

## A novel laccase from fresh fruiting bodies of the wild medicinal mushroom *Tricholoma matsutake*

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The knowledge about biological activities of constituents from medicinal mushrooms belonging to the genus *Tricholoma* is limited. A 59-kDa laccase has now been purified from fresh fruiting bodies of the mushroom *Tricholoma matsutake*. The purification protocol entailed ion exchange chromatography on DEAE-cellulose, affinity chromatography on Affi-gel blue gel, ion exchange chromatography on CM-cellulose, affinity chromatography on ConA-Sepharose, and gel filtration by fast protein liquid chromatography on Superdex 75. Of the various affinity and ion exchange chromatographic media employed, the laccase bound only on Con A-Sepharose. The activity of the laccase did not undergo major changes over the temperature range 20–80°C. However, all activity vanished following exposure to 100°C for 10 minutes. The enzyme activity varied only slightly over the pH range 3–5, with the optimal pH of 5, but exhibited a precipitous decline when the pH was increased to 6, and was undetectable at pH 8 and 9. The laccase showed activity in the decolorization of azo dyes without a mediator. Its N-terminal sequence demonstrated only slight resemblance to those of other mushroom laccases. The newly described laccase is distinctive from the previously isolated *Tricholoma* mushroom laccases in a number of aspects.

**Key words:** *Tricholoma matsutake*, wild mushroom, isolation, laccase, decolorization

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### INTRODUCTION

Laccase (EC 1.10.3.2) is a polyphenol oxidase, belonging to the family of blue multicopper oxidases (Kiiskinen *et al.*, 2002). Laccase was first discovered in exudates of the Japanese lacquer tree *Rhus vernicifera* in 1883 (Reinhammar, 1970). These enzymes are not only widespread in plants, but are also found in bacteria and fungi (Dwivedi *et al.*, 2011). Laccases oxidize a broad range of substrates such as polyphenols, methoxy-substituted phenols, diamines, and some inorganic compounds (Piontek *et al.*, 2002). Due to their broad substrate specificity, laccases are widely used in many industrial processes like lignin biodegradation, biosensor, biopulping, textile dye bleaching, phenolics removal, effluent detoxification, and others (Xu, 2005; Palmieri *et al.*, 1993; May, 1999).

Fungi are the main producers of laccases, especially basidiomycetes (Garzillo *et al.*, 2001; Coll *et al.*, 1993;

Shin & Lee, 2000; Cambria *et al.*, 2000; Wang & Ng, 2004a; Wang & Ng, 2004b; Zhang *et al.*, 2010; Li *et al.*, 2010). Fungal laccases are particularly abundant in many white-rot fungi, however, very few laccases have been reported from ectomycorrhizal fungi. *Tricholoma matsutake*, a well-known wild edible mushroom, is an ectomycorrhizal fungus belonging to Tricholomataceae family (Tong *et al.*, 2013). Due to its unique flavor and taste, it is a highly prized mushroom compared with the much less expensive *T. mongolicum* and *T. giganteum*. The knowledge about the constituents of mushrooms belonging to the genus *Tricholoma* is limited. Polysaccharopeptides with immunomodulatory and antitumor activities have been prepared from cultured mycelia of *T. mongolicum* and *T. lobayense* (Wang *et al.*, 1996; Wang *et al.*, 1995). A laccase has been purified from *T. mongolicum* (Li *et al.*, 2010). A nuclease (Hatakeyama *et al.*, 2010) and polysaccharide (Tong *et al.*, 2013) have been isolated from the fruiting body of *T. matsutake*. Lectins with antiproliferative, antitumor and immunoenhancing activities have been isolated from fruiting bodies of *T. mongolicum* (Wang *et al.*, 1995). One of these lectins produces hypotensive effects in rats (Wang *et al.*, 1996). Hence it would be a worthy undertaking to examine additional *Tricholoma* species for the presence of bioactive constituents. The present investigation disclosed that fresh fruiting bodies of *T. matsutake* produce a laccase with some unique characteristics and its potential application in the decolorization of various dyes has been examined.

