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ORIGINAL ARTICLE

Cadmium toxicity is alleviated by AtLCD and AtDCD in *Escherichia coli*

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Keywords
AtDCD, AtLCD, cadmium toxicity, hydrogen sulfide, oxidative stress.

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Abstract

Aims: *Arabidopsis thaliana* L- and D-cysteine desulfhydrases (AtLCD and AtDCD) are two important H_2S-generating enzymes. This study determined the effects of H_2S derived from AtLCD and AtDCD on cadmium (Cd) toxicity in *Escherichia coli*.

Methods and Results: *AtLCD* and *AtDCD* were cloned into pET28a vectors and transformed into wild-type *E. coli* strain BL21(DE3), named BL21(LCD) and BL21(DCD). In the induced BL21(LCD) and BL21(DCD) compared with wild type, significantly higher H_2S generation rates were observed. Additionally, higher survival rates, reduced contents of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2), decreased activities of superoxide dismutase and catalase under 220 μmol l^{-1} Cd stress were noted. We obtained similar results in the wild type treated with NaHS, a H_2S donor. The above changes were substantially counteracted by the mixture of ammonia and pyruvic acid potassium ($NH_3 + C_3H_3KO_3$), a synthetic inhibitor of H_2S.

Conclusions: AtLCD and AtDCD catalyse the H_2S production, generating an ameliorating effect against Cd-induced oxidative stress and resulting in *E. coli* resistance to Cd toxicity.

Significance and Impact of the Study: H_2S as a gasotransmitter is certified to have an ameliorating effect against Cd toxicity, thus providing information for further research regarding the role of H_2S in regulating resistance to the heavy metal stress in organisms.

Introduction

Cadmium (Cd) is a toxic metal (Gill and Tuteja 2011) and occurs in a variety of forms in soil, water, air and food (Metwally *et al.* 2003). Cd is nonessential to the growth of micro-organisms. The combination of Cd and mercaptane leads to short-term effects such as denaturation of proteins, malfunction of calcium metabolism and degradation of biomembranes. Moreover, Cd is a nonredox active metal, but suppresses DNA duplication and breaks single-strand DNA. It consequently destroys cells, reduces enzyme activities, and ultimately leads to the death of micro-organisms (Rensing *et al.* 1997; Nies 1999).

Hydrogen sulfide (H_2S) is emerging as an important gasotransmitter *in vivo* parallel to nitric oxide (NO) and Carbon Monoxide (CO) (Wang 2002). H_2S has been implicated in vasodilatation, hippocampal long-term potentiation, smooth muscle relaxation, anti-inflammatory activity in the gastrointestinal tract and cardio-protective processes (Wang 2010; Szabó *et al.* 2011). Research on H_2S in plants as a signal molecule is currently attracting considerable attention. *AtLCD* (At3g62130) and *AtDCD* (At1g48420) are known to

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catalyse L-cysteine and D-cysteine to generate sulfide, ammonia and pyruvate in Arabidopsis (Riemenschneider *et al.* 2005). In addition, the function of a series of genes, such as *AtDCD2* (At3g26115), *AtNFS1* (At5g65720), *AtNFS2* (At1g08490) (Papenbrock *et al.* 2007) and *AtDES1* (At5g28030) (Álvarez *et al.* 2010) are unconfirmed. At present, H_2S has been reported to be a primary messenger molecule, which participates in physiological processes in plants. Increased doses of the exogenous NaHS improve longevity of cut flowers (Zhang *et al.* 2011); Jin *et al.* (2011) reported that H_2S up-regulates the expression of several drought genes, such as *DREB2A*, *DREB2B*, *RD29A* and *CBF4*, to improve drought resistance in Arabidopsis. It is also documented that the synergic effect of H_2S and NO protects micro-organisms from oxidative stress induced by antibiotics (Shatalin *et al.* 2011).

Some studies reported that the prokaryotic expression could be applied to researching the eukaryotic gene functions (Evans *et al.* 1992; Suleman and Shakoori 2012). To further elucidate the relationship between *AtLCD* and *AtDCD* genes and tolerance to heavy metal, we cloned these two genes. We then constructed expression vectors in *Escherichia coli* and observed the effects of heavy metal stress on the two proteins in *E. coli*.

Materials and methods

Prokaryotic expression and purification of AtLCD and AtDCD

RNA was extracted from the leaves of Arabidopsis seedlings and transcribed into cDNA. According to the Genbank database, the following primer pairs were used: Primer 19 (5′-CGGGATCCATGGAGGCGGGAGAGCGGCGC-3′) and primer 20 (5′-ACGCGTCGACCTACAATGCAGGAAGGTTTTGAC-3′) were the primer pairs for *AtLCD* (1365 bp); primer 21 (5′-CGGGATCCATGAGAGGACGAAGCTTGACAC-3′) and primer 22 (5′-ACGCGTCGACCTAGAACATTTCCCAACACCAT-3′) were the primer pairs for *AtDCD* (1206 bp). Primer 19 and primer 21 had a *Bam*HI restriction site; primer 20 and primer 22 had a *Sal*I restriction site.

