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Purification and Characterization of a Laccase from the Edible Wild Mushroom *Tricholoma mongolicum*

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A novel laccase from *Tricholoma mongolicum* was purified by using a procedure that entailed ion-exchange chromatographies on DEAE-cellulose, CM-cellulose, and Q-Sepharose, and FPLC–gel filtration on Superdex 75. The purified enzyme was obtained with a specific activity of 1,480 U/mg-protein and a final yield of 15%. It was found to be a monomeric protein with a molecular mass of 66 kDa as estimated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Its N-terminal amino acid sequence was GIGPVADLYVGNRIL, similar to some but also different to other mushroom laccases. The optimum pH and temperature for the purified enzyme were pH 2 to pH 3 and 30°C, respectively. It displayed a low K_m toward 2,7-azinobis (3-ethylbenzothiazolone-6-sulfonic acid) diammonium salt (ABTS) and high k_{cat}/K_m values. The purified laccase oxidized a wide range of lignin-related phenols, but exerted maximal activity on ABTS. It was significantly inhibited by Hg^{2+} ions, and remarkably stimulated by Cu^{2+} ions. It inhibited HIV-1 reverse transcriptase and proliferation of hepatoma HepG2 cells and breast cancer MCF7 cells with an IC_{50} of 0.65 μM, 1.4 μM, and 4.2 μM, respectively, indicating that it is also an antipathogenic protein.

Keywords: Laccase, *Tricholoma mongolicum*, purification, characterization

Laccases (E.C.1.10.3.2) are polyphenol oxidases that contain copper atoms at the catalytic center and play a crucial role in lignin degradation [10, 20]. A laccase was first demonstrated in the exudates of the Japanese lacquer tree *Rhus vernicifera* in 1883 and purified in 1970 [28]. Laccases are widely distributed in fungi including *Agaricus*, *Podospora*, *Rhizoctonia*, *Polyporus*, *Pholiota*, *Coriolus*, *Neurospora*, and *Phlebia* species. They are glycoproteins with 10% to 80% carbohydrate contents, and their molecular masses range from 34 to 390 kDa [4, 19, 22, 32]. They catalyze the oxidation of phenolic compounds and aromatic amines with molecular oxygen as the electron acceptor. They find potential applications in lignin biodegradation [2], biosensors [25], textile dyes, and detoxification of polluted water, and use in other biotechnological processes [33].

In view of the importance of laccases, the present study aimed to isolate a laccase from *Tricholoma mongolicum*. Knowledge about this mushroom, especially its protein constituents, scarce, since only about a handful of proteins that occupy bioactive molecules have been reported to date.

T. mongolicum is an edible mushroom that was named by S. Imai in 1937. Two lectins, TML-1 and TML-2, were isolated from *T. mongolicum* [38, 39]. A polysaccharide–peptide from this mushroom exhibited immunoenhancing and antiproliferative activities [37]. The purified laccase was also tested for possible medicinal properties, including antiproliferative activity toward tumor cells and inhibitory activity toward HIV-1 reverse transcriptase. A comparison with previously reported mushroom laccases was also attempted in order to ascertain unique characteristics. Some laccases exhibit antiproliferative [40] and HIV-1 reverse transcriptase inhibitory [32–35] activities, indicating that these proteins possess antipathogenic activities, like other mushroom products [37, 39].

Materials and Methods

Fungal Strain and Culture Conditions

The *T. mongolicum* strain is kept in our laboratory, in China Agricultural University in Beijing. The complex liquid medium consisted of potato juice (20%) and glucose (2%). After inoculation, the cultures were incubated at 28°C on a rotary shaker at 150 rpm for 7 days. At

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the end of the incubation, the mycelial pellet was collected by centrifugation (4,000 ×*g*, 10 min) at 4°C and washed with deionized water. The mycelial extract rather than the supernatant was selected since laccase activity of the former was one order of magnitude higher than that of the latter.

