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Cadmium induced hydrogen peroxide accumulation and responses of enzymatic antioxidants in *Phanerochaete chrysosporium*

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ABSTRACT

The white-rot fungi *Phanerochaete chrysosporium* have been widely applied in heavy metal bioremediation, which commonly results in a stress exposure. Exposure studies showed that Cd bioaccumulation triggered H_2O_2 generation. Thereafter, the H_2O_2, as signal molecules, activated the enzymatic antioxidant response in the case of the conjunction of SOD and CAT. High levels of R (SOD/CAT) were found initially under Cd exposure, and then a significant decline occurred in time-course Cd exposure. Antioxidant activities also provided an alternative as an indication of the existence of antioxidant response upon Cd exposure, via coupling H_2O_2 level with the variation in antioxidant activities. This finding potentially has implications for the understanding of tolerance behavior and detoxification mechanisms, which is beneficial to expand the application of *P. chrysosporium* in bioremediation biotechnology.

1. Introduction

Heavy metals, such as cadmium, lead, copper, chromium, and arsenic, are widely occurring environmental pollutants. Among these, cadmium (Cd) is listed as a priority pollutant by the US environmental protection agency, due to its high water solubility, without known biological function, high toxicity and easy enrichment in food chain and pose a threat to human beings (Pérez-Chaca et al., 2014; Balestri et al., 2014; Xu et al., 2012a). Biosorption is an emerging cost-effective and ecofriendly technology that utilizes microorganisms to remove, transform, or stabilize a variety of heavy metals. Many workers have reported the application of *Phanerochaete chrysosporium* (*P. Chrysosporium)* in biosorption of heavy metals, in the case of high efficiency and low cost (Xu et al., 2012b, 2013; Iqbal and Edyvean, 2004; Çeribasi and Yetis, 2001). The biosorption capacity of *P. chrysosporium* partly depends on their ability to survive potentially toxic treatments. Studies carried out in different microorganisms have revealed that Cd is strongly toxic and causes growth inhibition and even cell death (Chen et al., 2008). Recent studies further reported that metals such as iron, copper, cadmium, chromium, lead and mercury exhibited the ability to produce reactive oxygen species (ROS), resulting in oxidative stress alteration of calcium homeostasis and DNA damage (Klaunig et al., 1998).

The question as how microorganisms may defend themselves against heavy metals is therefore receiving increasing attention. It has been widely reported that exposure to heavy metals led microorganisms to develop a series of defense mechanisms, including antioxidant defenses (Cadenas, 1997; Mishra et al., 2006). Fungi, like many other microorganisms, rely on antioxidant defense mechanisms to protect against oxidative damage (Chagas et al., 2008; Pacini et al., 2013). However, scarce study is underway to determine the antioxidant response under heavy metal exposure in *P. chrysosporium*. In order to prove the importance of using antioxidants in heavy metal detoxification, pertinent biochemical detoxification mechanisms contributing to stress tolerance is necessary to understand.

To understand tolerance and maximize the potential application of *P. chrysosporium* in bioremediation, it is essential to understand the toxicity of the heavy metals and the detoxification mechanisms of *P. chrysosporium*. The present research work was

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therefore focused on Cd induced H_2O_2 generation and the antioxidant response of *P. chrysosporium* on the metabolic and physiological level compared for cells cultured at Cd concentrations of 0, 20, 50 and 100 ppm. We further investigated the feasibility of the antioxidant mechanism of *P. chrysosporium* to Cd exposure, with particular attention given to the constitution and response of superoxide dismutase (SOD) and catalase (CAT). Meanwhile, effect of Cd on the antioxidant activity of *P. chrysosporium* was determined by detection the variations in total antioxidant activity (TAA) and radical scavenging activity (RSA).

2. Materials and methods

2.1. Strain and chemicals

The *P. chrysosporium* strain BKMF–1767 (ATCC 24,725) was purchased from the China Center for Type Culture Collection (Wuhan, China) and maintained by subculturing on potato dextrose agar (PDA) slants at 4 °C. All reagents used in the experiment were of or above analytical reagent grade.

2.2. Exposure to Cd

Spore suspensions of *P. chrysosporium* were prepared in the sterile distilled water at a concentration of 2.0×10^6 cell mL^{-1}. Liquid cultures, consisted of 100 mL growth medium as described by Kirk et al. (1986) were inoculated with 2 mL the defined spore suspensions with desirable concentrations of Cd. For the toxicity experiments, Cd in the mixture was controlled at the concentration of 0, 20, 50 and 100 ppm via the addition of $Cd(NO_3)_2{\cdot}4H_2O$. All experiments were conducted under constant stirring at 30 °C and 120 rpm and performed in three culture replicates.