PCR conditions were as follows: 38 amplification cycles of 30 s at 94°C, 30 s at 55°C and 180 s at 72°C. The amplified fragments were ligated into pGEM-T easy vectors (Promega Corporation, Taiyuan, Shanxi, China) and sequenced by the Promega Corporation.

Positive plasmid DNAs of the clones were identified by *Bam*HI and *Sal*I simultaneously. The *AtLCD* and *AtDCD* cDNAs were then cloned into the expression vector pET28a and introduced into wild-type *E. coli* strain BL21 (DE3), which allowed us to produce two target proteins. AtLCD and AtDCD proteins were expressed and purified according to Riemenschneider *et al.* (2005).

Measurement of H_2S formation

AtLCD and AtDCD activities were measured according to the method of Jin *et al.* (2011). Overnight cultures of wild type, BL21(LCD) and BL21(DCD) with and without the addition of isopropyl thiol-β-D-galactoside (IPTG) were collected and washed with 0·9% NaCl solution; then, 500 μl buffer (20 *m*mol l^{-1} 2-amino-2-(hydroxymethyl)-1, 3-propanediol (Tris), 500 *m*mol l^{-1} NaCl, 10 *m*mol l^{-1} Imidazole, 1% (v:v) Triton X-100, pH 7·8) was added, and the solutions were sonicated. The extracts were centrifuged at 10 000 ***g*** for 10 min at 4°C, and the supernatants were used to detect AtLCD and AtDCD protein activities.

Bacterial growth and phenotype observation

In the experiment on the effect of AtLCD and AtDCD on *E. coli* growth under Cd stress, wild type, BL21(LCD) and BL21(DCD) were first cultured in LB medium at 37°C with shaking to an OD_{600} of 0·8–1·0. After that, 1 *m*mol l^{-1} IPTG was added into BL21(LCD) and BL21 (DCD) medium for 1·5 h to induce protein expression followed by the addition of 2 *m*mol l^{-1} ammonia and pyruvic acid potassium (NH_3 and $C_3H_3KO_3$, termed as NC) for 0·5 h. For wild type only, NC was added. The culturing medium without IPTG or NC acted as negative control.

To detect the effect of exogenous NaHS on *E. coli* growth under Cd stress, for wild type, 100 μmol l^{-1} NaHS was added for 0·5 h then was followed by the addition of NC for 0·5 h. The culturing medium without NaHS or NC acted as negative control.

The above cultures were then diluted in fresh LB medium 1·25 : 1000. 40 μl of each mixture was transported to LB medium plates in the presence or absence of 220 μmol l^{-1} Cd, daubed equally and grown at 37°C for 15–16 h. LB medium plates without the addition of Cd were used as controls. After culturing, the colony-forming units (CFU) with a diameter >1 mm were counted and analysed.

Determination of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2)

Bacteria were cultured according to *Bacterium growth and phenotypic observation.* After related treats, Cd solution was added to the final concentration of 220 μmol l^{-1}. Protein content was determined according to the method of Bradford (1976). H_2O_2 was measured according to Alexieva *et al.* (2001). MDA was measured according to Heath and Packer (1968).

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Measurements of superoxide dismutase (SOD) and catalase (CAT) activities

SOD and CAT activities were measured according to Jiang and Zhang (2002).

Statistical analysis

We conducted the analysis of variance between different treatments and used SPSS statistical software to evaluate the significant differences between treatments by LSD multiple range tests ($P < 0.05$; ver. 17, IBM SPSS, Chicago, IL).

Results

Expression and function of AtLCD and AtDCD recombinant proteins

To identify the functions of *AtLCD* and *AtDCD* genes, we expressed the two corresponding full-length cDNAs into wild-type *E. coli*. The proteins were examined by SDS-PAGE after induction with IPTG (Fig. 1). As anticipated, there were two protein bands at the 46·4 kDa (Fig. 1a) and 41·7 kDa positions (Fig. 1b). This indicated that the exogenous proteins were correctly expressed in *E. coli*.

To detect AtLCD and AtDCD protein activities, we measured the H_2S generation rates in the crude extracts of induced *E. coli*, which contained pET28a-AtLCD and pET28a-AtDCD prokaryotic expression plasmids (Fig. 2). The experimental results indicated that the crude protein extract of BL21(LCD) decomposed L-cysteine into H_2S, whose production rate was 1·07 times that of wild type (Fig. 2a), and that BL21(DCD) decomposed D-cysteine into H_2S, whose production rate was 7·2 times that of wild type (Fig. 2b). This shows that AtLCD and AtDCD proteins were expressed in *E. coli* and had cysteine desulfhydrase activity.

