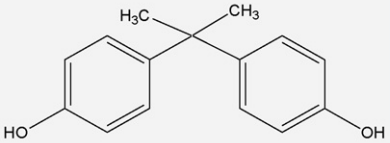
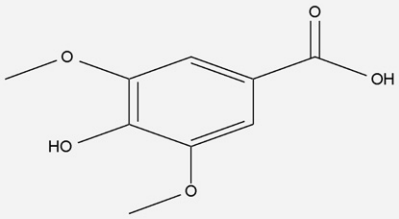
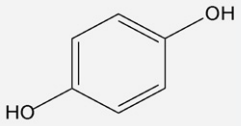
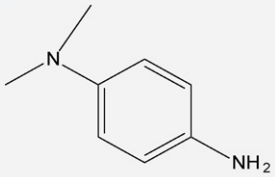
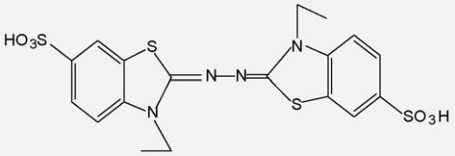
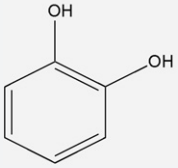
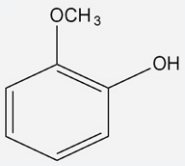
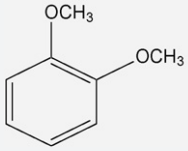
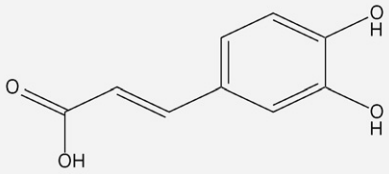
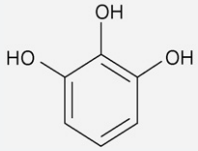
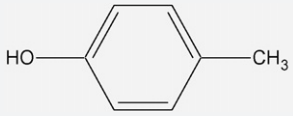
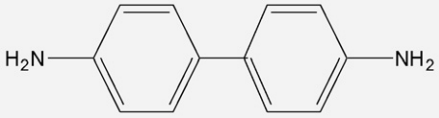
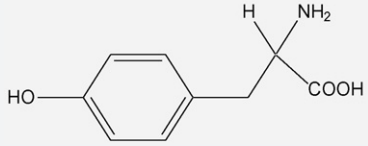
Substrates	Structures	λ_{max} (nm)	Specific activity[a]
o-Tolidine		366	0.242
Veratryl alcohol		310	0.134
Bisphenol A		278	0.097
Syringic acid		272	0.021
Hydroquinone		248	0.0097
N′N-dimethyl phenylenediamine		420	0.016
ABTS		420	0.003
Catechol		392	ND
Guaiacol		420	ND

Table 2 (*Continued*)

Substrates	Structures	λ_{max} (nm)	Specific activity[a]
Veratrole		310	ND
Caffeic acid		420	ND
Pyrogallol		450	ND
p-Cresol		400	ND
Benzidine		366	ND
Tyrosine		278	ND

ND: not detected.

[a] U mg^{-1} of protein min^{-1}.

gramming mode with a Resteck column (0.25 mm × 30 mm; XTI-5). The initial column temperature was 40 °C for 4 min, then increased linearly at 10 °C per min up to 270 °C and held for 4 min. The temperature of the injection port was 275 °C. GC–MS interface was maintained at 300 °C. The helium was used as carrier gas at 1 ml min^{-1} flow rate with 30 min run time. The compounds were identified on the basis of mass spectra and using the NIST library (version 1.10 beta Shimadzu) of the GC–MS.

3. Results and discussion

3.1. Isolation, screening and identification of microorganism

The efficient dye decolorizing bacterial isolate was identified as *Pseudomonas* sp. LBC1 on the basis of 16S rRNA sequence. The isolated organism was circular, small rods and gram negative in nature. It showed the positive test for catalase, oxidase, lysine decarboxylase, ornithine decarboxylase and urea hydrolysis.

