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# Purification and Characterization of a Novel Laccase from the Edible Mushroom *Hericium coralloides*

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**A novel laccase from the edible mushroom *Hericium coralloides* was purified by ion exchange chromatography on diethylaminoethyl (DEAE) cellulose, carboxymethyl (CM) cellulose, and Q-Sepharose columns followed by fast protein liquid chromatography gel filtration on a Superdex 75 column. Analysis by gel filtration and SDS-PAGE indicated that the protein is a monomer in solution with a molecular mass of 65 kDa. Its N-terminal amino acid sequence was AVGDDTPQLY, which exhibits partial sequence homology to previously isolated laccases. Optimum activity was observed at pH 2.2 and at 40°C. The enzyme showed activity toward a variety of substrates, the most sensitive of which was 2,2′-azinobis [3-ethylbenzothiazolone-6-sulfonic acid] diammonium salt (ABTS). The degradation activity toward substrates was ABTS > N,N-dimethyl-1,4-phenylenediamine > catechol > 2-methylcatechol > pyrogallol. The laccase did not exert any antiproliferative activity against Hep G2 or MCF 7 tumor cell lines at a concentration of 60 μM, unlike some previously reported mushroom proteins, but showed significant activity toward human immunodeficiency virus-1 (HIV-1) reverse transcriptase with an $IC_{50}$ of 0.06 μM.**

***Keywords***: laccase, mushroom, *Hericium coralloides*, purification

## Introduction

Laccase (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) is a multicopper blue phenoloxidase that catalyzes the oxidation of polyphenols, methoxy-substituted phenols, aromatic diamines, and a range of other compounds by a one-electron transfer mechanism using molecular oxygen as an oxidant (Sakurai, 1992; Xu, 1996; Xu *et al.*, 1996; Piontek *et al.*, 2002). Laccases are widely distributed in higher plants and fungi (Messerschmidt and Huber, 1990) and have also been found in insects and bacteria (Diamantidis *et al.*, 2000; Kramer *et al.*, 2001). Laccases have been the subject of intense research in recent decades because of their broad substrate specificity. Potential uses for laccases include textile-dye bleaching (Kierulff, 1997), pulp bleaching (Palonen and Viikari, 2004), food improvement (Minussi *et al.*, 2002), the bioremediation of soils and water (Li *et al.*, 1999; Wesenberg *et al.*, 2003), polymer synthesis (Marzorati *et al.*, 2005), the development of biosensors and biofuel cells (Trudeau *et al.*, 1997; Tayhas *et al.*, 1999), synthetic dye decolorization (Nagai *et al.*, 2002), bioremediation (Jaouani *et al.*, 2005) and chemical synthesis (Karamyshev *et al.*, 2003). Two of the most intensively studied areas in the potential industrial application of laccases are delignification and pulp bleaching and the bioremediation of environmental pollutants.

Because laccases are versatile biocatalysts in biotechnological processes, a major goal in the field has been the purification and characterization of a novel laccase. To date, more than 100 laccases have been purified from fungi and characterized to various degrees. Many edible mushrooms such as *Cantharellus cibarius* (Ng and Wang, 2004), *Pleurotus eryngii* (Wang and Ng, 2006a), *Hericium erinaceum* (Wang and Ng, 2004a), and *Tricholoma giganteum* (Wang and Ng, 2004b) can also produce laccases. However, the laccase of *Hericium coralloides*, a well-known wild edible mushroom, has not yet been identified. The objectives of this study were (1) to purify laccase from *H. coralloides* and (2) to characterize the enzymatic properties of this laccase.

## Materials and Methods

### Isolation of laccase

Dried fruiting bodies (100 g) of *H. coralloides* were extracted with distilled water (3 ml/g) using a Waring blender. The homogenate was centrifuged (13,000×g, 20 min), and Tris-HCl (1 M, pH 7.2) was added to the supernatant to a final concentration of 10 mM Tris. The supernatant was then loaded onto a DEAE-cellulose column (2.5×20 cm) that had been pre-equilibrated with 10 mM Tris-HCl buffer (pH 7.2). After elution of the flow through (D1), three adsorbed peaks, D2, D3, and D4, were eluted with step gradients of 50 mM NaCl, 150 mM NaCl, and 500 mM NaCl, respectively. Fraction D4 was subsequently subjected to ion exchange chromatography on a CM-cellulose column (1.0×15 cm, Sigma) that had been pre-equilibrated with 10 mM $NH_4OAc$ buffer (pH 5.2). After elution of the flow through (CM1), the adsorbed materials were eluted with step gradients of 50 mM NaCl, 150 mM NaCl, 500 mM, and 1 M NaCl. Three adsorbed

