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Effect of endophyte-infection on growth parameters and Cd-induced phytotoxicity of Cd-hyperaccumulator *Solanum nigrum* L.

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HIGHLIGHTS

- Endophyte LRE07 was inoculated into plant to improve Cd phytoremediation efficiency.
- Endophyte inoculation enhanced growth and total Cd-uptake of host plant.
- The beneficial effect of endophyte was more obvious at 10 μM Cd.
- Possible mechanisms are enhancement of nutrition uptake and enzymes activities.

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ABSTRACT

The aim of this work was to evaluate effects of endophytic bacterium inoculation on plant growth and assess the possible mechanism of endophyte in heavy metal phytoremediation. Seeds of *Solanum nigrum* L. were inoculated with endophyte *Serratia nematodiphila* LRE07 and were subjected to Cd in the growing medium. Cd produced a significant inhibition on plant growth and a reduction in the content of photosynthetic pigments. The inoculation of endophytic bacterium alleviated the Cd-induced changes, resulting in more biomass production and higher photosynthetic pigments content of leaves compared with non-symbiotic ones. The beneficial effect was more obvious at relatively low Cd concentration (10 μM). Based on the alteration of nutrient uptake and activated oxygen metabolism in infected plants, the possible mechanisms of endophytic bacterium in Cd phytotoxicity reduction can be concluded as uptake enhancement of essential mineral nutrition and improvement in the antioxidative enzymes activities in infected plant.

1. Introduction

Toxic metal pollution has received considerable attention over the years as a result of increased industrial activities. Heavy metals can be accumulated in organisms and finally threaten human health through food chains. Therefore, an effective and affordable solution is urgently needed to remove toxic metals from environment. Established technologies to remediate metal contaminated soils are frequently expensive and environmentally invasive.

In situ phytoremediation has been proposed as a low-cost, environmentally friendly and effective method to remove toxicant from contaminated soils (Salt et al., 1998). However, phytoremediation of heavy metal still has to deal with some important shortcomings such as phytotoxicity, slower than mechanical methods, and a limited contaminant uptake (Doty, 2008). To realize the full potential of this technology, several methods have been used to promote plant biomass and metal uptake in the presence of various environmental pollutants. A direct method is overexpression of genes whose products are related to metal uptake, transport, or sequestration in plants (Cherian and Oliveira, 2005). In addition, chelating agents such as EDTA, citric acid, and N-(2-hydroxyethyl)-ethylenediaminetriacetic acid have been tried to increase metal bioavailability and, hence, enhance phytoextraction (Turgut et al., 2004). However, some potential risks involved in transgenics and chelating agent were posed to environment (Nowack and VanBriesen, 2005; Andow and Zwahlen, 2006).

Plant-associated bacteria can be exploited to enhance phytoremediation (Newman and Reynolds, 2005; Chen et al., 2010; Glick, 2010). Plant growth promoting rhizobacteria (PGPR) have long been

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studied as inoculants for improvement of plant growth in metal contaminated soils (Glick, 2010). Certain heavy metal resistant PGPR can positively affect plants by increasing plant tolerance to various environmental stresses, facilitating root development, and improving growth and health (Glick, 2010). However, colonization of plants root with PGPR is often problematic, because the soil generally represents a hostile environment to microbial introductions and the inoculated microorganisms in soil are subjected to a range of adverse abiotic and biotic stresses (van Veen et al., 1997).

In contrast to rhizobacteria living on or around the plant surface, endophytic bacteria are the microbes inhabiting the interior of plant tissues without causing harm to the host (Kuklinsky-Sobral et al., 2004). The endophyte offers several advantages over PGPR. For instance, the endophyte is correlated more closely to plant as compared with PGPR, so more effective effects may exist in complementary niches of endophyte and its host. Furthermore, the host plant provides a ready-made environment so that the endophytic bacteria could be better protected from biotic and abiotic stresses than rhizobacteria (Newman and Reynolds, 2005). Simultaneously, endophyte can enhance plant growth and increase plant resistance to heavy metal stress through similar mechanisms described for PGPR such as nitrogen fixation, phosphate solubilization, siderophore and indole acetic acid production (Rajkumar et al., 2009). Hence, attention has been focused on the potential application of endophytic bacteria in phytoremediation (Newman and Reynolds, 2005; Doty, 2008). The endophytes isolated from hyperaccumulators show high tolerance to heavy metals and can enhance phytoremediation potential by reducing heavy metal phytotoxicity (Mastretta et al., 2009) and promoting the plant growth (Chen et al., 2010).

In this work, plant *Solanum nigrum* L., a Cd hyperaccumulator (Wei et al., 2004), was inoculated with an endophytic strain *Serratia nematodiphila* LRE07, a heavy metal resistant endophytic bacterium (Luo et al., 2011), and was exposed to different concentrations of $CdCl_2$. The physiological parameters and enzymatic antioxidants of growing plant were studied in order to know the possible mechanisms of endophyte in phytotoxicity reduction and growth promotion involved in phytoremediation.

2. Materials and methods

2.1. Culture condition and Cd treatment

S. nigrum L. plants were inoculated with endophytic bacterium *S. nematodiphila* LRE07. The strain had the capable of facilitating plant growth and could resist high concentrations of heavy metals. Fresh cultures of *S. nematodiphila* LRE07 were grown in Luria–Bertani (LB) medium which contained 10 g tryptone, 10 g NaCl and 5 g yeast extract in 1 L distilled water. Cultures were performed at 37 °C with 150 rpm shaking. After enrichment, cells were collected by centrifugation (6000 rpm) for 10 min at 4 °C, washed twice in 10 mM $MgSO_4$ and resuspended in 10 mM $MgSO_4$ to obtain an inoculum with approximate absorbance value (OD_{600}) of 1.

