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Metal bioaccumulation, oxidative stress and antioxidant defenses in *Phanerochaete chrysosporium* response to Cd exposure

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ABSTRACT

Exposure of *Phanerochaete chrysosporium* (*P. chrysosporium*) to heavy metals is a common phenomenon due to their environmental applications. In the present study, *P. chrysosporium* accumulated high intracellular contents of Cd when cultured in metal-enriched culture medium, and then built up of distinct inhibition of growth rate. Concomitantly, rapid accumulation of H_2O_2 occurred and then triggered an induced signal transduction in activation of non-enzymatic antioxidants (glutathione (GSH), ascorbate (Asc) and phenolics) (below 30 $\mu mol\ g^{-1}$, $R^2 > 0.6$). Promoted yield of antioxidants (GSH, Asc and phenolics) was observed in Cd-exposed *P. chrysosporium*, and a tight network of cooperation of those tested antioxidants was innovatively affirmed via Pearson correlation analysis ($p < 0.01$). Thereafter, antioxidant consumption occurred as a network, and caused progressive alleviation in growth inhibition and oxidative stress. Integrated biomarker response further confirmed a remarkable antioxidant response against the negative effects of Cd.

1. Introduction

The potential environmental application of *Phanerochaete chrysosporium* (*P. chrysosporium*) based on biosorption technology is of special importance, due to their high efficiency with low cost (Xu et al., 2012a; Huang et al., 2010). The use of *P. chrysosporium* as biosorbent is efficient for metal pollution control and at same time ease the burden of disposal costs ascribed to the widely distribution and high metal-sequestering property of the biosorbents (Yetis et al., 2000; Zeng et al., 2015). However, a common consequence of biosorption application is that they result, at some stage of metal exposure, in a heavy metal induced stress. Numerous studies have revealed that metals can accumulate and redistribute in some microorganisms, causes growth inhibition and even cell death (Huang et al., 2006; Liao et al., 2005; Xu et al., 2012b).

Nowadays, it has been found that Cd is a common contaminant which result in an oxidative stress with the production of reactive oxygen species (ROS), and it has been considered as a major mechanism for Cd-induced toxicity (Valko et al., 2005). ROS are highly reactive molecules or molecular fragments produced in cells, which encompass free radicals (such as superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$)) and non-radicals (such as hydrogen peroxide (H_2O_2)) (Khachatryan et al., 2011; Chen et al., 2008). Enhanced generation of ROS can overwhelm cells' intrinsic antioxidant defenses and exceed the capacity of the ROS suppressing mechanism. Excess ROS therefore initiate adverse health impacts via cross-linking of glycoproteins, or lipid peroxidation and membrane damage, in the case of a condition known as oxidative stress (Pwawlowska and Iris, 2004; Lamb and Dixon, 1997). A variety of oxidative damages are found to be related to the production of ROS induced by heavy metals, and therefore antioxidant defenses has an important role in the protection of organisms against metal-induced oxidative stress (Cadenas, 1997; Jomova and Valko, 2011; Brembu et al., 2011).

Fungi, like many other microorganisms, rely on antioxidant defense mechanisms for protection against oxidative damage (Liang et al., 2009). In order to improve fungi's environmental application, it is important to understand the mechanisms contributing to stress tolerance coping with adversity environments. Nowadays, much research attention has been focused on the antioxidant response in plants, algae and aquatic animals (Gomes et al., 2014; Yadav et al., 2013; Tripathi et al., 2013), however, researches in view

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of metal tolerance and detoxification mechanisms in white-rot fungi, with excellent biosorption ability to heavy metals, are quite scarce. The challenge is now to work toward a more comprehensive understanding of the biochemical basis of Cd bioaccumulation and tolerance.

The present research work was therefore focused on Cd toxicity and antioxidant response of *P. chrysosporium* on the metabolic and physiological level. Time-course of Cd-induced oxidative stress was detected and the feasibility of the antioxidant mechanism was further investigated, with particular attention given to the constitution and response of antioxidant network systems. Most importantly, mediating roles of as signals activation of antioxidant system or as noxious molecules triggering oxidative stress have been innovatively investigated. Bioassays based on antioxidant systems can provide more information about the response of *P. chrysosporium* coping with adversity environments during environmental application.

2. Materials and methods

2.1. Strain and chemicals

The *P. chrysosporium* strain BKMF-1767 (ATCC 24725) 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 solvents and other reagents were of the highest purity commercially available. All reagents used in the experiment were of or above analytical reagent grade.

2.2. Cd exposure and biomass analysis

Spore suspensions of *P. chrysosporium* were prepared in the sterile distilled water at a concentration of 2.0×10^6 CFU mL^{-1}. 2 mL of as prepared spore suspensions were inoculated into 100 mL growth medium as described by Kirk et al. (1986), with desirable concentrations of Cd at 0, 20, 50 and 100 ppm (as $Cd(NO_3)_2{\cdot}4H_2O$, 0 d) and cultured at 30 °C with constant stirring at 120 rpm (three culture replicates for each group). *P. chrysosporium* cultured at 0 ppm Cd was defined as control sample in the whole experiment (controls). Biomass was collected at selected intervals and washed three times in 20 mL phosphate buffer (PB, 50 mM, pH 7.0), and then centrifuged and filtered for monitoring the wet weight of *P. chrysosporium*.