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fractions, CM2, CM3, and CM4, were obtained. The active fraction CM4 was subsequently purified on an anion-exchange Q-Sepharose column (1.0×15 cm, Sigma) that had been pre-equilibrated with 10 mM Tris-HCl buffer (pH 7.0). After elution of the flow through, the adsorbed material was eluted by a linear concentration gradient of 0–1 M NaCl. Following dialysis, the active peak, designated Q1, was further purified by fast protein liquid chromatography (FPLC) by gel filtration on a Superdex 75 HR 10/30 column (GE Healthcare) in 0.15 M $NH_4HCO_3$ (pH 8.5) using an AKTA Purifier (GE Healthcare). The first peak (SU1) eluted constituted purified laccase.

### Assay of laccase activity

Laccase activity was determined using 2,2′-azinobis (3-ethylbenzothiazolone-6-sulfonic acid) diammonium salt (ABTS) as the substrate following the method of Shin and Lee (2000). 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 37°C. One unit of enzyme activity was defined as the amount of enzyme required to produce an increase in absorbance at 405 nm of one per min per ml under the aforementioned conditions (Wang and Ng, 2006b). All experiments were performed in triplicate.

### Determination of molecular mass

The molecular mass of purified laccase 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 (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 was carried out on a Superdex 75 column that had been calibrated with molecular mass standards (GE Healthcare).

### Analysis of N-terminal amino acid sequence

N-terminal sequencing of the protein was conducted on an HP G-1000A Edman degradation unit and an HP1000 HPLC system (Lam and Ng, 2001).

### Determination of pH and temperature optima

To determine the optimal pH for enzyme activity, a series of solutions of ABTS at various pH values was used. More than one buffer was used for such studies since a single buffer cannot cover the entire pH range. Enzyme activity was measured in potassium chloride/hydrochloricacid buffer (at pH 1.0, 1.6, 2.0, and 2.2) and $Na_2HPO_4$-citric acid buffer (at pH 2.2, 2.6, 3.6, 4.6, 5.6, 6.6, 7.6, and 8.0) at 37°C. To determine the optimal temperature for enzyme activity, the reaction mixture was incubated at 20°C to 100°C in the $Na_2HPO_4$-citric acid buffer used in the standard laccase assay above.

### Effect of metal ions on enzyme activity

The effect of metal ions and chemical reagents on enzyme activity was investigated by pre-incubating the compound of interest (5 μl) with the purified enzyme (5 μl) for 2 h at 4°C before the standard laccase assay. The following metal ions and chemical reagents were used at concentrations of 12.5 mM, 25 mM, 50 mM, and 100 mM: $Ca^{2+}$, $Mg^{2+}$, $Mn^{2+}$, $Zn^{2+}$, $Fe^{2+}$, $Cd^{2+}$, $Al^{3+}$, $Co^{2+}$, $K^{+}$, and $Hg^{2+}$.

### Determination of substrate specificity

Activity toward several aromatic substrates was tested to determine enzyme specificity. The following substrates were added to a final concentration of 5.0 mM: ABTS, N,N-dimethyl-1,4-phenylenediamine, pyrogallol, catechol, 2-methylcatechol, and tyrosine. The enzyme assay was performed as described above in 50 mM $Na_2HPO_4$-citric acid buffer (pH 2.2). The rate of substrate oxidation was determined by monitoring the change in absorbance of the substrate using the published molar extinction coefficient (ε) (Eggert *et al*., 1996; Galhaup *et al*., 2002).