Seeds of *S. nigrum* L. plant were surface sterilized using serial washing in 70% ethanol for 3 min, NaClO solution (2% available chlorine) for 3 min and rinsed three times in sterilized distilled water. The seeds were then planted in sterilized vermiculite and saturated with half-strength sterile Hoagland's nutrient solution to which the bacterial inocula were added to a final absorbance value (OD_{600}) of 0.1 (approximate 10^8 CFU mL^{-1}). Noninoculated seeds were used as controls. The Hoagland's solution contains per liter distilled water, 20 mL macroelements, 10 mL Ca solution, 1 mL Fe solution and 1 mL microelements (macroelements, containing 25.3 g KNO_3, 70.8 g, 6.8 g KH_2PO_4, 4 g $NH_4H_2PO_4$, 24.65 g $MgSO_4{\cdot}7\ H_2O$ per 1000 mL distilled water; Ca solution containing 47.25 g $Ca(NO_3)_2{\cdot}4\ H_2O$ per 500 mL distilled water; Fe solution containing 2.78 g $FeSO_4{\cdot}7\ H_2O$, 3.73 g EDTA-Na_2 per 500 mL distilled water; microelements 0.845 g $MnSO_4{\cdot}H_2O$, 1.395 g H_3BO_3, 0.718 g $ZnSO_4{\cdot}7H_2O$, 0.013 g $CuSO_4{\cdot}5\ H_2O$, 0.013 g $Na_2MoO_4{\cdot}2\ H_2O$ per 500 mL distilled water) solution the nutrient solution was buffered to pH 6.0 with HNO_3/KOH. After germinated at 25 °C in the dark for 7 d, the seedlings were grown at day/night conditions of 24/20 °C air temperature, 16/8 h under white light of 200 μmol m^{-2} s^{-1} and 60% relative humidity.

Pots were maintained in greenhouse for 4 wk and then LRE07 inoculated plants and noninoculated controls were taken out of the vermiculite carefully without damaging the adventitious root system and rinsed thoroughly in sterile water. Subsequently, plants were grown hydroponically in half-strength Hoagland solutions for 7 d. Treatments were started at 42 d (corresponding to day 0 of the experiment) by adding different $CdCl_2$ concentrations (0–100 μM) to the nutrient solutions. The nutrient solution was aerated, and changed every 3 d to avoid depletion of nutrient as well as Cd. Periodically up to 28 d, samples of different treated plants were collected and analyzed.

2.2. Recovery of inoculated bacteria

The presence of *S. nematodiphila* LRE07 in roots and shoots was tested in infected plants as well as its absence in non-infected plants. Plants were harvested on day 21 and day 70. Roots and shoots were treated separately. Fresh root and shoot materials were washed in distilled water, surface-sterilized by serial washing in 70% ethanol for 3 min, NaClO solution (2% available chlorine, w/v) for 3 min and rinsed three times in sterilized distilled water (Luo et al., 2011). A 100 μL sample of the sterile distilled water used in the final rinse was plated onto LB medium to verify the efficiency of disinfection process. After surface disinfection, the root or shoot tissue was cut and triturated in 10 mL of sterile PBS. Appropriate dilutions (100 μL) were plated onto LB medium and incubated at 28 °C for 2–14 d.

2.3. Determination of tiller number

After harvest on given days, the number of tillers per plant was determined. In order to eliminate the heterogeneity of the plants at the beginning of the experiment, the value thus obtained was the ratios of tillers on a given day and tillers on day 0.

2.4. Water content of the leaves

Fresh weight (FW) of the roots and fully expanded apical leaves was taken immediately after harvesting. Dry weight (DW) of plant tissue was determined after placing samples in hot air oven at 80 °C till they dried to constant weight.

2.5. Photosynthetic pigments

Fresh leaves (0.1 g) were taken from each treatment and used to quantify the photosynthetic pigments. The leaves were cut into pieces and placed in plastic tubes with lids containing 5 mL dimethylsulfoxide reagent as organic solvent. The mixture was incubated at 65 °C till the pigments extracted and then analyzed the extract at wavelengths of 480, 649 and 665 nm using the equations by Wellburn (1994) for carotenoids, chlorophyll *a* and chlorophyll *b*.

2.6. Elemental analyses

Fresh root and shoot samples were vigorously washed with distilled water to remove surface element traces (adsorbed culture medium) after harvest. Then samples were oven-dried at 80 °C for 48 h and subsequently crushed to a fine power with a mortar and

pestle. Dried samples (up to 0.1 g DW) of each organ were digested in a HNO_3–$HClO_4$ (3:1, v/v) mixture. After dilution and filtration, element concentrations of Mn, Zn, Cd and Fe were analyzed by flame atomic absorption spectrometry (Hitachi, Japan).