Assay of Laccase Activity

Laccase activity was determined by measuring the oxidation of 2,7'-azinobis (3-ethylbenzothiazolone-6-sulfonic acid) diammonium salt (ABTS). ABTS oxidation was detected by measuring the absorbance increase at 420 nm ($\varepsilon 420=3.6\times10^4\ M^{-1}cm^{-1}$) after 5 min using a spectrophotometer. A modification of the method of Shin and Lee [29] was used. An aliquot of the 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 420 nm of 1 per minute per milliliter of reaction mixture under the aforementioned conditions [34].

Purification of Laccase

For the purification of laccase, the mycelial pellet of *T. mongolicum* was extracted with distilled water (2.5 ml/g) in a Waring blender. The homogenate was centrifuged (13,000 ×*g*, 20 min) at 4°C, and the supernatant was saved. Phosphate buffer (PB; 1 M, pH 6.0) was added to the supernatant until the concentration of PB attained 10 mM. The supernatant was then passed through a column (2.5×20 cm) of DEAE-cellulose (Sigma) in 10 mM PB (pH 6.0). Unadsorbed proteins eluted with the starting buffer were collected as fraction D1, whereas adsorbed proteins eluted with 50 mM NaCl added to the starting buffer were collected as fraction D2. Laccase activity was concentrated in fraction D2. Fraction D2 was next subjected to ion-exchange chromatography on a CM-cellulose (Sigma) column (1.5 ×20 cm). Unadsorbed proteins were washed off the column with 10 mM HAc–NaAc (acetic acid/sodium acetate) buffer (pH 4.6), whereas absorbed proteins eluted with 50 mM NaCl added to the starting buffer were collected as fraction C2. Fraction C2 was applied to a 1.5×10 cm column of Q-Sepharose (GE Healthcare). Unadsorbed proteins were washed off with 10 mM HAc–NaAc buffer (pH 5.6). The column was eluted with a 0 to 1 M NaCl concentration gradient in the HAc–NaAc buffer. Fraction Q1 was dialyzed, lyophilized, and then further fractionated by fast protein liquid chromatography (FPLC) on a gel filtration Superdex 75 HR 10/30 column (GE Healthcare) using an AKTA Purifier System (GE Healthcare). The first eluted peak (SU1) represented purified laccase.

Molecular Mass Determination

Molecular mass was determined 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 [14], 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 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

The purified laccase was subjected to SDS–PAGE. After staining the single bard was excised and then eluted from the gel. Amino acid sequence analysis of the laccase was carried out by Edman degradation using an HP G1000A Edman degradation unit and an HP1000 HPLC system [17] .

Effects of pH and Temperature on Laccase Activity

To estimate the pH and temperature optima of the novel laccase, the above-mentioned assays were applied. The pH optimum was determined by measuring the steady-state velocities of laccase-catalyzed reaction in solutions with different pH values varied over pH 1.0–8.0 (67 mM) and plotting the steady-state velocities against the pH values of the reaction solutions. Potassium chloride/hydrochloric acid buffer (at pH 1.0, 1.4, 1.8, and 2.2) and phosphate/citrate buffer (at pH 2.2, 2.6, 3.0, 3.4, 3.8, 4.2, 4.6, 5.0, 5.4, 5.8, 6.2, 6.6, 7.0, 7.4, 7.8, and 8.0) were used. More than one buffer was used for such studies since a single buffer cannot cover the entire pH range [6]. The buffers themselves did not affect laccase activity. Similarly, the temperature optimum was determined by measuring the steady-state velocities in reaction solutions at different temperatures varied over 4°C–90°C and plotting the steady-state velocities against the temperatures of the reaction solutions [35, 36].

Kinetic Parameters

The Michaelis–Menten constant (K_m) was determined by measuring the steady-state velocities of the laccase-catalyzed reaction at different substrate concentrations and constructing double-reciprocal plots [8]. The concentrations of the ABTS substrate used were 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 mmol/l. The initial velocity at the optimum pH and optimum temperature was then determined for each substrate concentration. The reciprocals of the substrate concentrations and the reciprocals of the corresponding initial velocities were then used to generate the Lineweaver–Burk plot.