### MATERIALS AND METHODS

**Isolation of laccase.** Fresh fruiting bodies (900 g) of the mushroom *Tricholoma matsutake* were homogenized in distilled water (3 mL/g) using a Waring blender. Distilled water was used instead of physiological saline to obviate the need for subsequent dialysis. The homogenate was centrifuged at 18500 × *g* for 30 minutes. Tris-HCl buffer (1 M, pH 7.4) was added to the resulting supernatant to 10 mM. The supernatant was then loaded on a 5 × 20 cm column of DEAE-cellulose (Sigma) in 10 mM Tris-HCl buffer (pH 7.4). Unbound proteins were collected in the flowthrough fraction D1. Bound proteins were eluted with 0.8 M NaCl in the

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**Abbreviations:** CM, carboxymethyl; DEAE, diethylaminoethyl; ELISA, enzyme-linked immunosorbent assay; HPLC, high performance liquid chromatography; RT, reverse transcriptase; SDS, sodium dodecyl sulfate

starting buffer and collected in fraction D2. Fraction D1 was then subjected to affinity chromatography on a  $2.5 \times 20$  cm column of Affi-gel blue gel (Bio-Rad) in 10 mM Tris-HCl buffer (pH 7.4). Unbound proteins were eluted with the same buffer and collected as fraction B1. Adsorbed proteins were eluted with 1.5 M NaCl in 10 mM Tris-HCl buffer. Fraction B1 was fractionated on a  $2.5 \times 20$  cm column of CM-cellulose (Sigma) in 10 mM  $\text{NH}_4\text{OAc}$  buffer (pH 4.5). This step was in conformity with the frequently reported use of ion exchange chromatography at an acidic pH for laccase purification. The unbound fraction CM1 was eluted with the same buffer. The bound fraction CM2 was eluted with 1 M NaCl in starting buffer. Fraction CM1 was further purified on a  $2.5 \times 20$  cm column of Con A-Sepharose (GE Healthcare). Following elution of unbound proteins (fraction Con A1) with 50 mM Tris-HCl buffer (pH 7.4) containing 0.5 M NaCl, 10 mM  $\text{CaCl}_2$  and 10 mM  $\text{MgCl}_2$ , the bound proteins (fraction Con A2) were eluted with 0.4 M  $\alpha$ -methyl-D-glucopyranoside (Sigma) in the 50 mM Tris-HCl buffer.

Fraction ConA2 was dialyzed, lyophilized and then chromatographed on a Superdex 75 Hr 10/30 column (GE Healthcare) in 0.2 M  $\text{NH}_4\text{HCO}_3$  buffer (pH 8.5). The first eluted peak constituted purified laccase.

**Assay of laccase activity.** Laccase activity was assayed by measuring the oxidation of 2,2'-azinobis [3-ethylbenzothiazolone-6-sulfonic acid] diammonium salt (ABTS). A modification of the method of Shin and Lee (2000) was used. An aliquot of enzyme solution was incubated in 1.3 mL of 67 mM sodium acetate buffer (pH 4.5) containing 1.54 mM ABTS at 30°C. One unit of enzyme activity was defined as the amount of enzyme required to produce an absorbance increase at 405 nm of one per min per mL of reaction mixture under the aforementioned conditions. All determinations were performed in triplicate.

**Molecular mass determination by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and by FPLC gel filtration.** SDS-PAGE was carried out in accordance with the procedure of Laemmli and Favre (1973) using a 12% resolving gel and a 5% stacking gel. At the end of electrophoresis, the gel was stained with Coomassie brilliant blue. FPLC gel filtration in 0.2 M  $\text{NH}_4\text{HCO}_3$  buffer (pH 8.5) at a flow rate of 24 mL/h and with a fraction size of 0.8 mL was carried out using a Superdex 75 column which had been calibrated with molecular mass standards (GE Healthcare).

**Analysis of N-terminal amino acid sequence.** Amino acid sequence analysis was carried out using an HP G1000A Edman degradation unit and an HP1000 HPLC system (Lam & Ng, 2001).

**Assay of pH and temperature optima.** In the assay for optimal pH value, a series of solution of ABTS

in buffers with different pH values was used. The assay buffers were prepared in 0.1 M NaOAc buffer (pH 3.0, pH 4.0 and pH 5.0), 0.1 M Mes buffer (pH 5.0, pH 6.0 and pH 7.0), and 0.1 M Hepes buffer (pH 7.0, pH 8.0 and pH 9.0). The assay temperature was 37°C. To determine the optimal temperature, the reaction mixture was incubated at 20°C to 90°C in 0.1M NaOAc buffer (pH 5.0).