At the selected time intervals, *P. chrysosporium* biomass was collected and weighted, washed three times in 20 mL phosphate buffer solution (PB, 0.05 M, pH 7.0) solution, and then homogenized in 10 mL of phosphate buffer solution (PB, 0.05 M, pH 7.4). The homogenate was centrifuged at 10,000 rpm at 4 °C for 10 min, and the supernatant was filtered by 0.45 μm filter membranes and collected for further use. Removal of Cd was determined by detect the residual Cd in the growth medium by an atomic absorption spectrometer (AAS, Agilent 3510, USA). The concentrations of the Cd in the prepared *P. chrysosporium* extracts were tested to evaluate the bioaccumulation level of Cd.

2.3. H_2O_2 generation detection

During the chronic exposure time, the ROS and H_2O_2 generation, were frequently measured. H_2O_2 was detected according to H_2O_2 assay kit purchase from Beyotime institute of biotechnology.

2.4. Enzymatic antioxidant responses detection

Briefly, CAT activity was assayed by measuring the rate of decrease in H_2O_2 (100 mM) at 240 nm by UV–vis spectrophotometer (UV-2250, SHIMADZU, Japan). 0.1 mL extract was added to 2.5 mL PB solution (50 mM, pH 7.4), the reaction was started by addition of 0.5 mL H_2O_2. CAT activity was monitored through H_2O_2 consumption. Data were expressed in U mg $prot^{-1}$ (Nemmiche et al., 2007). The biological activities of SOD were tested in supernatants according to Marklund and Marklund (1974) by quantifying the inhibition of superoxide-dependent pyrogallol (PAPG) self-oxidation with the addition of *P. chrysosporium* extracts. One SOD unit is defined as the enzyme quantity that inhibits the autoxidation of PAPG by 50%. The reaction mixture contained 2.5 mL Tris–HCl buffer solution (100 mM, pH 8.2), 0.15 mL PAPG (10 mM) and 0.1 mL *P. chrysosporium* extracts. After intensive mixing, the variation in absorbance of the mixture was recorded via ultraviolet spectrophotometer at 320 nm for 3 min. Meanwhile, R (SOD/CAT) was defined as the ratios of enzyme activity between SOD and CAT. Protein concentrations were measured by the Bradford method (Bradford, 1976) using bovine albumin as the standard.

2.5. Antioxidant activity analysis

Antioxidant activity estimation was characterized as TAA, $O_2^{\bullet-}$ and $^{\bullet}OH$ RSA (Liu et al., 2014). Total antioxidant activity of *P. chrysosporium* extracts were conducted by measuring the variation in absorbance of $ABTS^{\bullet+}$ solution at 734 nm vs time for 3 min after the addition of 100 μl *P. chrysosporium* extracts. The $ABTS^{\bullet+}$ solution was prepared via the dilution of 2,2′-azino–bis(3-ethylbenzthiazoline-6- sulfonic acid) (ABTS) in acetate buffer to the absorbance of 0.70 (±0.02) at 734 nm using a UV–vis spectrophotometer. Meanwhile, the inhibition ratio between autoxidation and oxidation rate of pyrogallic acid, at the absence and presence of *P. chrysosporium* extracts, was detected to determine the $O_2^{\bullet-}$ scavenger activity. $^{\bullet}OH$ scavenger activity was performed by orderly mixing 1.5 mM $FeSO_4$, 6 mM H_2O_2, 20 mM sodium salicylate and *P. chrysosporium* extracts, and then incubated for 30 min in dark. The $^{\bullet}OH$ scavenger activity was conducted by calculation the variation in absorbance at the absence and presence of *P. chrysosporium* extracts at 562 nm.

2.6. Statistical analysis

All of the above analytical experiments were done in triplicate, and the results were analyzed as the mean value with the standard deviation. Correlation and regression analyses were carried out using the regression program in SPSS software (SPSS 18.0, Germany). All reported error bars represent one standard error of the arithmetic mean.

3. Results

3.1. Cd uptake and bioaccumulation in P. chrysosporium

P. chrysosporium has been identified as an effective bioremediation agent for its biosorption and degradation ability. Cd concentration curves shown in Fig. 1a demonstrated that Cd contents in all tested concentrations declined in the early calculation stage (first 4 days). As the concentration of Cd increased, there was a concomitant increase in Cd removal efficiency. It was mainly because that the initial concentration supplied a kind of important driving force, to overcome the existing mass transfer resistance of Cd, simultaneously reinforce the active uptake of *P. chrysosporium* (Xu et al., 2012b). Concomitantly, the disappearance of Cd was accompanied with a gradual bioaccumulation of Cd in *P. chrysosporium*. In all tested Cd concentrations, Cd bioaccumulation increased in relation to the Cd content of the growth medium as shown in Fig. 1b. A large increase in Cd bioaccumulation occurred in *P. chrysosporium* grown at high Cd concentration (100 ppm), while Cd contents increased slightly at concentrations of 50 ppm Cd. Interestingly, there was a distinct decrease in Cd contents after 6 d of growth at 20 ppm Cd, which might be ascribed to the active efflux mechanism.