Substrate Specificity of Isolated Laccase

The substrate specificity of the purified laccase was determined by measuring the steady-state velocities of the laccase-catalyzed reaction using different substrates at a final concentration of 5 mM, including N,N-dimethyl-1,4-phenylenediamine, ABTS, hydroquinone, 2-methylcatechol, pyrogallol, catechol, and tyrosine (Sigma). Tyrosine was used as a control [29]. The enzyme assay was performed as described above. The substrate oxidation rate was followed by measuring the absorbance change with the molar extinction coefficient (ε) obtained from the literature [5, 7].

Effects of Metal Ions on Laccase Activity

The effects of different metal ions including K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+}, Pb^{2+}, Hg^{2+}, Fe^{3+}, Al^{3+}, Fe^{2+}, and Cd^{2+} were tested at 1.25, 2.5, 5, and 10 mM concentrations. The purified laccase was incubated in the presence of metal ions for 60 min and the enzyme activity was assayed in triplicate as described above using 1 mM ABTS as substrate. Control samples were assayed without the metal ions [8].

Assay for HIV-1 RT Inhibitory Activity

The assay for HIV-1 reverse transcriptase (RT) inhibitory activity was carried out in view of the previous reports that some laccases exhibit this activity [32, 33, 35]. Details of the assay follow instructions supplied with the assay kit from Boehringer-Mannheim (Germany). The assay takes advantage of the ability of RT to synthesize DNA, starting from the template/primer hybrid poly(A) oligo(dT) 15. The digoxigenin- and biotin-labeled nucleotides in an optimized ratio are

Table 1. Yields and laccase activities of various chromatographic fractions derived from mycelial extract of *T. mongolicum*.

	Total protein (mg)	Specific activity (U/mg)	Total activity (U)	Yield (%)	Purification fold
Mycelial extract	606.4	11.3	6,852.3	100	1
D2	81.5	58.8	4,792.2	69.9	5.2
C2	9.9	299.5	2,965.1	43.3	26.5
Q1	1.1	1,099.0	1,208.9	17.6	97.3
SU1	0.7	1,480.0	1,036.0	15.1	131.0

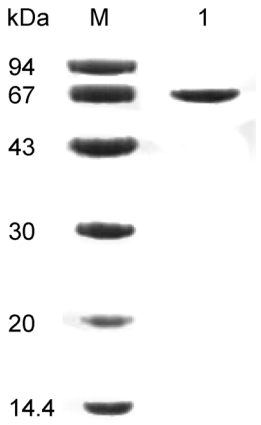

Fig. 1. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Lane 1: Purified *T. mongolicum* laccase (20 μg). M lane: Molecular mass marker standards (GE Healthcare). From top downward: phosphorylase b (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20 kDa), and lactalbumin (14.4 kDa).

incorporated into one of the same DNA molecule, which is freshly synthesized by the 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 modules that have been 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 enzyme catalyzes cleavage of the substrate, producing a colored reaction product. The absorbance of the samples at 405 nm, which can be determined using a microtiter plate (ELISA) reader, is directly correlated to the level of RT activity. A fixed amount (4–6 ng) of recombinant HIV-1 RT was used. The inhibitory activity of the isolated laccase was calculated as percent inhibition as compared with a control without the laccase [33, 35].

Assay of Antiproliferative Activity on Tumor Cell Lines

The isolated laccase was tested for antiproliferative activity since some mushroom proteins demonstrate this activity [38, 39]. Hepatoma HepG2 cells and breast cancer MCF7 cells (American Type Culture Collection) were cultured at 37°C in RPMI (Roswell Park Memorial Institute) medium supplemented with 10% (v/v) fetal bovine serum (FBS), 100 mg/l streptomycin, and 100 IU/ml penicillin, in a humidified atmosphere of 5% (v/v) CO_2. Cells were subsequently seeded into 96-well plates at a concentration of 2×10^3 cells/well, and incubated for 24 h at 37°C. Different concentrations of *T. mongolicum* laccase in 100 μl of complete RPMI medium were then added to the wells, and incubated for 72 h at 37°C [38]. After that, MTT quantification assays were carried out to measure the cell viability. Briefly, 20 μl of a 5 mg/ml solution of [3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) in phosphate-buffered saline (PBS) was spiked into each well, and the plates were incubated for 4 h. The plates were then centrifuged at 2,500 rpm for 5 min. The supernatant was carefully removed, and 150 μl of dimethyl sulfoxide was added to each well to dissolve the MTT formazan at the bottom of the well. After 10 min, the absorbance at 590 nm was measured with a microplate reader. PBS was added to the wells instead of protease, as control.