### Antiproliferative activity toward tumor cell lines

Tumor cell lines of human breast cancer (MCF7) and hepatoma (Hep G2) were purchased from the American Type Culture Collection (ATCC). The cell lines were maintained in Dulbecco modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 mg/L strep-

**Table 1. Yields and Laccase activities of aqueous extract and various chromatographic fractions (from 100 g dried *H. coralloides* fruiting bodies)**

| Chromatographic fractions | Protein yield (mg) | Laccase activity (U/mg) | Total activity (U) | Recovery of activity (%) | Purication fold |
|---|---|---|---|---|---|
| **Extract** | **685.4** | **4.3** | **2947.2** | **100** | **1** |
| D1 | 135.9 | **<0.1** | – | – | – |
| D2 | 55.2 | **<0.1** | – | – | – |
| D3 | 59.5 | 3.7 | 220.1 | 7.5 | 0.9 |
| **D4** | **174.1** | **12.9** | **2245.8** | **76.2** | **3.0** |
| CM1 | 8.6 | **<0.1** | – | – | – |
| CM2 | 14.4 | **<0.1** | – | – | – |
| CM3 | 30.2 | 4.7 | 141.9 | 4.8 | 1.1 |
| **CM4** | **32.3** | **52.1** | **1682.8** | **57.1** | **12.1** |
| Q1 | 3.4 | **<0.1** | – | – | – |
| **Q2** | **17.1** | **85.0** | **1453.5** | **49.3** | **19.8** |
| **SU1** | **11.6** | **104.5** | **1212.2** | **41.1** | **24.3** |

Laccase-enriched fractions are highlighted in boldface.

tomycin and 100 IU/ml penicillin at 37°C in a humidified atmosphere of 5% $CO_2$. Cells ($1\times10^4$) in their exponential growth phase were seeded onto a 96-well culture plate (Nunc, Denmark) and were incubated for 3 h before addition of the purified laccase solution. The cells were incubated with laccase for another 48 h. The radioactive precursor [$^3$H-methyl] thymidine (GE Healthcare) was then added to each well at a final concentration of 1 μCi, and the cells were incubated for 6 h. The cultures were then harvested by a cell harvester, and the incorporated radioactivity was determined by liquid scintillation counting (Li *et al.*, 2008).

### Assay for HIV-1 reverse transcriptase inhibitory activity

Inhibition of human immunodeficiency virus type 1 (HIV)-1 reverse transcriptase (RT) was assessed by an enzyme-linked immunosorbent assay (ELISA) kit from Boehringer Mannheim (Germany). The assay takes advantage of the ability of reverse transcriptase to synthesize DNA from the template/primer hybrid poly(A) oligo(dT)$_{15}$. Digoxigenin- and biotin-labeled nucleotides are incorporated in an optimized ratio into the freshly synthesized DNA molecule by the RT. The detection and quantification of synthesized DNA as a parameter for RT activity follows the sandwich ELISA protocol. Biotin-labeled DNA binds to the surface of microtiter plate modules that have been precoated with streptavidin. An antibody to digoxigenin conjugated to peroxidase (anti-DIG-POD) subsequently binds to the digoxigenin-labeled DNA. Finally, the peroxidase substrate is added, and the peroxidase enzyme catalyzes the cleavage of the substrate, which produces a colored reaction product. The absorbance at 405 nm is directly correlated to the level of RT activity and can be measured by a microtiter plate (ELISA) reader. A fixed amount (4–6 ng) of recombinant HIV-1 RT was used. The inhibitory activity of the laccase was calculated as the percentage of inhibition as compared with a control that did not contain laccase (Wang and Ng, 2004b).

### Protein sequence accession number

The new protein sequence data were deposited under EMBL with accession No. P87227.

## Results

Laccase from *Hericium coralloides* was purified by ion-exchange chromatography on DEAE-cellulose, CM-cellulose, and Q-Sepharose columns followed by fast protein liquid gel filtration on a Superdex 75 column. Fraction D4, which eluted from the DEAE-cellulose column at 500 mM NaCl, showed the highest laccase activity among the fractions eluted from the DEAE-cellulose column (Table 1). Fraction D4 was further separated on CM-cellulose into a small unadsorbed peak (CM1) and three adsorbed peaks, CM2, CM3, and CM4, which eluted at approximately 0.05 M, 0.15 M, and 0.5 M NaCl, respectively (Fig. 1A and Table 1). Laccase activity was observed in CM4. Subsequently, CM4 was fractionated into two peaks, Q1 and Q2, on a Q-Sepharose column (Fig. 1B and Table 1). Q2 was loaded onto a Superdex 75 HR gel filtration column (FPLC system;