2.7. Lipid peroxidation assay

Lipid peroxidation levels in the leaves were estimated in terms of malondialdehyde (MDA) formation according to the method of Heath and Packer (1968) with modifications. Approximately 0.1 g of fresh leaves was homogenized in 3 mL trichloroacetic acid (TCA) (0.1%) and centrifuged at 4000 rpm for 10 min. Two milliliter of supernatant was mixed with 2 mL of TCA (20%) containing 0.5% 2-thiobarbituric acid. The mixture was heated at 95 °C for 30 min and then quickly cooled in an ice bath. After centrifugation at 4000 rpm for 10 min, the absorbance of the supernatant was measured at 532 nm wavelengths. After subtracting the absorbance at 600 nm to correct the non-specific turbidity, the MDA concentration was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.

2.8. Enzymatic analyses

Fresh roots and expanded leaves (0.2 g) were ground with a mortar and pestle under chilled condition in 5 mL of 100 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 1% (w/v) polyvinylpyrrolidone. The homogenate was filtered through four layers of muslin cloth and centrifuged at 10000 rpm for 10 min at 4 °C (Mishra et al., 2006). The supernatant was used for enzyme determination.

Superoxide dismutase (SOD) activity was assayed at 25 °C by measuring the reduction of cytochrome (Cyt) *c* at 550 nm according to the method of McCord and Fridovich (1969) and modified by Schöner and Krause (1990). The reaction was performed in a total volume of 3 mL containing potassium phosphate (pH 7.8, 50 mM), 0.1 mM EDTA, 18 μM Cyt *c*, 0.1 mM xanthine, and extract. The reaction was started by the addition of xanthine oxidase to produce a rate of Cyt *c* reduction corresponding to 0.025 absorbance units per min in the assay mixture without protein sample. One unit of SOD activity was defined as the enzyme required to result in a 50% reduction of the rate of Cyt *c* under the specified conditions.

Catalase (CAT) activity was according to the method of Cakmak and Marschner (1992). The assay mixture contained 100 mM phosphate buffer, 6 mM H_2O_2 and 0.2 mL of tissue extract. At 25 °C, H_2O_2 was added at the end, and the decomposition of H_2O_2 was monitored at 240 nm for 3 min. CAT activity was calculated using an extinction coefficient of 39.4 mM^{-1} cm^{-1}.

For the estimation of glutathione peroxidase (GPX) activity, the oxidation of guaiacol was determined in a reaction mixture (3 mL) that contained 100 mM phosphate buffer, pH 7.0, 0.1 μM EDTA, 5.0 mM guaiacol, 15.0 mM H_2O_2 and 50 μL enzyme extract (de Azevedo Neto et al., 2006). The reaction was initiated by addition of enzyme extract and the increase in absorbance was recorded at 470 nm for 1 min. The enzyme activity was quantified using an extinction coefficient of 26.6 mM^{-1} cm^{-1}.

Ascorbate peroxidase (APX) activity was assayed as described by Koricheva et al. (1997). The reaction mixture contained 50 mM potassium phosphate (pH 7.0), 0.1 mM EDTA, 0.5 mM L-ascorbic acid, 0.1 mM hydrogen peroxide and 0.1 mL enzyme extract. The decrease in absorbance as ascorbate was oxidized was monitored at 290 nm using a UV–Vis spectrophotometer (extinction coefficient 2.8 mM^{-1} cm^{-1}).

2.9. Statistical analysis

All values reported in this work are the mean of at least three replicates. Data are presented as mean values ± standard deviation. To verify the statistical significances of differences among treatments, data were analyzed using SPSS statistical software by one-way ANOVA, MANOVA, and Duncan's multiple-range test. Differences were considered significant at $P < 0.05$.

3. Results and discussion

3.1. Colonization of endophytic bacterium LRE 07 in plants

After 21 and 70 d of growth, plant samples were harvested to determine the inoculation efficiency. Endophytic bacteria were isolated from roots and shoots separately and plated on LB medium. The colony of *S. nematodiphila* LRE07 could be identified distinctly due to its carmine pigment. As shown in Table 1, *S. nematodiphila* LRE07 was inoculated into plant successfully. The number of CFU that could be re-isolated showed a time-dependent growth in roots and shoots of their host plants and it was not inhibited by the presence of Cd. No endophytic *S. nematodiphila* LRE07 was isolated from control plants while some other bacteria existed, demonstrating that endogenous endophytic bacteria remained in the plants even though the seeds were surface sterilized. The aim of this work was evaluate the effect of inoculating a metal resistant endophytic bacterium on the growth and metabolism of plants exposed to toxic Cd concentrations. The effective recovery of inoculated bacteria suggested that the inoculation was successful in both roots and shoots (Table 1). The number of re-isolated CFU tended to increase with time, indicating that *S. nematodiphila* LRE07 could live concordantly with its host plants.

3.2. Effect of Cd treatment on plant growth

The increasing concentration of Cd produced growth inhibition of *S. nigrum* L., measured as tillers (Table 2), FW and shoot height (Fig. 1). Tiller number is considered to be an important agronomic parameter of productivity and persistence of grasslands (Bonnet et al., 2000). During the experiment, the tillers of the plants E+ and E− for different concentrations of Cd were numbered and the ratios (tillers of a given day/tillers of day 0) are shown in Table 2. At 0 and 10 μM Cd, the ratio increased over time for both infected and non-infected plants. However, at 50 and 100 μM Cd the ratio rapidly reached constant values and even decreased at the end of the experiment, reflecting the defoliation of plants. The higher the Cd concentration applied to the nutrient solution, the weaker the ratio of the tillers was obtained. It can be seen that the infected plants showed higher ratio values than noninfected controls in an equal Cd concentration as shown in Table 2 ($P < 0.05$, MANOVAs), that is, greater number of leaves were obtained in infected plants.

