Alleviation of heavy metal and silver nanoparticle toxicity and enhancement of their removal by hydrogen sulfide in *Phanerochaete chrysosporium*

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Abstract

Hydrogen sulfide (H_2S), an important cellular signaling molecule, plays vital roles in mediating responses to biotic/abiotic stresses. Influences of H_2S on metal removal, cell viability, and antioxidant response of *Phanerochaete chrysosporium* upon exposure to heavy metals and silver nanoparticles (AgNPs) in the present study were investigated. An enhancement in Pb(II) removal with an increase in concentration of the H_2S donor sodium hydrosulfide (NaHS) was observed, and the maximum removal efficiencies increased by 31% and 17% under 100 and 200 mg/L Pb(II) exposure, respectively, in the presence of 500 μM NaHS. Application of 500 μM NaHS increased the cell viability by 15%–39% under Pb(II) stress (10–200 mg/L) with relative to the untreated control. Increase in total Ag uptake and cell survival was also elicited by NaHS in a concentration-dependent manner under AgNP stress. Meanwhile, activities of superoxide dismutase and catalase were significantly enhanced with the introduction of NaHS under stresses of Pb(II), Cd(II), Cu(II), Zn(II), Ni(II), and AgNPs. The inhibition in lipid peroxidation and oxidative stress was observed in *P. chrysosporium* cells exposed to these toxicants following NaHS pretreatment, which could be attributed to the upregulation in antioxidant enzymes. The results obtained suggest that H_2S can alleviate heavy metals and AgNP-induced toxicity to *P. chrysosporium* and improve the removal efficiency of these toxicants from wastewater.

Keywords: Hydrogen sulfide; Heavy metals; Silver nanoparticles; Bioremediation; Antioxidant response; *Phanerochaete chrysosporium*

1. Introduction

Heavy metal contamination in water and soil has been one of the most concerned global environmental problems due to the increasing anthropogenic and industrial activities (Fang et al., 2016; Tang et al., 2018; Ye et al., 2017; Zhang et al., 2019). Excess exposure to heavy metals results in the deterioration of environmental quality and causes serious effects on the development of microorganisms, plants, animals, and humans because of their long-term toxic effects, carcinogenicity, and mutagenicity (Houda et al., 2016; He et al., 2018; Ren et al., 2018a and b; Wang et al., 2018). It is reported that lead (Pb), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), and zinc (Zn) are the major heavy metal toxicants (Macomber et al., 2011; Ye et al., 2017; Ye et al., 2017; Qin et al., 2018; Zhou et al., 2018; Wang et al., 2013). Trace amounts of these toxicants are able to cause colonization inhibition, membrane damage, oxidative stress, antioxidant enzyme upregulation, chromosome aberration, and even cell death (Gong et al., 2009; Huang et al., 2015; Ali et al., 2014). In addition, explosion in the use of silver nanoparticles (AgNPs), such as antimicrobial and sterile applications, also possesses environmental risks to human health and ecosystem (Choi et al., 2018; He et al., 2017a, 2018a and b). Both AgNPs themselves and released Ag^{+} can induce generation of reactive oxygen species (ROS) and cytotoxicity through direct damage to cell membrane of some aquatic organisms and microbes in biological wastewater treatment processes (Wu et al., 2017; Zhang et al., 2018; Yang et al., 2018). Meanwhile, it has been documented that Ag speciation and precipitation are potentially changed due to alteration in transport and fate of

AgNPs under environmental stress, thus influencing their toxicity against aquatic organisms (McGillicuddy et al., 2017; Yi et al., 2018; Yang et al., 2018; Xiong et al., 2018; Leng et al., 2019).

Bioremediation is an economic, efficient, and environmentally friendly alternative for removal of heavy metals (He et al., 2017b; Mir-Tutusaus et al., 2018). *Phanerochaete chrysosporium*, a typical species of white rot fungi, has been proven to be available for the treatment of wastewater containing heavy metals and AgNPs due to its admirable biosorption capacity (Xu et al., 2012a and b). However, the efficiency of *P. chrysosporium* in removal of heavy metals and AgNPs is still limited due to its lower resistance to toxic pollutants and longer bioremediation time. Hydrogen sulfide (H_2S) has been used to assist in 2,4-dichlorophenol (2,4-DCP) biodegradation by *P. chrysosporium* in our previous study (Chen et al., 2014). Therefore, it is worth further exploring whether this exogenous material can improve bioremediation capability of heavy metals and AgNPs.