**Assay of substrate specificity.** In order to determine the substrate specificity of the purified laccase, several aromatic substrates were added to the assay media in place of ABTS. The substrates used (5.0 mM) were ABTS, N,N-dimethyl-1,4-phenylenediamine, hydroquinone, catechol, 2-methylcatechol, pyrogallol, and tyrosine. The enzyme assay was performed as described above in 50 mM  $\text{Na}_2\text{HPO}_4$ -citric acid buffer (pH 3.0). The substrate oxidation rate was followed by measuring the absorbance change with the molar extinction coefficients ( $\epsilon$ ) obtained from the literature (EggertTemp & Eriksson, 1996; Galhaup *et al.*, 2002; Younes & Sayadi, 2011).

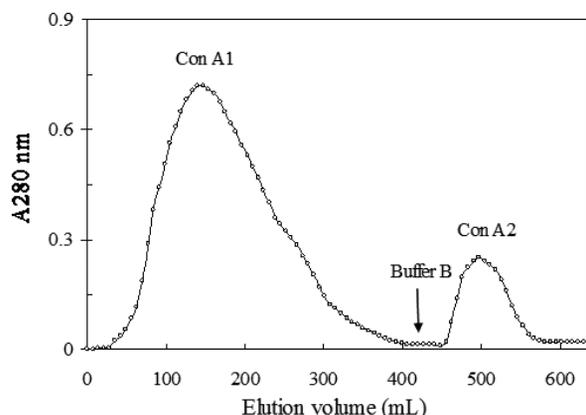
**Assay for HIV-1 reverse transcriptase inhibitory activity.** The assay for HIV-1 reverse transcriptase inhibitory activity was carried out according to instructions supplied with the assay kit from Boehringer Mannheim (Germany). The assay takes advantage of the ability of reverse transcriptase to synthesize DNA, starting from the template/primer hybrid poly (A) oligo (dT)<sub>15</sub>. Digoxigenin- and biotin-labeled nucleotides in an optimized ratio are incorporated into the DNA molecule synthesized by the reverse transcriptase (RT). The detection and quantification of synthesized DNA as a parameter for RT activity follows a sandwich ELISA protocol. Biotin-labeled DNA binds to the surface of microtiter plate precoated with streptavidin. In the next step, an antibody to digoxigenin, conjugated to peroxidase, binds to the digoxigenin-labeled DNA. In the final step, the peroxidase substrate is added. The peroxidase catalyzes the cleavage of the substrate, producing a colored product. The absorbance of the samples at 405 nm is determined by a microtiter plate (ELISA) reader and is directly proportional to the level of RT activity. A fixed amount (46 ng) of recombinant HIV-1 reverse transcriptase was used. The inhibitory activity of the isolated laccase was calculated as percent inhibition as compared to a control without the laccase.

**Decolorization of textile dyes using purified laccase.** Bromothymol blue, Congo red, and Eriochrome black T at a final concentration of 100 mg/L, and malachite green and Evans Blue with a final concentration of 10 mg/L were incubated with 10  $\mu\text{L}$  (0.2 U) enzyme in 10 mM NaAc-HAc buffer (pH 5.0) at 40°C for 3 h. Enzyme preparation boiled for 20 min was used as a

**Table 1.** Yields and laccase activities of aqueous extract and various chromatographic fractions (from 900 g of fresh fruiting bodies of *Tricholoma matsutake*).

Chromatographic fraction	Total activity (U)	Total protein content (mg)	Specific activity (U/mg)	Yield (%)	Purification Fold
Extract	39060	2100.0	18.6	100.0	1.0
D1	25728	670.0	38.4	65.9	2.1
B1	24444	420.0	58.2	62.6	3.1
CM1	18907	208.0	90.9	48.4	4.9
ConA2	15446	41.2	374.9	39.5	20.2
S1	11658	13.2	883.2	29.8	47.5

Laccase activity was determined at 30°C, pH 4.5.