2.3. Analysis of oxidative stress biomarkers

The collected biomass was homogenized in 10 mL of PB (50 mM, pH 7.4), and then centrifuged at 10,000 rpm at 4 °C for 10 min. Thereafter, the supernatant was filtered via 0.45 μm filter membranes for the preparation of *P. chrysosporium* extracts. The oxidative stress parameters, containing H_2O_2, malonaldehyde (MDA) and peroxide value (POV), were frequently measured. H_2O_2 in *P. chrysosporium* extracts was detected according to H_2O_2 assay kit purchase from Beyotime institute of biotechnology. MDA content was estimated using the procedure described by Aravind and Prasad (2003) with minor modifications. 1.5 mL *P. chrysosporium* extracts was boiled with 1.5 mL thiobarbituric acid (0.6%) for 20 min, and then centrifuged at 5000 rpm for 5 min. After that the absorbance of the mixture was measured at 450, 532 and 600 nm using the UV–visible spectrophotometry (Shimadzu 2550). Additionally, POV, characterizing the degree of oxidation of the cells, was determined according to the food quality standards in China (GB/T 5009.37-2003). Briefly, 0.05 mL of $FeCl_2$ solution (3.5 g L^{-1}) were mixed with 0.25 mL the prepared *P. chrysosporium* extracts, and then diluted to 4.0 mL using trichloromethane–methanol solution, thereafter 0.05 mL potassium thiocyanate was added and stewing for 5 min at 30 °C. The absorbance of the mixture was tested at 500 nm by UV–visible spectrophotometry.

2.4. Analysis of antioxidant components

GSH content was determined according to our previous study (Xu et al., 2014). Asc was determined using the method of Kampfenkel et al. (1995). Asc was determined in a reaction mixture containing 150 μL *P. chrysosporium* extracts, 300 μL ultrawater, 300 μL phosphate buffer 0.2 M (pH 7.4), 750 μL 10% trichloroacetic acid, 600 μL of 42% phosphoric acid, 600 μL of dipiridil dissolved in 70% ethanol and 300 μL of 3% phosphoric acid, shaking and incubating at 42 °C for 40 min, the absorbance of the mixture solutions was recorded at 525 nm by UV–visible spectrophotometry. Total phenolics were determined by Folin–Ciocalteau method according to the previous literature with minor modifications, using gallic acid as a standard (Cheung et al., 2003). An aliquot of 0.4 mL *P. chrysosporium* extracts was mixed with 0.4 mL of Folin–Ciocalteau agent (50%) for 3 min, and 2 mL of sodium carbonate solution (2%) was then added to the mixture. The absorbance of the mixture was read at 765 nm after incubation at room temperature (25–30 °C) for 45 min.

2.5. Integrated biomarker response analysis

Integrated biomarker response (IBR), as a method considering all the tested biomarker responses to one general stress index, was applied to evaluate an integrated response to Cd exposure (Qu et al., 2014; Wang et al., 2010). The procedure of IBR calculation is briefly described here: (1) Calculation of mean value and SD for each Cd concentration; (2) Standardization of data for each station: $Y_i' = (Y_i - \text{mean } Y)/S$, where Y_i' is the standardized value of the biomarker, Y_i is the mean value of a biomarker from each Cd concentration, mean Y is the mean of the biomarker calculated from all Cd concentrations, and S is the standard deviation of the biomarker; (3) Defined a standardized data Z, determined as $+Y_i'$ in the case of activation and $-Y_i'$ in the case of inhibition activation; (4) Calculation of the score B_i as $B_i = |\min Y_i'| + Z$, $|\min Y_i'|$ is the absolute value of minimum Y_i'. For all the biomarkers treated this way, a star plot radius coordinate represents the score of a given biomarker. Star plot areas are calculated by multiplication of the obtained value of each biomarker (Bi) with the value of the next biomarker, arranged as a set and summing-up of all values. The corresponding IBR value is: $\{[(B_1 \times B_2)/2]+[(B_2 \times B_3)/2]+[(B_{n-1} \times B_n)/2]+[(B_n \times B_1)/2]\}$. In the present study, two kind of IBR was calculated in the case of Cd-induced toxicity and detoxification response of *P. chrysosporium*.