3.2. Cd-induced H_2O_2 accumulation in P. chrysosporium

It is known that Cd exposure is inevitably connected with an internal oxidative stress, even though Cd is a non redox-active element (May et al., 1998). The excessive production of ROS, via consumption of oxygen and antioxidants result in a so-called

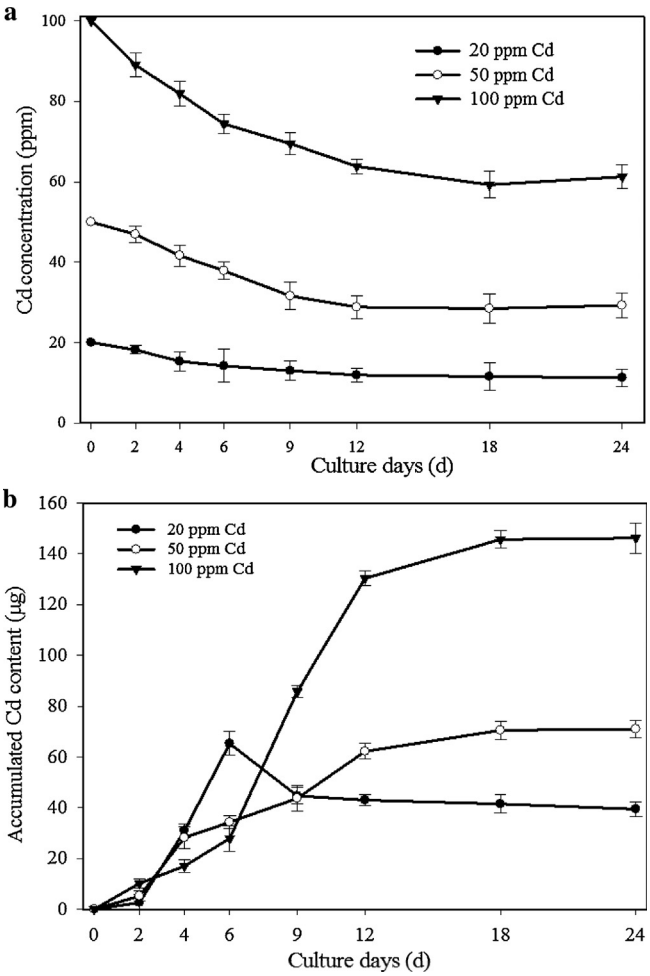

Fig. 1. Cd uptake (a) and bioaccumulation of Cd (b) in *P. chrysosporium* exposed to 20, 50, 100 ppm Cd. Data are means of three replicates (±SD). Vertical bars show standard deviation.

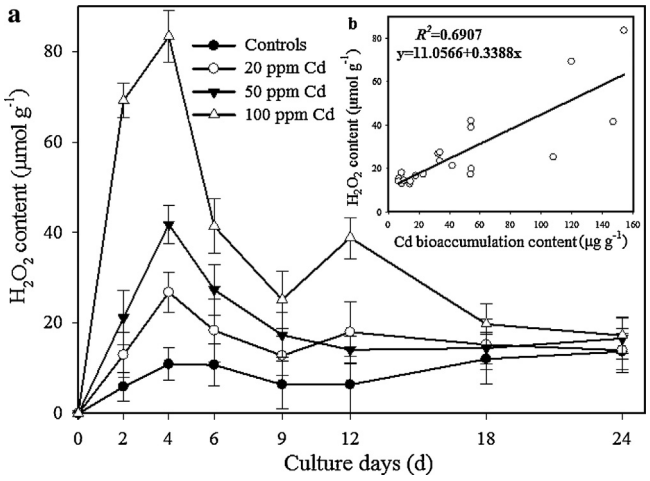

Fig. 2. Time-course of intracellular H_2O_2 accumulation in *P. chrysosporium* exposed to 0, 20, 50 and 100 ppm Cd; the inserted Fig.(b) showed the significant correlation between accumulated Cd contents and H_2O_2 during the whole growth stage.

oxidative burst, is one of the cellular toxicity. In the present study, real-time of Cd-induced oxidative stress in forms of H_2O_2 was quantified in *P. chrysosporium* (Fig. 2). It is apparent that H_2O_2 plummeted after 4 d of growth, in addition to an unexpected increase at 12 d. After 24 d of growth, H_2O_2 in all the tested concentrations tends to be similar at a detectable level. Obviously, H_2O_2 accumulation was strongly induced with Cd at the initial growth stage (4 d). H_2O_2 were induced by 1.98, 4.12 and 7.58 fold in the presence of 20, 50 and 100 ppm Cd at 4 d while compared with the control samples (Fig. 2a).

To find out the data basis of this increased H_2O_2 accumulation, correlation between the Cd content and H_2O_2 content is demonstrated in Fig. 2b. The high Cd concentration presumably led to an increased oxidative stress relative to that during normal metabolism, subjecting the fungi to a ROS-rich environment. Such an oxidative event might be induced in a regulated fashion by the activation of Cd bioaccumulation, in the case of positive correlation between intracellular Cd contents and H_2O_2 (Fig. 2b, $R^2 = 0.6907$).