**Figure 1. Affinity chromatography on Con A-Sepharose.**

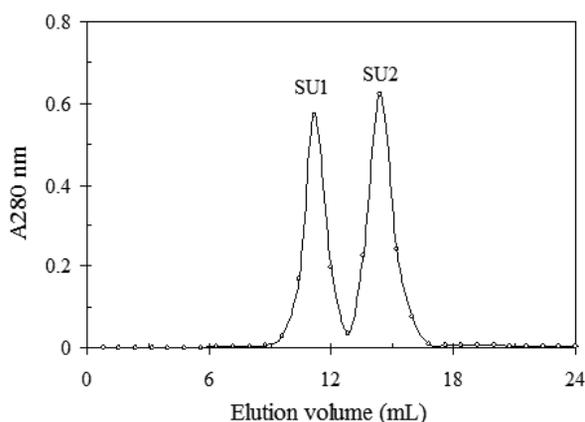
Sample: *Tricholoma matsutake* fruiting body extract unbound on DEAE-cellulose, Affi-gel blue gel and then on CM-cellulose. Column dimensions: 2.5×20 cm. Starting buffer for eluting fraction Con A1: 50 mM Tris-HCl (pH 7.4) containing 0.5 M NaCl, 10 mM CaCl<sub>2</sub> and 10 mM MgCl<sub>2</sub>. Buffer B for eluting fraction Con A2: 0.4 M  $\alpha$ -methyl-D-glucopyranoside in starting buffer. Laccase activity resided in fraction Con A2.

negative control. The decolorization was monitored at  $\lambda_{max}$  of the respective dye using a UV-visible spectrophotometer.

## RESULTS

### Isolation of laccase

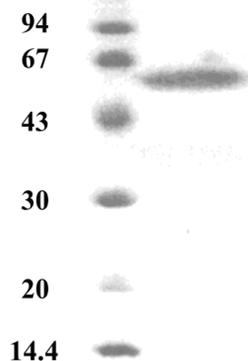
The protocol utilized in the present investigation was efficacious for purifying *T. matsutake* laccase, as summarized in Table 1. Proteins with little or no activity were removed from the laccase-enriched fraction in each of the chromatographic steps on DEAE-cellulose, Affi-gel blue gel, and CM-cellulose. Affinity chromatography of CM-cellulose flow-through on Con A-Sepharose yielded a large unbound peak (fraction Con A1) and a much smaller bound peak (fraction Con A2) (Fig. 1). Laccase activity resided in fraction Con A2 only (Table 1). Con A2 was resolved on Superdex 75 into two peaks of similar size (Fig. 2). Laccase activity was enriched in the first



**Figure 2. Gel filtration by fast protein liquid chromatography on a Superdex 75 HR 10/30 column using an AKTA Purifier System (GE Healthcare).**

Sample: fraction Con A2. Eluent: 0.2 M NH<sub>4</sub>HCO<sub>3</sub> (pH 8.5). Fraction SU1 represents purified laccase.

### kDa marker laccase



**Figure 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis.**

**Left lane:** Molecular mass standards (GE Healthcare). From top downward: phosphorylase b (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20 kDa) and  $\alpha$ -lactalbumin (14.4 kg). **Right lane:** *T. matsutake* laccase (fraction SU1) (12  $\mu$ g).

peak SU1. The second fraction SU2 had minimal activity (Table 1). A 47.5-fold purification and activity recovery of 29.83% were achieved.

### Molecular mass determination and analysis of the N-terminal amino acid sequence

The purified laccase appeared as a single band with a molecular mass of 59 kDa in SDS-PAGE (Fig. 3) and as a single peak with the same molecular mass upon re-chromatography on Superdex 75 (not shown). The N-terminal amino acid sequence of *T. matsutake* laccase was ADPVGIVGD, which does not resemble previously isolated mushroom laccases to any appreciable extent. A comparison of the N-terminal amino acid sequence of the *T. matsutake* laccase with other fungal laccases is shown in Table 2.

### Substrate specificity of isolated laccase

The enzyme oxidized a number of substrates, comprising polyphenols (hydroquinone, pyrogallol, catechol), methoxy-substituted phenols (2-methylcatechol), aromatic diamines (N,N'-dimethyl-1,4-phenylenediamine) and the non-phenolic heterocyclic compound ABTS. The highest activity was demonstrated toward ABTS, about half as much activity toward N,N'-dimethyl-1,4-phenylenediamine, about 1/10 as much activity toward hydroquinone, 2-methylcatechol and catechol, approximately 8% as much activity toward pyrogallol, and no activity toward tyrosine (Table 3).

### Effect of temperature and pH on laccase activity

With ABTS as substrate, the activity of the enzyme rose slowly but steadily (about 30% increase) when the temperature was increased from 20 to 60°C. The activity fell when the temperature was further raised to 70 and 80°C (Fig. 3). Incubation of the enzyme at 100°C for 10 minutes brought about a total destruction of activity. The optimal pH value of the purified laccase was about 5.0, with a slow increase in activity as the pH value moved from 3 to 5. Negligible laccase activity was detected at pH 7 and no activity at pH 8 and 9 (Fig. 4, Table 4).

