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Growth and Cadmium Accumulation of *Solanum nigrum* L. Seedling were Enhanced by Heavy Metal-Tolerant Strains of *Pseudomonas aeruginosa*

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Abstract Some heavy metal-tolerant bacteria recognized as plant growth-promoting bacteria (PGPB) could improve plant growth. Here, the growth and Cd accumulation of *Solanum nigrum* seedling inoculated by soaking the roots in a dilute suspension of the Cd-tolerant strains ZGKD5 or ZGKD2 were investigated. The results showed that both ZGKD5 and ZGKD2 exhibited the characterization of producing IAA, siderophores, ammonia, and biosurfactant, and solubilizing phosphate and fixing nitrogen. The siderophores produced by both strains could chelate various heavy metals, such as Cu, Cd, Zn, and Ni. The shoot height, root length, number of fibrous root, and dry weight of *S. nigrum* seedling were significantly increased by inoculation with ZGKD5 or ZGKD2 in the absence or presence of Cd stress. The Cd concentration and translocation from root to shoot in seedlings were remarkably increased, indicating that both strains could improve the growth and Cd phytoextraction of *S. nigrum*. The activities of SOD, POD, CAT, and APX in both inoculated and uninoculated plants were increased under Cd stress, indicating that these antioxidative enzymes could alleviate oxidative stress induced by Cd. Furthermore, activities of antioxidative enzyme in inoculated plants exposed to Cd stress was lower than that in uninoculated Cd-stressed plants, which might be due to the decreasing metabolism caused by high levels of Cd. These results indicated that strains ZGKD5 and ZGKD2 are PGPB and have the potential for improving the phytoremediation of *S. nigrum* in Cd-contaminated farmland soil.

Keywords Cadmium · *Pseudomonas aeruginosa* · *Solanum nigrum* L. · Antioxidant enzymes

1 Introduction

Heavy metals in soil are difficult to be removed, and could be absorbed by plant roots and then accumulate in various plant tissues. The excessive Cd concentrations in the soil of farmland caused a considerable concern to human health through the food chain (Nawrot et al. 2010). For decades, more attention has been paid to phytoremediation due to its environmental friendliness and cost effectiveness (Wei et al. 2005). The efficiency of phytoremediation was influenced by many factors, such as slow growth of plant, small amount of biomass, and limited tolerance to heavy metal stress (Jiang et al. 2008). Therefore, the most available way to improve efficiency of phytoremediation might be the use of

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heavy metal-tolerant or accumulator plant in combination with plant growth-promoting bacteria (PGPB).

A variety of PGPB with the characteristics of producing indole acetic acid (IAA), siderophores, biosurfactant, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase could increase the plant biomass production; however, heavy metal concentration accumulated in plant tissue varies among plants and bacterial species and their interactions. For instance, the Cu-resistant bacterium *Providencia vermicola* with several plant growth-promoting traits could promote the growth of lentil and reduce oxidative stress and uptake of copper in plant tissues (Islam et al. 2016). Losses of plant biomass and Zn/Cd accumulation in shoot of sunflower in Zn- and Cd-contaminated soils were reduced by inoculation with the metal-resistant and plant growth-promoting rhizosphere strain *Chrysiobacterium humi* (Marques et al. 2013). Inoculation with metal tolerance PGPB strain *Bacillus* sp. SC2b elevated the shoot and root biomass of the Zn/Cd accumulator *Sedum plumbizincicola*, and increased Cd and Zn accumulation in the shoot and root, indicating that SC2b could serve as a biofertilizer and a metal mobilizing bioinoculant for rhizoremediation of metal-polluted soils (Ma et al. 2015). Moreover, Cu-tolerant *Pseudomonas putida* CZ1 acted as a plant growth-promoting rhizobacterium, significantly enhanced the growth of the Cu-tolerant plant *Elsholtzia splendens*, and promoted the accumulation and translocation of Cu from roots to shoots (Xu et al. 2015).