RESULTS

When the mycelial pellet extract was chromatographed on DEAE-cellulose, five fractions (D1–D5) were obtained. The unadsorbed fraction D1 exhibited no laccase activity. The tiny adsorbed fraction D2 that eluted with 50 mM NaCl exhibited the highest laccase activity (Table 1). Fraction D2 was then fractionated on CM-cellulose. It yielded a small unadsorbed peak C1 devoid of laccase activity, a small adsorbed peak enriched with activity (C2), and two

Table 2. Comparison of characteristics data of laccases.

Laccase	Molecular mass (kDa)	Optimum pH	Optimum temperature (°C)	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m ($\mu M^{-1}\cdot s^{-1}$)	References
Tricholoma mongolicum	66	2–3	30	2.3	1,480	643.5	This study
Agaricus blazei	66	2.3	20	63	21	0.3	[31]
Pleurotus ostreatus[a]	58	5	30–40	8–80	18–1,565	-	[8]
Pleurotus pulmonarius	46	4.0–5.5	50	210	1,520	7.2	[19]
Panus tigrinus	69.1	3.8	55	31	185.7	6.0	[27]
Lentinula edodes I	72.2	4	40	108	43.7	0.4	[23]
Lentinula edodes II	58	3	40	127	22	0.2	[23]

[a]Data from 6 strains.

Table 3. N-Terminal sequence comparison of laccases from *T. mongolicum* and other mushrooms/fungi.

Microorganism/Fungi	Reference	N-Terminal amino acid sequence
Tricholoma mongolicum	This study	G I G P V A D L Y V G N R I L
Tricholoma giganteum	[33]	D D P Q Q A V T D D
Trametes versicolor II	[3]	G I G P V A D L T I T D A
Trametes versicolor III	[3]	G I G P V A D L T I T D A
Coriolus hirsutus II	[29]	G I G T K A N L V I
Pycnoporus cinnabarinus	[5]	A I G P V A D L T L T N A
Trametes versicolor I	[3]	A I G P V A S L V V A N A
Lentinula edodes	[23]	A I G P V T D L H I V N
Pleurotus ostreatus	[8]	A I G P T G N M Y I V N E
Coriolus hirsutus	[29]	A I G P T A D L T I S N A
Pleurotus eryngii	[34]	A V G P V L G P D A
Rigidoporus lignosus	[8]	A T V A L D L H T L N
Ganoderma lucidum	[32]	G Q N G D A V P
Agaricus bisporus	[26]	D T K T F N F D L V N T R L A

Identical corresponding residues are underscored.

large peaks without activity (C3 and C4). Fraction C2 was next applied to Q-Sepharose and eluted with a linear gradient of 0–1 M NaCl. Laccase activity was concentrated in Q1. The more strongly adsorbed fraction Q2 lacked laccase activity (Table 1). Q1 was separated on Superdex 75 into a large peak, SU1, with laccase activity and a small peak, SU2, without laccase activity (Table 1). There was an about 131-fold increase in specific activity when the final purifed enzyme was obtained (Table 1). SU1 appeared in SDS–PAGE as a single peak with a molecular mass of 66 kDa (Fig. 1). Its molecular mass was close to those of most laccases (Table 2). The N-terminal amino acid sequence was GIGPVADLYVGNRIL, demonstrating the highest similarity to laccase from *Trametes versicolor* (Table 3).

