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ORIGINAL ARTICLE

Production and characterization of laccase from *Pleurotus ferulae* in submerged fermentation

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Abstract *Pleurotus ferulae* is a mushroom typically found in arid steppe that is distributed widely in the Junggar Basin of Xinjiang, China. In this work, laccase production by *P. ferulae* JM30X was optimized in terms of medium composition and culture conditions. After optimization, the highest laccase activity obtained was 6,832.86 U/L. A single isozyme with a molecular weight of 66 kDa was observed by SDS-PAGE and native-PAGE. Optimum pH and temperature were 3.0 and 50–70 °C, respectively. The best laccase substrate was ABTS, for which the Michaelis-Menten constant (K_m) and catalytic efficiency (K_{cat}/K_m) value for *P. ferulae* laccase were 0.193 mM and 2.73×10^6 $(mM\ s)^{-1}$, respectively. The activity of purified laccase was increased by more than four-fold by Cu^{2+}, Mn^{2+} and Mg^{2+}, while it was completely inhibited by Fe^{2+} and Fe^{3+}. The production of laccase was influenced by the initial pH and K^+ concentration, and the activity of purified laccase was enhanced by Cu^{2+}, Mn^{2+} and Mg^{2+}. This *Pleurotus* genus laccase from *P. ferulae* JM30X was analyzed by MS spectrum and the results are conducive to furthering our understanding of *Pleurotus* genus laccases.

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Keywords *Pleurotus ferulae* · Laccase · Submerged fermentation · Metal ion

Introduction

Laccases (benzenediol:oxygen oxidoreductase, EC 1.10.3.2) are a group of blue multicopper oxidases that are capable of oxidizing mono-, di- and polyphenols, aminophenols, methoxyphenols, aromatic amines and ascorbic acid. Due to its catalytic properties, laccase is used in several industrial applications, including dye effluent decolorization (Chhabra et al. 2008; Jadhav et al. 2008), in the pulp and paper industry (Oudia et al. 2008; Virk et al. 2012) and in removal of herbicides (Coelho et al. 2010a, b). Recently, laccase was used as the cathode reducing oxygen to water in enzymatic biofuel cells. This process can produce a stable current with the use of sustainable and renewable resources.

Laccase is secreted either constitutively or can be induced during fungal growth and is involved in lignin degradation. It has been reported that laccase is produced mainly by white-rot fungi (WRF), such as *Phanerochaete chrysosporium* (Srinivasan et al. 1995), *Coriolus versicolor* (Arockiasamy et al. 2008) and *Pycnoporus cinnabarinus* (Meza et al. 2005). Recently, mushrooms have attracted much attention because of their laccase production and excellent enzymatic properties. Various species of the genus *Pleurotus*, including *P. ostreatus* (Tinoco et al. 2011), *P. sajor-caju* (Zucca et al. 2011), *P. florida* (Palvannan and Sathishkumar 2010), *P. eryngii* (Wang and Ng 2006) and *P. pulmonarius* (Tychanowicz et al. 2006), possess the ability to produce laccase.

Pleurotus ferulae is a typical mushroom of the arid steppe and is distributed widely in the Junggar Basin of Xinjiang, China (Liu et al. 2011). It grows mostly on the roots of *Ferula communis* and its fruiting body contains some active components, including carbohydrates, polysaccharides,

crude fiber, crude proteins, crude fat, ash, mineral elements and amino acids (Guo et al. 2007). *P. ferulae* is reported to contain nematicidal metabolites and to have nematicidal activity (Li et al. 2007). Some studies of *P. ferulae* have focused on the production of basidiocarp using agricultural wastes as substrate (Akydz and Yildiz 2007; Kirbag and Akyuz 2008). Punelli et al. (2009) reported detection of laccase activity in two *Pleurotus* spp. However, there are few reports focusing on the promotion of laccase production by optimization of culture conditions in submerged fermentation.

In this study, *P. ferulae* laccase was produced in submerged fermentation with lignocellulosic materials. The enzymatic properties and MS spectrum of the purified laccase were analyzed and the characterized laccase was compared with other *Pleurotus* laccases. This study adds to the characterization of *Pleurotus* laccase and will be beneficial for industrial applications.

Methods and materials

Fungal strain and culture media

The fungal strain, *Pleurotus ferulae* JM30X, was screened from the Junggar Basin (Xinjiang, China) and stored in our laboratory. Slants were inoculated and incubated at 25 °C for 16 days, then stored at 4 °C. The seed medium contained 20 g glucose, 10 g wheat bran, 10 g corn meal, 3 g KH_2PO_4 and 2 g $MgSO_4 \cdot 7H_2O$ per liter. The composition of the basic fermentation medium was similar to that of the seed medium and was used as control medium (pH 5.2) in the following experiments.