3. Results and discussion

3.1. Cd bioaccumulation and growth inhibition in P. chrysosporium

Cd amount curves shown in Fig. 1a plotted out that Cd bioaccumulation in *P. chrysosporium* occurred in all tested concentrations. After 6 d of exposure, a significant accumulation of Cd was observed in *P. chrysosporium*. As the Cd concentration increased, there was a concomitant promotion in Cd bioaccumulation content. The highest Cd accumulation ((154.29 μg g^{-1} at 6 d) was observed in *P. chrysosporium* exposed to 100 ppm Cd. Simultaneously, growth inhibition, as a common symptom of metal toxicity, occurred accompanied with Cd bioaccumulation. Period of exponential growth of *P. chrysosporium* shown in Fig. 1b demonstrated that Cd exerted an inhibition effect on the growth of *P. chrysosporium*. At higher Cd concentrations, *P. chrysosporium* grew significantly slower while compared with controls in the early exponential growth phase. As compared with the controls, biomass treated with 50 and 100 ppm Cd significantly decreased (0.25 and 0.083 fold of

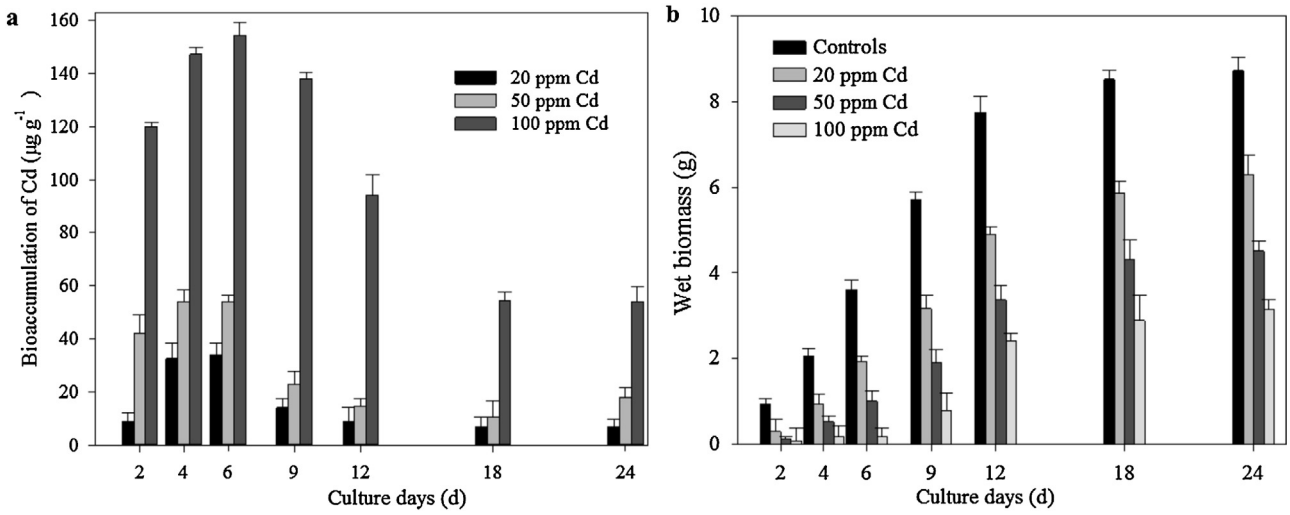

Fig. 1. Bioaccumulation of Cd in *P. chrysosporium* (a) and wet biomass of *P. chrysosporium* (b) exposed to 0, 20, 50, 100 ppm Cd. *P. chrysosporium* were grown in Kirk liquid culture under the exposure to 0, 20, 50, 100 ppm Cd at 30 °C. Vertical bars show standard deviation. Data are means of three replicates (±SD).

Table 1
Pearson correlation analysis correlated to intracellular Cd (Cd_{intra}), Cd-induced growth status (biomass), oxidative stress (H_2O_2, MDA, POV) and antioxidant responses (GSH, Asc, phenolic) in *Phanerochaete chrysosporium*.

	Cd_{intra}	Biomass	H_2O_2	MDA	POV	GSH	Asc	Phenolic
Cd_{intra}	1							
Biomass	−**0.640****	1						
H_2O_2	**0.385***	−0.409*	1					
MDA	**0.598****	−0.570**	**0.666****	1				
POV	**0.812****	−0.617**	**0.470***	**0.656****	1			
GSH	0.841**	−0.670**	0.397*	**0.627****	**0.944****	1		
Asc	0.882**	−0.699**	0.305*	**0.738****	**0.954****	**0.916****	1	
Phenolic	0.829**	−0.615*	0.238	**0.718****	**0.957****	**0.893****	**0.960****	1

* Correlation is significant at the 0.05 level (two-tailed).
** Correlation is significant at the 0.01 level (two-tailed).

controls at 4 d, respectively). This is in line with the general mechanism of Cd-induced inhibition of growth under Cd exposure, that is, Cd insertion into the *P. chrysosporium* intracellularly (Table 1, $p < 0.01$). However, a distinct decrease of bioaccumulation levels in *P. chrysosporium*, occurred after 6 d, and then kept a gradual decrease till 18 d of Cd exposure, might be attributed to the desorption of Cd. As a consequence, remittent growth inhibition was found under continuous Cd exposure, along with a distinct decreased cell inhibition ratio in the Cd-containing culture on day 24 (Fig. 1b).