3.3. Cd induced enzymatic antioxidant response in P. chrysosporium

As shown in Fig. 3a, Cd treatment initially triggered the activation of SOD activities. SOD activities increased with prolonged Cd exposure and generally proportional to the levels of the stress. Particularly, SOD in *P. chrysosporium* treated with 100 ppm Cd increased significantly compared with those found in controls till at the end of the experiment. At 12 d, the activity of SOD reached maximum at 100 ppm Cd (19.07 U mg $prot^{-1}$). In contrast to SOD, CAT activities were initially suppressed under Cd exposure. At 4 d, Cd-induced relative changes in CAT activity were −20.61%, −43.12% and −89.61%, respectively, while compared with controls. After 6 d of growth, increase in CAT activity has been found in Cd-exposed *P. chrysosporium*, which is supposed to be an adaptive trait possibly helping to overcome the damage to cell metabolism by reducing toxic levels of H_2O_2. However, unlike SOD, CAT activities elevated remarkably exposed to low concentration Cd (20 ppm), while the induction was less pronounced in *P. chrysosporium* exposed to Cd at 50 and 100 ppm (Fig. 3b). CAT activity was calculated at the value of 0.38, 1.36, 0.58 and 0.96 U mg $prot^{-1}$ at 0, 20, 50 and 100 ppm Cd, respectively. Meanwhile, the stimulation synthesis of those antioxidants possibly performed a prior role as an adaptive response to heavy metals, rather than initially participating in antioxidant activities. Therefore, time-adaptive antioxidants aggravate an imbalance in ROS and antioxidant levels, in turn triggers oxidative stress in cells initially (Fig. 3b).

3.4. Cooperation of SOD and CAT as antioxidant network response to Cd

To better understand Cd-induced oxidative effects and detoxification mechanisms in *P. chrysosporium*, we calculated the ratio between SOD and CAT activity during the Cd exposure (Fig. 4). Relative high values of R (SOD/CAT) have been observed at 2 d, companied with high levels of H_2O_2 initially (Figs. 2 and 4), which is recognized as a key intracellular messenger at subtoxic levels in certain important signal pathways. A gradual decrease in R (SOD/CAT) has been found significantly dependent on Cd, their concentration and the time of exposure, contributing to a remittent in H_2O_2 accumulation (Fig. 4a). The positive correlation between the ratio R (SOD/CAT) and H_2O_2 ($R^2 = 0.8617$, $p = 0.0134$) in *P. chrysosporium* was further observed in Fig. 4b, indicating the cooperation of SOD and CAT in H_2O_2 regulation in *P. chrysosporium*.

3.5. Cd induced variation in antioxidant activities

Metabolic activation significantly increased the potency of the fungi response to Cd. To find out whether the observed alleviation in oxidative stress is dependent on the antioxidant defense of *P. chrysosporium*, quantitative estimates of the antioxidant capacity and radical scavenging activity are necessary. As a first step

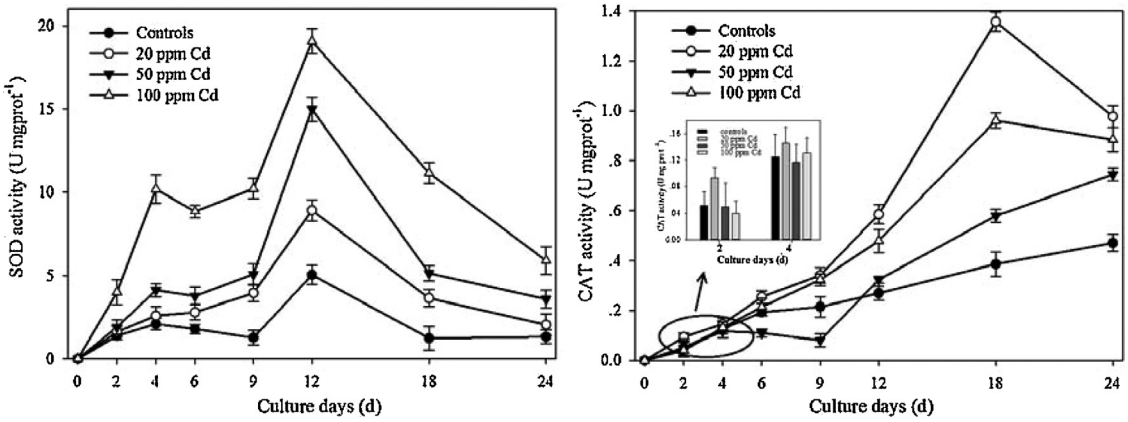

Fig. 3. Time-and-concentration dependent of intracellular SOD (a) and CAT (b) activities in *P. chrysosporium* exposed to 0, 20, 50 and 100 ppm Cd.