In order to determine the optimized composition of fermentation medium for laccase production, the effects of nutrients in the culture medium were investigated. The effect of lignocellulosic materials on laccase production was investigated using different materials at a concentration of 10 g/L instead of wheat bran. The effect of the initial pH of the medium on laccase production was monitored using pH values ranging from 3.0 to 10.0 (regulated by 2 M NaOH and 2 M HCl). To assess the effect of metal ions on laccase synthesis, eight metal salts were tested at respective concentrations of 10 mg/L.

Laccase assay and protein determination

Laccase activity was determined spectrophotometrically as described previously (Hou et al. 2004). A 1 mL reaction mixture contained 880 μL 100 mM sodium acetate buffer (pH 4.5), 100 μL ABTS stock (final assay concentration 1 mM) and 20 μL appropriately diluted crude culture broth or purified enzyme, and the reaction time was 4 min. The enzyme activity was calculated using the molar extinction coefficient of oxidized ABTS ($\varepsilon_{420}=3.6\times10^4$ mol^{-1} cm^{-1}) with one unit of activity defined as the amount of enzyme required to oxidize 1 μmol ABTS per minute. Protein content in cell extracts was measured as described by Bradford (1976).

Purification of laccase

The crude laccase was purified from culture broth using the optimized medium. All purification steps were carried out at 4 °C, with the laccase activity and protein content at each step. Laccase in the culture broth was precipitated using a 90 % saturated solution of $(NH_4)_2SO_4$. The precipitated protein was dissolved in 50 mM sodium acetate buffer (pH 6.0) and then dialyzed with the same buffer. The desalted protein was loaded on a Mono Q 5/50 GL strong anion exchange column and the column was equilibrated with 50 mM sodium acetate buffer (pH 6.0). The absorbed proteins were eluted with a linear salt gradient (0–0.8 mol NaCl) in 50 mM sodium acetate buffer (pH 6.0) over 20 column volumes at a flow rate of 0.5 mL/min. Laccase active fractions were pooled, dialyzed and then concentrated by ultramembrane centrifugation. A volume of 0.5 mL enzyme sample was loaded onto Superdex™ 75 10/300 GL and eluted with 50 mM sodium acetate buffer (pH 5.0) at 0.2 mL/min. The laccase-rich fractions were pooled and stored for subsequent analysis.

Polyacrylamide gel electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with 10 % resolving gels and 5 % stacking gels as described by Laemmli (1970). The SDS-PAGE gel was stained with Coomassie Brilliant Blue R-250 and the molecular mass was calculated using standard protein markers. Native-PAGE was performed similarly to SDS-

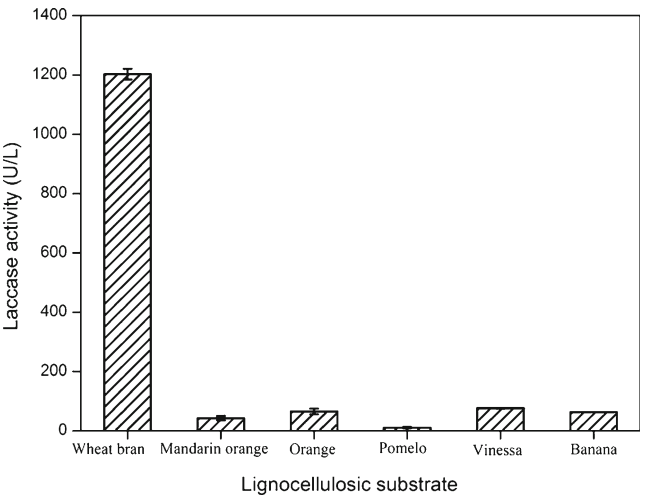

Fig. 1 Effect of different lignocellulosic materials on laccase production by *P. ferulae* in submerged fermentation

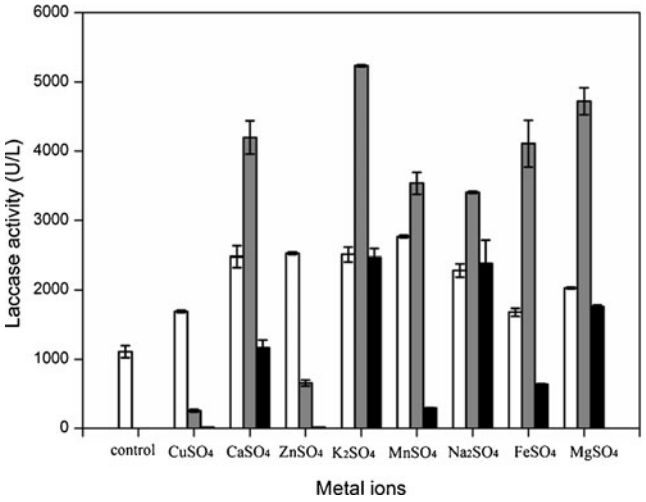

Fig. 2 Effect of different concentrations of metal salts on laccase production by *P. ferulae* in submerged fermentation: *white bars* 0.1 mM, *gray bars* 1.0 mM, *black bars* 5.0 mM

PAGE without SDS. Activity staining of laccase was carried out by incubating the native-PAGE gel in 50 mM sodium acetate buffer solution (pH 4.5) containing 1 mM ABTS at room temperature.