T. mongolicum laccase exhibited the smallest K_m and a high k_{cat} value when compared with a number of other mushroom laccases (Table 2). The optimum pH and temperature for the purified enzyme were pH 2 to 3 and 30°C, respectively (Fig. 2 and Table 2). The activity rose abruptly when the pH was increased from slightly below 2.0. The activity declined sharply when the pH was raised from 4 to 5 and was indiscernible at pH 6 to 8. Its optimum pH resembled those of *Agaricus blazei* laccase and *Lentinula edodes* laccase II but lower than those of other laccases. The enzyme activity increased by about 70% to its maximum at 30°C when the temperature was elevated from 5°C, and then fell to about 10% of the maximal value when the temperature was raised to 70°C. It had a low optimum temperature like that of *A. blazei* laccase but lower than those of most other laccases. The enzyme oxidized a range of substrates, including polyphenolic substrates (hydroquinone, pyrogallol, catechol), methoxy-

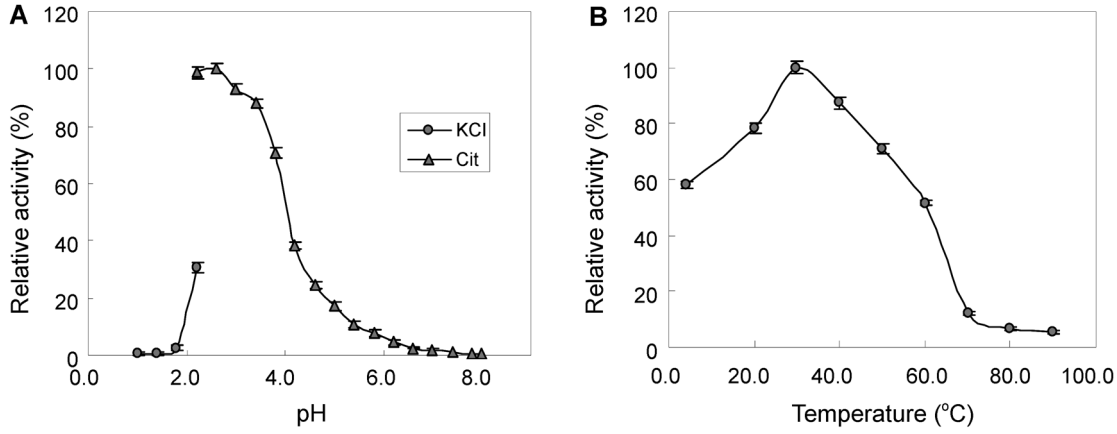

Fig. 2. Optimal conditions for activity of *T. mongolicum* laccase.
A. effect of pH on activity of *T. mongolicum* laccase. KCl=0.2 M potassium chloride/hydrochloric buffer; Cit=0.2 M phosphate/citrate buffer. **B**. effect of temperature on activity of *T. mongolicum* laccase. The profiles were reproducible when the experiments were repeated two more times. Results represent mean ± SD (n=3).

Table 4. Activities of laccase from *T. mongolicum* toward various substrates.

Substrate	Wavelength (nm)	Relative activity (%)
N,*N*-Dimethyl-1,4-phenylenediamine	515	49.4
ABTS	420	100.0
Hydroquinone	248	13.5
2-Methylcatechol	436	8.6
Pyrogallol	450	3.5
Catechol	450	11.5
Tyrosine (negative control)	280	0.0

Table 6. Comparison of *T. mongolicum* laccase with other mushroom laccases on potencies in inhibiting HIV-1 reverse transcriptase.