**Table 2. N-terminal sequence comparison of *Tricholoma matsutake* laccase and other mushroom laccases.**

Mushroom laccase	N-terminal sequence
<i>Tricholoma matsutake</i> laccase	ADPVGIVGD
<i>Tricholoma giganteum</i> laccase	DDPQQAVIDD
<i>Tricholoma mongolicum</i> laccase	GIGPVADLYVGNRIL
<i>Clitocybe maxima</i> laccase	DIGPVTLAI
<i>Ceriporiopsis subvermispora</i> laccase I	AIGPVTDL
Basidiomycete PM1 laccase	SIGPVADLT
<i>Phlebia radiata</i> laccase	SIGPVDFH
<i>Pycnoporus cinnabarius</i> laccase	AIGPVADLT
<i>Trametes versicolor</i> laccase I	AIGPVASLV
<i>Trametes versicolor</i> laccase II	GIGPVADLT
<i>Trametes versicolor</i> laccase III	GIGPVADLT
<i>Agaricus bisporus</i> laccase I	KTRTFDFDL
<i>Agaricus bisporus</i> laccase II	DTKTFNFDL
<i>Pleurotus eryngii</i> laccase I	AXKKLDFHI
<i>Pleurotus eryngii</i> laccase II	ATKKLDFHI
<i>Pleurotus ostreatus</i> laccase	SIGPRGLN
<i>Ganoderma lucidum</i> laccase	GQNGDAVP

Identical corresponding amino acid residues were underlined. N-terminal sequence of laccases other than *Tricholoma matsutake* laccase were taken from Refs (Coll *et al.*, 1993; Wang & Ng, 2004a; Zhang *et al.*, 2010; Li *et al.*, 2010; Wang & Ng, 2006a; Garzillo *et al.*, 2001).

### Effect of laccase on HIV-1 reverse transcriptase inhibitory activity

No inhibitory effect of the isolated laccase on the activity of HIV reverse transcriptase was observed at a laccase concentration of 10  $\mu$ M (Table 4).

### Dye decolorization by the purified laccase

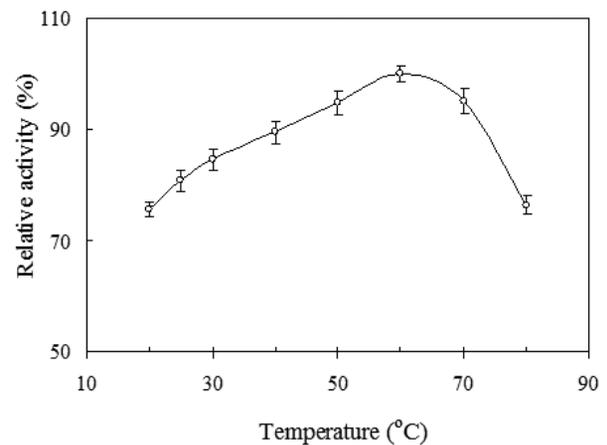
The purified laccase decolorized structurally different dyes with a variable decolorization rate from 10% to 40% (Table 5).

## DISCUSSION

Previously most laccases were purified from culture broths and not from fruiting bodies of mushrooms. The laccase purified from the fruiting bodies of *T. matsutake* differs from the laccases isolated from other *Tricholoma* species. The protocol utilized in the present investigation was efficacious for purifying *T. matsutake* laccase. Pro-

**Table 3. Activities of laccase from *Tricholoma matsutake* toward various substrates**

Substrate	Wavelength (nm)	Relative activity (%)
ABTS	420	100.0
N,N'-Dimethyl-1,4-phenylenediamine	515	48.7
Hydroquinone	248	15.3
2-Methylcatechol	436	10.8
Pyrogallol	450	10.0
Catechol	450	8.2
Tyrosine	280	0.0

**Figure 4. Dependence of activity of *Tricholoma matsutake* laccase on temperature.**

Buffer used: 0.1M NaOAc buffer (pH 5.0). Duration of incubation: 5 min. Substrate: 1.54 mM ABTS in sodium acetate buffer (67 mM, pH 4.5). All determinations were performed in triplicate.