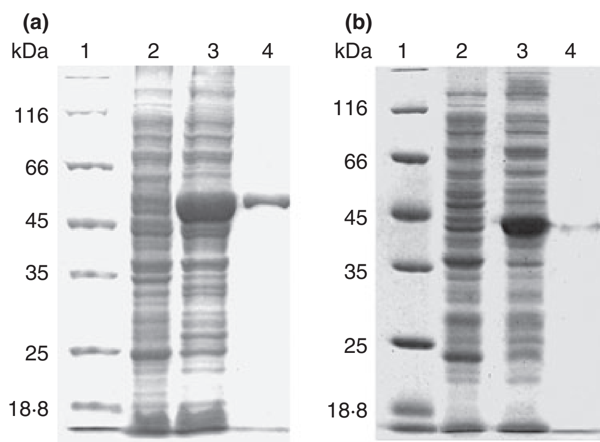

Figure 1 SDS-PAGE analysis of AtLCD and AtDCD prokaryotic expression. Lane 1: protein molecule marker; Lane 2: protein extracts of *Escherichia coli* strains BL21(LCD) (a) and BL21(DCD) (b) shortly before induction; Lane 3: protein extracts of *E. coli* strains BL21(LCD) (a) and BL21(DCD) (b) 1·5 h after induction; Lane 4: AtLCD (a) and AtDCD (b) proteins purified by Ni^{2+}-affinity chromatography. The molecular masses of the marker proteins are given in kDa on the left.

Effect of AtLCD and AtDCD on *Escherichia coli* growth under Cd stress

To determine the effect of AtLCD and AtDCD proteins in *E. coli* under Cd stress, we recorded and analysed the colony number of BL21(LCD) and BL21(DCD). The pre-experiments showed that 220 μmol l^{-1} Cd caused a significant inhibition of the growth of wild-type bacterium (data not shown), which included a significant reduction in CFU and a reduction in colony size. Therefore,

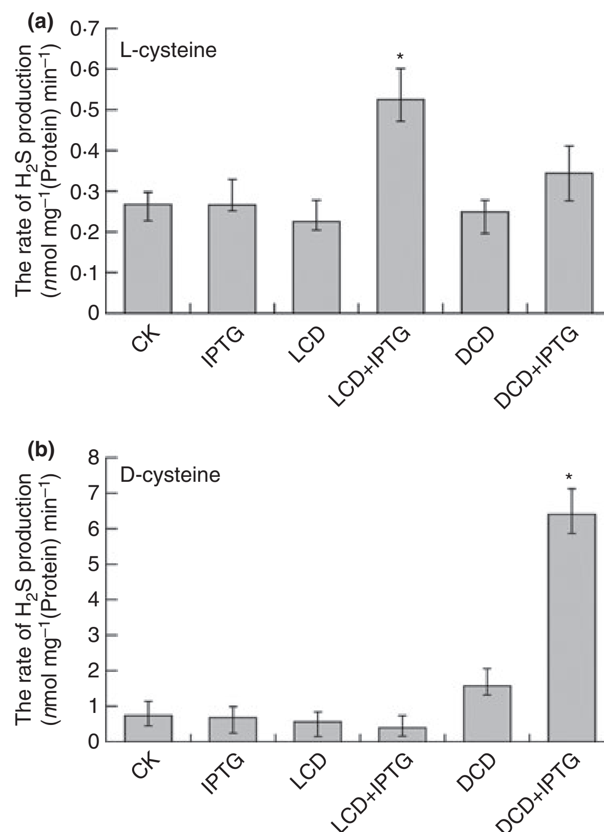

Figure 2 Effect of AtLCD and AtDCD proteins on the rate of H_2S production in *Escherichia coli*. The rate of H_2S production on L-cysteine (a) and D-cysteine (b) as a substrate is denoted in the Figure. CK: wild-type *E. coli* strain BL21(DE3); isopropyl thiol-β-D-galactoside (IPTG): addition of induced IPTG concentration on wild type and as a control; LCD: *E. coli* strain BL21(LCD); LCD+IPTG: after induction in BL21(LCD); DCD: *E. coli* strain BL21(DCD); DCD+IPTG: after induction in BL21(DCD). The same designations were used in the following figures. The data are expressed as mean ± SE ($n = 3$ independent experiments), * is expressed as significant differences ($P < 0.05$).