H_2S, a new gaseous signal molecule, has been recommended for mediating a variety of physiological processes and defense responses against to biotic and abiotic stresses including heavy metals (Chen et al., 2018). More recent evidences have indicated that H_2S can exert antiinflammatory, antioxidant, antiapoptotic, cytoprotective, and organ-protective effects, further improving environmental stress tolerance of cells (Yuan et al., 2017). The protective roles of H_2S in plants could be attributed to the decreased influx and transport of metals and the elevated antioxidant enzymatic systems, including superoxide dismutase (SOD), catalase (CAT),

peroxidase, and non-enzymatic constituents (He et al., 2018). Enhancement in enzyme activities, such as SOD, CAT, and reduced glutathione, leads to the decreases in malonyldialdehyde (MDA) content and ROS production. In addition to the improvement of antioxidant enzymes, H_2S, as a reductive substance, can also scavenge ROS directly, such as superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (Geng et al., 2004; Mitsuhashi et al., 2006). However, little information is available on the introduction of H_2S to bioremediation using microorganisms, especially fungi. Moreover, whether H_2S can alleviate the toxic effects induced by heavy metals and nanomaterials, and improve the biological treatment efficiency remains an open question. Thus, the aim of this study was to investigate the effects of H_2S on the bioremediation efficiency, cell viability, oxidative damage, and antioxidant enzyme activities of *P. chrysosporium* under stresses of Pb(II), Cd(II), Cu(II), Ni(II), Zn(II), and AgNPs. *P. chrysosporium* was pretreated with the H_2S donor sodium hydrosulfide (NaHS) prior to exposure to these toxicants.

2. Materials and methods

2.1. Strain culture

P. chrysosporium strain BKMF-1767 (CCTCC AF96007) obtained from the China Center for Type Culture Collection (Wuhan, China) was maintained on malt extract agar slants at 4 ℃. Spore suspensions were prepared by gently scraping the spores from the agar surface into sterile ultrapure water and blending them evenly. The fungal spore suspensions at a concentration of 2.0×10^6 CFU/mL were inoculated into the culture medium and cultivated at 37 ℃ under 150 rpm. After 3 days of

incubation, *P. chrysosporium* mycelia were harvested and rinsed several times for further use.

2.2. Characterizations of AgNPs

Polyethylene glycol-coated AgNPs (PVP-AgNPs) used in this work were obtained from NanoAmor (Houston, TX). The PVP-AgNP powders were suspended in ultrapure water, mixed with ultrasonic agitation, and stored at 4 ℃ in dark for further use. The as-prepared AgNPs were monodispersed and spherical in shape with an average particle diameter of 57.3 ± 15.5 nm on the basis of transmission electron microscopy (TEM) observations (Fig. 1). The mean hydrodynamic diameter of AgNPs was also determined with the value of 85.1 ± 2.7 nm by dynamic light scattering (DLS) with a Zetasizer Nano-ZS (Malvern Instrument, U.K.). Difference in size distributions obtained from TEM and DLS methods arises from their different measurement principles (Huang et al., 2017). Besides, the zeta-potential of AgNPs showed a negative value of −10.8 ± 0.8 mV, and the dissolved fractions of AgNP stock suspensions were found to be less than 1% by monitoring the filtrates after ultrafiltration centrifugation using ICP-MS. After digestion of the samples using HNO_3 and H_2O_2, total Ag concentration in aqueous solutions was determined by a flame atomic absorption spectroscopy (FAAS, PerkinElmer AA700, USA) (Huang et al., 2018a and b).