Effect of pH and temperature on laccase activity and stability

The influence of pH on laccase activity was determined over the pH range 2.0–8.0. Different pH gradients were obtained using KCl-HCl buffer (2.0), sodium acetate buffer (3.0–6.0) and sodium phosphate buffer (6.0–8.0). To determine the optimum temperature, the activity of laccase was examined at temperatures ranging from 20 °C to 80 °C. The stability of purified laccase at various pH levels was evaluated by incubating the reaction mixture in 100 mM sodium acetate buffer

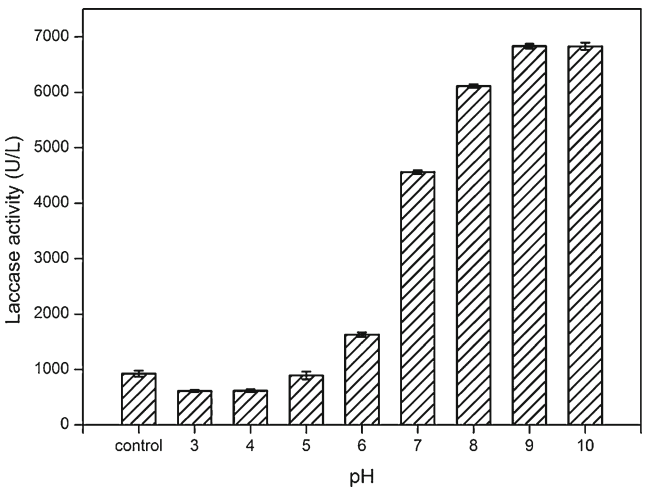

Fig. 3 Effect of different initial pH of the medium on laccase production by *P. ferulae* in submerged fermentation

Table 1 Summary of laccase purification from *P. ferulae*

Purification step	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Yield (%)	Purification fold
Culture supernatant	2,171	99.68	21.78	100	1
$(NH_4)_2SO_4$ precipitation	1,769	65.01	27.21	81.48	1.25
Mono Q	881.6	20.91	42.16	40.60	1.94
Superdex 75	584.2	5.53	105.6	26.91	4.85

(pH 2.0–8.0). The thermostability of the purified laccase was analyzed in 50 mM sodium acetate buffer (pH 4.5) at 20–80 °C. The relative activity of laccase was measured using ABTS as described above.

Effect of metal ions and inhibitors on laccase activity

To determine the effect of inhibitors, purified laccase was incubated with EDTA, DTT, PMSF and NaN_3 at various concentrations, and the enzyme activity was then tested. The influence of different metal ions on laccase activity was investigated as follows: the enzyme was incubated at different metal ion concentrations of 0.5, 1 and 5 mM for 1 h. Laccase activity was assessed with 1 mM ABTS as described above and each experiment was performed in triplicate.

Kinetic parameters of laccase

The activity of purified laccase under varying concentrations of ABTS, DMP, guaiacol, veratryl alcohol and tyrosine were

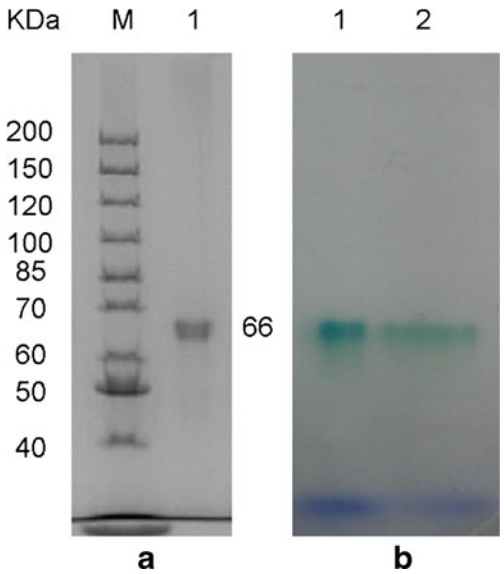

Fig. 4 SDS-PAGE and zymogram analysis of laccase purified from *P. ferulae*. **a** SDS-PAGE gel stained with Coomassie blue R-250. **b** Native-PAGE gel stained with 1 mM ABTS. Lanes: *M* Low molecular mass protein marker, *1* purified laccase, *2* laccase crude extract

Table 2 Comparison of the enzymatic properties of different laccase isolated from *Pleurotus* spp.