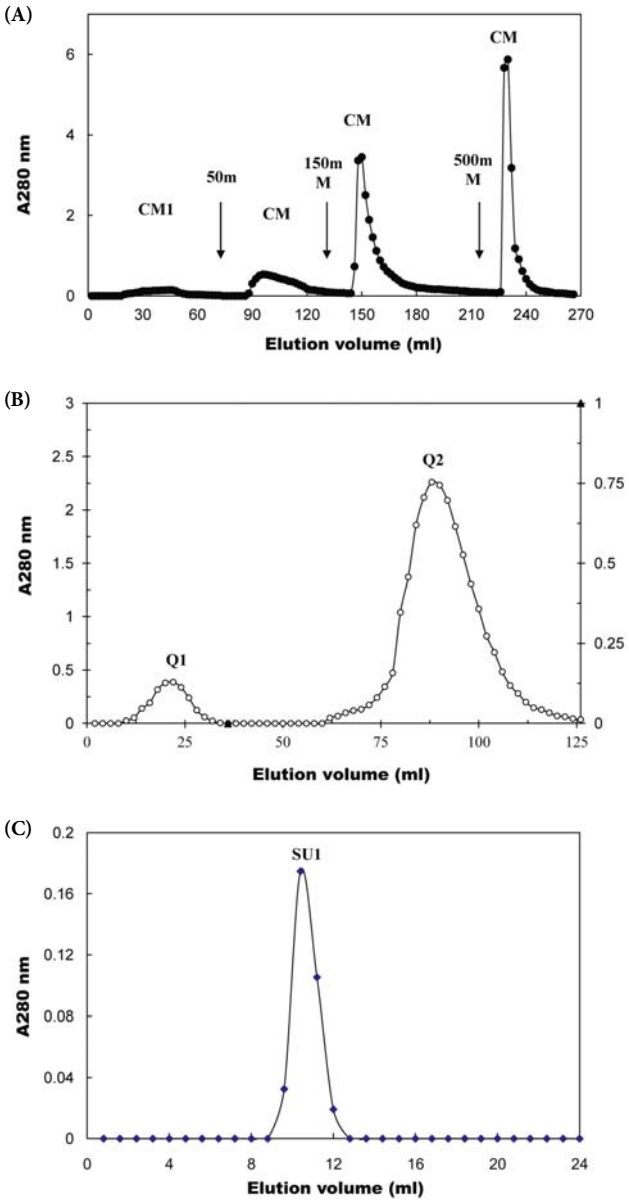


**Fig. 1. Elution profiles of laccase from *H. coralloides*.** (A) Cation exchange chromatography of fraction D4 (adsorbed on DEAE-cellulose) on a CM-cellulose column (1.0×15 cm, Sigma). The column was initially eluted with 10 mM $NH_4OAc$ buffer (pH 5.2) and subsequently with 50 mM NaCl, 150 mM NaCl, and 500 mM. The flow rate was 1.0 ml/min. (B) Anion exchange chromatography of fraction CM4 (adsorbed on CM-cellulose) on a Q-Sepharose column (1.0×15 cm, Sigma). The column was initially eluted with 10 mM Tris-HCl buffer (pH 7.0) and subsequently with a linear gradient of 0–1 M NaCl. The flow rate was 1.0 ml/min. **(C)** Gel filtration of fraction Q1 from the Q-Sepharose column on a Superdex 75 column, which was eluted with 0.15 M $NH_4HCO_3$ buffer (pH 8.5). The flow rate was 0.8 ml/min.

Pharmacia), and laccase activity was enriched in SU1 (Fig. 1C and Table 1). The increase in specific activity after chromatographic step demonstrates the increase in enzyme purity after each step (Table 1). The peak containing laccase activity could be readily identified by comparing the specific activities of the various chromatographic fractions (Table 1).

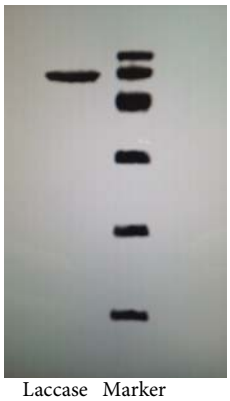


**Fig. 2. SDS-PAGE results.** Left lane: purified laccase from *H. coralloides* (65 kDa). Right lane: molecular mass standards from Amersham Biosciences, from top downward: phosphorylase b (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20 kDa) and α-lactalbumin (14.4 kDa).