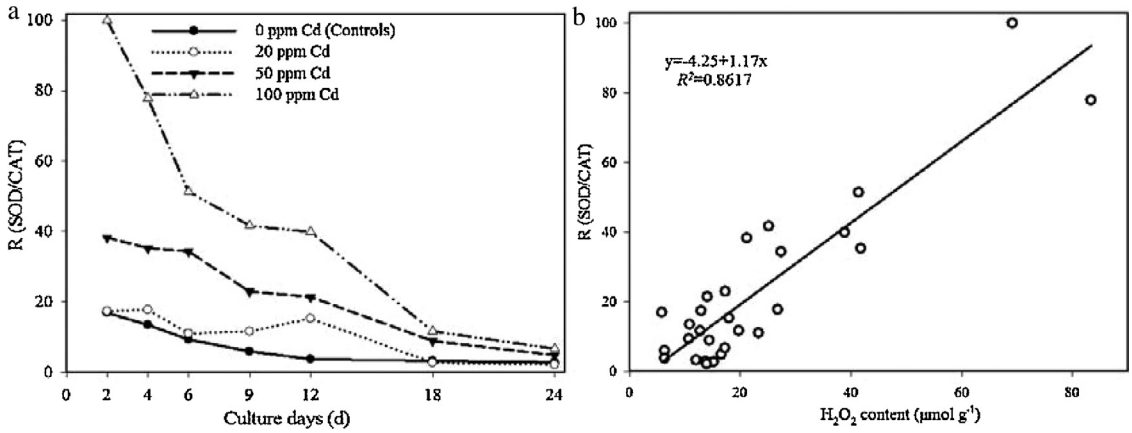

Fig. 4. (a) Time-and-concentration dependent of R (SOD/CAT) (ratios of SOD/CAT) in *P. chrysosporium* exposed to 0, 20, 50 and 100 ppm Cd; (b) correlations between intracellular H_2O_2 levels and R (SOD/CAT).

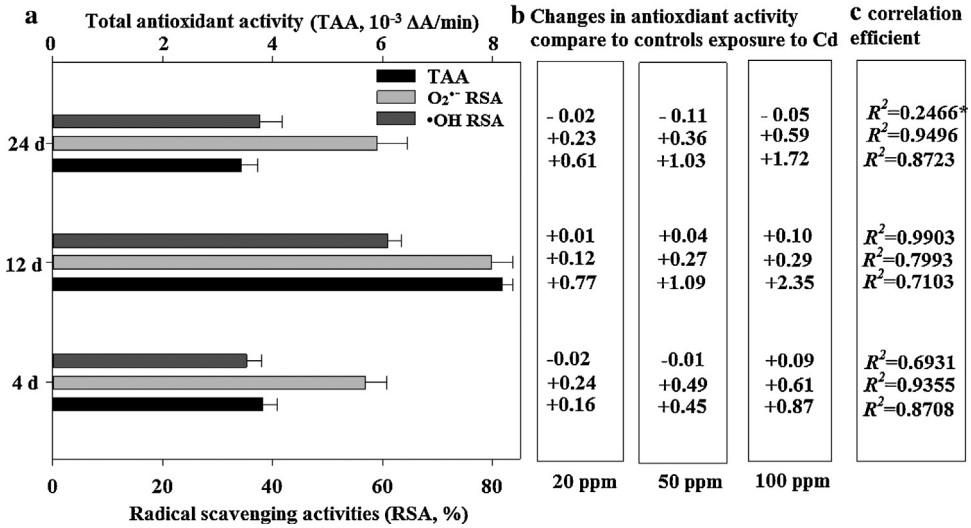

Fig. 5. (a) Total antioxidant activities (TAA) and radical scavenging activities (RSA) of *P. chrysosporium* in the absence of Cd after various growth stage (4, 12 and 24 d); (b) changes in antioxidant activities relative to controls after exposure to 0, 20, 50 and 100 ppm of Cd. Relative change was calculated as $(A_{sample} - A_{control})/A_{control}$; (c) relationships between tested antioxidant activities and initial exposed Cd. * Mark means negative correlation.

towards an assessment of volume-related activities of defense enzymes and antioxidant activity in healthy *P. chrysosporium* were calculated (Fig. 5a). It was obviously that *P. chrysosporium* exhibited admirable antioxidant activity and radical scavenging activity. As examples, the *P. chrysosporium* extract after 4 d of exposure could scavenge almost 56.91% and 35.29% of $O_2^{\bullet-}$ and

•OH, respectively. At 12 d, total antioxidant and radical scavenging activity tend to peak values, accompanied with the high SOD and CAT activities.

In a second modeling step, a situation was envisaged under Cd exposure (as indicated for 4, 12 and 24 d of Cd-treatment in Fig. 5b). Indeed, the antioxidant activities were influenced by time and Cd exposure. TAA showed largest changes after Cd-exposure. When the fungi were grown under Cd exposure conditions, a significant strengthen of TAA was predicted, which is consistent with the alleviation in oxidative stress. The $O_2^{\bullet-}$ scavenging activity was significantly correlated to the Cd levels, especially short-term exposure, due to the preferential dismutation of $O_2^{\bullet-}$ by initial stimulation in SOD. Positive linear correlations were found response to various Cd levels (Fig. 5c), given that the *P. chrysosporium* exhibited a Cd-dependent antioxidant response at all of the tested concentrations.