Source of laccase	Optimal temperature (°C)	Optimal pH	Molecular mass (kDa)	$t_{1/2}$	K_m (mM)	K_{cat}/K_m	Reference
Pleurotus ostreatus PO XA3a	35	ABTS=3.6 Syringaldazine=6.2 DMP=5.5	85	6 h	ABTS=7.0×10^{-2} Syringaldazine=3.6×10^{-2} DMP=14	ABTS=6.3×10^{7} (mM min)$^{-1}$ Syringaldazine=4.7×10^{6} (mM min)$^{-1}$ DMP=1.0×10^{5} (mM min)$^{-1}$	Palmieri et al. 2003
Pleurotus ostreatus PO XA3b	35	ABTS=3.6 Syringaldazine=6.2 DMP=5.5	83	14 h	ABTS=7.4×10^{-2} Syringaldazine=7.9×10^{-2} DMP=8.8	ABTS=1.3×10^{8} (mM min)$^{-1}$ Syringaldazine=8.9×10^{6} (mM min)$^{-1}$ DMP=1.4×10^{5} (mM min)$^{-1}$	
Pleurotus florida	50	o-Bianisidine=5 Guaiacol=5	77	o-Bianisidine=0.056 s Guaiacol=92.4 s	o-Bianisidine=0.13 Guaiacol=30.0	o-Bianisidine=6.32×10^{7} (mM s)$^{-1}$ Guaiacol=1.92×10^{3} (mM s)$^{-1}$	Das et al. 2000
Pleurotus sajor-caju	40	ABTS=4.5–5.0	61	n.d.[a]	ABTS=5.6×10^{-2} Syringaldazine=4.2×10^{-2}	ABTS=9.2×10^{5}(M s)$^{-1}$ Syringaldazine=8.7×10^{5} (M s)$^{-1}$	Murugesan et al. 2006
Pleurotus eryngii	70–80	ABTS=3–5	34	n.d.	n.d.	n.d.	Wang and Ng 2006
Pleurotus ostreatus D1	n.d.	Syringaldazine=7.0 Pyrocatechol=8.0 ABTS=4.0 DMP=4.0	64	n.d.	Syringaldazine=0.0087 Pyrocatechol=3.65 ABTS=0.11 DMP=0.43	n.d.	Pozdnyakova et al. 2006
Pleurotus pulmonarius	50	Syringaldazine=6.2–6.5 ABTS=4.0–5.5 Guaiacol=6.0–8.0	46	n.d.	Syringaldazine=1.2×10^{-2} ABTS=2.1×10^{-1} Guaiacol=5.5×10^{-1}	Syringaldazine=54.50 (mM s)$^{-1}$ ABTS=7.23(mM s)$^{-1}$ Guaiacol=0.56(mM s)$^{-1}$	De Souza and Peralta 2003
Pleurotus ostreatus strain 10969	4.0	ABTS=50	40	n.d.	ABTS=0.31	n.d.	Liu et al. 2009
Pleurotus florida	50	n.d.	70	*o*-dianisidine=0.012 s Guaiacol=1.47 s	*o*-dianisidine=0.045 Guaiacol=2.81	*o*-dianisidine=4.13×10^{7} (mM s)$^{-1}$ Guaiacol=3.31×10^{5} (mM s)$^{-1}$	Das et al. 2001
Pleurotus ostreatus	n.d.	n.d.	55	n.d.	ABTS=15×10^{-2} DMP=27×10^{-2} Syringaldazine=5.5×10^{-2}	n.d.	Lettera et al. 2010
Pleurotus ostreatus	n.d.	n.d.	71	n.d.	*o*-dianisidine=1.72×10^{-2} Guaiacol=4 ABTS=5.52×10^{-2}	n.d.	Freixo et al. 2012
Pleurotus cornucopiae	40	4	66	n.d.	n.d.	n.d.	Wong et al. 2010
P. ferulae JM30X	50	3	66	50 °C=7.59 h	ABTS=0.19 DMP=3.77 Veratryl alcohol=70.21 Guaiacol=22.24 Tyrosine=6.87	ABTS=2.73×10^{6}(mM s)$^{-1}$ DMP=4.54×10^{4} (mM s)$^{-1}$ Veratryl alcohol=6.85×10^{4} (mM s)$^{-1}$ Guaiacol=1.87×10^{3} (mM s)$^{-1}$ Tyrosine=1.21×10^{5} (mM s)$^{-1}$	This work