*H. coralloides* laccase exhibited only one band with a molecular mass of 65 kDa by SDS-PAGE, as shown in Fig. 2. The molecular mass was also determined to be 65 kDa by gel filtration (data not shown). The N-terminal amino acid sequence of the purified laccase (10 residues) was AVGDDTPQLY. A comparison of the N-terminal amino acid sequence of *H. coralloides* laccase with those of other mushroom laccases is shown in Table 2.

The optimal pH value of the purified laccase was pH 2.2. The enzyme activity declined sharply as the pH was increased, and activity was nearly undetectable above pH 6.0 (Fig. 3). At pH 6.6, *H. coralloides* laccase showed no activity (data not shown). The optimal temperature of the laccase was 40°C. At 100°C, 24% of the activity remained whereas at 20°C, 65% of the activity remained (Fig. 4). Laccase activity was completely abolished when the enzyme was boiled for 10 min (data not shown).

The effect of different ions (12.5–100 mM) on the enzyme activity was also investigated. $Mg^{2+}$ and $Al^{3+}$ ions significantly

**Table 2. Comparison of N-terminal amino acid sequence of laccase from *H. coralloides* with those of laccases encoding recently described for other mushroom**

| Mushroom laccase | N-terminal sequence |
|---|---|
| *H. coralloides* laccase | AVGDDTPQLY |
| *Hericium erinaceum* laccase | AVDDDAEQIP |
| *Agaricus bisporus* laccase I | KTRTFDFDLVN |
| *Agaricus bisporus* laccase II | DTKTFNFDLVN |
| Basidiomycete PM1 laccase | SIGPVADLTI |
| *Cantharellus cibarius* laccase | GCCNCGHA |
| *Ceriporiopsis subvermispora* laccase I | AIGPVTDLEI |
| *Ganoderma lucidum* laccase | GQNGDAVP |
| *Clitocybe maxima* laccase | DIGPVTPLAI |
| *Lentinula edodes* SR-1 laccase | AIGPVTDLHIVN |
| *Phlebia radiata* laccase | SIGPVTDFHI |
| *Pleurotus eryngii* laccase I | AXKKLDFHIIN |
| *Pleurotus eryngii* laccase II | ATKKLDFHIIN |
| *Pleurotus ostreatus* lacase | AIGPDGNMYI |
| *Pycnoporus cinnabarinus* laccase | AIGPVADLTL |
| *Trametes versicolor* laccase I | AIGPVASLVV |
| *Trametes versicolor* laccase II | GIGPVADLTI |
| *Trametes versicolor* laccase III | GIGPVADLTI |

Identical amino acid residues are underlined. Data are taken from references (Wang and Ng, 2004a).

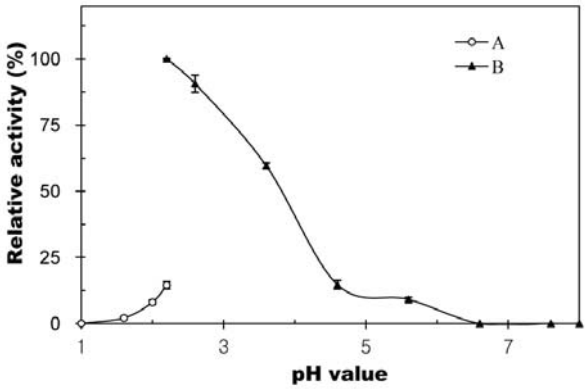


**Fig. 3. pH dependence of activity of purified laccase from *H. coralloides*.** Effect of pH on laccase activity. Enzyme activity was assayed at various pH values. A, 50 mM potassium chloride/hydrochloric buffer (pH 1.0–2.2); B, 50 mM $Na_2HPO_4$-citric acid buffer (pH 2.2–8.0). Results represent the mean±SD (n=3).

increased the activity of laccase at concentrations of 12.5–100 mM, whereas $Fe^{2+}$ and $Hg^{2+}$ ions significantly inhibited the laccase activity at 12.5–100 mM. $Ca^{2+}$, $Mn^{2+}$, $Cd^{2+}$, $Co^{2+}$ and $K^{+}$ ions inhibited enzyme activity at 100 mM but increased activity at 12.5–50 mM. Laccase activity was unaffected by $Zn^{2+}$ at a concentration of 12.5–50 mM whereas 100 mM $Zn^{2+}$ decreased activity to 78.3±1.2% of the level without $Zn^{2+}$ (Table 3).