Similar beneficial effects of endophytic bacterium LRE07 on plant growth were observed in FW, shoot height, and root weight (Fig. 1). The FWs of plants were determined after harvested on day 28. Whether the plants infected or not, there was a decrease in FW with the increase of Cd (Fig. 1a). However, the weight values of infected plants were higher than non-infected ones ($P < 0.05$, MANOVAs), especially in 10 μM. Similar trends were observed in the shoot heights (Fig. 1b) and root weights (Fig. 1c) while no obvious change was obtained with water content (Fig. 1d). The inoculation of *S. nematodiphila* LRE07 was highly effective at protecting its host plants from growth inhibition caused by the presence of cadmium, especially at low concentrations (10 μM) (Fig. 1). At 10 μM Cd, no visible growth inhibition was observed for infected plants and the FW of these plants increased by 58% compared with non-infected ones.

Unlike the remediation of organic pollutants, which are enzymatically degraded, the heavy metals must be sequestered

Table 1
Number of bacterial colonies (g^{-1} FW) isolated from roots and shoots of *Solanum nigrum* L.[a]

	Plant part	14 d [b]	70 d
Non-exposed plants	Root	8.2×10^3	8.6×10^5
	Shoot	2.5×10^3	1.7×10^3
10 μM $CdCl_2$	Root	8.2×10^3	5.0×10^6
	Shoot	2.5×10^3	4.0×10^5
100 μM $CdCl_2$	Root	8.2×10^3	3.4×10^5
	Shoot	2.5×10^3	2.1×10^4

[a] *Solanum nigrum* L. plants were inoculated with *S. nematodiphila* LRE07. As controls, plants without inoculum are not shown.
[b] These were the number of CFUs isolated from plants pre-exposed to cadmium.

Table 2
Effect of *S. nematodiphila* LRE07 on the ratio tillers of *Solanum nigrum* L. exposed to cadmium.

	Day 8		Day 14		Day 28	
	E+	E−	E+	E−	E+	E−
Controls	2.1 ± 0.6*	1.5 ± 0.3	2.7 ± 0.2*	2.0 ± 0.1	4.5 ± 0.4	4.6 ± 0.5
10 μM	2.1 ± 0.4*	1.4 ± 0.1	2.3 ± 0.1*	1.6 ± 0.1	4.0 ± 0.1*	2.4 ± 0.5
50 μM	2.0 ± 0.4*	1.1 ± 0.3	2.1 ± 0.1*	1.4 ± 0.2	2.2 ± 0.3*	1.1 ± 0.1
100 μM	1.3 ± 0.1	1.1 ± 0.3	1.7 ± 0.2*	0.9 ± 0.1	1.5 ± 0.4*	0.8 ± 0.0

Values are the ratio tillers on a given day/tillers on day 0 of *Solanum nigrum* L. cultivated with a nutrient solution containing 0, 10, 50, 100 μM $CdCl_2$.Values are the means of four replicates. E+, plants infected by *S. nematodiphila* LRE07; E−, plant not infected. Asterisks represent significant differences from each control value for a given day.

and removed physically from the contaminated site. This feature implies that being taller can be particularly important for a bioaccumulator plant, because the increased biomass means it can pick up more contaminants. The results obtained above showed that endophyte inoculation can be beneficial for plant growth under heavy metal exposure, suggesting that the isolate has the potential to improve phytoremediation efficiency. Endophytes can act as bio-fertilizers to promote its host plant productivity (Vessey, 2003). The mechanisms involved in plant growth promotion by endophytes inoculation include phosphate solubilization activity, indole acetic acid production, siderophore production and nitrogen fixation (Ryan et al., 2008). In our previous study, we researched the endophyte *S. nematodiphila* LRE07 and found that the isolate possesses some of these features (Luo et al., 2011).

3.3. Effect of Cd on photosynthetic pigment contents

The growth inhibition produced by Cd could be mainly due to the effect of heavy metal on the photosynthetic pigment contents. Photosynthetic pigment, including chlorophyll *a*, chlorophyll *b* and carotenoid, is the basis for photosynthesis. The degradation of chlorophyll or the inhibition of its biosynthesis has been proposed as being responsible for the growth reduction produced by Cd