[a] Not detected

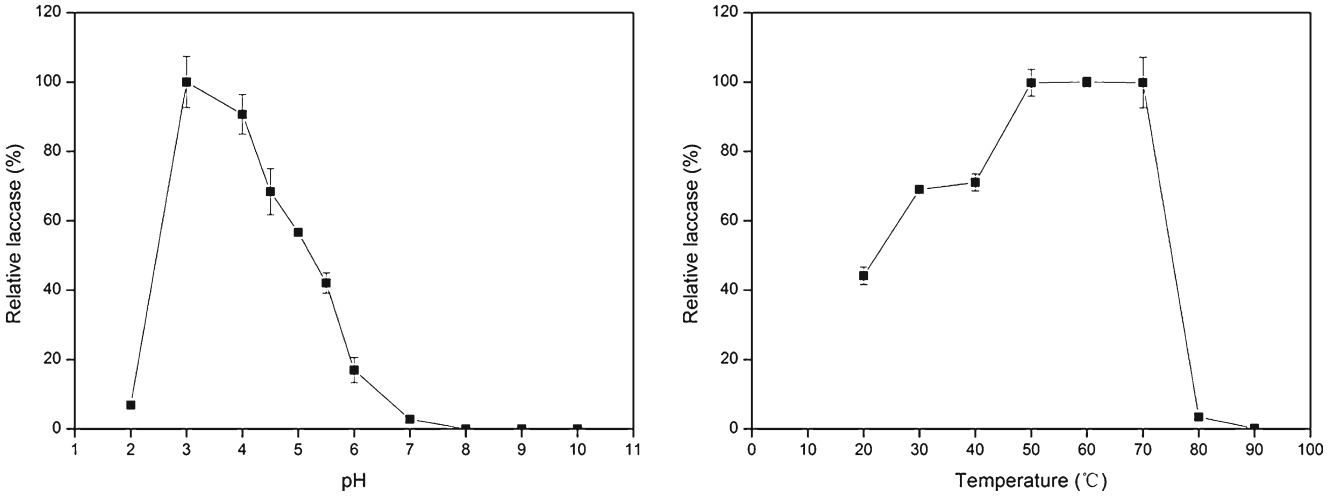

Fig. 5 Effect of pH and temperature on the activity of laccase purified from *P. ferulae*. **a** Effect of pH on laccase activity at 30 °C using ATBS as a substrate. **b** Effect of temperature on laccase activity at pH 3.0 using ATBS as a substrate

determined, and the Michaelis-Menten constant (K_m) and catalytic constants (K_{cat}) were calculated by a Lineweaver-Burk plot using the Michaelis-Menten equation.

Analysis of protein sequence by mass spectrometry

The laccase band in the SDS-PAGE gel was excised and subjected to in-gel trypsin digestion as previously described (Finnie et al. 2006). The peptides in the digest mixture were analyzed by Ultraflex™ MALDI-TOF-TOF-MS (matrix-assisted laser desorption/ionization-time of flight-mass spectrometry, Bruker-Daltonics, Germany). The experimental mass values were compared with the NCBInr database at the National Center for Biotechnology Information.

Statistic analysis

Each experiment was performed in triplicate. The results shown are the average of three replicates, with error bars indicating standard deviation.

Results

Effect of culture conditions on laccase production by *P. ferulae*

The effect of five different lignocellulosic materials on laccase production was investigated at concentrations of 10 g/L (Fig. 1). The results showed that all tested materials had a

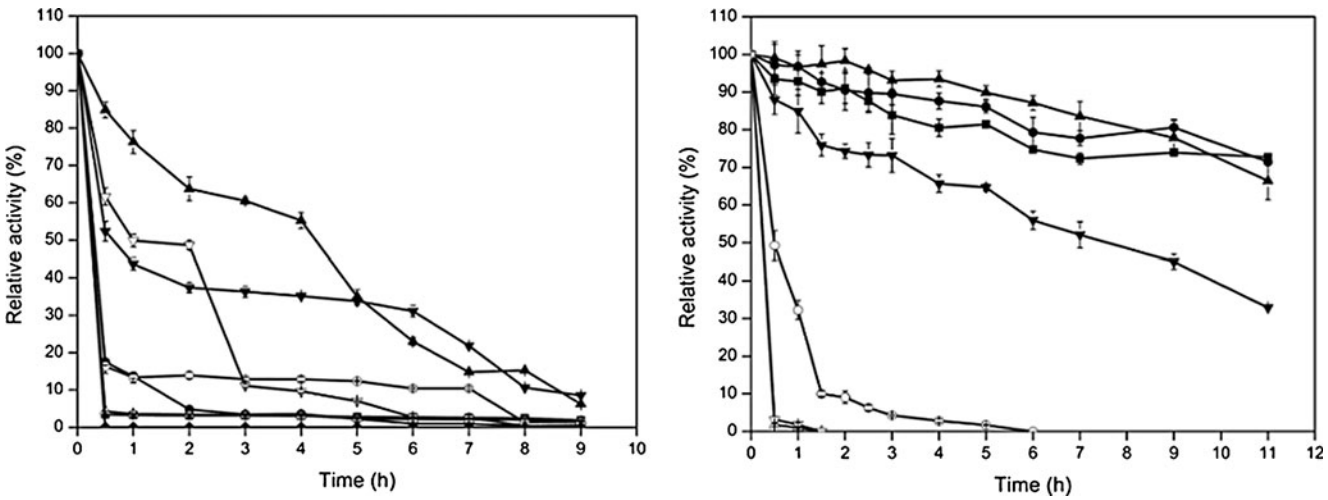

Fig. 6 The pH and thermal stability of laccase purified from *P. ferulae*. **a** pH stability over 9 h using ATBS as a substrate: ■ pH 2.0, ● pH 3.0, ▲ pH 4.0, ▽ pH 4.5, ▼ pH 5.0, ○ pH 6.0, △ pH 7.0, ◆ pH 8.0. **b** Thermal stability over 9 h using ATBS as a substrate: ■ 20 °C, ● 30 °C, ▲ 40 °C, ▼ 50 °C, ○ 60 °C, △ 70 °C, ▽80 °C