Laccase	IC_{50} (μM)
Tricholoma mongolicum laccase	0.65
Tricholoma giganteum laccase	2.2
Cantharellus cibarius laccase	No activity
Hericium erinaceum laccase	9.5
Pleurotus eryngii laccase	2.2
Ganoderma lucidum laccase	1.2
Albatrella dispansus laccase	No activity

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, half as much activity toward *N*,*N*-dimethyl-1,4-phenylenediamine, about 1/10 as much activity toward catechol and hydroquinone, very little activity (<10%) toward 2-methylcatechol and pyrogallol, and no activity toward tyrosine (Table 4). The laccase was significantly inhibited by Hg^{2+} ions, and remarkably stimulated by Cu^{2+} ions, whereas Fe^{2+}, Pb^{2+}, and Al^{3+} ions were weakly inhibitory. The % inhibition caused by Hg^{2+}, Fe^{2+}, Pb^{2+}, and Al^{3+} ions and % stimulation caused by Cu^{2+} ions were respectively 11.2%, 58.3%, 73.7%, 73.7%, and 1347.8%. The inhibitory activity of *T. mongolicum* laccase toward HIV-1 reverse transcriptase, HepG2 cells, and MCF7 cells was dose-dependent. The IC_{50} values were 0.65 μM, 1.4 μM, and 4.2 μM, respectively (Table 5). A comparison of inhibiting HIV-1 reverse transcriptase from other mushroom laccases is shown in Table 6. Its antiproliferative activity toward normal cells was also tested. No inhibitory activity against the hepatic fetal human epithelial cell line (WRL-68) was found when the laccase concentration was up to 1 mM (data not shown).

DISCUSSION

In the present study, a novel laccase was successfully isolated from the mycelial pellet of the edible mushroom *T. mongolicum*. Ion-exchange chromatographies on DEAE-cellulose, CM-cellulose, and Q-Sepharose, and gel filtration on Superdex 75 were used successively to remove inactive proteins from the laccase-containing chromatographic fraction. Its chromatographic behavior on DEAE-cellulose was similar to that of *Pleurotus ostreatus* laccase [8] but differed from laccases from *Cantharellus cibarius*, *Hericium erinaceum*, and *Ganoderma lucidum* [24, 32, 35]. Some mushroom laccases were unadsorbed on the ion exchanges DEAE-cellulose and CM-cellulose and also unabsorbed on the affinity chromatographic Affi-gel blue gel. They could only be adsorbed on Con A-Sepharose [24, 32]. Since the same buffers, which have different pH values, were used in these studies [32, 35], it can be deduced that different mushroom laccases may have different pI values.

T. mongolicum laccase appeared as a single band with a molecular mass of 66 kDa in SDS–PAGE. The molecular mass fell within the range of molecular masses reported for other mushroom laccases [13, 27, 31]. On the other hand, *P. eryngii* laccase has a small molecular mass of 34 kDa [32] and *L. edodes* laccase I has a large molecular mass of 72.2 kDa [22, 23].

The N-terminal amino acid sequence of *T. mongolicum* laccase manifested the highest homology to laccase from *Trametes versicolor* [3]. However, there was only slight similarity to the N-terminal sequence of *Agaricus bisporus* laccase [26]. This is noteworthy since both *T. mongolicum* and *A. bisporus* belong to the Agaricales, whereas *T. versicolor* belongs to the Aphyllophorales. In this regard, it deserves mention that laccases from different *Pleurotus* species may have distinct N-terminal sequences [8].

The optimum pH and temperature for the purified enzyme were pH 2 to 3 and 30°C, distinctly lower than

Table 5. Dose-dependent inhibitory activity of *T. mongolicum* laccase toward the activity of HIV-1 reverse transcriptase and proliferation of HepG2 cells and MCF7 cells.

	% Inhibition					
	5 μM	2.5 μM	1.25 μM	0.6 μM	0.3 μM	IC_{50} (μM)
HIV-1 RT	96.1±5.6	90.8±4.7	74.2±5.0	48.4±4.3	29.8±3.2	0.65
HepG2 cells	92.7±5.8	76.1±4.4	44.6±3.2	20.4±2.4	11.5±1.7	1.4
MCF7 cells	62.9±4.0	24.5±2.8	8.7±1.2	ND	ND	4.2

Results represent mean ± SD (n=3). ND=not determined.

those of other mushroom laccases. The activity underwent a decline at pH 4 and 5 and was indiscernible at pH 6 and 7. *T. mongolicum* laccase had a pH optimum close to those of *L. edodes* laccase II (3.0) and *A. blazei* laccase (2.3) [22, 31], and a temperature optimum resembling those of *P. ostreatus* laccase and *L. edodes* laccase [8, 23]. The purified *T. mongolicum* laccase exhibited activity to typical substrates for laccases including various phenolic compounds and ABTS, but not tyrosine. The highest activity was observed toward ABTS. The laccase was significantly inhibited by Hg^{2+} ions, and considerably stimulated by Cu^{2+} ions. It is a novel protein with possible biotechnological, industrial, and environmental applications, especially for the enzymatic degradation of aromatic pollutants.