Cd-hyperaccumulator *Solanum nigrum* L. was used in the phytoremediation of Cd-contaminated soils due to its short growing duration and large biomass (Gao et al. 2010). Pot-culture experiments demonstrated that Cd concentrations in stems and leaves of *S. nigrum* were 103.8 and 124.6 mg/kg after Cd treatment for 88 days, respectively (Wei et al. 2005). Hence, *S. nigrum* is a promising plant for Cd phytoextraction in Cd-contaminated soils. There are several reports describing the effects of PGPB on growth and Cd accumulation of *S. nigrum*. Cd-tolerant endophytic bacteria *Serratia* sp. RSC-14 could alleviate the Cd toxicity in *S. nigrum* by significantly increasing plant biomass and decreasing malondialdehyde (MDA) (Khan et al. 2015). Both biomass and Cd accumulation of *S. nigrum* were significantly enhanced by the Cd-resistant fungi *Paecilomyces lilacinus* NH1 isolated from Cd-polluted soil in a waste landfill field (Gao et al. 2010). Heavy metal-tolerant bacteria that acted as PGPB such as *Chrysiobacterium*, *Bacillus*, *Pseudomonas*, and *Streptomyces* were used in plants and micro-organisms combined bioremediation. However, to the best of our knowledge, the effects of Cd-tolerant *Pseudomonas aeruginosa* in enhancing plant growth and Cd accumulation of *S. nigrum* have not been reported, and the responses of antioxidative enzyme activities of *S. nigrum* by inoculated *P. aeruginosa* to Cd stress have also not been reported. Two *P. aeruginosa* strains, ZGKD5 and ZGKD2, isolated from soil contaminated by gangue pile of a coal mine area exhibited high levels of tolerance to Cd, Cu, Zn, Ni, and Pb in our previous study (Zhang et al. 2010). The objective of this study was to investigate the effects of *P. aeruginosa* strains ZGKD5 and ZGKD2 on the plant growth, Cd accumulation, and the responses of antioxidative enzyme activity of *S. nigrum* exposed to Cd stress.

2 Materials and Methods

2.1 Plant Growth-Promoting Characteristics of Bacteria

The Cd-tolerant bacteria *P. aeruginosa* ZGKD5 and ZGKD2 were isolated from gangue pile of a coal mine area (Zhang et al. 2010). Cells were cultured in 100-mL Erlenmeyer flasks containing 50 mL of nutrient broth medium containing 3.0 g of beef extract, 10.0 g of peptone, and 5.0 g of sodium chloride per liter with an initial pH of 7.0 and incubated at 37 °C until the late exponential phase (seed inoculums). The inoculums were used for studying the plant growth-promoting characteristics of strains. For inoculation, the cell suspensions were centrifuged at 12,000 rpm for 10 min at 4 °C, the pellets were washed twice with phosphate butter (pH 7.0), and then re-suspended in sterilized water to form an optical density (OD_{600}) of 0.02 and 0.04 at 600 nm, respectively. The diluted cell suspension was used for the later root soaking.

Several plant growth-promoting traits of the strain, including production of IAA, siderophore, ammonia, and biosurfactant, and potential of phosphate solubilization and nitrogen fixation were measured according to the methods described elsewhere. Briefly, IAA production was assayed using Salkowski's colorimetric method (Sheng et al. 2008). Ammonia production activity was tested by Nessler's reagent (Dye. 1962). Biosurfactant production was tested on blood agar plates containing 5% (*v*/*v*) blood (Hassanshahian. 2014). The phosphate solubilization ability and the nitrogen fixing ability of the strains were determined in Pikovskaya's agar medium (Sundara-Rao and Sinha 1963) and Burk's medium (Wilson and Knight 1952), respectively.

Production of siderophore by stains were detected and quantified by the "universal" chrome azurol-S (CAS) analytical method (Schwyn and Neilands 1987). Briefly, cell-free culture supernatant, was incubated on CAS assay agar plates for 4–8 h at 37 °C in the dark. The orange halo surrounding the disc indicates the production of siderophore. Affinity of siderophore for multiple heavy metals was assayed as follows, aliquots of cell-free culture 5 ml of the supernatant were transferred to 10-ml tubes containing heavy metal solutions (Fe^{3+}, Cd^{2+}, Cu^{2+}, Zn^{2+}, Ni^{2+}, or Pb^{2+}) at different concentrations (0, 20, 40, 60, or 80 μM) and allowed to stand at 30 °C for 30 min to allow metal chelation by siderophores. Then, tubes were centrifuged at 12,000 rpm for 5 min and 1.5 ml of supernatant was transferred to another tube containing 1.5 ml of CAS dye and allowed to stand at 37 °C for 30 min. Siderophores were quantified based on absorbance at the wavelength of 630 nm after a color change from blue to orange.