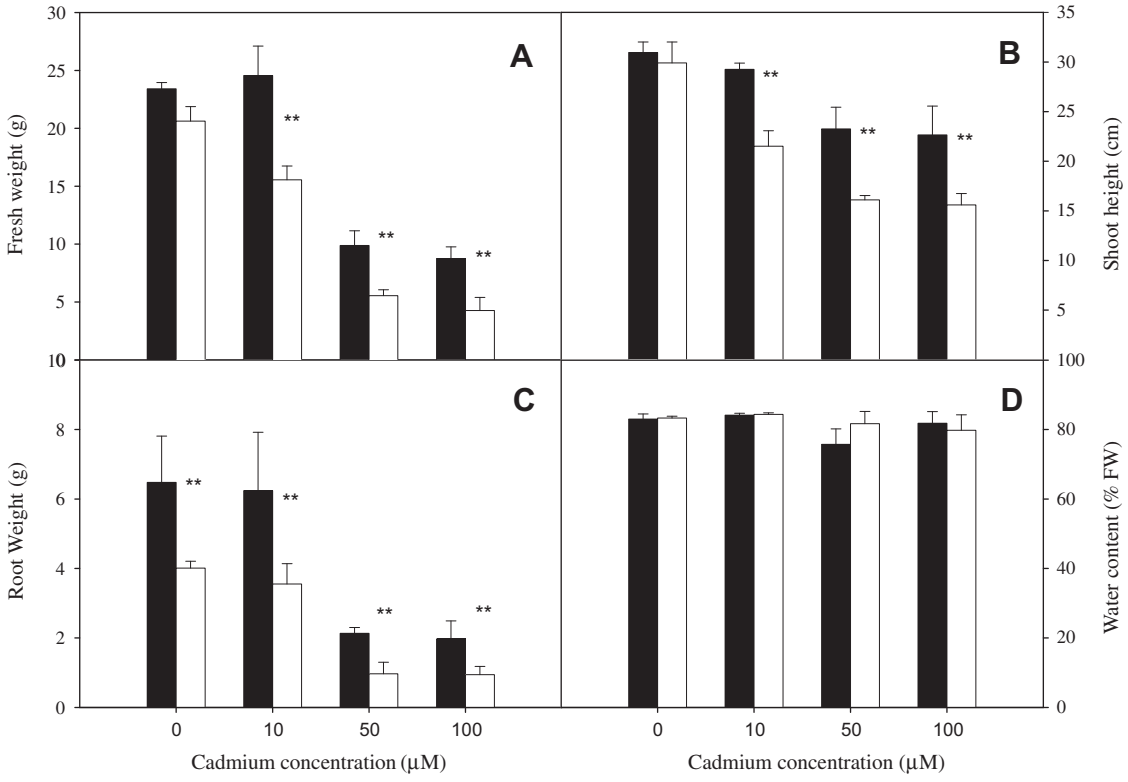

Fig. 1. Effect of cadmium treatment on fresh weights (A), shoot height (B), root weight (C) and plant water content (D) of *Solanum nigrum* L. infected with *S. nematodiphila* LRE07 (solid bars) and uninfected (open bars). Each rectangle represents the means ± standard deviations of four replicates. $^{**}P < 0.05$ indicate significant differences.

(Somashekaraiah et al., 1992). In this study, the photosynthetic pigment contents were measured during the whole experiment period. There was a net decrease in pigment content from day 0 to the end of the experiment with increasing Cd concentration whether the plants were infected or not (Fig. 2). However, endophyte-infection positively influenced the photosynthetic pigment contents of its host plant. It can be seen that for all Cd concentrations (except for day 3 at 100 μM), chlorophyll *a*, chlorophyll *b*, and carotenoid of infected plants leaves were higher than non-infected ones ($P < 0.05$, MANOVAs). In addition, the decreases of photosynthetic pigment caused by Cd exposure in infected plants were much less than that of in endophyte-free plants (Fig. 2).

The results obtained above are in agreement with some previous studies, which have also reported that endophyte inoculation could influence positively to photosynthetic pigment contents of host plant under abiotic stresses (Hunt et al., 2005; Zhang et al., 2010). Cd content in the growing medium could suppress the Fe uptake by the plants (Das et al., 1997), while Fe is an indispensable cofactor for cellular activities in photosynthesis (Briat et al., 1995). The uptake efficiency of Fe maintained by the presence of the endophyte (Table 3) could contribute to the higher level photosynthetic pigments of endophyte-infection plants.

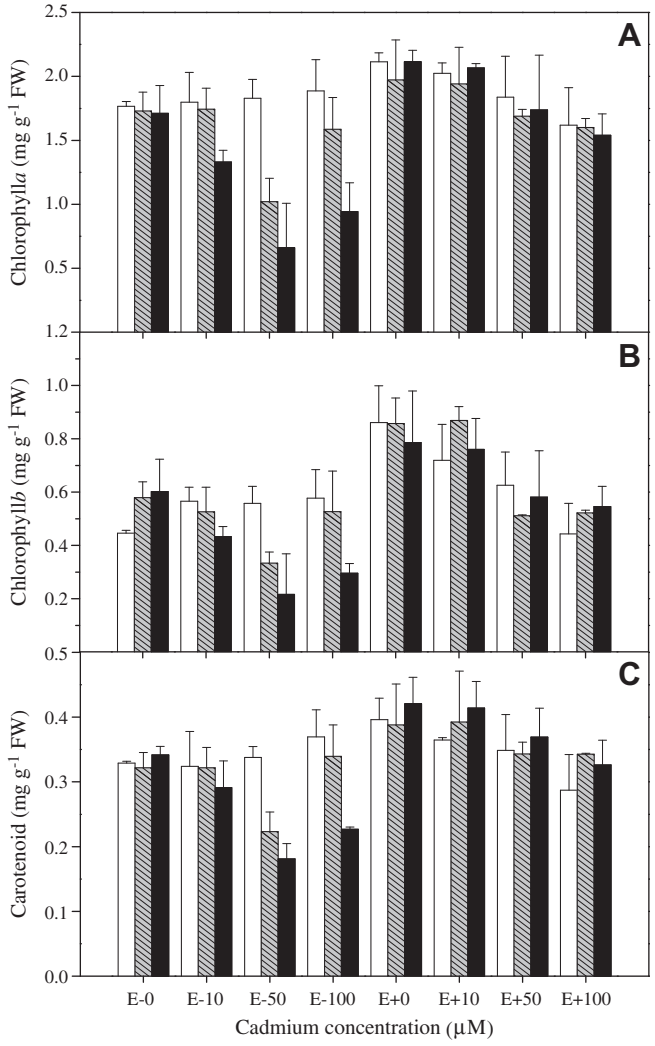

Fig. 2. Changes of pigment content of *Solanum nigrum* L. infected with *S. nematodiphila* LRE07 (E+) and uninfected (E−) exposed to 0, 10, 50, 100 μM $CdCl_2$ (3 d, open columns; 8 d, shaded columns; 14 d, closed columns). Data presents the means ± SD of four replicates.