negative influence on laccase synthesis compared to the control medium (1,002.62 U/L). The effect of metal ions on laccase production was studied by adding different salts (Fig. 2). All metal ions at 0.1 mM enhanced laccase production. The highest laccase activity of 5,231.65 U/L was obtained by adding 1 mM potassium salt, resulting in a 4.71-fold enhancement. The effects of an initial pH between 3.0 and 10.0 on laccase production were investigated compared to the control (pH 5.2). After fermentation, the culture broth with different initial pH had a terminal pH value of 4.5. The result indicated that an initial alkalic pH was beneficial for laccase production, and 6,832.86 U/L was obtained using an initial pH of 9.0 (Fig. 3).

Purification and gel electrophoresis of *P. ferulae* laccase

Pleurotus ferulae laccase was purified by $(NH_4)_2SO_4$ precipitation, anion exchange and gel filtration chromatography, as summarized in Table 1. The purified laccase was analyzed by SDS-PAGE and native-PAGE, and one major band was observed after PAGE under denaturing and non-denaturing conditions (Fig. 4). The results indicated a molecular weight of *P. ferulae* laccase of 66 kDa, and that a single laccase isoform was isolated in the purified enzyme extract.

Kinetic properties of *P. ferulae* laccase activity

The kinetic parameters of *P. ferulae* laccase using ABTS, DMP, guaiacol, tyrosine and veratryl alcohol as substrate are shown in Table 2. Among the tested substrates, the lowest K_m value of 0.19 mM and the highest catalytic efficiency (K_{cat}/K_m) were obtained using ABTS, which exhibited high affinity with *P. ferulae* laccase.

Effect of pH and temperature on *P. ferulae* laccase activity and stability

The effects of pH and temperature on laccase activity are shown in Fig. 5. The optimal pH for laccase activity was 3.0, and high laccase activity was obtained over a temperature range of 50 °C–70 °C. *P. ferulae* laccase exhibited the interesting property that its highest activity was maintained over this wide range of tested temperatures.

The pH stability of the purified laccase was investigated by testing residual laccase activity after incubating the enzyme for 9 h at pH values between 2.0 and 8.0. The *P. ferulae* laccase showed higher stability under acidic conditions and remained 60 % active at a pH of 3.0 for 4 h. The thermal properties of *P. ferulae* laccase are depicted in Fig. 6b. The laccase remained active at more than 70 % during a 9-h incubation below 40 °C, and 54 % residual activity was obtained under 50 °C in a 7-h incubation. The enzyme was completely inactivated after 3 h as the temperature was increased to 80 °C.

Table 3 Effect of inhibitors on purified laccase activity from *P. ferulae*

Inhibitor	Inhibition (%)		
	0.1 mM	0.5 mM	1 mM
EDTA	53±1.70	68±3.34	72±3.03
DTT	100±0	100±0	100±0
PMSF	74±4.75	78±0.78	100±0
NaN_3	98±0.64	100±0	100±0

Effect of metal ions and inhibitors on *P. ferulae* laccase activity

The effect of various concentrations of inhibitors and metal ions was investigated and the results are shown in Tables 3 and 4. The results revealed that laccase activity was greatly enhanced by most metal ions and that an increase of 488.51 % was obtained by adding 0.1 mM Cu^{2+}. However, increasing the metal ion concentration from 0.1 mM to 1.0 mM decreased the enhancement of laccase activity. The laccase activity was completely inhibited by all concentrations of Fe^{2+} and Fe^{3+}. Of four potential inhibitors, laccase activity was completely inhibited by DTT and NaN_3 at all concentrations; however, 53 % laccase activity was obtained when adding 1.0 mM EDTA.

Analysis of protein sequence by mass spectrometry

The amino acid sequence of *P. ferulae* laccase was determined by MALDI-TOF-TOF-MS. Figure 7 shows the MS spectrum of *P. ferulae* laccase, with three peptides matching sequences in the database. As shown in Table 5, these three peptide sequences were identical with the laccase from *P. ostreatus* (57.64 kDa, gi|15594026) and *P. sapidus* (57.67 kDa, gi|67508841). This result indicated that we had isolated a *Pleurotus* genus protein. However, some differences

Table 4 Effect of metal ions on purified laccase activity from *P. ferulae*

Metal ions	Relative activity (%)		
	0.1 mM	1.0 mM	5.0 mM
$CuSO_4$	488.51±3.80	211.27±4.17	87.88±5.52
$CaSO_4$	15.75±8.38	186.73±9.12	109.52±10.96
$ZnSO_4$	214.37±14.77	168.47±8.17	66.22±5.44
$MnSO_4$	424.78±7.30	224.10±11.12	79.88±12.69
$MgSO_4$	406.57±1.29	191.10±1.72	66.20±4.55
$FeSO_4$	0	0	0
$Fe_2(SO_4)_3$	0	0	0
K_2SO_4	327.99±14.08	182.79±11.00	78.03±8.17