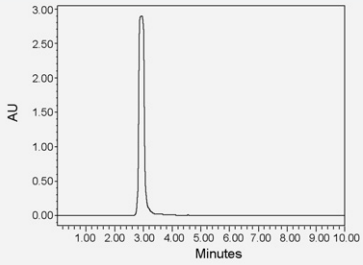
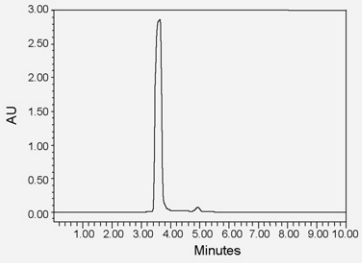
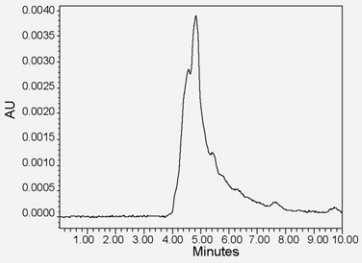
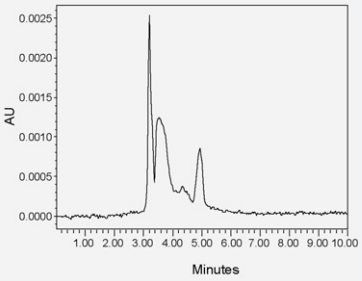
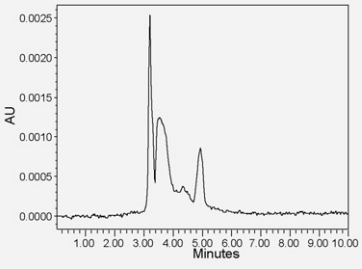
3.2. Purification and properties of laccase

Pseudomonas sp. LBC1 secretes an extracellular laccase in the synthetic medium after 24 h of growth while the fungal strain secretes laccase after 7 days of growth [25]. The extracellular laccase was purified using acetone precipitation and DEAE-cellulose an anion-exchange chromatography, whereas most of the eukaryotic laccases required more than two steps for the purification [26]. DEAE-cellulose an anion exchange elution profile of proteins was shown in Fig. 1. The procedure yielded 1.54 mg of purified enzyme from 900 ml of culture supernatant. The recovery of total laccase

Table 3
Assessment of *o*-tolidine oxidation rate of *Pseudomonas* sp. LBC1 laccase and eukaryotic laccases.

Enzyme source	Rate of oxidation of *o*-tolidine at 366 nm *A* min^{-1} mg^{-1} of protein	Reference
Commercial enzymes		
Pyricularia oryzae	0.127	
Agaricus bisporus tyrosinase	0.071	Miller et al. [16]
Crude extract		
Agaricus bisporous	0.0046	
Crimping	0.0032	Ratcliffe et al. [26]
Enoki	0.0024	
Oyster	0.0019	
Shiitake	2.081	
Extracellular fluid		
Agaricus bisporus laccase	1.149	Miller et al. [16]
Extracellular purified enzyme		
Pseudomonas sp. LBC1 laccase	0.649	

Table 4
HPLC analysis of bisphenol A and metabolites obtained after degradation of bisphenol A.

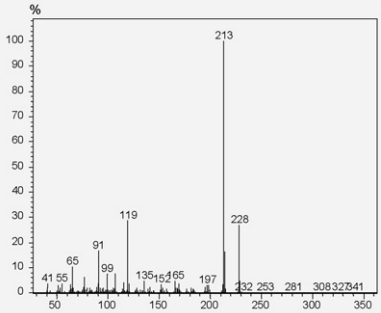
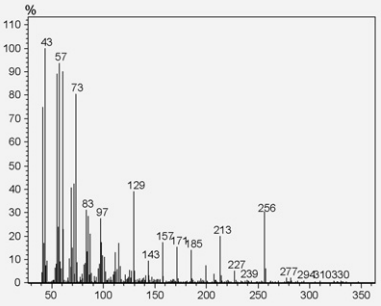
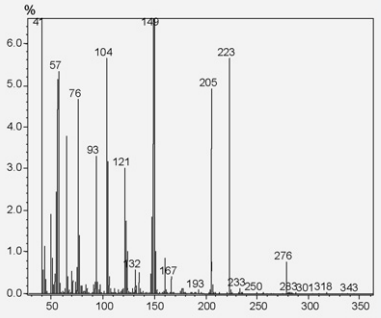
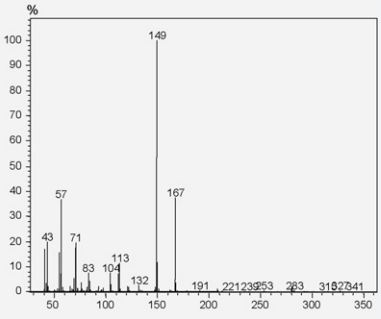
Time (h)	Bisphenol A	Rt. time (min)	Area (%)	Absorbance spectrum
0	100[a]	2.937	99.70	
1	2.0	3.650	95.70	
		4.930	2.0	
3	0.7	4.569	41	
		4.850	58	
5	00	3.201	37.96	
		3.534	44.24	
		4.928	17.80	
10	00	3.201	37.96	
		3.534	44.24	
		4.928	17.80	

[a] Initial concentration (160 μM) at 0 h was defined as 100%.

activity was 2% with 9.1-fold purification (Table 1). The purified laccase appeared as a single protein band both on SDS-PAGE and nondenaturing PAGE (Fig. 2). The molecular weight of *Pseudomonas* sp. LBC1 laccase (70 kDa) as determined by SDS-PAGE was consistent with the molecular weight of eukaryotic laccases (60–75 kDa) [27].