2.3. Effect of H_2S on removal of heavy metals and AgNPs

The influences of different concentrations of H_2S on Pb(II) and total Ag removal were investigated. After pretreatment with NaHS (0, 50, 100, and 500 μM) for 4 h, *P.*

chrysosporium pellets were transferred to aqueous solutions containing 10, 50, 100, and 200 mg/L Pb(II) and AgNPs separately for another 78 h. Furthermore, a comparative experiment on removal of various heavy metals was also performed. The fungal pellets were pretreated with 100 μM NaHS for 4 h and then exposed to 100 mg/L Cd(II), Cu(II), Ni(II), and Zn(II), separately. Cultures that were treated with 0–500 μM NaHS but without heavy metals or AgNPs were used as controls. The culture media were taken at predetermined intervals for analysis of residual heavy metal concentrations. Concentrations of heavy metals in the aqueous solutions were monitored by using FAAS.

2.4. Physiological assays

After pretreatment with NaHS (0–100 μM), *P. chrysosporium* pellets, that were exposed to Pb(II), Cd(II), Cu(II), Ni(II), Zn(II), and AgNPs at initial concentrations of 100 mg/L for 24 h, were harvested and washed three times with ultrapure water for physiological analyses, including cell viability, lipid peroxidation, $O_2^{\cdot-}$ generation, and antioxidant enzymes. For cell viability assays, prolonged exposure (78 h) to 10–200 mg/L Pb(II) and AgNPs with and without NaHS pretreatments was also carried out. Cell viability was assessed by MTT method according to Chen et al. (2014).

The content of malondialdehyde (MDA), a cytotoxic product of lipid peroxidation, was measured following our previous procedures (Zeng et al., 2012; Huang et al., 2018c). $O_2^{\cdot-}$ generation was detected according to Chen et al. (2014) with minor modifications. Briefly, fungal samples (0.2 g) were homogenized in 2.5 mL of phosphate buffer (50 mM, pH 7.8). 1 mL of the extracts was added into 0.9 mL

of 50 mM phosphate buffer and 0.1 mL of 10 mM hydroxylamine hydrochloride. After 20 min of reaction at 25 ℃, 1 mL of 17 mM p-aminobenzenesulfonic acid and 1 mL of 7 mM a-naphthylamine were introduced into the mixture, which was incubated at 25 ℃ for another 20 min. Absorbance of the mixture was recorded at 530 nm by spectrophotometry. *P. chrysosporium* viability and $O_2^{\cdot-}$ levels were expressed as relative percentages to the untreated control.

The activities of antioxidant enzymes SOD and CAT were measured following the method described by Zeng et al. (2012) and Huang et al. (2018). SOD activity was detected by monitoring 50% inhibition of nitroblue tetrazolium chloride reduction. CAT activity was tested by monitoring the absorbance of H_2O_2 at 240 nm and one unit of CAT was defined as a decrease of 0.1 unit of A_{240} per min.

All assays were conducted in triplicate. The data were statistically analyzed by SPSS 22.0 software and expressed as the means ± standard deviations. Statistical differences between the experimental groups were determined using one-way analysis of variance, followed by Tukey post-hoc test. Differences of $p < 0.05$ were considered to be statistically significant.

3. Results and discussion

3.1. Promoting effects of H_2S on Pb(II) removal

Effects of H_2S on Pb(II) removal by *P. chrysosporium* were investigated under stress of 10–200 mg/L Pb(II). As shown in Fig. 2A-D, no obvious changes in Pb(II) removal are observed for low-concentration NaHS pretreatments (0–100 μM). Further increase in NaHS concentration (up to 500 μM) results in a significant promotion in

Pb(II) removal efficiency. Although the maximum removal efficiencies for NaHS (0–500 μM) pretreatments all arrived at 100% at the initial Pb(II) concentration of 10 and 50 mg/L, 500-μM NaHS pretreatment elicited higher Pb(II) removal efficiency for short-term exposure (1–24 h) (Fig. 2A and B). The removal efficiencies under 100 and 200 mg/L Pb(II) treatments were also markedly increased by higher-dose NaHS during the whole adsorption process, with an increase of 31% and 17%, respectively, in the maximum removal efficiency of Pb(II) relative to the samples without NaHS (Fig. 2C and D). These findings indicated that Pb(II) removal by *P. chrysosporium* was enhanced after NaHS pretreatment, especially at higher concentrations, and the time to achieve higher removal efficiencies was shortened greatly. Similar results were reported for promotion of 2,4-DCP degradation by 50–100 μM NaHS (Chen et al., 2014). Besides, our previous studies found that the pH levels increased with increasing reaction time (Huang et al., 2013 and 2017). Thus, another possibility for higher Pb(II) removal could be the formation of the precipitation of Pb(II) ions.