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220 μmol l^{-1} was chosen as an appropriate Cd stress concentration in the following experiments. We assessed the CFU of *E. coli* under different treatments and noted growth at 0 and 220 μmol l^{-1} Cd, respectively. Under Cd stress, the survival rates of induced BL21(LCD) and BL21 (DCD) were 11·6 and 6·94 times that of wild type, respectively (Treatments 1, 4 and 7 in Fig. 3). Tolerance of the bacterium to Cd was markedly enhanced. NC and H_2S are both enzyme-catalysed products of AtLCD and AtDCD; hence, adding exogenous NC (2 *m*mol l^{-1}) inhibited the generation of H_2S and consequently led to a decrease in CFU and colony size of BL21(LCD) and BL21 (DCD) (Treatments 4, 5, 7 and 8 in Fig. 3). We may conclude from the above findings that exogenous NC can eliminate the positive effects AtLCD and AtDCD proteins have on enhancing tolerance of the bacterium to heavy metal. This indicates that the generation of H_2S catalysed by AtLCD and AtDCD in *E. coli* is the primary reason for the enhanced tolerance of this bacterium to Cd.

Effect of AtLCD, AtDCD and exogenous NaHS on H_2O_2 and MDA production under Cd stress

Previous studies have shown that exposure to Cd significantly increases MDA and H_2O_2 accumulation (Flora *et al.* 2008; Cuypers *et al.* 2010). To explore whether Cd-induced oxidative damage was associated with AtLCD and AtDCD proteins in *E. coli*, we measured H_2O_2 and MDA contents under different treatments (Fig. 4). A decrease in H_2O_2 and MDA production was detected in induced *E. coli* strains BL21(LCD) and BL21(DCD) under Cd stress ($P < 0{\cdot}05$). The H_2O_2 and MDA contents in BL21(LCD) were 0·49 and 0·67 times that of wild type, respectively (Treatments 3 and 7 in Fig. 4); The H_2O_2 and MDA contents of BL21(DCD) were 0·40 and 0·81 times that of wild type, respectively (Treatments 3 and 8 in Fig. 4). This implied that the expression of AtLCD and AtDCD proteins reduced the reactive oxygen species (ROS) effect and oxidative damage on the bacterium under Cd stress. We further treated wild-type bacterium with exogenous 100 μmol l^{-1} NaHS (Treatments 12 in Fig. 4), and the result obtained was similar to those for BL21(LCD) and BL21(DCD). The addition of 2 *m*mol l^{-1} NC reversed the effect of AtLCD, AtDCD and NaHS, which caused an increase in H_2O_2 content and MDA accumulation in BL21(LCD), BL21(DCD) and wild type (Treatments 9, 10 and 13 in Fig. 4). We conclude from the above experiments that H_2S was the primary cause of the quenching of ROS. This indicates that AtLCD and AtDCD catalyse the generation of H_2S in *E. coli* and therefore quench the production of ROS.

Effect of AtLCD, AtDCD and exogenous NaHS on ROS metabolism under Cd stress

The redox balance in *E. coli* is regulated by ROS metabolism. Therefore, we measured the scavenging activities of SOD and CAT under different treatments to determine the effects of H_2S on this process. The activities of SOD and CAT in wild type, BL21(LCD) and BL21(DCD)

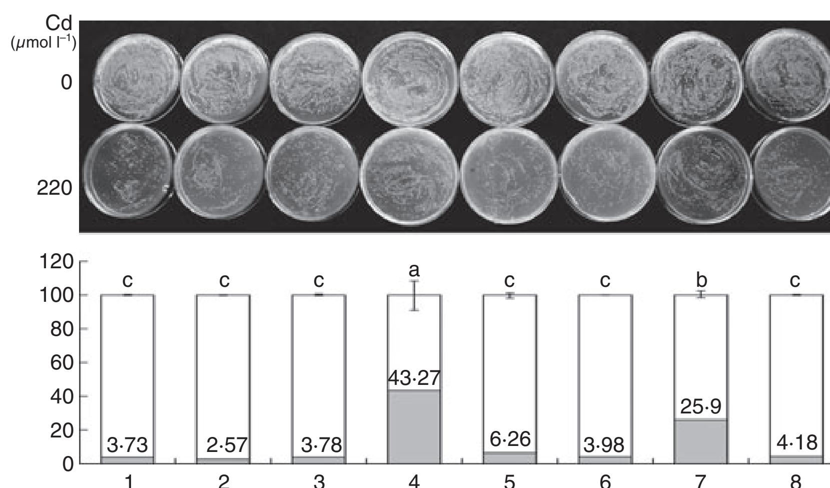

Figure 3 Effect of AtLCD and AtDCD on *Escherichia coli* growth under Cd stress. *Escherichia coli* colonies grew on 0 μmol l^{-1} Cd and 220 μmol l^{-1} Cd plates, and the survival rate on 220 μmol l^{-1} Cd plates was calculated. Survival (%) = (CFU on 220 μmol l^{-1} Cd plates/CFU on 0 μmol l^{-1} Cd plates) × 100%. Colony-forming units (CFU) on 0 μmol l^{-1} Cd plates were 100%. 1: wild-type; 2: NC, addition of NC into LB medium when OD_{600} nm of wild type was 0·8–1·0. 3: LCD; 4: LCD+isopropyl thiol-β-D-galactoside (IPTG); 5: LCD+IPTG+NC, addition of NC on induced BL21(LCD); 6: DCD; 7: DCD+IPTG; 8: DCD+IPTG+NC, addition of NC on induced BL21(DCD). Data are expressed as mean ± SE of three independent experiments. Means with *different letters* within a column are significantly different ($P < 0{\cdot}05$) with regard to treatment.