3.2. *Cd-induced oxidative stress in* P. chrysosporium

Under physiological steady state conditions, cells or tissues are in a stable state in an essentially balance between the rates of ROS production and scavenging capacity. H_2O_2 derived from radicals exist in biological cells and tissues at low but measurable concentrations (Fig. 2a, controls). However, the equilibrium between production and scavenging of ROS may be perturbed by a number of adverse environmental factors, such as heavy metal exposure (Apel and Hirt, 2004). The monitored real-time oxidative stress in *P. chrysosporium* following sequential exposure to Cd is presented in Fig. 2. It was obviously that oxidative biomarkers of samples exposed to various levels of Cd were all higher than controls during the whole growth stage. As the concentration of Cd in the external medium increased, there was a concomitant increase in intracellular oxidative stress. H_2O_2 content in 20, 50 and 100 ppm Cd treated cells was 1.98, 4.12 and 7.58 fold of the control (4 d), respectively. The formed H_2O_2, unlike the oxygen radicals, can readily diffuse across biological membranes; consequently cause oxidative stress far from the site of formation, resulting in the attack of cellular components, particularly cell membranes. The cell membranes, containing a phospholipid bilayer that embedded a variety of biological lipids, proteins, phospholipids and glycolipids, were susceptible to oxidation and caused ROS-induced lipid peroxidation (Valko et al., 2006). Products of lipid peroxidation, such as MDA, are capable of inactivating many cellular proteins by forming protein cross-linkages, therefore used as indirect biomarkers of oxidative stress (Valko et al., 2004, 2005). Remarkable increased levels of MDA were observed in the Cd exposed *P. chrysosporium* at the initial culture stage (4 d), at the values of 0.011, 0.016 and 0.025 μmol g^{-1} at the Cd concentration of 20, 50 and 100 ppm, respectively (Fig. 2b). In addition, Cd exposure could also change the redox status of the cell. Significant stimulation of POV was also detected in the *P. chrysosporium* intracellularly while exposed to higher levels of Cd (Fig. 2c).

Meanwhile, oxidative stress based on these tested symbiotic indexes was found to be directly proportional to Cd bioaccumulation levels (Fig. 2d, $R^2 > 0.6$). For example, H_2O_2 contents were found to be significant correlated with the Cd contents in *P. chrysosporium* during the whole exposure stage at various Cd concentrations (Fig. 2d, $R^2 = 0.6189$). The assays corroborated that *P. chrysosporium* was experiencing an increased rate of lipid peroxidation initially accompanied with Cd bioaccumulation. However, it was also important to note that exposure time significantly affected the oxidative stress, which decreased as a result of growth time course, possibly due to the provocative detoxification mechanism of *P. chrysosporium*.