3.2. Effects of H_2S on total Ag removal

A promoting effect of H_2S was also observed on total Ag removal at NaHS concentration of 50 μM. The maximum percentages of total Ag removal were 56%, 68%, 90%, and 70.0% at the AgNPs concentration of 10, 50, 100, and 200 mg/L, respectively (Fig. 3). It was found that higher Ag removal efficiencies were obtained at moderate concentrations of AgNPs. In addition, it was also observed that exposure to 200 mg/L AgNPs showed higher removal efficiency of total Ag under 100-μM NaHS pretreatment. However, pretreatments with higher NaHS concentrations (500

μM) induced a clear dose-dependent efficiency reduction in total Ag removal, reaching undetectable levels in all AgNP-treated groups. The findings indicated that Ag uptake by *P. chrysosporium* mycelium could be closely related to the concentrations of NaHS and AgNPs. AgNPs with higher initial concentrations tended to maintain better dispersion and stability, and oxidative dissolution and precipitations of nanoparticles could occur at lower concentrations of NaHS. These would result in greater Ag diffusion into fungal mycelium, enhancing the treatment efficiency of *P. chrysosporium* (Guo et al., 2016a and b). Additionally, the undetectable levels in total Ag removal efficiency in the presence of 500 μM NaHS could be explained by the fact that AgNPs might be directly converted to the nanoparticle aggregates and/or larger-sized Ag_2S-NPs through a solid-fluid sulfidation reaction at higher NaHS concentrations, resulting in higher retention of Ag content in the media (Wirth et al., 2012; Guo et al., 2016a; Wang et al., 2015).

3.3. Removal of various heavy metals and AgNPs with NaHS pretreatment

Pb(II), Cd(II), Cu(II), Ni(II), and Zn(II) at the same initial concentrations of 100 mg/L were adopted as models of heavy metal ions. Fig. 4A shows that the capture percentages of 27% and 51% for Pb(II) and Cd(II) are observed after 24 h of exposure, whereas no removal is detected for Cu(II), Ni(II), Zn(II), and AgNP treatments when *P. chrysosporium* cells are pretreated with 100 μM NaHS. Predictably, diverse heavy metals with different adsorption sites and metal-binding energies in *P. chrysosporium* may result in different degrees of removal performance. The undetectable removal efficiency implied that *P. chrysosporium* might have

212 weaker binding affinities to Cu(II), Ni(II), and Zn(II) than Pb(II) and Cd(II). Coupled
213 with the observations of total Ag removal in Fig. 3, unlike heavy metals, the
214 hindrance of Ag uptake into cells could be possibly due to NaHS-incuded AgNP
215 aggregation being blocked outside the cells (Wirth et al., 2012). Moreover, effect of
216 H_2S on Cd(II) removal was evaluated under 100-mg/L Cd(II) (Fig. 4B). Similar to
217 Pb(II) removal, application of NaHS (0, 50, and 100 μM) enhanced removal of Cd(II)
218 in a dose-dependent manner, with maximum efficiencies of 49%, 50%, and 55%,
219 respectively.

220 *3.4. Effects of H_2S on cell viability under heavy metal and AgNP stress*

221 After exposure to different concentrations of Pb(II) and AgNPs for 78 h, the
222 effects of H_2S on the tolerance of *P. chrysosporium* to Pb(II) and AgNP toxicity were
223 investigated (Fig. 5A and B). The results showed that a concentration-dependent
224 decrease in *P. chrysosporium* cell viability without NaHS pretreatment was observed
225 after introduction of Pb(II) (10, 50, 100, and 200 mg/L), causing the death of
226 approximately 25%, 40%, 55%, and 60%, respectively (Fig. 5A). A significant
227 decrease in cell viability might be due to inhibition of cell division imparted by Pb(II)
228 in cell wall (Ali et al., 2014). However, Pb(II)-induced cell death was dramatically
229 reversed by NaHS pretreatment in a concentration-dependent manner. Application of
230 500 μM NaHS caused the most significant increase in cell viability, approximately
231 27%, 15%, 27%, and 39% higher viability than the untreated control, respectively.
232 The enhancement in cell viability induced by NaHS demonstrated that H_2S exerted a
233 strong protective effect against Pb(II) toxicity.