2.2 Plant Growth and Inoculation

Seeds of *S. nigrum* used in the study were kindly provided by Professor Wei Shuhe from the Institute of Applied Ecology, Chinese Academy of Sciences. The seeds were germinated and cultivated according to Liu et al. (2013). The sterilized seeds were sown in 1/2 Murashige and Skoog solidified medium and germinated in a growth chamber with a relative humidity range of 60–70%, a temperature of 22 ± 3 °C, and a daily photoperiod of 16 h with a photosynthetic photon flux density of 165 μmol m^{-2} s^{-1}. The seedlings were transferred into plastic pots with half-strength Hoagland solution (Hoagland and Arnon 1938) and grew for 5 days. Then, the roots of 2-week-old seedlings were soaked in bacterial suspension with an OD_{600} of 0.04 or 0.02 or sterilized water (uninoculation control) for 1.5 h. Afterwards, seedlings were transplanted into the half-strength Hoagland solution with 0 or 50 μM of $CdCl_2$. The pH of the solution was adjusted to 6.5 and the nutrient solutions were changed every 3 days. Plants were collected and separated into roots and shoots after 5 days treatment; the shoot height, root length, fibrous root number and dry weight were recorded, respectively.

2.3 Cd Concentrations and Translocation

The roots and shoots were washed twice with deionized water and were dried at 80 °C until a constant weight was reached. The dried plant samples were ground into a fine powder with an agate mortar and completely digested with a mixture acid of HNO_3 and $HClO_4$ (4/1, *v/v*). The Cd concentrations were determined by inductively coupled plasma optical emission spectrometry (ICP-OES). Cd translocation efficiency from the root to shoot was represented by the translocation factor (TF): $TF = C_{shoot}/C_{root}$, where C_{shoot} and C_{root} are the Cd concentrations of shoot and root, respectively.

2.4 Antioxidant Enzyme Activities

Extraction of enzyme and evaluation of enzyme activity were conducted according to Liu et al. (2013). Briefly, fresh leaves and roots (0.5 g) of *S. nigrum* seedlings were homogenized in ice-cold 50 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 1% polyvinypyrrolidone (*w/v*). The homogenate was centrifuged at 12,000 rpm for 10 min at 4 °C; the supernatant was used to determine the activity of enzyme. Activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) were determined according to Beauchamp and Fridovich (1971), Cakmak and Horst (1991), Nickel and Cunningham (1969), and Jiang and Zhang (2001), respectively. The protein content in plant tissues was estimated according to the method of Bradford using bovine serum albumin as a standard (Bradford 1976).

2.5 Statistical Analyses

All data were analyzed by SPSS 16.0. Analysis of the values are means of three replicates. Statistical analyses were performed by one-way ANOVA, and the least significant difference (LSD) was used to compare treatment means ($P < 0.05$).

3 Results

3.1 Plant Growth-Promoting Traits of Strains ZGKD5 and ZGKD2

ZGKD5 and ZGKD2 showed characters of producing IAA, siderophores, ammonia, and biosurfactant, the activity of inorganic phosphate solubilization and nitrogen fixation (Table 1), indicating that both ZGKD5 and ZGKD2 were plant growth-promoting bacteria and had the capability of promoting the growth of plants. The two strains were therefore selected for further analysis based on their PGP traits as well as their tolerance to Cd.

3.2 The Affinity of Siderophores Produced by Strains ZGKD5 and ZGKD2 for Various Heavy Metals

ZGKD5 and ZGKD2 could produce siderophores; the affinity of siderophores for heavy metals was tested (Fig. 1). The results showed that siderophores produced by both strains could chelate a variety of heavy metal ions, such as Fe^{3+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+} and Pb^{2+}, especially for Cu^{2+} and Fe^{3+}. The affinity of siderophores produced by ZGKD5 and ZGKD2 for the metals decreased in the order $Cu^{2+} > Fe^{3+} > Cd^{2+} > Zn^{2+} > Ni^{2+} > Pb^{2+}$ and $Cu^{2+} > Fe^{3+} > Ni^{2+} > Zn^{2+} > Cd^{2+} > Pb^{2+}$, indicating that affinity of siderophores produced by ZGKD5 and ZGKD2 is different among heavy metal ions. In addition, siderophores produced by ZGKD2 have stronger affinity for Cd than that by ZGKD5. These results indicated that strains ZGKD5 and ZGKD2 have the potential in alleviating the toxicity of heavy metals and improving the resistance of plants to heavy metals through the chelation of metals by siderophores.