teins with little or no activity were removed from the laccase-enriched fraction in each of the chromatographic steps on DEAE-cellulose, Affi-gel blue gel, CM-cellulose, Con A-Sepharose and Superdex 75. Unlike some of the previously reported laccases, e.g., those from *Tricholoma mongolicum* (Li *et al.*, 2010), *Lentinus tigrinus* (Xu *et al.*, 2012) and *Abortiporus biennis* (Zhang *et al.*, 2011), which were bound on cationic and anionic exchangers, the *T. matsutake* laccase did not bind to DEAE- or CM-cellulose (Table 4). Its adsorption on Con A-Sepharose revealed that it is a glycoprotein like the previously reported *Ganoderma lucidum* laccase (Wang & Ng, 2006a). Its molecular mass was 59 kDa, within the range of molecular masses reported for fungal laccases (Garzillo *et al.*, 2001; Giardina *et al.*, 1999; Shin & Lee, 2000; Zhang *et al.*, 2011). But was different from those of other *Tricholoma* sp. laccases: *T. mongolicum* (66 kDa) and *T. giganteum* (43 kDa) (Hyoung *et al.*, 2004; Li *et al.*, 2010). Its N-terminal sequence showed only a slight similarity to those of *T. mongolicum* and *T. giganteum* (Hyoung *et al.*, 2004; Li *et al.*, 2010). Similar to other fungal laccases, the best substrate for the enzyme was ABTS (Li *et al.*, 2010; Zhang *et al.*, 2010; 2011).

The optimum pH for the purified enzyme was pH 5, which was higher than that of laccases reported before, such as *T. mongolicum* (pH 23) (Li *et al.*, 2010), *T. giganteum* (pH 4) (Wang & Ng, 2004a), *C. maxima* (pH 3) (Zhang *et al.*, 2010), *L. tigrinus* (pH 4) (Xu *et al.*, 2012) and *A. bi-*

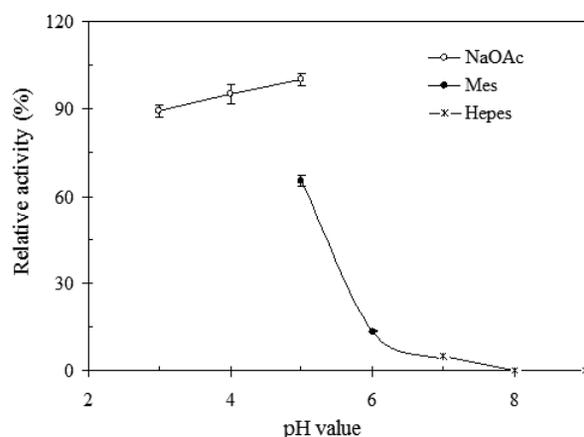
**Table 4. Comparison of biochemical characteristics and activities of *Tricholoma matsutake* laccase with other mushroom laccases.**

	<i>Tricholoma matsutake</i>	<i>Tricholoma mongolicum</i>	<i>Tricholoma giganteum</i>	<i>Lentinus tigrinus</i>	<i>Lentinus edodes</i>	<i>Abortiporus biennis</i>	<i>Pleurotus eryngii</i>	<i>Ganoderma lucidum</i>
Molecular mass (kDa)	59	66	43	59	67	56	34	75
Chromatographic behavior on								
DEAE-ion exchange	UN	BD	UN	BD	UN	BD	BD	UD
CM-ion exchange	UN	BD	BD	BD	UN	BD	UD	–
Q-ion exchange	–	UN	–	–	–	BD	BD	–
Affi-gel blue gel	UN	–	BD	–	UN	–	–	UD
Con A-Sepharose	BD	–	–	–	–	–	–	BD
Optimum pH	5	2-3	4	4	4	3-4	–	3-5
Optimum temperature (°C)	60	30	70	60	70	60	70	70
IC <sub>50</sub> (μM) of inhibiting HIV-1 RT	No activity	0.65	2.2	2.4	7.5	9.2	2.2	1.2

BD, Bound; UN, Unbound; —: not determined or not attempted. References: *Tricholoma mongolicum* (Li *et al.*, 2010), *Tricholoma giganteum* (Hyoung *et al.*, 2004), *Lentinus tigrinus* (Xu *et al.*, 2012), *Lentinus edodes* (Sun *et al.*, 2011), *Abortiporus biennis* (Zhang *et al.*, 2011), *Pleurotus eryngii* (Wang & Ng, 2006b), *Ganoderma lucidum* (Wang & Ng, 2006a).