However, *P. chrysosporium* viability in the presence of AgNPs alone was slightly enhanced, rather than inhibited with the increasing AgNP concentrations (Fig. 5B). This could be possibly because of nanoparticle aggregation greatly reducing the direct contact/interaction between AgNPs and *P. chrysosporium* cells. Meanwhile, an obvious increase in cell viability was noticed after pretreatment with 50 μM NaHS, except for 10-μM AgNP treatments, indicating H_2S-exerted protection against AgNP stress. With further increasing the concentrations of NaHS, however, *P. chrysosporium* viability was remarkably depressed, resulting in substantial cell death (38%–41%) under exposure to 50–200 mg/L AgNPs following pretreatment with 500 μM NaHS. The inhibitory effects of higher concentrations of NaHS suggested that excess H_2S released might be toxic to *P. chrysosporium*, in agreement with the findings of previous studies (Chen et al., 2013; Chen et al., 2014).

Furthermore, the data regarding effects of H_2S on the viability of *P. chrysosporium* after exposure to various heavy metals and AgNPs for 24 h are shown in Fig. 5C. In the absence of NaHS pretreatment, Pb(II), Cd(II), Cu(II), Ni(II), and Zn(II) at initial concentration of 100 mg/L led to reduction in cell viability by 36%, 57%, 78%, 28%, and 56%, respectively, as compared to the untreated control. By comparison, Cd(II), Cu(II), and Zn(II) exerted more toxic effects to *P. chrysosporium* cells. The differential responses of cell viability to various heavy metals could be due to different sensitivities and repair abilities of *P. chrysosporium*. After pretreatment with 50 and 100 μM NaHS, cell viability under heavy-metal stress was not significantly affected, in line with the finding reported by Shahbaz et al. (2014) who

found that H_2S had little influence on Cu toxicity in *Brassica pekinensis*. By contrast, stimulation of AgNPs and NaHS on cell survival was observed when *P. chrysosporium* cells were exposed to 100 mg/L AgNPs for 24 h. This could be attributed to the increased nanoparticle sizes and agglomeration under high-dose AgNPs and/or in the presence of NaHS (Gliga et al., 2014).

In contrast to short-term exposure (24 h), H_2S exerted the stimulatory effects on *P. chrysosporium* survival in a concentration-dependent manner following 78 h of exposure to Pb(II) (Fig. 5A and C). The phenomena could be associated with effective removal of Pb(II) by *P. chrysosporium* after prolonged exposure, as illustrated in Fig. 2. More enzymes were probably activated to defend against oxidative damage and to recover cell growth and replication during long-term exposure (Huang et al., 2018c).

To verify the effects of H_2S promotion on heavy metal removal and cell viability induced by NaHS, physiological analyses of *P. chrysosporium* pretreated with and without NaHS were carried out under the stresses of various heavy metals and AgNPs.

3.5. Effects of H_2S on contents of MDA and $O_2^{\cdot-}$

MDA content was determined to estimate the extent of lipid peroxidation under different treatments of NaHS and heavy metals (Fig. 6). Significant MDA accumulation was evoked by Pb(II), Cd(II), Cu(II), and AgNP treatments, especially in the case of Pb(II) stressed group. A higher MDA content in *P. chrysosporium* exposed to Pb(II) demonstrated that Pb(II) could not enter into cells, but might be distributed onto the cell walls and plasma membranes (Wang et al., 2010; Xu et al., 2012b). It led to a decrease in the concentrations of unsaturated fatty acids, thus