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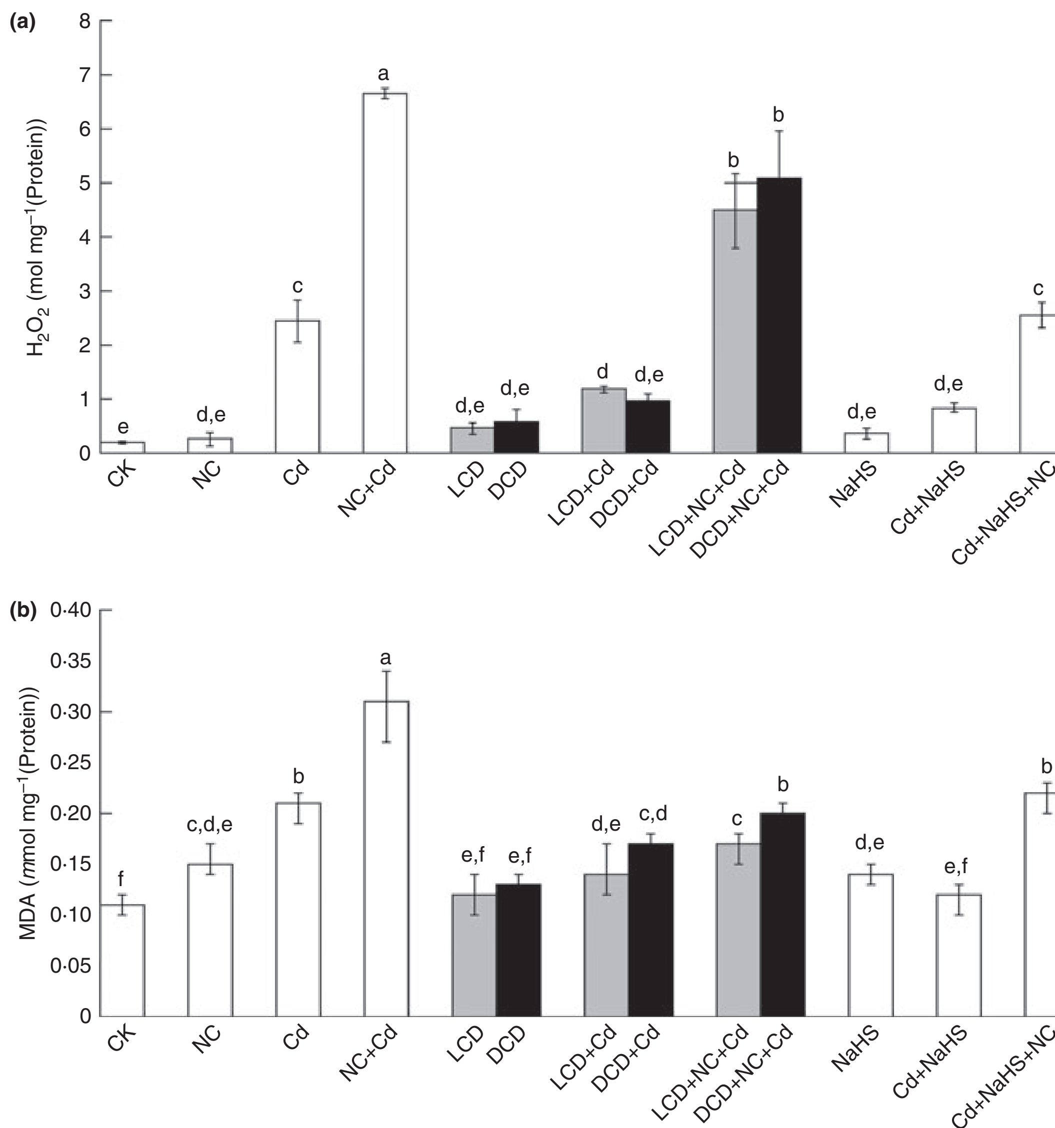

Figure 4 Effect of AtLCD, AtDCD and exogenous NaHS on the 220 μmol l^{-1} Cd-induced H_2O_2 (a) and malondialdehyde (b) contents in *Escherichia coli*. Cd: addition of Cd into the LB medium when OD_{600} nm of wild type, BL21(LCD) and BL21(DCD) was 0·8–1·0; NaHS: wild-type was treated with 100 μmol l^{-1} NaHS. Data are expressed as mean ± SE of three independent experiments. Means with *different letters* within a column are significantly different ($P < 0{\cdot}05$) with regard to treatment.