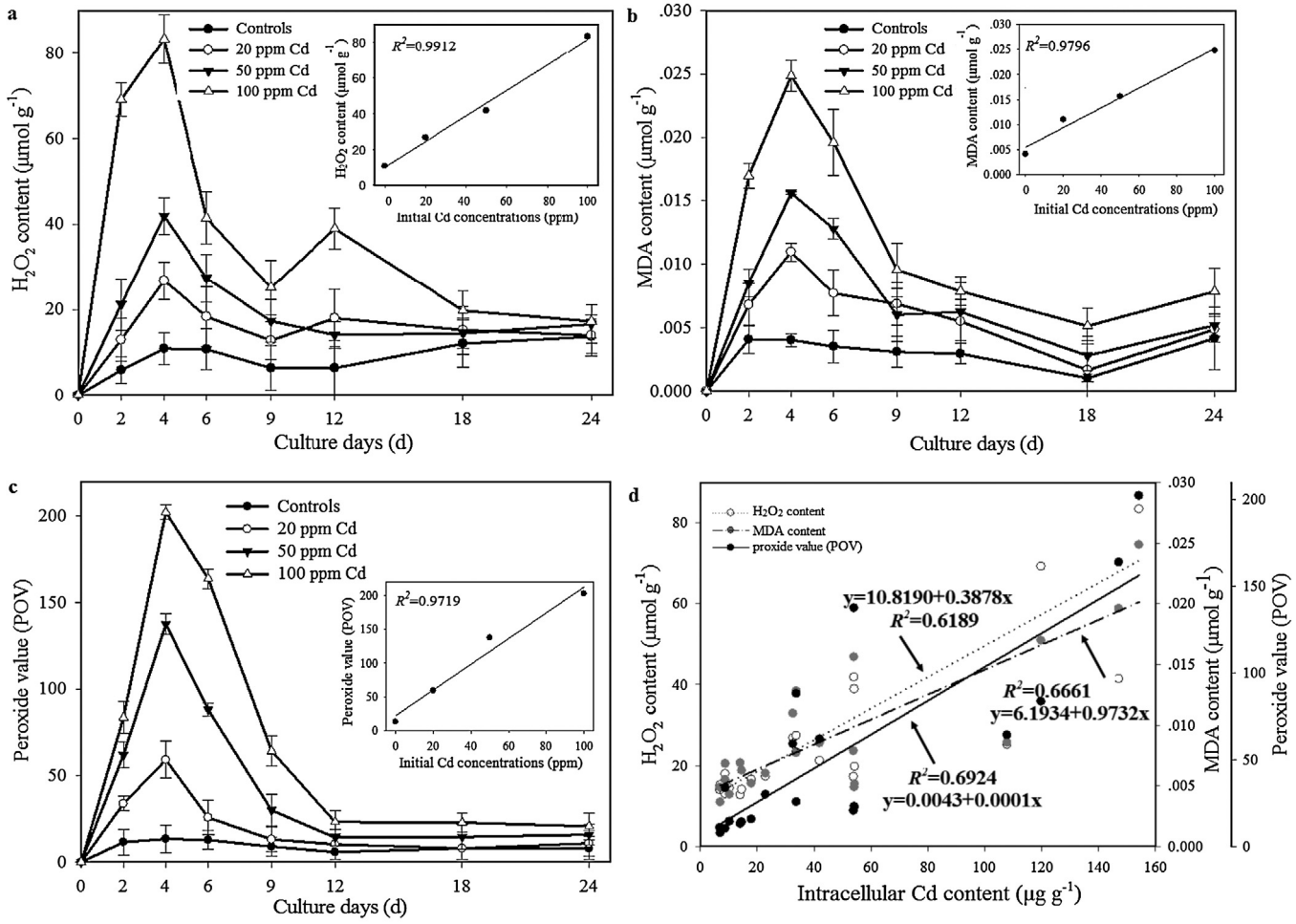

Fig. 2. Time-course of intracellular H_2O_2 accumulation (a), malondialdehyde (MDA) accumulation (b) and peroxide value (POV) (c) of *P. chrysosporium* exposed to 20, 50 and 100 ppm Cd; The inserted figure showed the significant correlation between initial Cd concentration and the oxidative biomarkers (H_2O_2, ROS, MDA, POV) in *P. chrysosporium* at 4 d. (d) Correlations between intracellular Cd content and intracellular H_2O_2, MDA, POV in *Phanerochaete chrysosporium* intracellularly.

3.3. Cd-induced antioxidant response and cooperation of antioxidant network

When oxidative stress occurs, cells attempt to counteract the oxidant effects and restore the redox balance by activation of antioxidants. Fig. 3 shows GSH, Asc and phenolic bioaccumulation in *P. chrysosporium* exposed to 0, 20, 50 and 100 ppm concentrations of Cd, respectively. Apparently, Cd exposure initially resulted in a rapid induction of antioxidant response within the first 4 days of Cd exposure in *P. chrysosporium* (Fig. 3). Results of intracellular GSH bioaccumulation revealed that GSH at the initial stage (4 d) ranged from 0.45 to 3.68 μmol g^{-1}, with a dose-dependent increase in external Cd concentration (Fig. 3a). Peak value of GSH (4.87 μmol g^{-1}) was found at 100 ppm concentration at day 6. Thereafter, GSH depletion occurred, and the depletion rate was found quite coincided with ROS scavenging rate and lipid peroxidation remission from 2 to 4 d (Figs. 2 and 3). The consumption of GSH might be ascribed to the possible antioxidant roles of GSH as general reductant or substrate participating in control of ROS levels (Schützendübel and Polle, 2002; Pinto et al., 2003). The present data confirmed that Cd-induced induction of GSH bioaccumulation (Fig. 3d, R^2 = 0.7079) may serve as an early inducible protective mechanism against toxic heavy metals, particularly at Cd concentrations that arise during environmental or occupational exposure. Generally, thiol group of GSH donated a reducing equivalent ($H^+ + e^-$) to ROS, initiating the decomposition reaction. And then the formed reactive glutathione radicals readily reacted with another GSH molecular with the generation of glutathione disulfide (GSSG) (Kidd, 1997).

Likewise, higher resistance to Cd may be concerning to the comparatively drastic depletion of Asc and phenolics (Fig. 3). Pronounced increases were found at the initial stage for Asc and phenolics, especially at higher levels of Cd. Analysis of Asc amounts in the presence of 20 and 100 ppm Cd showed that 2.15- and 7.12-fold more Asc has been found at 4 d, respectively, than those of samples in the absence of Cd (Fig. 3b). Meanwhile, the constitutive concentrations of phenolic also increased proportional to the accumulated Cd content (Fig. 3d, R^2 = 0.6982). Cd at 100 ppm caused a most significant promotion of total phenolic content (6.12 fold) at 4 d. After reach peak values, plummeted depletion of the tested antioxidants occurred, and it was found quite coincided with ROS scavenging rate and lipid peroxidation remission.

