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

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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 to 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.

Keywords: *Phanerochaete chrysosporium*; Lead; Oxidative stress; Antioxidant response; Tolerance

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; 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; Huang et al., 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 et al., 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 ℃ and transferred to PDA plates at 37 ℃ 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 ℃ 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 ℃ 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 ℃ 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 ℃ 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 dinucleotide phosphate (NADPH, final concentration, 0.2 mM) and glutathione reductase (final concentration, 0.1 U mL^{-1}) was prepared and the absorbance at 412 nm was monitored for the analysis of the total glutathione. The content of GSSG was measured from the difference between the total glutathione and GSH.

2.5. Statistical analysis

Results were given as the means and standard deviation of three replicates. The differences between the treatments were analyzed by one-way analysis of variance (ANOVA) with a multiple comparison test (LSD) at a 5% significant level. Pearson correlation analysis was performed to assess the degree of relationship between the Pb level and biological data. All the above analysis was performed by SPSS 18.0 (SPSS Inc, Chicago, IL, USA). A value of $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Pb tolerance in *P. chrysosporium*

The impact of Pb exposure on the growth of *P. chrysosporium* is shown in Fig. 1, the biomass (dry weight) in all trials displayed the similar trend that increased from 2 to 120 h and decreased from 120 to 168 h. There was no significant difference between the biomass of groups with the Pb concentration range from 0 to 100 mg L^{-1} during the first 24 hours. *P. chrysosporium* could survive in high Pb concentration of up to 400 mg L^{-1}. However, the growth was significantly inhibited in high concentration of Pb (≥ 200 mg L^{-1}) during the whole exposure period. The maximum values of biomass were 1.72 ± 0.02, 1.64 ± 0.02, 1.63 ± 0.02, 1.49 ± 0.02, and 1.16 ± 0.03 g L^{-1} for the treatment with 0, 50, 100, 200, and 400 mg L^{-1} Pb, respectively.

3.2. Total removal rate, adsorption and bioaccumulation of Pb

As shown in Fig. 2a, the removal of Pb was rapid during the first 72 hours of incubation and became slow thereafter. The treatment with 50 mg L^{-1} Pb showed the highest value of Pb removal rate among all treatments during the whole exposure period, while that with 400 mg L^{-1} Pb showed the lowest one. No obvious difference was found between the 100 mg L^{-1} and 200 mg L^{-1} Pb treatments. After 168 h, the removal rate of Pb by *P. chrysosporium* with the increase of the tested Pb concentration was 91.3% (50 mg L^{-1}), 84.1% (100 mg L^{-1}), 82.6% (200 mg L^{-1}), and 63.2% (400 mg L^{-1}).

To investigate the detailed manner in Pb removal by *P. chrysosporium*, we examined the extracellular adsorption and intracellular accumulation of Pb which were presented in Fig. 2b and c. It was shown that both of them were Pb concentration dependent. The extracellular

adsorption content increased during the first 8 hours and decreased after 24 h. The maximum value (123.8 mg g^{-1} dry biomass weight) was observed in treatment with 400 mg L^{-1} Pb at 24 h. Differed from the extracellular adsorption, the intracellular accumulation content increased until 72 h after exposure, followed by slight variations. The maximum value (162.6 mg g^{-1} dry biomass weight) was found in treatment with 400 mg L^{-1} Pb at 168 h.

3.3. Pb-induced oxidative stress in *P. chrysosporium*

The oxidative stress in *P. chrysosporium* in exposure to Pb was evaluated by measuring the content of H_2O_2 and MDA (Fig. 3). It was observed that Pb exposure for 2 h and 24 h both caused an increase in the content of H_2O_2, and the effect was found to follow a Pb concentration-dependent trend (Fig. 3a). H_2O_2 content was promoted in the range of 119–238% and 132–229% after 2 h and 24 h, respectively, in exposure to 50–400 mg L^{-1} Pb (Fig. 3a). In addition, the level of H_2O_2 after 24 h was higher than that after 2 h in all trials. The exposure to 400 mg L^{-1} Pb resulted in a substantial increase in the H_2O_2 content when compared with the other trials.

As a major end-product of lipid peroxidation, MDA was determined to evaluate the oxidative damage of fungus by Pb. After 2 h of Pb exposure, MDA content increased in a Pb concentration-dependent manner which was similar to the H_2O_2 (Fig. 3a and b). It was observed to be 2.22 μM g^{-1} and 2.75 μM g^{-1}, respectively, at the 200 mg L^{-1} and 400 mg L^{-1} Pb treatment, which were 1.57- and 1.95-fold higher than the control (0 mg L^{-1}) (Fig. 3b). A significant decrease in MDA content occurred in all trials after exposure for 24 h. However, no significant difference was found between treatments with Pb concentration at 0, 50, and 100 mg L^{-1}. But when Pb concentration increased to 200–400 mg L^{-1}, an obvious increase in

MDA content (1.29–1.61 μM g^{-1}) was observed.