3.4. Metal contents of roots and shoots

The metal contents in shoots and roots were altered by the addition of cadmium in the culture medium whether the plants were infected or not, but the content variations were different between both types of plants (Table 3). Increasing concentrations of Cd in nutrient solutions led to an increase of Cd contents in shoots and roots. Nevertheless, no significant differences were observed between infected and non-infected plants (except in stem: Cd 10 and 100 μM). The content of Mn in plant tissues decreased significantly as the increase of Cd concentration in nutrient solutions ($P < 0.05$, MANOVAs). The absorbed Mn in infected plants was higher than that of non-infected plants in the presence of Cd, especially in high Cd concentrations (50 and 100 μM). The Fe contents in roots and leaves increased as a result of Cd treatment, but in stems a decrease was detected. Zn contents were decreased in leaves and stems while increased in root, although differences were not statistically significant. The Cd-induced content variations of Fe and Zn were alleviated in infected plants. In other words, the change was smaller in infected plants.

S. nigrum L. is a Cd hyperaccumulator with high phytoextraction efficiency (Chen et al., 2010). In a pot-culture experiment, *S. nigrum* L. could accumulate up to 124.6 μg Cd g^{-1} DW in leaves, indicating no phytotoxic symptoms and reduction in growth (Wei et al., 2004). But in this study, Cd produced a significant inhibition on *S. nigrum* L. growth, especially in high Cd concentrations (Fig. 1). And the root Cd concentration of *S. nigrum* L. is much higher than shoot and stem (Table 3). The differences of culture mediums for *S. nigrum* L. growth could be one possible reason. In the studies of Wei et al. (2004) and Chen et al. (2010), plants were cultured in Cd-contaminated soil or vermiculite which can absorb or bind heavy metals (Bradl, 2004; Malandrino et al., 2006). Meanwhile, rhizobacteria in soils have the ability to reduce heavy metal phytotoxicity. Therefore, *S. nigrum* L. cultured in soil or vermiculite can be protected under Cd exposure. In this study, plants were grown hydroponically in half-strength Hoagland solutions containing different Cd concentrations. Cd stressed the plant roots directly. Therefore, *S. nigrum* L. accumulated a large amount of Cd in its tissues, especially in its root. Consequently, Cd produced a significant inhibition on *S. nigrum* L. growth.

With the increasing concentrations of cadmium in nutrient solutions, the plant accumulated a mass of cadmium in its shoot and root whether the plant were infected or not. On a dry-weight basis, the plants infected and non-infected with *S. nematodiphila* LRE07 took up approximately the same amount of cadmium. Compared with noninoculated controls, endophyte inoculation did not greatly influence the cadmium concentrations in plant tissues, but achieved a higher biomass production, thus resulting in more total Cd-uptake per plant (72 ± 5% increase at 10 μM), reflecting the promotion of phytoextraction efficiency.

Cd produced increase in the uptake of Fe in tissues, while the uptake of Mn and Zn were decreased except no significant changes of Zn in the root. These results indicated that Cd induced alteration on translocation of other heavy metals in plant tissues. Fe is of great importance for plant life due to its involvement in photosynthesis, hormone biosynthesis, and production and scavenging of reactive oxygen species (Hänsch and Mendel, 2009). Mn is essential for plant metabolism and enzyme activation. Zinc is important as a component of enzymes for protein synthesis and seed development (Hänsch and Mendel, 2009). The presence of Cd in the nutrient solution influenced the nutritional status of leaves and roots probably by inhibiting the transporters of loading other metals into the aerial part of the plant and influencing in the production of phytochelatins (Sandalio et al., 2001). The inoculation of endophyte *S. nematodiphila* LRE07 alleviated this Cd-induced changes on metal contents (Table 3). Mn content in infected plant

Table 3
Metal contents of shoots and roots of *Solanum nigrum* L. subjected to Cd treatment.

Element	Cd concentration (μM)							
	0		10		50		100	
	E+	E−	E+	E−	E+	E−	E+	E−
	Tissue metal content (mg kg^{-1} DW)							
Leaves								
Cd	19 d	22 d	504 c	497 c	468 b	471 b	603 a	599 a
Mn	594 b	745 a	707 a	728 a	565 bc	527 c	425 d	385 d
Fe	148 de	170 cd	198 b	158 cde	225 a	138 e	235 a	182 bc
Zn	89 c	115 a	120 a	100 b	82 c	66 d	85 c	66 d
Stems								
Cd	14 f	12 f	290 e	372 d	394 c	431 bc	568 a	449 b
Mn	218 b	289 a	273 a	210 b	145 c	91 d	133 c	78 d
Fe	94 e	98 e	238 a	150 d	183 b	177 b	166 bcd	154 d
Zn	100 c	116 a	114 ab	107 bc	70 d	52 e	67 d	37 f
Roots								
Cd	36 d	30 d	847 c	867 c	1013 b	1022 b	1115 a	1160 a
Mn	1103 ab	1254 a	1100 ab	993 bc	883 c	591 d	917 c	662 d
Fe	810 e	882 e	1305 d	1181 d	1610 c	2255 a	1829 b	2175 a
Zn	272 c	362 b	261 c	280 c	261 c	402 a	261 c	386 a