Actually, the potential for heavy metal ion toxicity has forced attention on its early evolution to develop metal ion homoeostasis factors and metal-resistance determinants (Jaleel et al., 2008; Ahmad et al., 2010). A tightly controlled metal homeostasis network to adjust to fluctuations is therefore a necessity for all organisms, to maintain the overall redox/oxidative balance. Both phenol-coupled-APX reaction and Asc/GSH cycle serve the removal of H_2O_2, which are inevitably formed as by-products of the normal metabolism or as a consequence of various exogenous environmental insults (Polle, 2001). Asc and GSH attributed to their

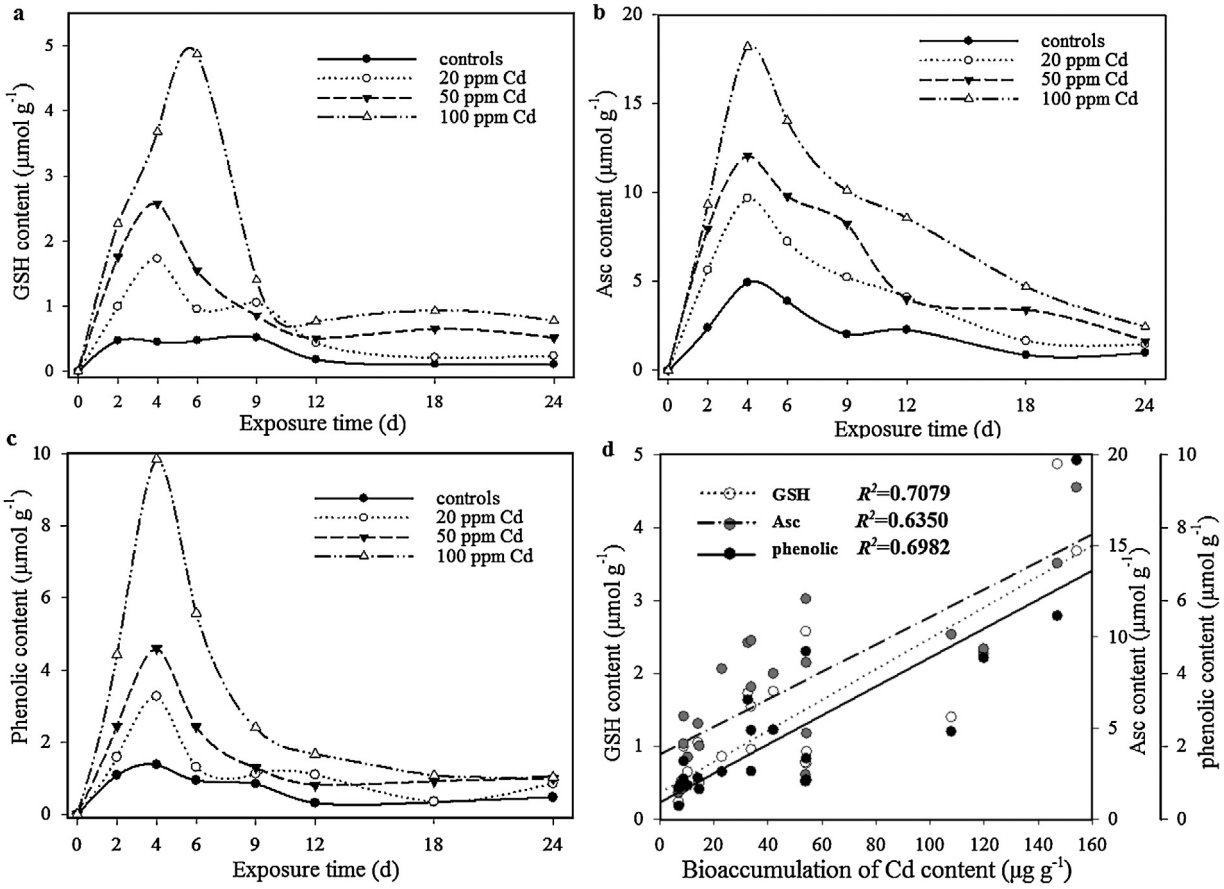

Fig. 3. Time-and-concentration dependent of intracellular GSH (a), Asc (b) and phenolic (c) accumulation in *P. chrysosporium* exposed to 0, 20, 50 and 100 ppm Cd; (d) Correlation between Cd bioaccumulation intracellularly and GSH, Asc and phenolic during the whole exposure stage of 24 days.

most prominent and best established functions via Asc/GSH cycle. Causal relationships between certain components of the cycle and stress tolerance have been demonstrated with relatively high correlation coefficient among GSH, Asc and phenolics (Table 1, $p < 0.01$). After initial accumulation of H_2O_2, GSH, Asc and phenolics were rapidly consumed and H_2O_2 accumulated to nontoxic concentrations under standard conditions (Figs. 2 and 3). Plummeted depletion of GSH occurred from 4 d, which contributed to the decomposition of the accumulated H_2O_2 in *P. chrysosporium* ($H_2O_2 + GSH \rightarrow GSSG + 2H_2O$). Besides depletion of GSH, Asc was oxidized to MDA resulting in a slightly dampened increase in MDA at 4 d ($H_2O_2 + 2Asc \rightarrow 2MDA + 2H_2O$). However, the produced MDA can be reduced in a reaction catalyzed by MDA reductase via complete conversion of Asc into DHA ($MDA + MDA \rightarrow Asc + DHA$) (Hossain and Asada, 1985), and the DHA could further reduced by GSH ($DHA + GSH \rightarrow Asc + GSSG$) (Foyer and Halliwell, 1976). The depletion of GSH and subsequently that of Asc were onset (Fig. 3), net accumulation of H_2O_2 and MDA decreased sharply (Fig. 2). Therefore, despite the intense initial stress, the cells exhibited resistance to Cd exposure in late exponential phase, with waning of IBR in growth inhibition and oxidative stress (Fig. 4).