We also analyzed the activity of *H. coralloides* laccase toward various substrates, including polyphenolic substrates (pyrogallol, catechol), methoxy-substituted phenols (2-methylcatechol), aromatic diamines (N,N-dimethyl-1,4-phenylenediamine), and the non-phenolic heterocyclic compound ABTS to determine substrate specificity (Table 4). The highest activity was found with ABTS which was then taken as the reference substrate. Approximately 35% relative activity toward N,N-dimethyl-1,4-phenylenediamine and catechol was observed whereas approximately 20% as much activity

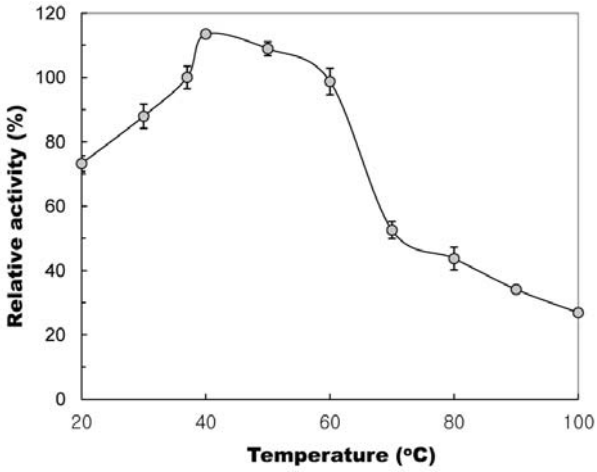


**Fig. 4. Temperature dependence of activity of purified laccase from *H. coralloides*.** Enzyme activity was assayed at various temperatures from 20 to 100°C for 5 min. Results represent the mean±SD (n=3).

**Table 3. Effect of some metals on the activity of *H. coralloides* laccase**

| Metal ion | Relative activity (% of control) | | |
|---|---|---|---|
| | 100 mM | 50 mM | 25 mM |
| $Ca^{2+}$ | 84.3±2.3 | 106.5±1.2 | 120.0±2.5 |
| $Mn^{2+}$ | 96.9±2.8 | 127.5±0.2 | 140.7±1.4 |
| $Mg^{2+}$ | 263.6±1.6 | 292.4±3.8 | 305.2±2.5 |
| $Zn^{2+}$ | 78.3±1.2 | 100.6±0.5 | 100.7±1.0 |
| $Cd^{2+}$ | 80.3±1.0 | 88.8±2.5 | 100.9±1.4 |
| $Fe^{2+}$ | 31.4±2.1 | 46.7±0.7 | 70.4±2.7 |
| $Co^{2+}$ | 99.6±2.8 | 150.3±2.3 | 158.5±2.1 |
| $K^{+}$ | 93.6±3.6 | 141.0±1.8 | 141.3±2.4 |
| $Al^{3+}$ | 398.4±0.8 | 362.4±2.7 | 229.5±2.2 |
| $Hg^{2+}$ | 4.6±0.2 | 6.5±1.0 | 5.3±1.1 |

The laccase activity in the absence of metal ions was regarded as 100%

toward 2-methylcatechol was observed, and very little activity (~5%) toward pyrogallol was observed. No activity toward tyrosine was observed (Table 4).

The purified laccase did not inhibit proliferation of HepG2 cells or MCF7 cells, at concentrations of 2.5, 5.0, 10, 20, and 60 μM. The laccase showed activity against human immunodeficiency virus-1 (HIV-1) reverse transcriptase with an $IC_{50}$ of 0.06 μM, the percent inhibition at 0.032, 0.16, 0.8, and 20 μM was 37.6, 74.6, 94.5, and 99.2%, respectively. A comparison of inhibiting HIV-1 reverse transcriptase from other mushroom laccases is shown in Table 5.