Solanum nigrum L. infected with *S. nematodiphila* LRE07 (E+) and uninfected (E−) were treated with 0, 10, 50, 100 μM $CdCl_2$ and harvested on day 28. Values are means of four replicates. For the same metal contents in leaves, stems or roots, values followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple range test.

tissues (roots, leaves and stems) was higher, either at 10, 50 or 100 μM, than that of in non-infected controls. Compared with non-symbiotic controls, symbiotic plants assimilated more Fe and Zn in leaves and stems. Similarly, Malinowski et al. (2000) reported that endophyte present in the leaf of tall fescue could enhance mineral uptake rate. Considering the beneficial effect of Fe, Zn and Mn on plant life, the increased concentration of these metals in plant tissue caused by endophyte infection may be the indirect mechanism involving in biomass production enhancement of infect-plants.

3.5. Changes of lipid peroxidation contents

Cd is known to result in extensive lipid peroxidation (Sandalio et al., 2001) which affects cell membrane functionality and integrity and can produce irreversible damage to the cell function (Halliwell and Gutteridge, 1989). As an indicator of lipid peroxidation the content of MDA in plant leaves was measured. No surprisingly, the MDA content showed a linear enhancement with time and with the increase in concentration of Cd whether the plants infected or not (Supplemental Material (SM), Fig. SM-1). However, it can be seen that the MDA contents in infected plants were lower than that of in the noninfected plants, although differences were not statistically significant. In addition, the increment value of MDA content with time was smaller in infected plants than those in noninfected plants. Similarly, *Piriformospora indica*, a root-endophytic basidiomycete, significantly attenuated the content of salt-induced lipid peroxidation in *Hordeum vulgare* (Baltruschat et al., 2008). The lower level of lipid peroxidation in infected plant suggested that endophytic bacteria may cooperate with its host plants to protect from oxidative damage under cadmium stress.

3.6. Effect of Cd on activated oxygen metabolism

Activities of enzymes (SOD, GPX, CAT and APX) detoxifying the cells from active oxygen species were measured as representative enzymes involves in antioxidant metabolism upon Cd exposure (Figs. 3 and 4). Cadmium concentrations of 10 and 50 μM produced slight increases in the content of enzymes in leaves compared with Cd-free plants, but decreases were observed with 100 μM whether the plants were infected or not (Figs. 3 and 4a). Fig. 4 represents the total activity of SOD measured in roots and leaves of infected and non-infected plants. It can be seen that Cd induced obvious increase in the content of SOD, and SOD activity is higher in infected plant tissues compared to non-infected plants for Cd concentration of 10 and 50 μM. CAT activity in leaves showed no significant differences between both types of plants (Fig. 4a). Leaves of infected plants showed higher activity of GPX and APX compared with non-symbiotic plants at 10 and 50 μM (Fig. 4a). In roots, increasing cadmium concentrations caused a linear enhancement of GPX in both infected and non-infected plants (Fig. 4b), while for 50 and 100 μM Cd the GPX activity of infected plants were significantly higher compared with non-infected ones. The activity of the antioxidative enzymes CAT and APX in roots increased at low Cd concentration (10 μM) while depressed with high concentration of Cd (Fig. 4b). CAT and APX activity did not show significant difference between symbiotic and the non-symbiotic plants although a disparity could be observed for CAT activity with 10 μM Cd.

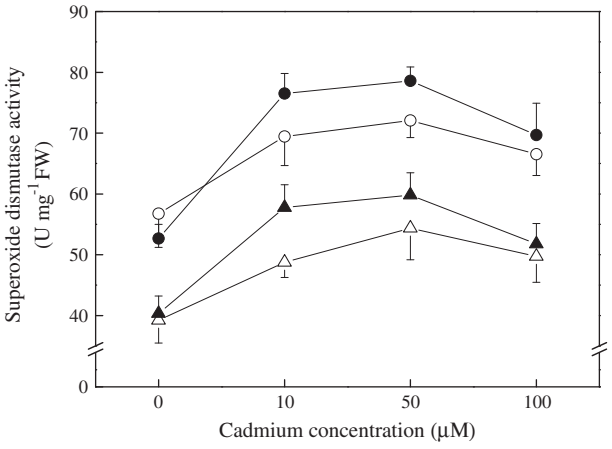

Fig. 3. Changes of superoxide dismutase activity in roots and leaves of *Solanum nigrum* L. infected by *S. nematodiphila* LRE07 (E+) and not infected controls (E−) exposed to 0, 10, 50, 100 μM $CdCl_2$. (●) E + root; (○) E− root; (▲) E + leaf; (△) E− leaf. Each value is the mean of four replicates (±SD).