3.4. Mediating roles of H_2O_2 as signal factor between oxidative stress and antioxidant systems

Commonly, reactive oxygen species have been considered mainly as dangerous molecules, whose levels need to be kept as low as possible. Nowadays it has been realized that ROS might play important roles in the defense system, acting as intermediate signaling molecules to induce antioxidant systems and/or regulate the expression of genes (Qu et al., 2014). The lack of correlations of the GSH, Asc and phenolic concentrations among the whole H_2O_2 levels indicate the dual role of H_2O_2 in mediating the balance between oxidative stress and antioxidant response (Table 1). However, considering a concentration of 30 μmol g^{-1} as the boundary between inducible antioxidant signals and oxidative stress dangerous molecules, the dual relationship between intracellular concentrations of H_2O_2 and the test non-enzymatic antioxidants was shown in Fig. 4a. Gradual increase in GSH, Asc and phenolics up to a certain level of intracellular H_2O_2 (30 μmol g^{-1}) was observed (Fig. 4b, $r > 0.65$). Results showed that dietary H_2O_2 concentrations below 25 μmol g^{-1} are found to be proportional to non-enzymatic antioxidants, due to the signaling trait of H_2O_2 responsible for the onset of cellular protection mechanisms (Torres et al., 2006).

However, further increase in H_2O_2 concentration caused decrease in non-enzymatic antioxidant contents in *P. chrysosporium*, indicating the potential toxicity of excessive H_2O_2. Actually, Cd enters the cell through membrane induces conformational changes in compounds located in the membrane and consequently interfere with oxidative phosphorylation and alter its membrane permeability further causing a free-radical mediated propagation of oxidative insult to lipid peroxidation. Consequently, H_2O_2-dependent MDA accumulation and POV increment were observed in Fig. 4b, with high correlation coefficients ($R^2 = 0.7963$ and 0.6983, respectively), indicating the H_2O_2 derived lipid peroxidation and oxidation/redox imbalance in *P. chrysosporium*. High correlations among those three indicators suggested that ROS production, lipid peroxidation and peroxide value provided a convenient, noninvasive method of assessing oxidative stress in response to various contaminants.

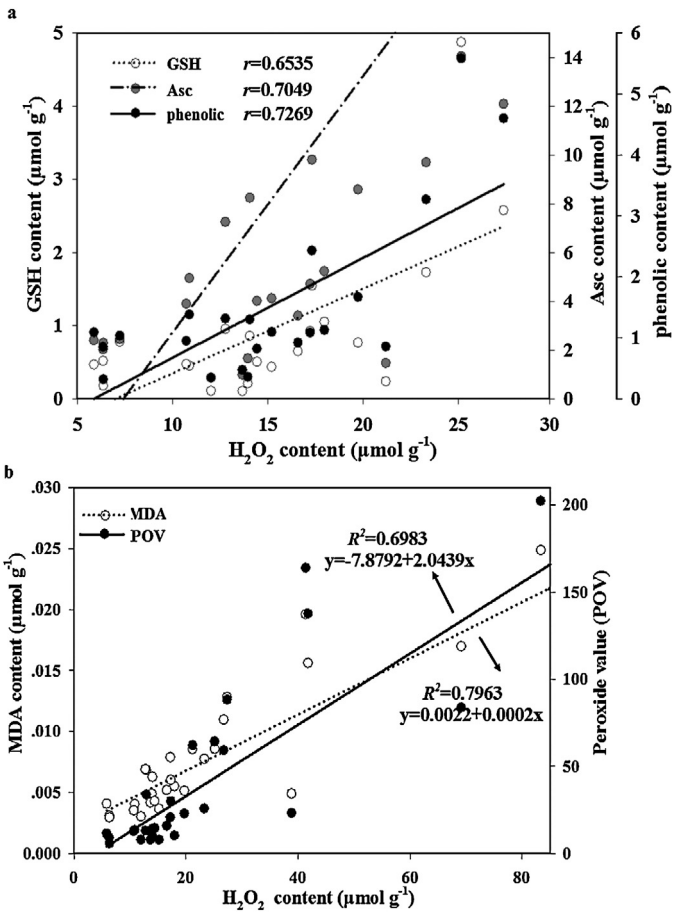

Fig. 4. (a) Relationship between dietary intracellular H_2O_2 contents (below 30 μmol g^{-1}) and non-enzymatic antioxidants; (b) Correlations between H_2O_2 content and MDA, POV in *Phanerochaete chrysosporium* intracellularly.

Overall, since the uncontrolled levels of H_2O_2 lead to •OH formation and lipid peroxidation, and critical for their roles in redox-dependent signaling, antioxidant systems exist to regulate intracellular ROS levels spatially and temporally are necessary (Reczek and Chandel, 2015). The phenomenon of cross-response to various concentrations of H_2O_2 suggests the existence of a common factor, which provides crosstalk between signaling pathways and potential toxicity. However, further studies are necessary to accurate evaluate the critical roles of H_2O_2 wandering between the signaling roles and oxidative stress sources.