278 enhancing the peroxidation of membrane lipid markedly (Wang et al., 2010).
279 Interestingly, in spite of the highest MDA level caused by Pb(II) treatment, *P.*
280 *chrysosporium* viability was not strongly inhibited. The higher tolerance of *P.*
281 *chrysosporium* to Pb(II) was likely ascribed to its highly effective antioxidant defense
282 mechanisms. However, a significant reduction in lipid peroxidation occurred in
283 Pb(II)-, Cd(II)-, Cu(II)-, Zn(II)-, and AgNP-stressed cells following H_2S application
284 compared with their corresponding treatments without NaHS. The results indicated
285 that H_2S depressed heavy metal and AgNP-induced plasma membrane damage
286 significantly.
287 The production of $O_2^{\cdot-}$ was also measured to evaluate the role of NaHS in
288 mediating heavy metal-induced oxidative stress. Fig. 6B shows that NaHS
289 pretreatments have no significant influence on $O_2^{\cdot-}$ production in the control and the
290 groups exposed to Pb(II), Ni(II), and Zn(II). However, $O_2^{\cdot-}$ production in the NaHS-
291 incubated *P. chrysosporium* cells under stress of Cd(II), Cu(II), and AgNPs reduced
292 42%, 34%, and 46%, respectively, as compared to those without NaHS incubation,
293 indicating the relieving effect of NaHS on $O_2^{\cdot-}$ accumulation. Under Cu(II) stress,
294 although NaHS-induced inhibitory effects on $O_2^{\cdot-}$ generation occurred, the prominent
295 production of $O_2^{\cdot-}$ was still observed. The overproduction of free radicals, not being
296 eliminated effectively, would give rise to oxidative damage to fungal cells, resulting
297 in a significant reduction in cell survival, as illustrated in Fig. 5C.

298 Taken together, H_2S seemed to be an important antioxidant signaling molecule
299 involved in the mechanisms of tolerance against lipid peroxidation and oxidative
300 stress induced by heavy metals and AgNPs.
301 *3.6. Promotion of exogenous H_2S on antioxidant enzyme activities*
302 To observe the role of H_2S on antioxidant defense system of *P. chrysosporium*,
303 the activities of enzymes SOD and CAT under heavy-metal and AgNP stress were
304 measured when *P. chrysosporium* was pretreated with NaHS. Higher SOD activities
305 in the range of 136.1–214.2 U/g Fw were obtained after introduction of Pb(II), Cd(II),
306 Cu(II), and AgNPs, whereas, for Ni(II) and Zn(II) exposure, there was no significant
307 difference in SOD activity relative to the untreated control (Fig. 7A). Accordingly, in
308 comparison with Ni(II) and Zn(II), *P. chrysosporium* was more sensitive to the other
309 metal ions and AgNPs. It has been documented that SOD activity could be stimulated
310 by the introduction of toxic pollutants directly, such as heavy metals and AgNPs, or be
311 increased by upregulating the expression of genes encoding SOD indirectly, in
312 response to compensation of excess $O_2^{\cdot-}$ generation (Zeng et al., 2012; Ma et al.,
313 2015; Huang et al., 2018c). Importantly, SOD activities were enhanced by 102.7,
314 53.3, 34.3, 93.5, 61.5, 45.0, and 32.0 U/g Fw in cells pre-incubated with NaHS under
315 the control, Pb(II), Cd(II), Cu(II), Ni(II), Zn(II), and AgNP stress, respectively, when
316 compared to those without NaHS pre-incubation. A similar dose-dependent
317 stimulatory effect of NaHS on CAT activity was observed when *P. chrysosporium* was
318 subjected to the treatments with heavy metals and AgNPs (Fig. 7B). CAT activities

were greatly activated in the NaHS-pretreated groups, with an increase of 35.5–100.3 U/g Fw relative to those of the untreated groups.

Besides, it should be noted that in the absence of NaHS pretreatment, there was no significant difference in CAT activity of *P. chrysosporium* between the control and treatments with heavy metals and AgNPs. CAT activities under the control and stresses of Pb(II), Cd(II), Cu(II), Ni(II), Zn(II), and AgNPs were 64.8, 67.7, 77.2, 56.7, 55.4, 32.8, and 52.7 U/g Fw, respectively. By contrast, changes in antioxidant enzyme activities induced by heavy metals and AgNPs had a clear difference in SOD and CAT in absence of NaHS. SOD is well-known to a key enzyme in an active oxygen scavenger system and act as the first defense line against toxic ROS for cells to adapt to biotic and abiotic stresses, catalyzing the dismutation of $O_2^{\cdot-}$ to O_2 and H_2O_2 (Tan et al., 2015). CAT plays a vital key role in scavenging or detoxifying H_2O_2 into H_2O and O_2 (Huang et al., 2018c). Therefore, it was speculated that the lower CAT activity was probably be concerned with the higher SOD activity provoked by heavy metal and AgNP stress and that accumulation of H_2O_2 was increased due to SOD overexpression, resulting in the suppression in CAT activity (Pacini et al., 2013; Huang et al., 2018c). Another possibility for the depression of CAT was that subunits assembly and/or biosynthesis of CAT had been adversely affected by a variety of toxic pollutants. Furthermore, the metal-enzyme complexes formed perhaps led to alterations in the structure and enzyme activity of CAT (Sun et al., 2009).