Atomic absorption spectroscopy revealed the presence of 2.9 mol copper atoms per mol of purified enzyme. Purified protein showed the absorbance maxima at 260 nm (ε_{260} was $3.4 \times 10^{-4}\,M^{-1}\,cm^{-1}$). The UV–vis spectrum of the purified laccase did not show any absorbance peak near 600 nm for type 1 blue copper site characteristic of eukaryotic laccases. Thus, *Pseudomonas* sp. LBC1 laccase is similar to the eukaryotic laccases that lack type 1 copper site [20,28,29]. *Pseudomonas* sp. LBC1 laccase belongs to the group of low redox potential laccases since cyclic voltammogram determined the redox potential of the purified lac-

Table 5
Mass spectral characteristics of bisphenol A and metabolites obtained after degradation of bisphenol A.

Metabolites	Rt. (min)	Mw (*m*/*z*)	Mass spectra
[I]	24.50	228	
[II]	22.04	256	
[III]	22.24	276	
[IV]	27.70	167	

[I] = Bisphenol A.
[II] = 2,3′-bis(4-hydroxyphenyl)-2-hydroxypropionaldehyde.
[III] = 2,3′-bis(4-hydroxyphenyl)-2,3-dihydroxypropionaldehyde.
[IV] = *p*-1,2-dihydroxyisopropyl phenol.

case as 0.30 V. The laccases are classified as high redox potential laccases (above 0.56 V) and low redox potential laccases (below 0.56 V) [27,30,31]. The *Pseudomonas* sp. LBC1 laccase showed optimum enzyme activity at pH 4.0 and 40 °C temperature, respectively, which was consistence with the optimum conditions of eukaryotic laccases [4].

The enzyme lost complete activity at below pH 3.0 and above 50 °C within 2 and 3 h, respectively (Fig. 3A and B). The *Pseudomonas* sp. LBC1 laccase oxidized a several compounds, including the methyl and methoxy group substituted phenolic and nonphenolic compounds viz. *o*-tolidine, ABTS, veratryl alcohol, hydroquinone, syringic acid, N,N′-dimethyl phenylene diamine and bisphenol A (Table 2). Introduction of OH, OCH_3, or CH_3 groups into the aromatic system renders the compound more easily oxidized by laccase [32]. The purified enzyme showed highest substrate specificity towards *o*-tolidine as compared to eukaryotic laccases (Table 3) [18]. Blue laccases did not oxidize the nonphenolic compound (veratryl alcohol) in a reaction mixture containing buffer, substrate and enzyme [33]. Oxidation of veratryl alcohol was observed when the reaction mixture was supplemented with suitable electron transfer mediator (ABTS and HBT). Also, the lignin peroxidase oxidized the nonphenolic compound (veratryl alcohol) without redox mediators. The veratrylaldehyde was the product of oxidation of this compound. *Pseudomonas* sp. LBC1 laccase oxidized the veratryl alcohol with the formation of veratrylaldehyde in the absence of suitable electron transfer mediator in the reaction mixture. No product of the reaction was found in the reaction mixture without enzyme or when purified enzyme was boiled for 20 min prior addition to reaction mixture. The ability of the purified enzyme to react with phenolic (hydroquinone and syringic acid) and nonphenolic compounds (veratryl alcohol and *o*-tolidine) explains the existence of bacterial phenolic and nonphenolic oxidation system without lignin peroxidase [34]. The K_m, V_{max} and K_{cat} for purified laccase were 0.25 mM, 2.09 U mg^{-1} of enzyme min^{-1} and 2.71 min^{-1}, respectively. The *Pseudomonas* sp. LBC1 laccase did not produce the hydrogen peroxide as a reaction product after oxidation of phenolic and nonphenolic substrates. This suggests the oxidation of substrate with concomitant reduction of O_2 to water [29].

3.3. Effect of metal salts, salinity and inhibitor on laccase activity

$CuSO_4$ showed marginal increase (3%) in the *o*-tolidine oxidation rate. The activation of laccase by $CuSO_4$ could be due to filling of type-2 copper binding sites with copper ions. The earlier report also showed reconstitution of the activity by the incubation with Cu^{2+} under aerobic conditions caused by the removal of type-2 copper of the laccase during purification [35]. However, $CaCl_2$ (12%), $MnCl_2$ (12%), $CoCl_2$ (4%), $NiSO_4$ (27%), $CdCl_2$ (23%), KCl (12%) and $ZnSO_4$ (4%) decreased the *o*-tolidine oxidation rate of purified laccase as compared to control. The purified laccase catalyzed the oxidation of *o*-tolidine at 50 mM salinity without decrease in the oxidation rate. However, 30% reduction in the *o*-tolidine oxidation rate of laccase was observed at 100 mM salinity. The *Pseudomonas* sp. LBC1 laccase was inhibited by EDTA and sodium azide as described in case of eukaryotic laccase [36].

