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# A novel laccase with inhibitory activity towards HIV-I reverse transcriptase and antiproliferative effects on tumor cells from the fermentation broth of mushroom *Pleurotus cornucopiae*

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**ABSTRACT: A novel laccase with a molecular mass of 67 kDa was isolated from the fermentation broth of *Pleurotus cornucopiae* through ion exchange chromatography and gel filtration. The optimal pH and temperature for the laccase was pH 4.2 and 30°C, respectively. The laccase activity was remarkably inhibited by $Fe^{3+}$ and $Hg^{2+}$, while it was stimulated by $Cu^{2+}$ and $Pb^{2+}$. It inhibited proliferation of the hepatoma cells HepG2 and the breast cancer cells MCF-7, and the activity of HIV-I reverse transcriptase with $IC_{50}$ values of 3.9, 7.6 and 3.7 μM, respectively. Copyright © 2013 John Wiley & Sons, Ltd.**

**Keywords:** mushroom; *Pleurotus cornucopiae*; fermentation broth; isolation; laccase; FPLC

## Introduction

Laccase (benzenediol: oxygen oxidoreductases, EC 1.10.3.2), which belongs to a group of blue multicopper oxidases, oxidizes many aromatic substrates coupled with reduction of $O_2$ to water (Solomon *et al*., 1996; Giardina *et al*., 2010). It was first found in the exudates of the Japanese lacquer tree in 1883 (Yoshida, 1883) and was later discovered distributed widely in plants, fungi and bacteria (Claus, 2003; Baldrian, 2006; Kumar and Srikumar, 2011). Fungal laccases were revealed to play roles in lignin degradation, morphogenesis, fungal virulence and stress defense (Gianfreda *et al*., 1999). Owing to their wide substrate range, laccases have been recently applied in various industry fields such as pulp, paper, bioremediation, textiles and biosensors, and they even have potential in the synthetic chemistry and cosmetics in the future (Rodríguez Couto and Toca Herrera, 2006).

Mushrooms are distinguished for their lignin degradation ability and are thus considered typical sources of laccases. A large number of laccases from them have been isolated and characterized (Li *et al*., 2010; Ng, 2004; Sun *et al*., 2012; Wang and Ng, 2004). *Pleurotus comucopiae* is a commercially cultivated edible mushroom in China and Japan. Previous research mainly focused on the metabolites with medicinal effects of this mushroom including D-mannitol, perhydrobenzannulated 5,5-spiroketal sesquiterpenes and lectin (Oguri *et al*., 1996; Hagiwara *et al*., 2005; Wang *et al*., 2013). A laccase with a molecular mass of 66 kDa was isolated from the fruiting body of this mushroom (Wong *et al*., 2010). As most of the fungal laccases are extracellular and found in the mycelium fermentation rather than fruiting bodies, the fermentation broth of *P. cornucopiae* was used as a source in the present study to isolate a laccase. Its chromatographic behavior, N-terminal amino acid sequences, optimal pH and temperature, and medicinal activities were compared in detail with some other mushroom-originated laccases.

## Experimental

### Preparation of crude laccase fermentation broth

The strain of *P. cornucopiae* (collection no. CCMSSC00297), obtained from the China Center for Mushroom Spawn Standards and Control was inoculated into potato dextrose medium and incubated at 25°C on a reciprocal shaker with speed of 170 rpm. After 7 days, the fermentation broth, which exhibited laccase activity, was collected for filtration to remove the mycelium. Then the cultures were applied to ultrafiltration to obtain the crude laccase fermentation broth.

### Isolation and purification

Ion exchange chromatography and gel filtration were applied to purify the laccase. Different concentrations of saline solutions were used to elute proteins from the column and all the eluent fractions were determined for the absorbance at 280 nm and tested for laccase activity. The fractions which exhibit laccase activity were collected and applied

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**Abbreviations used:** ABTS, 2,7′-azinobis (3-ethylbenzothiazolone-6-sulfonic acid) diammonium salt; HIV-I RT, HIV-I reverse transcriptase; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; SDS–PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

 