3.4. The enzymatic and nonenzymatic antioxidants response of *P. chrysosporium*

Antioxidant enzymes have been considered as the first line of defense in response to oxidative stress (Li et al., 2008). In the present study, we examined the activities of SOD, CAT, and POD, which was reported to be important in the antioxidative defense system in fungi (Bai et al., 2003). As shown in Fig. 4a, a concentration-dependent increase in SOD activity was observed and reached the maximum at 400 mg L^{-1} Pb treatment. Moreover, SOD activity was significantly enhanced after Pb exposure for 24 h than for 2 h in all trials. Unlike SOD, the activity of CAT was inhibited after exposure to Pb for 2 h (Fig. 4b). However, a slightly enhanced CAT activity was noticed after 24 h at low Pb concentrations (50–100 mg L^{-1}), but it was restrained at high Pb concentrations of 200 and 400 mg L^{-1} which were respectively 0.88- and 0.82-fold of the control. POD activity was reduced upon treatment with the increasing levels of Pb concentration (Fig. 4c). At 400 mg L^{-1} Pb exposure, the activity of POD decreased to 0.67- and 0.61-fold over the control after exposure for 2 h and 24 h, respectively.

The responses of GSH and GSSG in *P. chrysosporium* under Pb stress, as well as the ratio of GSH/GSSG, were also evaluated as presented in Fig. 5. Considerable amounts of GSH were accumulated in Pb-exposed *P. chrysosporium*, and the maximum GSH content was reached at 200 mg L^{-1} Pb treatment with the values of 82.7 μM g^{-1} protein and 84.5 μM g^{-1} protein after Pb exposure for 2 h and 24 h, respectively. However, a slight decrease in GSH content was observed when Pb concentration reached up to 400 mg L^{-1}. GSSG content was found to maintain stable in a concentration range from 0 to 100 mg L^{-1} Pb, but it increased

significantly when Pb concentration increased to 200–400 mg L^{-1} and the maximum GSSG content obtained at 400 mg L^{-1} Pb was 1.75- and 1.89-fold over the control for 2 h and 24 h of Pb exposure, respectively. The GSH/GSSG ratio increased with increasing Pb concentrations ranged from 0 to 100 mg L^{-1}, and began to decline with further increases in Pb concentrations.

3.5. Correlation analysis among the chemical and biochemical parameters

Relationships between tested parameters are presented in Table 1. Intracellular accumulation of Pb (Pb_{intra}) correlated significantly only with GSH after 2 h of Pb exposure, while with both the growth (biomass), oxidative stress (H_2O_2, MDA), and antioxidants (SOD, CAT, POD, GSSG) of *P. chrysosporium* after 24 h. Biomass was found to be significantly correlated to the all tested parameters except GSH. A good correlation was observed between the oxidative stress and enzymatic antioxidants parameters after 2 h. In addition, GSSG had a strong correlation with all tested parameters except Pb_{intra} and GSH after 2 h, and except only GSH after 24 h.

4. Discussion

In recent years, biotechnology tends to be an attractive alternative for the treatment of heavy metal pollution in wastewater. *P. chrysosporium* is of particular concern for its powerful ability in the removal of heavy metals (such as Pb) from wastewater through biosorption and uptake (Xu et al., 2014). In this study, we explored Pb tolerance and accumulation in *P. chrysosporium*, as well as the oxidative stress and antioxidant response under the different concentrations of Pb. The results indicated that the exposure to Pb would affect the growth of *P. chrysosporium* cells and induce the oxidative stress as well as the

defense response against toxicity.

Trace amounts of essential heavy metals such as Mn, Zn, and Cu are usually required for the growth of fungi, but an excess of heavy metals are toxic which can inhibit the growth, reproduction, cause morphological and physiological changes (Tang et al., 2012; Wang et al., 2013). In this study, *P. chrysosporium* demonstrated a high degree of tolerance to Pb and could survive at a Pb concentration of up to 400 mg L^{-1} (Fig. 1). This may be attributed to the defense mechanism of *P. chrysosporium*, primarily based on immobilizing Pb ions outside the hyphae by adsorption or precipitation in the cell wall (Li et al., 2015). However, Pb concentration over 200 mg L^{-1} significantly inhibited the biomass growth ($P < 0.05$), which was similar to the behavior of other fungi like *Aspergillus lentulus* FJ172995 (Mishra and Malik, 2012) and *Pleurotus ostreatus* HAU-2 (Zhang et al., 2016b), and simultaneously caused an obviously higher intracellular accumulation (Fig. 2c). It was suggested that a high concentration of Pb stimulated the intracellular accumulation of Pb and resulted in damage to *P. chrysosporium* which was evidenced by a relatively high content of MDA (Fig. 3b).