3.4. Bisphenol A degradation using purified laccase and analysis of metabolites obtained after degradation of bisphenol A

The purified laccase removes (100%) the bisphenol A (160 μM) within 5 h without any redox mediator, whereas the eukaryotic laccases required 3 h for the degradation of 2.2 μM of bisphenol A [37]. The peroxidases required the redox mediators for the complete removal of bisphenol A [14]. The *Pseudomonas* sp. LBC1 laccase is catalytically efficient for the degradation of endocrine disruptor (bisphenol A) as compared to eukaryotic laccases. The difference in FTIR spectrum of bisphenol A and metabolites obtained after its degradation suggests biodegradation of bisphenol A (Fig. 4A and B). This observation suggests the unusual rearrangement of stable carbon–carbon bond. HPLC analysis of bisphenol A showed the absorbance peak at 2.937 min. The metabolites formed after 1 h incubation showed the absorbance peaks at 3.650 and 4.930 min. The metabolites formed after 3 h incubation showed the absorbance peaks at 4.569 and 4.850 min. The metabolites formed after 5 and 10 h incubation showed the absorbance peaks at 3.201, 3.534 and 4.928 min (Table 4) indicating no further metabolism after 5 h. The difference in retention time of bisphenol A and metabolites obtained after degradation of bisphenol A suggests the biodegradation of bisphenol A. The difference in the retention time of metabolites obtained after 1, 3 and 5 h incubations indicates the laccase reacts with the reaction intermediates. GC–MS analysis of metabolites obtained after degradation of bisphenol A suggest the 2,3′-bis(4-hydroxyphenyl)-2-hydroxy propionaldehyde [II], 2,3′-bis(4-hydroxyphenyl)-2,3-dihydroxypropionaldehyde [III] and *p*-1,2-dihydroxyisopropyl phenol [IV] or isomers of these compounds as possible metabolites formed after degradation of bisphenol A (Table 5).

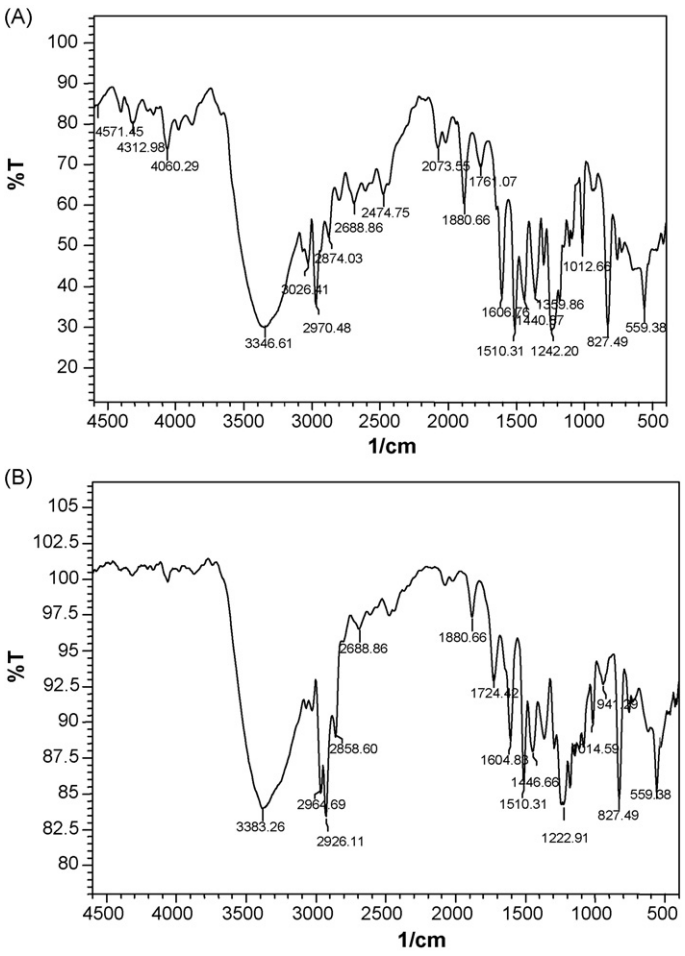


Fig. 4. FTIR analysis of bisphenol A (A) and metabolites formed after degradation of bisphenol A (B).

4. Conclusion

The *Pseudomonas* sp. LBC1 produces an extracellular laccase. It is an efficient biocatalyst for the complete removal of endocrine disruptors without redox mediators as compared to eukaryotic laccases and peroxidase.

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