## Discussion

The N-terminal sequence of the laccase isolated from *H. coralloides* fruiting bodies in this investigation shows some resemblance to the N-terminal sequence of the *H. erinaceum* laccase (Wang and Ng, 2004a). However, it differs markedly from other mushroom laccase N-terminal sequences (Bourbonnais and Paice, 1992; Galhaup *et al.*, 2002; Nagai *et al.*, 2002; Wang and Ng, 2004b). As these data demonstrate, the laccase from *H. coralloides* may be a novel laccase and encoded by different laccase genes. Different mushroom laccases may have different pH and temperature optima and chromatographic behavior on ion exchange resins. *H. erinaceum* laccase has its own specific characteristics. Fungal laccases are generally active at low pH values (pH 3 to 5) (Fukushima and Kirk, 1995; Slomczynski *et al.*, 1995; Munoz *et al.*, 1997; Quaratino *et al.*, 2007). In this study, the *H. coralloides* laccase exhibited maximum activity at pH 2.2 and had a temperature optimum between 40 and

**Table 4. Substrate specificity of the purified *H. coralloides* laccase**

| Substrate | Wavelength (nm) | Relative activity (%) |
|---|---|---|
| ABTS | 405 | 100.0 |
| N,N-Dimethyl-1,4-phenylenediamine | 515 | 34.9 |
| Catechol | 450 | 34.2 |
| 2-Methylcatechol | 436 | 17.6 |
| Pyrogallol | 450 | 3.1 |
| Tyrosine (negative control) | 280 | 0.0 |

The laccase activity towards ABTS was regarded as 100%.

**Table 5. Comparison of *H. coralloides* laccase with other mushroom laccases on potencies in inhibiting HIV-1 RT activity**

| Laccase | $IC_{50}$ (μM) of inhibiting HIV-1 RT |
|---|---|
| *H. coralloides* laccase | 0.06 |
| *Tricholoma mongolicum* laccase | 0.65 |
| *Ganoderma lucidum* laccase | 1.2 |
| *Hericium erinaceum* laccase | 9.5 |
| *Pleurotus eryngii* laccase | 2.2 |
| *Tricholoma giganteum* laccase | 2.2 |

Data are taken from references (Li *et al.*, 2010).

50°C, the optimum pH and temperature were lower than those of other mushroom laccases. Furthermore, this enzyme showed higher temperature stability than other laccases purified from a number of mushrooms (Bourbonnais *et al.*, 1992; Galhaup *et al.*, 2002; Nagai *et al.*, 2002; Wang and Ng, 2004b).

*H. coralloides* laccase, like other fungal laccases (Call and Mücke, 1997), non-specifically oxidized a wide range of substrates, but not tyrosine. The nature and substitution of the phenolic ring affected the oxidation activity of the laccase. The degradation activity toward substrates was ABTS > N,N-dimethyl-1,4-phenylenediamine > catechol > 2-methylcatechol > pyrogallol. In general, increasing the number of substituted methoxy groups increased the oxidation activity. The laccase was significantly inhibited by $Fe^{2+}$ and $Hg^{2+}$ ions, and considerably stimulated by $Mg^{2+}$ and $Al^{3+}$ ions. Thus, the enzyme can potentially be applied in various detoxification processes such as the enzymatic degradation of aromatic pollutants.

Laccases have been shown to possess antiproliferative activity toward tumor cells and inhibitory activity toward HIV-1 reverse transcriptase (Wang and Ng, 2004a, 2004b, 2006a). *H. coralloides* laccase, unlike lectins and antifungal proteins, which are defense or antipathogenic proteins, did not exhibit antiproliferative activity toward tumor cells but showed significant activity toward human immunodeficiency virus-1 (HIV-1) reverse transcriptase.

*H. coralloides* laccase resembles that of *H. erinaceum* (Wang and Ng, 2004a) in that both are adsorbed on diethylaminoethyl (DEAE) cellulose, carboxymethyl (CM) cellulose, and Q-Sepharose and can be further purified fast protein liquid chromatography–gel filtration on a Superdex 75 column. Other laccases from the mushrooms *Pleurotus eryngii* (Wang and Ng, 2006a), *Rigidoporus lignosus* (Garzillo *et al.*, 2001), and *Coriolus hirsutus* (Shin and Lee, 2000) have been purified in similar manners. By using a similar isolation procedure, we purified a laccase from *H. coralloides* with a purification factor of 40-fold and a 24% yield (Table 1). In comparison, the laccase from the edible mushroom *H. erinaceum* was purified with a purification factor of 15-fold and a 12% yield (Wang and Ng, 2004a). On the other hand, *H. coralloides* laccase resembled laccases from other genera including *Tricholoma mongolicum* (Li *et al.*, 2010) and *Agaricus blazei* (Ullrich *et al.*, 2005) laccase in optimum pH and molecular mass, and *Lentinula edodes* I and *Lentinula edodes* II (Nagai *et al.*, 2002) laccase in optimum temperature.