The enzymatic and antiproliferative activities of *T. mongolicum* laccase are highly potent. Previously, some mushroom laccases have been reported to have HIV-1 reverse transcriptase inhibitory activity [32, 33, 35, 36]. The mechanism with which *T. mongolicum* laccase inhibited HIV-1 reverse transcriptase was possibly protein–protein interaction, similar to the mechanism of inhibition of the retroviral reverse transcriptase by the homologous protease [1]. *T. mongolicum* laccase manifested a more potent antiproliferative activity toward HepG 2 cells than toward MCF 7 cells. Differences in antiproliferative activity toward different cancer cell lines have been reported for various proteins. For example, a 38 kDa caper protein has a 60-fold higher antiproliferative potency toward HepG 2 cells than toward MCF 7 cells [16]. Ribosome-inactivating proteins displayed differential inhibitory activity on different tumor cell lines [30]. Hence, different cancer cells have different sensitivities to different antitumor proteins. However, a protein may be equipotent against different cancer cells [15].

It is noteworthy that laccase isolated from *T. mongolicum* in the present investigation differed from *T. giganteum* laccase reported earlier [33] in a number of aspects including N-terminal sequence, molecular mass, optimum pH, optimum temperature, chromatographic behavior on DEAE-cellulose and inhibitory potency toward HIV-1 reverse transcriptase. On the other hand, *T. mongolicum* laccase resembled laccases from other genera including *A. blazei* laccase in optimum pH and molecular mass, and *P. ostreatus* laccase in optimum temperature (Table 2).

To recapitulate, the distinctive features of *T. mongolicum* laccase encompass an N-terminal amino acid sequence different from that of the closely related *Agaricus bisporus*, a very low K_m value, extremely high k_{cat} and k_{cat}/K_m values, and exploitable activities other than laccase activities including HIV-1 reverse transcriptase inhibitory activity and antiproliferative activities toward cancer cells. The former is absent in some mushroom laccases [36] and weaker than that of *T. mongolicum* laccase (IC_{50}=2.2 to 12 μM) in other mushroom laccases [32–35, 40]. The latter activity has been reported only for *Pleurotus cornucopiae* laccase [40]. It also differs from the closely related *T. giganteum* laccase a number of aspects, such as N-terminal sequence, molecular mass, pH optimum, temperature optimum, and HIV-1 reverse transcriptase inhibitory activity.

Not too many biomolecules have been isolated from mushrooms belonging to the *Tricholoma* genus. A kinin-inactivating enzyme from *T. conglobatum* [12], a metalloenzyme from *T. saponaceum* [11], a laccase from *T. giganteum* [33], two lectins from *T. mongolicum* [38], a peptide with angiotensin-I-converting enzyme inhibitory activity from *T. giganteum*, an antifungal peptide from *T. giganteum* [9], a heteroglycan from *T. giganteum* [21], polysaccharide-peptides from *T. mongolicum* [37] and *T. lobayense* [18], and an α-glucan–protein complex from *T. matsutake* have been reported. The present report furnishes additional information about *Tricholoma* mushrooms.

Two lectins and a polysaccharide–peptide with antiproliferative activity toward tumor cells and antitumor activity in tumor-bearing mice have been isolated from *T. mongolicum* [37–39]. Taken together with the finding of a laccase with antiproliferative and HIV-1 reverse transcriptase inhibitory activities in the same mushroom species, the data support the contention that mushrooms are a source of biomolecules beneficial to health.

Acknowledgments

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