It is widely reported that white-rot fungi are capable of removing heavy metals by extracellular adsorption and intracellular accumulation based on the active functional groups such as carboxyl and the chelating compounds (Petr, 2003; Xu et al., 2015). Our study showed that *P. chrysosporium* was very efficient in Pb removal. After Pb exposure for 168 h, most of Pb (over 80%) was removed by *P. chrysosporium* in all trials except at 400 mg L^{-1} (63.2%). The highest Pb removal rate of 91.3% observed at 50 mg L^{-1} was greater than that of 79.3% by *Rhizopus arrhizus* observed at 10 mg L^{-1} (Uslu et al., 2003), and was similar with our previous report (Xu et al., 2014). In addition, it seemed that the high content of

extracellular adsorption contributed to the fast increase in removal rate during the first 24 h of Pb exposure, indicating the extracellular adsorption was a fast process in Pb removal which was in accordance with the report by Li et al. (2015). However, the extracellular adsorption of Pb decreased obviously after 24 h (Fig. 2b). In contrast, a great increase was observed in intercellular accumulation of Pb. This implied that both the two mechanisms played a part in Pb removal by *P. chrysosporium* and the extracellular adsorption of Pb could be transferred to the intercellular accumulation of Pb during the later exposure period. Once metals are trapped inside cells by the functional binding sites, the affinity with cell components is enhanced compared with those at the cell surface. Alternatively, bioaccumulation is generally preferred to adsorption in treatment of metal-polluted wastewater. Pb stress (50–400 mg L^{-1}) caused a concentration-dependent increase of the two approaches. The maximal intracellular accumulated Pb level reached 162.6 mg g^{-1} dry weight (Fig. 2c), which was slightly higher compared with that of 125.2 mg g^{-1} dry weight obtained by Çeribasi and Yetis (2001) and was similar with that of another potential metal biosorbent *Pleurotus ostreatus* HAU-2 (165.0 mg g^{-1} dry weight) (Zhang et al., 2016b). The above observations demonstrated that *P. chrysosporium* can immobilize Pb effectively and is a promising alternative to the treatment of Pb-polluted wastewater.

ROS are highly reactive and are capable of initiating chain reactions that may cause ultrastructural and functional changes in cell nuclei, DNA, lipids, and proteins (Ashraf and Tang, 2017). Pb stress often triggers the production of free radicals as well as ROS and leads to oxidative stress in cells. The cell membranes, composed of phospholipid bilayers, are susceptible to oxidation by ROS and thus lead to lipid peroxidation (Catalá, 2009). MDA, the

major product of lipid peroxidation, are usually used as the indirect biomarkers of oxidative stress (Valavanidis et al., 2006). However, it is challenging to detect the ROS species in aqueous media since their half-lives are as short as 10^{-9} s for •OH and 10^{-6} s for $RO^{\bullet -}$ (Khachatryan et al., 2011; Cheng et al., 2016). It is also hard to determine the production of $O_2^{\bullet -}$ in *P. chrysosporium* as a result of a high SOD activity, which catalyzes the dismutation of $O_2^{\bullet -}$ to O_2 and H_2O_2 (Xu et al., 2015). The formed H_2O_2, unlike the oxygen radicals, can diffuse freely across biological membranes and therefore cause oxidative stress far from its production site, resulting in an attack on cellular components, particularly cell membranes. Previous researches on Pb accumulation and its oxidative stress were mainly concentrated on plants, particularly hyperaccumulating plants (Li et al., 2016; Sidhu et al., 2016). In the present study of white-rot fungus *P. chrysosporium*, we observed a Pb dose-dependent accumulation of H_2O_2 and MDA, indicating that Pb stress induced the overaccumulation of H_2O_2 which caused membrane damage by lipid peroxidation (Fig. 3). The results were consistent with the recent reports by Zhang et al. (2016b) and Long et al. (2017), who observed a Pb-induced overgeneration of ROS and consequent oxidation stress in *Pleurotus ostreatus* and *Aspergillus oryzae*, respectively.