Collectively, the activities of antioxidant enzymes can be stimulated by heavy-metal and AgNP-induced ROS generation, which in turn will be scavenged by

antioxidant enzymes to maintain the oxidative balance in *P. chrysosporium*, further protecting against oxidative damage to the cellular components. The levels of MDA and $O_2^{\cdot-}$ were markedly lowered when the mycelia were pretreated with 50 and 100 μM NaHS, probably because of the remarkable enhancement in activities of SOD and CAT enzymes under NaHS pretreatments. Predictably, H_2S mitigated the oxidative stress triggered by heavy metal ions and AgNPs via enhancing the expression of ROS scavenging enzymes (SOD and CAT). Similar results on NaHS-promoted tolerance to oxidative stress caused by toxicants in bacteria and fungi were also reported (Chen et al. 2014; Mironov et al., 2017; He et al., 2018).

Furthermore, many evidences indicate that low-dose H_2S has a positive effect on growth, development, and abiotic/biotic stress resistance of animals, plants, and microorganisms (García-Mata and Lamattina, 2010; Mironov et al., 2017; Zhu et al., 2018). For example, pretreatment with NaHS (a H_2S donor) in plants can increase stress tolerance to toxic heavy metals, such as Pb, Cd, Cu, Cr, Zn, Al, and As (Chen et al. 2014; Guo et al., 2016b), and decrease the accumulation of heavy metals (Liu et al., 2016; Han et al., 2018), thereby alleviating heavy metal-induced toxicity. Conversely, in the present study, H_2S application was proven to be rewarding for improvement in removal efficacy of heavy metals Pb, Cd, and Ag. Further studies must be conducted to explore high-efficiency removal of other heavy metals and the simultaneous removal of various toxic pollutants by *P. chrysosporium* through H_2S-based technologies. Activation of *P. chrysosporium* cells could be stimulated due to the effective removal of these toxicants, and the surviving cells might induce an

363 increase in the production of enzymes against membrane-damaging lipid peroxidation
364 and oxidative stress. So, NaHS-induced significant increase in *P. chrysosporium*
365 viability may be precisely due to the alleviation of oxidative stress under heavy metal
366 and AgNP stress. Consequently, it could be concluded that H_2S plays a vital role in
367 cell growth, antioxidant defense systems, and efficient removal of toxic pollutants in
368 wastewater treatment.

369 **4. Conclusions**

370 In the present study, H_2S pretreatment improved the removal of Pb(II), Cd(II),
371 and total Ag by *P. chrysosporium* and ameliorated heavy metal-induced growth
372 inhibition significantly. H_2S-promoted enhancement in the activities of antioxidant
373 enzymes was observed. Furthermore, lipid peroxidation and oxidative stress evoked
374 by heavy metals and AgNPs were also alleviated by H_2S. Stimulation of H_2S on *P.*
375 *chrysosporium* viability under heavy metal and AgNP stress could be ascribed to the
376 upregulation of antioxidant enzymes, as well as the efficient biological removal of
377 these toxicants. The insights in this work provide the evidence of potential
378 applications of H_2S in bioremediation of wastewater and have great significance for
379 advancing the mechanistic understanding of H_2S-facilitated toxicant tolerance in
380 fungal cells.

381 **Acknowledgements**

382 This work was financially supported by the National Natural Science Foundation of
383 China (51579099, 51521006, 51508186, and 51879105), the Program for Changjiang
384 Scholars and Innovative Research Team in University (IRT-13R17).

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