4. Discussion

Apparently, exposure to Cd led to increases in Cd bioaccumulation and H_2O_2 production at all tested Cd concentrations (Figs. 1 and 2). Generally, the natural ROS play an essential role in normal functioning as important signaling molecules modulating the activity of specific defense enzymes (Pinto et al., 2003). Under physiological steady state conditions, H_2O_2 existed in biological cells at low but measurable concentrations in *P. chrysosporium* (Fig. 2, controls). However, Cd-induced NADPH oxidase production has been reported, which function as multicomponent enzymes, and use electrons derived from intracellular NADPH to generate $O_2^{\bullet-}$ from O_2 (Cuypers et al., 2010), result in ROS generation and signaling to the onset of cellular protection mechanisms. Besides, some experiments also confirmed the generation of (non-radical) H_2O_2 and oxidative stress under Cd exposure which itself in turn may be a significant source of radicals via Fenton chemistry (Valko et al., 2006; Watanabe et al., 2003). Detection of ROS species in aqueous media is challenging, as their half-lives, depending on the media, could be as short as 10^{-9} s for •OH, 10^{-6} s for RO• (Khachatryan et al., 2011). It is also difficult to detect the occurrence of the $O_2^{\bullet-}$ in *P. chrysosporium*, most probably in consequence of the presence of high SOD activity therein, which enzymatically converts $O_2^{\bullet-}$ to O_2 and to the less-reactive species H_2O_2. Therefore, we choose H_2O_2 as biomarkers for reactive oxygen stress. As expected, pronounced H_2O_2 accumulation was proportional to Cd bioaccumulation levels in *P. chrysosporium* (Fig. 2b, $R^2 = 0.6907$).

In response to toxic levels of heavy metals, a cell may develop metal resistance systems in an attempt to protect sensitive cellular components. When oxidative stress occurs, cells attempt to counteract the oxidant effects and maintain redox balance by activation of antioxidants. This potential for heavy metal toxicity has forced attention on its early evolution to develop metal ion homoeostasis factors and antioxidant determinants (Jaleel et al., 2008; Shi and Zhu, 2008). In *P. chrysosporium,* treatment with Cd initially resulted in increased SOD activities. SOD appears to be crucial for protection of fungi with elevated levels of ROS as well as an increased tolerance, which has been deemed to be the first defense mechanism against ROS, catalyzes the disproportionation of $O_2^{\bullet-}$ to O_2 and H_2O_2. However, the up-regulation of SOD is implicated in combating oxidative stress, due to the findings that induction of SOD also results in the activation of H_2O_2 in the case of the $O_2^{\bullet-}$ elimination. Such a situation inevitably leads to the formation of H_2O_2 (Scott et al., 1987). Thereafter, promoted activation of CAT occurred, contributing to H_2O_2 scavenging. Catalase, existing as the peroxisomes in nearly all aerobic cells, serves to protect the cell by catalyzing H_2O_2 without the production of free radicals. The mechanisms of catalysis process of CAT has not been precisely clarified yet, but possible pathway, similar as cytochrome c peroxidase, has been proposed, based on the monomer structure which containing a heme prosthetic group and one NADH molecule. The catalytic process is reported to occur in two stages (Morita et al., 1994; Bhaduri and Fulekar, 2012):

$$H_2O_2 + \text{Fe(III)-E} \rightarrow H_2O + O = \text{Fe(IV)-E(+)} \quad (1)$$

$$H_2O_2 + O = \text{Fe(IV)-E(+)} \rightarrow H_2O + \text{Fe(III)-E} + O_2 \quad (2)$$

where Fe-E represents the iron center of the heme attached to the enzyme (E).

Accordingly, H_2O_2 might play important roles in the defense system, acting as intermediate signaling molecules and cellular messenger for the induction of antioxidant systems (Egan et al., 2013). It is recently reported that antioxidant defenses tend to operate as a balanced and coordinated system, a whole array of enzymes is therefore needed for the regeneration of the active forms of the antioxidants (Sies et al., 2005). The capacity to regenerate other antioxidants is driven by the redox potentials of the (Red/Ox) couple, correlating to increased levels of ROS and/or disturbed activities of enzymatic antioxidants (Valko et al., 2006). Analysis of CAT activities showed that low Cd exposure (20 pm) contributed to a 1.17-fold of increase in CAT activity (18 d). A likely explanation for the lower induction ratio of 100 ppm Cd to CAT is that the high level of Cd resulted in the elevated SOD activity, which promoted increased H_2O_2 formation derived from SOD catalyze reaction, further aggravated the CAT depletion. Therefore, in contrast to induction of SOD or CAT, H_2O_2 acted as signal molecules induced the antioxidant network in the conjunction of SOD and CAT balance. Our results declared that H_2O_2 concentrations were proportional to R (SOD/CAT), due to the signaling trait of H_2O_2 to the onset of cellular protection mechanisms (Fig. 4b, $R^2 = 0.8617$). In this case, H_2O_2 was probably of enzymatic origin considering the low concentration in the system and initiated the various H_2O_2 derived antioxidants. In fact, prolonged Cd exposure induced SOD and CAT activities have been found compared with samples without Cd (Fig. 3) and thus were shown to be well acclimatized to the Cd-exposed environment. It is reasonably deduce that cells may trigger two different mechanisms to regulate their intracellular ROS concentrations and oxidation/redox balance: one that will enable the fine modulation of low levels of ROS for signaling purposes, and one that will enable the scavenging of excess ROS. A fast decomposition of H_2O_2 has been found after 4 d of incubation representing an important adaptive response to withstanding adverse conditions with pronounced CAT activities. As a result, despite the intense initial stress, the cells exhibited hypertolerance to Cd exposure in late exponential phase, with waning of stimulation ratios in H_2O_2 accumulation.