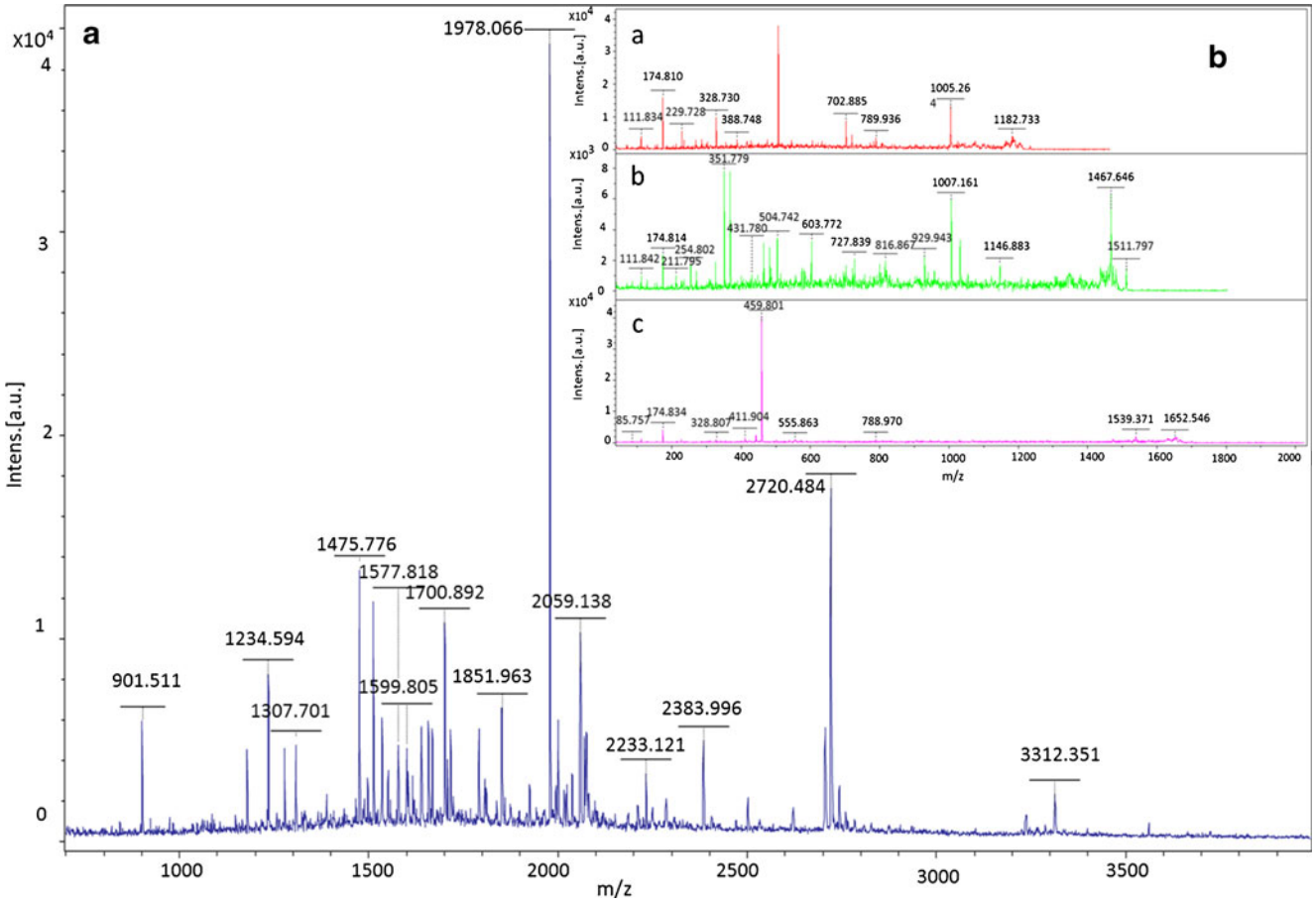

Fig. 7 MALDI-TOF-TOF-MS (matrix-assisted laser desorption/ionization-time of flight-mass spectrometry) spectra of the laccase band from *P. ferulae*. **a** MALDI-TOF mass spectrum of peptides generated by tryptic digestion of laccase. **b** MALDI-TOF/TOF spectrum of the precursor ion with different m/z: *a* 1,234.59, *b* 1,511.80, *c* 1,700.89

from other *Pleurotus* laccases indicated that the *P. ferulae* laccase isolated here is a novel laccase.

Discussion

It has been reported that some strains of *Pleurotus* genus produce laccase during mycelium growth; however, few studies have yet revealed laccase production by *P. ferulae*. In this work, the laccase-producing strain *P. ferulae* JM30X was investigated.