3.5. Integrated biomarker response analysis

The star plots of IBR for Cd-induced toxicity and antioxidant responses were shown in Fig. 5. Based on the IBR, it was apparent that Cd at higher concentrations led to severer toxicity to *P. chrysosporium*, accompanied with higher values of IBR related to growth inhibition and oxidative stress biomarkers (Fig. 5a). IBR values were at the maximal values at 100 ppm Cd at 4 d, and then IBR decreased at 12 d and 24 d, on account of the tolerance with alleviation of Cd-induced toxicity. Meanwhile, intense antioxidant responses were also found at higher Cd concentrations. However, decreases in IBR values in the case of antioxidant response were observed during the exposure stage. The IBR analysis was quite agreed with the above results that an early inducible detoxification mechanism in *P. chrysosporium* against Cd bioaccumulation. A remarkable protection of fungi performance against the negative effects of Cd therefore could be predicted, indicating the tolerance of *P. chrysosporium* to Cd in the case of the inherent detoxification mechanisms. Results proved that *P. chrysosporium* may trigger two different mechanisms to regulate their intracellular Cd concentrations and oxidation/redox balance: one that will enable the fine modulation of low levels of ROS for signaling purposes enabling the detoxification of excess ROS, and one that high concentrations of H_2O_2 up the steady-state level can pose a threat to cells, causing the inactivation of the antioxidant response.

4. Conclusions

Taking all these observations together, the following hypothetical framework could be confirmed: Cd ions entering the cells induced a free-radical mediated propagation of oxidative insult with the production of H_2O_2 and triggered lipid peroxidation. The rapidity of H_2O_2 production and the potential of freely diffuse across membranes suggested that H_2O_2 could function as an intercellular or intracellular second messenger and set off a sequence of reactions with up-regulation of GSH, Asc and phenolics.

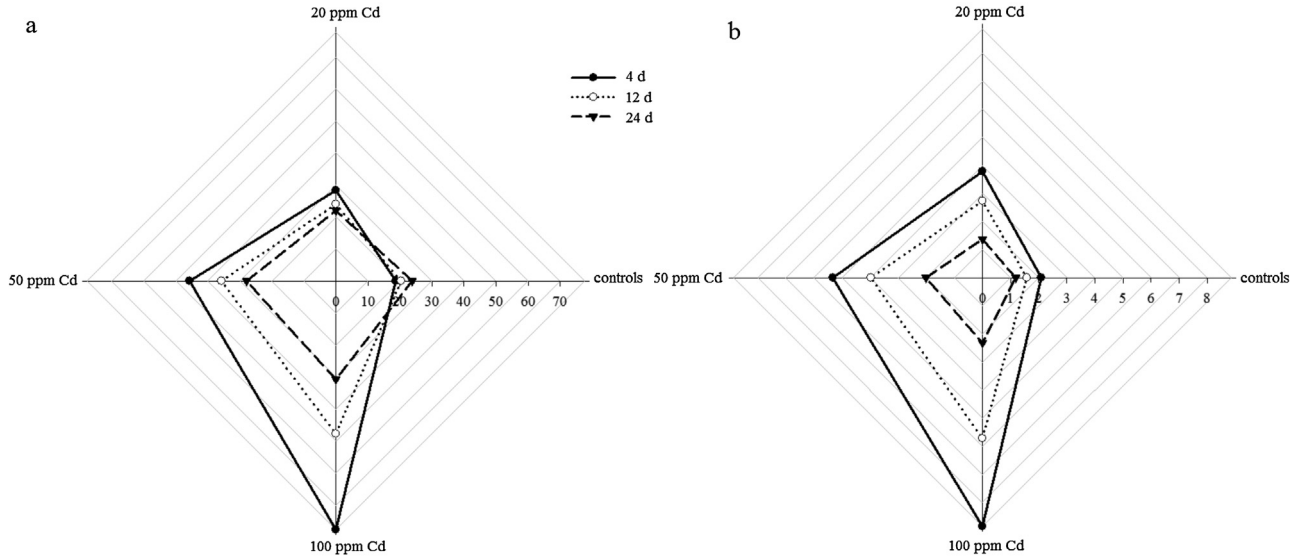

Fig. 5. Integrated biomarker response (IBR) index of Cd-induced toxicity (a) and antioxidant response (b) in *P. chrysosporium* exposed to 0, 20, 50 and 100 ppm Cd.

Cooperation of antioxidant network system response to Cd exposure has been determined with significant correlations via Pearson correlation analysis ($p < 0.01$). Consequently, alleviation in growth inhibition and oxidative stress was observed and further confirmed by integrated biomarker response analysis. In conclusion, we suggest that the superior tolerance to Cd may be related to the higher antioxidant accumulation and, even most importantly, the cooperation of non-enzymatic network.

Acknowledgments

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