Furthermore, bioassays based on antioxidant activities can provide more information about the response of *P. chrysosporium* coping with adversity environments during environmental application. Indeed, maintenance of a high antioxidant capacity in cells has been linked to increased tolerance against different kinds of environmental stress, due to the ability to ROS scavenging. Promoted levels of cellular antioxidants therefore allow cells to acclimatize to ROS under Cd stress, resulting in the decrease of H_2O_2. It is tempting to speculate that the observed increase of antioxidant activities after Cd-exposure might be necessary to the provocative antioxidant responses. Enhanced levels of cellular antioxidant activity could allow cells to acclimatize to ROS under Cd stress, contributing to the alleviation of oxidative stress in *P. chrysosporium*. It also provided an alternative as a better indication of the existence of oxidative stress upon Cd exposure via coupling

ROS level with the change in activity of antioxidant systems and antioxidant responses.

5. Conclusions

In conclusion, heavy metal stress is one of the major problems affecting efficiency of environment microbiology. Our results suggest that Cd bioaccumulation in *P. chrysosporium* promotes H_2O_2 generation, and then triggers a consecutively signal transduction of antioxidant response as antioxidant network cooperation with SOD and CAT. SOD enzymes work in conjunction with CAT, based on the positive correlations between H_2O_2 accumulation and R (SOD/CAT) ($R^2 = 0.8617$). Cd exposure also promoted antioxidant activities in *P. chrysosporium*, attributed to the hypertolerance of *P. chrysosporium* to Cd. Distinct remission H_2O_2 accumulation was consequently found after an adaptive antioxidant response in *P. chrysosporium*. Results from this study will serve as useful references for the physiological responses of fungi to metal exposure during environmental application.