Laccase production by *Ganoderma lucidum* was significantly induced by peels of plants of the *Rutaceae* family (Ding et al. 2012), while banana peel was found to enhance production more effectively than mandarin peel in *Pleurotus florida* (Sathishkumar et al. 2010). A range of lignocellulosic materials, including vinessa, the peels of *Rutaceae* fruits and banana peel, were applied in this work to promote the synthesis of laccase by *P. ferulae* JM30X. Unlike in previous reports, the *Rutaceae* fruit and banana peels had no stimulatory effect on laccase production compared to the control medium containing wheat bran.

It has been reported that laccase production by *Ganoderma applanatum* and *Peniophora* sp. was stimulated 49.2-fold and 19.7-fold, respectively, by adding copper (Fonseca et al. 2010). The promoting effect of copper was attributed to the regulation

Table 5 Mass spectrometry of *P. ferulae* laccase

Peptide residues start-end	Sequence	m/z	Matched information		
			Organism	Accession no.	Mr (kDa)
30–40	R.NDVVSPDGFER.R	1,234.59			
62–76	R.VQINTINELTDPGMR.R	1,511.80	*Pleurotus ostreatus*	gi|15594026	57.64
432–445	R.TSNSDVVNLVNPPR.R	1,700.89	*Pleurotus sapidus*	gi|67508841	57.67

of laccase gene transcription (Collins and Dobson 1997). However, the addition of copper at various concentrations did not lead to the expected improvement of laccase production in *P. ferulae* JM30X. Potassium at 1.0 mM yielded the highest production of laccase, which was similar to a previous report in *Schizophyllum commune* (Irshad and Asgher 2011). The initial pH of the culture medium strongly influenced laccase production, with highest activity obtained at a pH of 9.0. Laccase production was stimulated more effectively by initial alkalic pH conditions, which has previously not been reported.

Many *Pleurotus* spp. laccases have been purified and characterized and their enzymatic properties are summarized in Table 2. Some species of *Pleurotus* possess a number of different laccase isozymes with molecular masses ranging from 34 kDa to 85 kDa (Palmieri et al. 2003; Pozdnyakova et al. 2006; Wang and Ng 2006). In this study, a single isoform laccase of 66 kDa was purified from *P. ferulae* fermentation; the molecular weight of this laccase was similar to that of *P. cornucopiae* laccase (Wong et al. 2010). Laccase is considered to be non-specific, oxidizing a range of phenolic and non-phenolic substrates. Among the selected substrates, ABTS was the ideal substrate for *P. ferulae* laccase, in accordance with other fungal laccases (Hildén et al. 2007; Wang et al. 2010a).

The optimal pH for laccase activity varied with different substrates, whereas some fungal laccases exhibit optimal catalytic activity under acidic pH conditions when using ABTS as a substrate (Liu et al. 2009; Halaburgi et al. 2011). Our results are in accordance with many previous studies indicating that *P. ferulae* laccase is stable over a narrow pH range (Hildén et al. 2007; Halaburgi et al. 2011). Optimal temperatures for *P. florida* and *P. sajor-caju* laccases are reported as 50 °C and 40 °C, respectively. Wang and Ng (2006) reported that 70–80 °C was beneficial to *P. eryngii* laccase. In this study, high laccase activity was obtained between 50 °C and 70 °C, which differed from previous reports. Therefore, given the extensive range of thermal stability of *P. ferulae* laccase, it would be more suitable for the textile and paper industry. The properties of the new *P. ferulae* laccase are attractive and the application of this laccase will be developed in further research.

Thermal stability is an important enzyme property for industrial applications, and many thermostable laccases have been found in various fungi (Wang et al. 2010b). Vite-Vallejo et al. (2009) reported that the half life of *Pycnoporus sanguineus* laccase was 2 h at 60 °C, with similar results obtained from *G. lucidum* (Murugesan et al. 2007). Laccase produced by *Pleurotus* spp. have been reported to possess thermostability, whereas the thermal properties of *P. ferulae* laccase remained unclear.

Recently, laccases have been studied for use in the treatment of effluents from the textile industry, which contain abundant dyes as well as metal ions. The activity of some fungal laccase is highly sensitive to heavy metal ions. Liu et al. (2009) revealed that *P. ostreatus* laccase was inhibited by all metal ions tested, especially Ag^{+}. However, laccase activity was enhanced by other metal ions, such as Ca^{2+}, Co^{2+}, Cu^{2+} and Zn^{2+} for *G. lucidum* (Murugesan et al. 2009), and $Cu^{2+}$and Mg^{2+} for *Pycnoporus* sp. SYBC-L1 (Wang et al. 2010a). In our work, *P. ferulae* laccase exhibited much higher improvement by metal ions than other fungal laccases, with the highest enhancement of 488.51 % being obtained by adding 0.1 mM Cu^{2+}.

Conclusions

In this work, the production of laccase was influenced greatly by initial pH levels, while laccase activity was also enhanced by some metal ions. Sequence analysis indicated that the purified laccase was a *Pleurotus* spp. protein. This work was focused on the laccase production by *P. ferulae* in submerged fermentation and extends the range of laccase production by *Pleurotus* fungi.

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