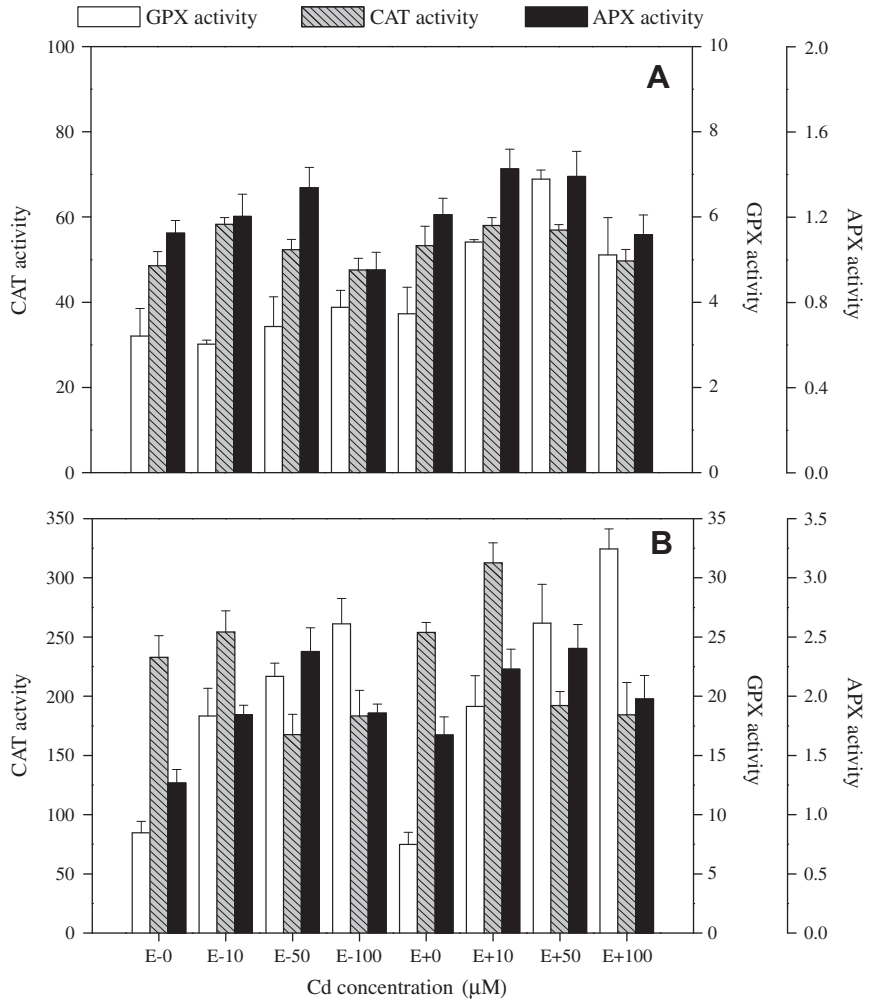

Fig. 4. Changes of enzymatic antioxidant in leaves (A) and roots (B) of *Solanum nigrum* L. infected by *S. nematodiphila* LRE07 (E+) and not infected controls (E−) exposed to 0, 10, 50, 100 μM $CdCl_2$. Catalase is expressed as μmol min^{-1} g^{-1} FW, guaiacol peroxidase and ascorbate peroxidase as mmol min^{-1} g^{-1} FW. Each rectangle represents the mean ± SD of four replicates.

Cd exposure can induce severe oxidative stress indirectly by disturbing cellular equilibria between the generation and the neutralization of reactive oxygen species (ROS) (Schützendübel and Polle, 2002). It is important and necessary to control the level of ROS due to their cellular damage activities. Plants employ defense antioxidative systems, consists of low molecular weight antioxidants and several enzymatic scavengers of activated oxygen such as SOD, CAT, APX and POD, to metabolize ROS (Rao et al., 1996). Antioxidative ability plays an important role in the tolerance and accumulation of Cd in plants. Uraguchi et al. (2006) showed that *Avena strigosa*, a novel Cd-accumulating crop, exhibited higher activities of antioxidative enzymes such as SOD and APX than common crops. A significant enhancement on enzymatic activity of CAT and APX in tissues of three Cd-hyperaccumualtors was observed with Cd treatment (Pinto et al., 2009). In the present study, when plants were submitted to different levels of $CdCl_2$ (0, 10, 50 μM), enhancements in the activities of CAT, APX and GPX were observed with the increasing concentration of Cd (Figs. 3 and 4). Moreover, the antioxidative capabilities of the infected plants were found increased compared with the non-infected plants (Figs. 3 and 4). This indicated that endophyte-infection could benefit host plant against Cd phytotoxicity by improving antioxidative enzymes activities. Similar effects of endophytes on plant antioxidative system have been reported for Zn in ryegrass (Bonnet et al., 2000) and Cd in drunken horse grass (Zhang et al., 2010). However, the mechanisms involved in antioxidative system enhancement by endophytes inoculation are not yet well understood. Colonizing the internal tissues of plants, endophytes are likely to interact much closely with their host and therefore influence the physiology of the plant easily. Some traits, such as production of antioxidative enzymes and siderophore, of bacteria may be the possible reason of enhancing the activities of antioxidative enzymes in plants. More molecular techniques should be introduced to investigate this mechanism involved in plant-endophytes interaction in the further studies.

4. Conclusions

Cd produced a significant inhibition on *S. nigrum* L. growth as well as a reduction in the content of photosynthetic pigments. The inoculation of endophytic bacterium LRE07 alleviated these Cd-induced changes, resulting in promotion of plant growth and increase of total Cd-uptake per plant. The uptake enhancement of essential mineral nutrition and improvement in antioxidative enzymes activities could be the possible mechanisms of endophyte involved in Cd phytotoxicity reduction. The beneficial effect of endophyte was more obvious at relatively low Cd concentration (10 μM), suggesting practical applications in using endophyte to improve the efficiency of phytoremediating low concentration cadmium contaminants.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2012.07.005.

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