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References

Balestri, M., Bottega, S., Spanò, C., 2014. Response of Pteris vittatato different cadmium treatments. Acta. Physiol. Plant 36, 767–775.
Bhaduri, A.M., Fulekar, M.H., 2012. Antioxidant enzyme responses of plants to heavy metal stress. Rev. Environ. Sci. Biotechnol. 11, 55–69.
Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.
Cadenas, E., 1997. Basic mechanisms of antioxidant activity. Biofactors 6, 391–397.
Çeribasi, I.K., Yetis, U., 2001. Biosorption of Ni(II) and Pb(II) by *Phanerochaete* chrysosporium from a binary metal system–kinetics. Water SA 27, 15–20.
Chagas, R.F., Bailão, A.M., Pereira, M., Winters, M.S., Smullian, A.G., Deepe, G.S., de Almeida Soares, C.M., 2008. The catalases of *aracoccidioides brasiliensis* are differentially regulated: protein activity and transcript analysis. Fungal Genet. Biol. 45, 1470–1478.
Chen, H.Y., Yao, J., Zhou, Y., Chen, H.L., Wang, F., Gai, N., Zhuang, R.S., Ceccanti, B., Maskow, T., Zaray, G., 2008. Investigation of the toxic effect of cadmium on *Candida humicola* and *Bacillus subtilis* using a microcalorimetric method. J. Hazard. Mater. 159, 465–470.
Cuypers, A., Plusquin, M., Remans, T., Jozefczak, M., Keunen, E., Gielen, H., Opdenakker, K., Nair, A.R., Munters, E., Artois, T.J., 2010. Cadmium stress: an oxidative challenge. Biometals 23, 927–940.
Egan, S., Fernandes, N.D., Kumar, V., Gardiner, M., Thomas, T., 2013. Bacterial pathogens: virulence mechanism and host defence in marine macroalgae. Environ. Microbiol. 16, 925–938.
Iqbal, M., Edyvean, R.G.J., 2004. Biosorption of lead: copper and zinc ions on loofa sponge immobilized biomass of *Phanerochaete chrysosporium*. Miner. Eng. 17, 217–223.
Jaleel, C.A., Gopi, R., Manivannan, P., Panneerselvam, R., 2008. Exogenous application of triadimefon affects the antioxidant defense system of *Withania somnifera* Dunal. Pestic. Biochem. Physiol. 91, 170–174.
Khachatryan, L., Vejerano, E., Lomnicki, S., Dellinger, B., 2011. Environmentally persistent free radicals (EPFRs).1. Generation of reactive oxygen species in aqueous solutions. Environ. Sci. Technol. 45, 8559–8566.
Kirk, T.K., Croan, S., Tien, M., 1986. Production of multiple ligninases by *Phanerochaete chrysosporium:* effect of selected growth conditions and use of a mutant strain. Enzyme Microb. Technol. 8, 27–32.
Klaunig, J.E., Xu, Y., Isenberg, J.S., Bachowski, S., Kolaja, K.L., Jiang, J., Stevenson, D.E., Walborg Jr., S.I., 1998. The role of oxidative stress in chemical carcinogenesis. Environ. Health Perspect. 106, 289.
Liu, L., Xu, P., Zeng, G.M., Huang, D.L., Zhao, M.H., Lai, C., Chen, M., Li, N.J., Huang, C., Wang, C., Cheng, M., He, X.X., Lai, M.Y., He, Y.B., 2014. Inherent antioxidant activity and high yield production of antioxidants in *Phanerochaete chrysosporium*. Biochem. Eng. J. 90, 245–254.
Marklund, S., Marklund, G., 1974. Involvement of superoxide anion radical in the auto oxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem. 47, 469–474.
May, M.J., Vernoux, T., Leaver, C., Van Montagu, M., Inzé, D., 1998. Glutathione homeostasis in plants: implications for environmental sensing and plant development. J. Exp. Bot. 49, 649–667.
Mishra, S., Srivastava, S., Tripathi, R.D., Kumar, R., Seth, C.S., Gupta, D.K., 2006. Lead detoxification by coontail (*Ceratophyllum demersum* L.) involves induction of phytochelatins and antioxidant system in response to its accumulation. Chemosphere 65, 1027–1039.
Morita, S., Tasaka, M., Fujisawa, H., Ushimaru, T., Tsuji, H., 1994. A cDNA clone encoding a rice catalase isozyme. Plant Physiol. 105, 1015–1016.
Nemmiche, S., Chabane-Sari, D., Guiraud, P., 2007. Role of α-tocopherol in cadmium-induced oxidative stress in Wistar rat's blood, liver and brain. Chem. Biol. Interact. 170, 221–230.
Pacini, N., Elia, A.C., Abete, M.C., Dörr, A.J.M., Brizio, P., Gasco, L., Righetti, M., Prearo, M., 2013. Antioxidant response versus selenium accumulation in the liver and kidney of the Siberian sturgeon (*Acipenser baeri*). Chemosphere 93, 2405–2412.
Pérez-Chaca, M.V., Rodríguez-Serrano, M., Molina, A.S., Pedranzani, H.E., Zirulnik, F., Sandalio, L.M., Romero-Puertas, M.C., 2014. Cadmium induces two waves of reactive oxygen species in *Glycine max* (L.) roots. Plant Cell Environ. 37, 1672–1687.
Pinto, E., Sigaud-kutner, T., Leitao, M.A., Okamoto, O.K., Morse, D., Colepicolo, P., 2003. Heavy metal-induced oxidative stress in algae. J. Phycol. 39, 1008–1018.
Scott, M.D., Meshnick, S.R., Eaton, J.W., 1987. Superoxide dismutase-rich bacteria: paradoxical increase in oxidant toxicity. J. Biol. Chem. 262, 3640–3645.
Shi, Q., Zhu, Z., 2008. Effects of exogenous salicylic acid on manganese toxicity: element contents and antioxidative system in cucumber. Environ. Exp. Bot. 63, 317–326.
Sies, H., Stahl, W., Sevanian, A., 2005. Nutritional, dietary and postprandial oxidative stress. J. Nutr. 135, 969–972.
Valko, M., Rhodes, C., Moncol, J., Izakovic, M., Mazur, M., 2006. Free radicals: metals and antioxidants in oxidative stress-induced cancer. Chem. Biol. Interact. 160, 1–40.
Watanabe, M., Henmi, K., Ogawa K. i. Suzuki, T., 2003. Cadmium-dependent generation of reactive oxygen species and mitochondrial DNA breaks in photosynthetic and non-photosynthetic strains of *Euglena gracilis*. Comp. Biochem. Physiol. C 134, 227–234.
Xu, P., Zeng, G.M., Huang, D.L., Feng, C.L., Hu, S., Zhao, M.H., Lai, C., Wei, Z., Huang, C., Xie, G.X., 2012a. Use of iron oxide nanomaterials in wastewater treatment: a review. Sci. Total. Environ. 424, 1–10.
Xu, P., Zeng, G.M., Huang, D.L., Lai, C., Zhao, M.H., Wei, Z., Li, N.J., Huang, C., Xie, G.X., 2012b. Adsorption of Pb (II) by iron oxide nanoparticles immobilized *Phanerochaete chrysosporium*: equilibrium kinetic, thermodynamic and mechanisms analysis. Chem. Eng. J. 203, 423–431.
Xu, P., Zeng, G., Huang, D., Hu, S., Feng, C., Lai, C., Zhao, M., Huang, C., Li, N., Wei, Z., 2013. Synthesis of iron oxide nanoparticles and their application in *Phanerochaete chrysosporium* immobilization for Pb (II) removal. Colloid. Surf. A 419, 147–155.