*ennis* (pH 3.5) (Zhang *et al.*, 2011). It is noteworthy that a dramatic decrement in laccase activity ensues when the ambient pH is raised to 6 and that there is complete loss of activity at pH 8 and 9.

The *T. matsutake* laccase manifested considerably high thermal stability and maintained most of its activity when the temperature was varied from 20°C to 80°C. It required an ambient temperature of 60°C for maximal activity, similar to other laccases from *L. tigrinus* (60°C) (Xu *et al.*, 2012), *G. lucidum* (60°C) (Wang & Ng, 2006a), and *C. maxima* (60°C) (Zhang *et al.*, 2010). Its optimal temperature was higher than that of laccase from *T. mongolicum* (30°C) (Li *et al.*, 2010), *Hericium erinaceus* (40°C) (Wang & Ng, 2004b) and *L. edodes* (40°C) (Nagai *et al.*,

**Figure 5. Dependence of activity of *Tricholoma matsutake* laccase on pH.**

Temperature used: 37°C, NaOAc buffer (0.1 M) at pH 3.0, pH 4.0 and pH 5.0, Mes buffer (0.1 M) at pH 5.0, pH 6.0 and pH 7.0, and Hepes buffer (0.1 M) at pH 7.0, pH 8.0 and pH 9.0 were used. Substrate: 1.54 mM ABTS in sodium acetate buffer (67 mM, pH 4.5). All determinations were performed in triplicate.

2002). On the other hand, *T. giganteum* (Hyoung *et al.*, 2004), *L. edodes* (Sun *et al.*, 2011), *P. eryngii* (Wang & Ng, 2006b), and *G. lucidum* (Wang & Ng, 2006a) laccases demonstrated a higher optimal temperature of 70°C.

Mushroom laccases, such as those from *T. mongolicum* (Li *et al.*, 2010) and *T. giganteum* (Wang & Ng, 2004a), have shown HIV-1 reverse transcriptase inhibitory activity. Although *T. matsutake* has been widely used for medicinal or health functional purpose since ancient times in Asian countries (Tong *et al.*, 2013), the laccase isolated in the present study was devoid of an HIV-1 reverse transcriptase inhibitory activity.

Compared with laccases from other sources (Telke *et al.*, 2011; Forootanfar *et al.*, 2011), the purified *T. matsutake* laccase manifested a considerably high decolorization activity towards Eriochrome black T but low activity towards other assayed dye. Notably, the laccase from *Bacillus* sp. ADR showed no decolorization activity (Telke *et al.*, 2011). The *T. matsutake* laccase differs from its counterparts from *T. giganteum* and *T. mongolicum* in a number of aspects, such as molecular mass, pH and temperature optima, N-terminal amino acid and HIV-1 reverse transcriptase inhibitory activity (Table 4). The chromatographic behavior of *T. matsutake* laccase was quite different from other *Tricholoma* mushroom laccases. It was unbound on DEAE-cellulose, CM-cellulose or Affi-gel blue gel. In contrast, *T. mongolicum* laccase was bound on DEAE- and CM-cellulose, while *T. giganteum* laccase was bound on CM-cellulose and Affi-gel blue gel. Its

**Table 5. Decolorization of structurally different azo dyes by purified *Tricholoma matsutake* laccase.**

Dyes	$\lambda_{\max}$ (nm)	Decolorization (%)
Eriochrome black T	600	40
Bromothymol blue	430	13
malachite green	614	10
Evans Blue	610	15

lack of inhibitory effect on the activity of the HIV enzyme is in contrast to the inhibitory action of *T. giganteum* and *T. mongolicum* laccases. It deserves attention that not all *Tricholoma* mushroom laccases exhibit an inhibitory effect towards HIV-1 reverse transcriptase.

In summary, we have reported the purification and characterization of a novel laccase from the fruiting body of *T. matsutake*. The purified enzyme manifested a molecular mass of 59 kDa, N-terminal amino-acid sequence of ADPVGIVGD, a pH optimum of 5 and temperature of 60°C. It showed activity in the decolorization of azo dyes without a mediator. The present enzyme was the first laccase purified from *T. matsutake*. It represents an addition to the growing list of mushroom laccases (Leonowicz & Malinowska, 1982; Shin & Lee, 2000; Dedeyan *et al.*, 2000; Cambria *et al.*, 2000; Fukuda *et al.*, 2001; Baldrian, 2006; Tinoco *et al.*, 2001).

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