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Lead-induced oxidative stress and antioxidant response provide insight into the tolerance of *Phanerochaete chrysosporium* to lead exposure

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HIGHLIGHTS

- *P. chrysosporium* showed good Pb tolerance and could accumulate Pb up to 162.6 mg g^{-1}.
- Pb induced oxidative stress by elevating the accumulation of H_2O_2 and MDA.
- *P. chrysosporium* evolved an antioxidant system including SOD and GSH against ROS.
- Pearson correlation analysis revealed the cooperation mechanism for antioxidants.

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ABSTRACT

The present work investigated the effect of lead (Pb) on the growth, metal accumulation, oxidative stress, and antioxidant response in *Phanerochaete chrysosporium*, which is a well-known hyperaccumulating species for heavy metal with appreciable bioaccumulation capacity. Results revealed that *P. chrysosporium* exhibited a good ability in Pb accumulation and tolerance over a concentration range of 50–100 mg L^{-1} Pb. The removal rate of Pb decreased with the increasing levels of Pb and reached a maximum of 91.3% at 50 mg L^{-1}. Both extracellular adsorption and intracellular bioaccumulation contributed to the removal of Pb, with the maximum of 123.8 mg g^{-1} and 162.5 mg g^{-1} dry weight, respectively. Pb may exert its toxicity to *P. chrysosporium* by impairing oxidative metabolism, as evidenced by the enhanced accumulation of hydrogen peroxide (H_2O_2) and lipid peroxidation product malonaldehyde (MDA). *P. chrysosporium* evolved an antioxidant system by elevating the activity of superoxide dismutase (SOD) and the level of reduced glutathione (GSH) in response to Pb stress, whereas decreasing the activities of catalase (CAT) and peroxidase (POD). Moreover, Pearson correlation analysis demonstrated a good correlation between oxidative stress biomarkers and enzymatic antioxidants. The preset work suggested that *P. chrysosporium* exhibited an outstanding accumulation of Pb and tolerance of Pb-induced oxidative stress by the effective antioxidant defense mechanism.

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1. Introduction

Rapid industrialization, injudicious use of agricultural fertilizers, faulty mining activities, and improper management of solid wastes pose a threat to ecosystem and human health due to the persistent release of toxic heavy metals (Zeng et al., 2013a; Tang et al., 2014; Wang et al., 2015; Lai et al., 2016; Sidhu et al., 2016; Zhang et al., 2016a). The treatment of heavy metal contaminated wastewater is a world-wide concern, while conventional methods such as chemical precipitation, chemical oxidation or reduction, ion exchange, reverse osmosis, filtration, and electrochemical treatment are often cost prohibitive and may cause secondary pollution (Feng et al., 2010; Fu and Wang, 2011; Hu et al., 2011; Xu et al., 2012;

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Huang et al., 2015; Liang et al., 2017a). Biosorption using the biomass of microorganisms is an emerging effective and economical technology for the removal, transformation or stabilization of a variety of heavy metals from wastewater (Gong et al., 2009; Zeng et al., 2013b; Khan et al., 2016; Huang et al., 2017a). White-rot fungi are characterized by their unique ability to adsorb and accumulate heavy metals, as well as the excellent mechanical properties of mycelial pellets, which makes them a potential biosorbent for treatment of heavy metals contaminated wastewater (Petr, 2003). Particularly, *Phanerochaete chrysosporium*, a typical white-rot fungus with excellent performance for heavy metal adsorption, has been most extensively studied in recent years (Xu et al., 2014; Li et al., 2015).

Lead (Pb) is well-recognized as a potent heavy metal pollutant and has severe eco-toxicological manifestations with prolonged persistence in the environment due to its non-biodegradable nature (Fan et al., 2008; Flora et al., 2012; Liang et al., 2017b). Previous studies have reported the capacity of *P. chrysosporium* in the removal of Pb from wastewaters (Yetis et al., 2000; Zeng et al., 2015). However, the biosorption capacity of *P. chrysosporium* is affected by their ability to survive oxidative stress arising from the production of reactive oxygen species (ROS) during heavy metal exposure. The exposure of microorganisms to heavy metals will inhibit microbial growth and physiological metabolism (Kapoor et al., 2015; Huang et al., 2016, 2017b). Specifically, it will enhance the production of ROS including superoxide radicals ($O_2^{\bullet -}$), hydroxyl radicals (•OH), and hydrogen peroxide (H_2O_2), which can cause lipid peroxidation on the membrane lipids and lead to oxidative damage to fungal cells (Tan et al., 2015).