increased significantly ($P < 0{\cdot}05$) under Cd exposure. The activities of SOD and CAT in the wild-type bacterium were 2·17, 4·52 times that of wild type without the addition of Cd, respectively (Treatments 1 and 3 in Fig. 5); in BL21(LCD), this figure was 1·55, 3·15 times that of the control group, respectively (Treatments 5 and 7 in Fig. 5); in BL21(DCD), this figure was 1·58, 3·02 times that of the control, respectively (Treatments 6 and 8 in Fig. 5). It suggests that the scavenging enzymes take effect and work against oxidative damage in *E. coli* under Cd stress.

However, compared with wild type (Treatments 3 in Fig. 5), BL21(LCD) and BL21(DCD) had lower SOD and CAT activities under Cd stress. The SOD and CAT activities in BL21(LCD) were 0·77 and 0·69 times that of wild type, respectively (Treatments 3 and 7 in Fig. 5); in BL21(DCD) were 0·8 and 0·7 times that of wild type, respectively (Treatmeats 3 and 8 in Fig. 5). The above results indicate that AtLCD and AtDCD did not reduce Cd-induced oxidative damage by regulating the antioxidant enzyme system. The result of further experiment on the exogenous NaHS (Treatments 12 in Fig. 5) obtained was similar to those for BL21

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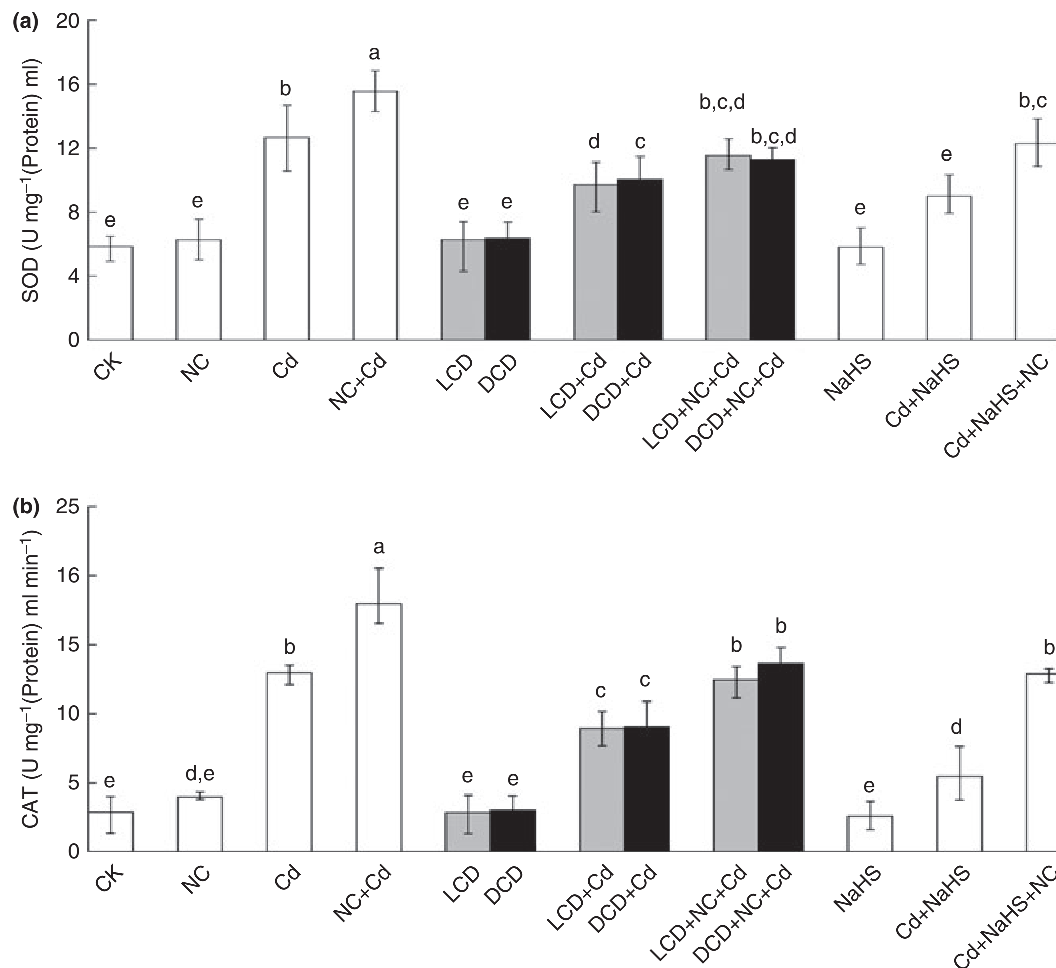