In response to oxidative stress caused by heavy metals, biomass must assure a well-adjusted antioxidant system to protect sensitive cellular components, generally by enzymatic antioxidant, including SOD, CAT, and POD, and nonenzymatic antioxidant, such as GSH, flavonoids, and phenolics. SOD acts as the first line of the enzymatic antioxidant defense system against ROS, which seems to play an essential role in protection of fungi against heavy metal toxicity (Xu et al., 2015). It converts the highly reactive $O_2^{\bullet -}$ to O_2 and

H_2O_2 and the latter can be further reduced to H_2O by CAT and POD (Mostofa et al., 2017). In the present study, the three enzymatic antioxidants (SOD, CAT, and POD) exhibit different responses to Pb stress (Fig. 4). The desynchronized activities of SOD, CAT, and POD, as evidenced by continual increase of SOD activity and decrease of CAT and POD activities, contribute to the accumulation of H_2O_2 in *P. chrysosporium*. The up-regulation of SOD in *P. chrysosporium* under Pb stress is implicated in counteracting the oxidative burden and thus leads to $O_2^{\bullet -}$ scavenging. The Pb-induced inhibition of CAT and POD activity may be due to the enhanced production of H_2O_2 in *P. chrysosporium* that can suppress the CAT and POD activity (Sidhu et al., 2016). Gajewska et al. (2006) also found that Pb ions in cells might inactivate CAT activity through inactivating the enzyme-bound heme group, suggesting a direct inhibition of this enzyme.

GSH, the most abundant intracellular non-protein thiols, is involved in maintaining the balance of the cellular redox status and protecting cells against the oxidative stress caused by heavy metals through chelation and detoxification soon after they enter the cell (Gutiérrez-Alcalá et al., 2000). In *P. chrysosporium*, a significant increase in GSH accumulation was observed in exposure to Pb (Fig. 5) and intracellular accumulation of Pb after 2 h exposure was significantly positively correlated with GSH as evidenced by Pearson correlation analysis, indicating the potential role of GSH in the alleviation of oxidative stress such as the decomposition of the accumulated H_2O_2. GSH could react with H_2O_2 to produce GSSG under the catalyzation of glutathione peroxidase enzymes, which was confirmed by the incremental accumulation of GSSG (Fig. 5). Excess Pb exposure (400 mg L^{-1}) triggered the decrease in GSH accumulation, probably because of the toxicity of Pb at this concentration to

microbes. The result was similar with the previous study on *Pleurotus ostreatus* that 100–200 mg L^{-1} Pb significantly promoted the GSH accumulation (Zhang et al., 2016b). The synthesis of GSH from inorganic sulfate is demand-driven by sulfur assimilation and cysteine biosynthetic pathways, influenced by various factors such as heavy metal exposure, oxidative stress, and sulfur or nitrogen deficiency (Mendoza-Cózatl et al., 2005). Heavy metal ions such as Hg^{2+} and Pb^{2+} have also been demonstrated to induce the accumulation of GSH in fishes (Thomas and Juedes, 1992) and plants (Gutiérrez-Alcalá et al., 2000; Sun et al., 2007). But in other reports, it has shown that heavy metal accumulation in the cells can contribute to the depletion of GSH by forming insoluble sulfide adducts and excreting these complexes (de Almeida et al., 2004). In addition, the GSH-dependent synthesis of metal-binding peptides like phytochelatins can sequester and detoxify heavy metals by forming stable GSH-metal complexes intracellularly, which further aggravates the GSH depletion (Estrella-Gómez et al., 2012). However, there was not an obvious depletion of GSH in *P. chrysosporium* during 24 h of Pb exposure, suggesting that GSH responds to the Pb exposure in the manner of demand-driven GSH synthesis via sulfate depletion, rather than forming GSH-Pb complexes by chelation. Nevertheless, the detailed molecular mechanism and transformation of GSH in response to Pb needs further study.

5. Conclusion

In the present work, *P. chrysosporium* was tested in terms of its tolerance and antioxidant ability towards Pb exposure and consequent oxidative stress. The results revealed that *P. chrysosporium* had a good tolerance towards low concentrations of Pb (< 200 mg L^{-1}) and possessed a considerable ability to remove Pb from solution by both extracellular adsorption

and intracellular accumulation. However, Pb exposure enhanced the intracellular accumulation of H_2O_2 and triggered lipid peroxidation, which caused oxidative stress in *P. chrysosporium*. The upregulation of SOD activity and downregulation of CAT and POD activity might contribute to the accumulation H_2O_2. The Pb-induced elevation of GSH indicated its role in the scavenging of ROS. Pearson correlation analysis revealed the cooperation mechanism for antioxidant defense system in response to Pb stress. The findings indicate that *P. chrysosporium* has a superior capacity to withstand and tolerate Pb-induced oxidative stress by an effective defense mechanism, and is a promising candidate for environmental applications in wastewater treatment.

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