To alleviate the damage caused by metal toxicity, on one hand, fungi often initiate active defense systems such as exclusion and binding by cell-wall components to avoid the access of metal into the cell. For Pb, binding by extracellular polymeric substances is one of the major mechanisms for detoxification (Li et al., 2015). On the other hand, the enzymatic and non-enzymatic antioxidative defense in fungi may play a crucial part in detoxifying the oxidative damage. It was reported that both the enzymatic antioxidants, including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), and non-enzymatic antioxidants such as reduced and oxidized glutathione (GSH and GSSG) were involved in the defense response against the attack of ROS during Pb exposure for fungus *Pleurotus ostreatus* HAU-2 (Zhang et al., 2016b). Nevertheless, much attention was paid to the antioxidant response in plants (Ashraf and Tang, 2017) and aquatic animals (Javed et al., 2016; Morcillo, 2016), and the information of metal tolerance and detoxification mechanisms in white-rot fungi was limited. Hence, there is a need to improve the understanding of the antioxidant defense mechanism of *P. chrysosporium* to Pb exposure.

The present work aims to evaluate: (i) the adsorption and accumulation of Pb in *P. chrysosporium*, (ii) the effect of different concentrations (0–400 mg L^{-1}) of Pb on the growth, oxidative stress, as well as the enzymatic and non-enzymatic antioxidant response of *P. chrysosporium*, and (iii) the Pb tolerance mechanism in *P. chrysosporium*. In addition, the relationship among Pb accumulation, the biomass, oxidative stress, and antioxidants was performed to examine the antioxidative mechanisms, which were involved in the detoxification of Pb by *P. chrysosporium*.

2. Materials and methods

2.1. Strain and culture condition

The fungus *P. chrysosporium* (BKM-F-1767) was supplied by China Center for type Culture Collection (Wuhan, China). The strain was maintained on potato dextrose agar (PDA) slants at 4 °C and transferred to PDA plates at 37 °C for 48 h before inoculation. The spore suspensions were prepared by scraping fungal spores from plates, diluting the spores in sterile water and then adjusted to a concentration of 2.0×10^6 CFU mL^{-1} according to Huang et al. (2008). 2 mL of spore suspension were inoculated into 200 mL of sterile potato dextrose broth (PDB) in 500-mL flasks. Cultures were incubated at 30 °C with shaking at 150 rpm.

2.2. Determination of biomass and Pb removal

After 41 h cultivation, the fungus cells entered the exponential growth phase according to our previously work (Li et al., 2015), Pb was spiked in the form of $Pb(NO_3)_2$ to a final concentration of 50, 100, 200, and 400 mg L^{-1}, respectively. Cultures without Pb were used as the controls. Each treatment was conducted in at least triplicate. The mycelia in flasks were collected at selected intervals, then washed three times with phosphate-buffered saline (PBS) and heated at 80 °C for 24 h to determine the biomass.

To measure the Pb removal efficiency, the liquid culture was filtered and the supernatant was analyzed by flame atomic absorption spectrometry (AAS700, PerkinElmer, USA). To estimate the extracellular adsorption of Pb, the mycelia in flasks were collected and then resuspended in 100 mL of 0.2 M HNO_3 solution for 1 h to desorb superficially bound metals (Zhang et al., 2016b). The washed solution was digested with nitric acid and perchloric acid (4:1, v/v) before analysis by AAS700. To estimate the intracellular accumulation of Pb, the washed mycelia were rinsed twice with distilled water, then homogenized in a glass homogenizer, digested and analyzed by AAS700 (Zhang et al., 2016b). The extracellular adsorption and intracellular accumulation of Pb were expressed as mg g^{-1} dry mycelia weight.

2.3. Oxidative stress analyses

The harvested mycelia were washed with PBS buffer (50 mM, pH 7.8) and homogenized in a glass homogenizer, the extract was centrifuged at 12,000 g for 10 min at 4 °C and the supernatant was used for the analyses of the oxidative stress parameters including H_2O_2 and malonaldehyde (MDA). The H_2O_2 content was determined by monitoring the titanium-peroxidase complex formation (Brennan and Frenkel, 1977). MDA was estimated according to the method of Aravind and Prasad (2003).

2.4. Antioxidant analyses

To determine the activities of antioxidative enzymes, 0.5 g fresh mycelia were ground in liquid nitrogen and suspended in 3 mL of extraction buffer composed of 50 mM sodium phosphate buffer (pH 7.5), 1% (w/v) polyvinylpyrrolidone (PVP), and 0.1 mM EDTA. Total protein concentrations were analyzed using bovine serum albumin as a standard. The enzyme activities of SOD, CAT, and POD were measured following the method of Zhang et al. (2016b). The results were expressed as Unit mg^{-1} protein.

Glutathione content was determined by the method of Rehman and Anjum (2011). Briefly, the mycelia were suspended in 0.1 M phosphate buffer (pH 7.0) containing 0.5 mM EDTA and sonicated for 2 min. After centrifugation at 14,000 g for 10 min at 4 °C the supernatant was used for analysis. GSH was estimated by monitoring the reduction of 5, 5′-dithiobis (2-nitrobenzoic acid) (DTNB) to 5-thio-2-nitrobenzoate (TNB) at 412 nm. The 3 mL of reaction mixture was composed of 0.5 mL of the above supernatant, 2.0 mL of reaction buffer, and 0.5 mL of 3 mM DTNB. After 5 min, the change in absorbance at 412 nm was monitored. Besides, the same reaction mixture supplemented with nicotinamide adenine

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