Figure 5 Effect of AtLCD, AtDCD and exogenous NaHS on specific activity of superoxide dismutase (SOD) (a) and catalase (CAT) (b) under Cd stress. Orders of the columns were the same as in Fig. 4. One SOD unit was the amount of enzyme required to inhibit photoreduction of nitroblue tetrazolium chloride by 50% at 25°C. One CAT unit was the amount of enzyme required to decompose 1 μmol of H_2O_2 min^{-1} at 25°C (pH 7.0). Consumption of H_2O_2 was measured as the decrease in absorbance at 240 nm. Data are expressed as mean ± SE of three independent experiments. Means with *different letters* within a column are significantly different ($P < 0{\cdot}05$) with regard to treatment.

(LCD) and BL21(DCD). The addition of 2 *m*mol l^{-1} NC reversed the effect of AtLCD, AtDCD and NaHS, which caused an increase in SOD and CAT activities in BL21 (LCD), BL21(DCD) and wild type (Treatments 9, 10 and 13 in Fig. 5). This confirms that the generation of H_2S catalysed by AtLCD and AtDCD plays an important role in ROS metabolism.

Effect of exogenous NaHS on *Escherichia coli* growth under Cd stress

To confirm that tolerance to Cd in *E. coli* was promoted by H_2S, we assessed the growth of wild-type bacterium in the presence of the exogenous H_2S donor, NaHS. Addition of 100 μmol l^{-1} NaHS significantly restored CFU number and colony size; at the same time, addition of NC eliminated the effect of NaHS on the bacterium (Fig. 6).

Discussion

Previous studies have reported that the methods of prokaryotic expression could be applied to the research in eukaryotic gene function. The *Tetrahymena Cd metallothionein 1* (*TMCd1*) gene has been expressed in *E. coli* to accumulate more Cd (Suleman and Shakoori 2012). The pea metallothionein-like gene (*PsMTA*) has been expressed in *E. coli* and Arabidopsis to induce more copper accumulation (Evans *et al.* 1992). The same principal is applied in our research to determine that bacterium resistance to Cd toxicity parallels the function of AtLCD and AtDCD proteins expressed in Arabidopsis.

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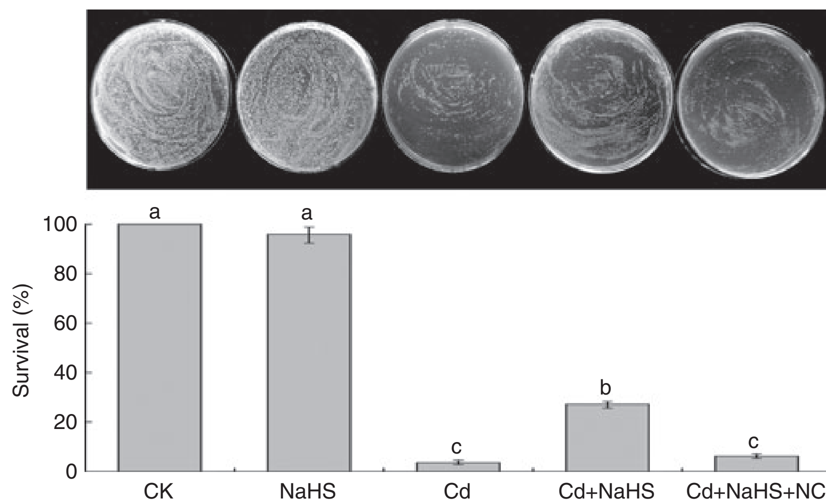

Figure 6 Effect of exogenous NaHS on *Escherichia coli* growth under Cd stress. Wild-type *E. coli* colonies grew on 0 μmol l^{-1} Cd, 100 μmol l^{-1} NaHS, 220 μmol l^{-1} Cd, 220 μmol l^{-1} Cd + 100 μmol l^{-1} NaHS and 220 μmol l^{-1} Cd + 100 μmol l^{-1} NaHS + 2 *m*mol l^{-1} NC plates; the survival rates on the above plates were recorded. The survival rate was in terms of colony-forming units (CFU), and CFU on 0 μmol l^{-1} Cd plates were 100%. The data are expressed as mean ± SE ($n = 3$ independent experiments). Means with *different letters* within a column are significantly different ($P < 0{\cdot}05$) with regard to treatments.