Characteristics of *H. coralloides* and *H. erinaceum* laccases (Wang and Ng, 2004a) were compared in table 6. Both the two

**Table 6. Characteristic comparison of *H. coralloides* and *H. erinaceum* laccases (Wang and Ng, 2004a)**

| Characteristics | *H. coralloides* laccase | *H. erinaceum* laccase |
|---|---|---|
| Chromatographic behavior on | | |
| DEAE-cellulose ion exchanger | adosorbed, eluted with 500 mM NaCl in pH 7.2 Tris-HCl buffer | adosorbed, eluted with 500 mM NaCl in pH 7.2 Tris-HCl buffer |
| CM-cellulose ion exchanger | adosorbed, eluted with 0-1 M NaCl linear gradient in pH 7.0 Tris buffer | unadsorbed, in pH 4.5 $NH_4OAc$ buffer |
| Q-Sepharose | | adosorbed, eluted with 0–1 M NaCl linear gradient in pH 9.2 $NH_4OAc$ buffer |
| Recovery rate (%) | 41.1 | 12 |
| Molecular mass (kDa) | 65 | 63 |
| Molecular structure | monomeric | monomeric |
| N-terminal sequence | AVGDDTPQLY | AVDDDAEQIP |
| Optimal pH | 2.2 | 3–5 |
| Optimal Temperature | 40°C | 50–80°C |
| Antiproliferative activity | no apparent acitvity towards HepG2 and MCF7 in a concentration of 60 μM. | not determined |
| HIV-1 RT inhibitory activity | $IC_{50}$=0.06 μM | $IC_{50}$=9.5 μM |

species belongs to genus *Hericium* and possesses laccases. The two laccases were both monomeric proteins with similar size of molecular mass (65 kDa and 63 kDa, respectively), but different N-terminal amino acid sequences (AVGDDTPQLY and AVDDDAEQIP). *H. coralloides* laccase was adsorbed on DEAE-cellulose and eluted with 500 mM NaCl in pH 7.2 Tris-HCl buffer, just like that of *H. erinaceum* laccase. But they were extremely different in the CM-cellulose and Q-Sepharose chromatographic behavior. *H. coralloides* laccase was strongly adosorbed on CM-cellulose and eluted with 1 M NaCl in pH 5.2 $NH_4OA_C$ buffer, while *H. erinaceum* laccase was unadsorbed even in pH 4.5 $NH_4OAc$ buffer. *H. erinaceum* laccase can adsorbed on Q-Sepharose in pH 9.2 $NH_4OAc$ buffer, but *H. coralloides* laccase can adsorbed in it with pH 7.0 $NH_4OAc$ buffer. Optimal pH of *H. coralloides* laccase (pH 2.2) was lower than that of *H. erinaceum* laccase (pH 3–5), while the last one manifested a high temperature optimum (50–80°C) than that of *H. coralloides* laccase (40°C). The two laccases can both inhibit HIV-1 RT activity, but *H. coralloides* laccase possessed a lowest $IC_{50}$ value ever reported.

In this study, a novel laccase possessing some features different from those of reported mushroom laccases. was isolated and purified from the edible mushroom *H. coralloides*. Characterization studies show the enzyme has a molecular mass of 65 kDa, an N-terminal amino acid sequence of AVGDDTPQLY, a pH optimum of 2.2, and a temperature optimum of 40°C. It manifests activity toward a variety of phenolic compounds. The most sensitive substrate for the enzyme is ABTS. The laccase did not exert any antiproliferative activity against Hep G2 or MCF 7 tumor cells at a concentration of 60 μM, unlike some previously reported mushroom proteins but showed higher activity toward human immunodeficiency virus-1 (HIV-1) reverse transcriptase with an $IC_{50}$ of 0.06 μM.

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