Three H_2S-generating enzymes have been characterized in bacteria: 3-mercaptopyruvate sulfurtransferase (3MST), cystathionine-synthase (CBS) and cystathionine-lyase (CSE). 3MST along with cysteine aminotranferase (CAT) produces H_2S using cysteine as a substrate. CBS and CSE produce H_2S predominantly from L-cysteine (Shatalin *et al.* 2011). 3MST is the critical enzyme in *E. coli* to generate H_2S. In addition, there are several other candidate cysteine-degrading enzymes such as LCDes and DCDes (Nagasawa *et al.* 1985). After the exogenous plasmids pET28a-AtLCD/AtDCD were introduced into wild-type *E. coli*, 3MST, LCDes and DCDes also catalysed the formation of H_2S simultaneously. Compared to wild type, the H_2S generation rates in BL21(LCD) and BL21(DCD) increased markedly (Fig. 2). This implies that AtLCD and AtDCD induced greater production of enzyme proteins than the endogenous H_2S-generating proteins in *E. coli*. Therefore, we conclude that *AtLCD* and *AtDCD* genes contribute to the major production of H_2S.

Although the function of the D-forms of most amino acids in living organisms remains unclear, a certain percentage of D-amino acids exists in different plant species, for example D-cysteine. The amount of D-amino acids is far less than the 0·5–3% of L-amino acids in unprocessed vegetables and fruits. The concentration of D-cysteine cannot be determined because of its small amount, while the quantity of L-cysteine is about 10 μmol l^{-1} in cells (Riemenschneider *et al.* 2005). In Fig. 2, it can be seen that the H_2S generation rate in BL21(DCD) was higher than that in BL21(LCD) before the addition of Cd. In Fig. 3, however, under Cd stress, the survival percentage of BL21(DCD) was lower than that of BL21(LCD). The possible reason for this reverse situation could be that there is a larger amount of L-cysteine than D-cysteine in *E. coli*.

It is reported that the physiological concentration of H_2S detected in animals and plants ranges from 1 to 100 μmol l^{-1} (Jin *et al.* 2011). However, few reports on the H_2S physiological concentration in micro-organisms are available. Induced expression of AtLCD and AtDCD in BL21(LCD) and BL21(DCD) caused a mass generation of H_2S (Fig. 2). Under such H_2S concentration, no harmful effects on *E. coli* growth were observed (Fig. 3). Therefore, we suggest that the H_2S concentration fell to its normal range in *E. coli*. The mass generation of H_2S also significantly quenched ROS in cells, which implies that H_2S plays a more important role as a gasotransmitter than a chemical reductant. More specifically, H_2S serves to activate the mechanism of response to heavy metals and therefore increases tolerance to heavy metals in *E. coli*. In Fig. 6, wild-type was treated with 100 μmol l^{-1} NaHS, and the results showed no interference in the growth of the bacterium. In Fig. 4 and 5, MDA and H_2O_2 contents as well as SOD and CAT activities were similar before and after NaHS was added to the wild type. Thus, it is suggested that H_2S concentration was within its normal range in *E. coli*.

In Figs 4 and 5, it can be seen that an increase in the contents of MDA and H_2O_2 and in the activities of SOD and CAT in wild-type bacterium occurred under Cd stress. We may conclude that the antioxidant enzyme system takes effect and works against oxidative damage in *E. coli* cells. In Fig. 5, however, the activities of SOD and CAT showed an unexpected decrease when wild-type *E. coli* was treated with NaHS. Singh *et al.* (2009) reported that a reduction in oxidative damage led to a similar decrease in NO. H_2S is either presumed to have a direct quenching effect on ROS or can reduce the generation of MDA and H_2O_2 by regulating the nonenzymatic antioxidant system, and so scavenges the ROS

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generated under Cd stress. Therefore, a possible explanation for the decreased activities of SOD and CAT is that ROS-scavenging systems may have various responses according to different Cd concentrations. Research on the function of NO in plants under Cd stress supports the above viewpoint (Arasimowicz-Jelonek *et al.* 2011).

In conclusion, although we cannot be sure whether there is an interaction between H_2S and Cd in *E. coli*, our results suggest that AtLCD and AtDCD provide significant resistance in *E. coli* against Cd-induced toxicity and have an ameliorating effect on Cd-induced oxidative stress.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1 The observation of H_2S and $CdCl_2$ in double distilled water (ddH_2O) and liquid LB medium. The solvent of 1–3 is ddH_2O and 4–6 is liquid LB medium. ddH_2O: 10 ml ddH_2O as a control; LB: 10 ml liquid LB medium as a control; NaHS (0 h): addition of 100 μmol l^{-1} NaHS to ddH_2O/liquid LB medium as 0 h; $CdCl_2$ (0 h): addition of 100 μmol l^{-1} NaHS and 220 μmol l^{-1} $CaCl_2$ to ddH_2O/liquid LB medium simultaneously; $CdCl_2$ (1.5 h): addition of 100 μmol l^{-1} NaHS to ddH_2O/liquid LB medium, and 1.5 h of shake culture, then addition of 220 μmol l^{-1} $CdCl_2$; +: addition of this solution; –: not add this solution.

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