3.3 Effects of ZGKD5 and ZGKD2 on Growth of *S. nigrum* Seedlings Under Cd Stress

ZGKD5 and ZGKD2 have plant growth-promoting properties. *S. nigrum* seedling inoculated by soaking the roots in a suspension with an OD_{600} of 0.04 or 0.02 of ZGKD5 or ZGKD2 was subjected to 0 or 50 μM Cd for 5 days. The results showed that plant growth was obviously inhibited in uninoculated *S. nigrum* plants under Cd stress compared to control plants, while inoculation with ZGKD5 or ZGKD2 significantly improved the shoot height, root length, fibrous root number, and dry weight of *S. nigrum* seedlings in the absence or presence of Cd ($P < 0.05$) (Fig. 2). For example, in comparison with the control plants, the shoot height, root length, fibrous root number, and dry weight of *S. nigrum* seedling inoculated with an OD_{600} of 0.04 or 0.02 ZGKD5 under normal growth conditions were increased by 19.7–29.5%, 76.2–77.8%, 73.1–87.4%, and 48.8–58.7%, respectively. The shoot height, root length, fibrous root number, and dry weight of *S. nigrum* seedling inoculated with ZGKD5 in response to 50 μM Cd were increased by 28.3–36.7%, 63.3–91.3%, 104.8–144.6%, and 17.0–19.8%, respectively, as compared to the uninoculated Cd treatment plants. The effect of ZGKD2 plant growth promotion was similar to that of ZGKD5, indicating that both ZGKD5 and ZGKD2 could promote the growth of *S. nigrum* seedlings in the absence or presence of Cd stress.

3.4 Effects of ZGKD5 and ZGKD2 on Cd Accumulation and Translocation from Roots to Shoots in *S. nigrum* Seedlings

The uptake and phytoextraction of heavy metals was positively related to plant biomass and heavy metal accumulation in plant tissues. ZGKD5 and ZGKD2 could enhance the growth of *S. nigrum* seedling; Cd concentration in *S. nigrum* seedling was detected. The Cd concentration of shoots and roots in uninoculated plant were 107.8 and 250.4 mg/kg, respectively. Inoculation with ZGKD5 or ZGKD2 of a density of OD_{600} of 0.04 or 0.02 increased Cd concentrations by 41.8–121.1% in shoots, and Cd concentration in roots was increased by 20.3% at the OD_{600} of 0.04 (Fig. 3a), indicating that both strains could enhance the Cd accumulation in *S. nigrum*. The Cd translocation from roots to shoots can be described by the translocation factor (TF). Figure 3b showed that TF of Cd in uninoculated *S. nigrum* plant was 0.43, while TF in plant inoculated with ZGKD5 and ZGKD2 was increased by 1.67- to

Table 1 Plant growth-promoting traits of strains ZGKD5 and ZGKD2

Bacterial strains	IAA production	Siderophore production	Ammonia production	Biosurfactant	P-solublizing	Nitrogen fixation
ZGKD5	+	+	+	+	+	+
ZGKD2	+	+	+	+	+	+

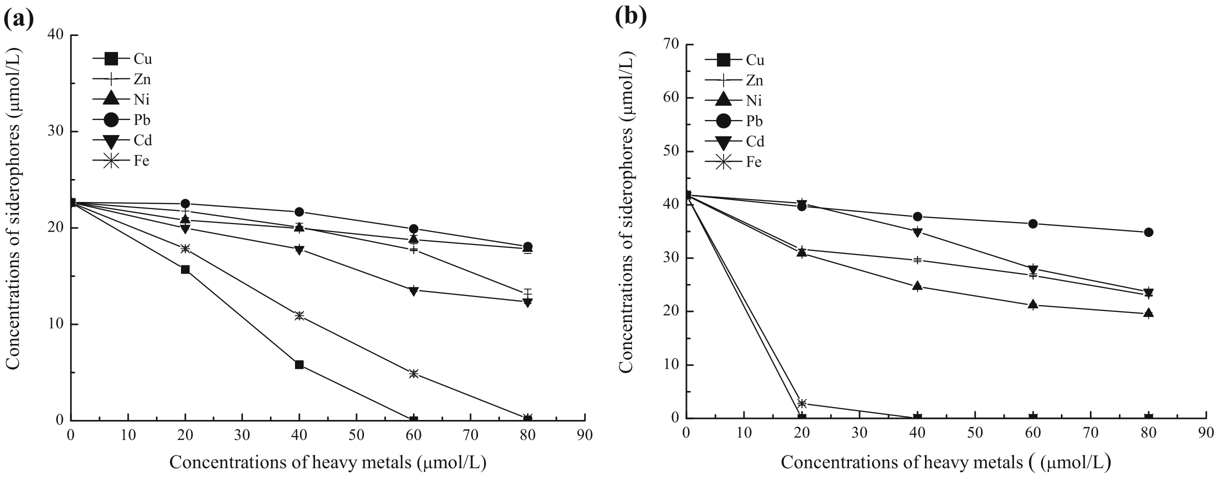

Fig. 1 The affinity of siderophores produced by strains ZGKD5 (**a**) and ZGKD2 (**b**) for heavy metals

1.59-fold and by 1.55- to 1.46-fold, respectively. The results suggested that ZGKD5 and ZGKD2 could also enhance the Cd accumulation and translocation from root to shoot in *S. nigrum* seedlings.

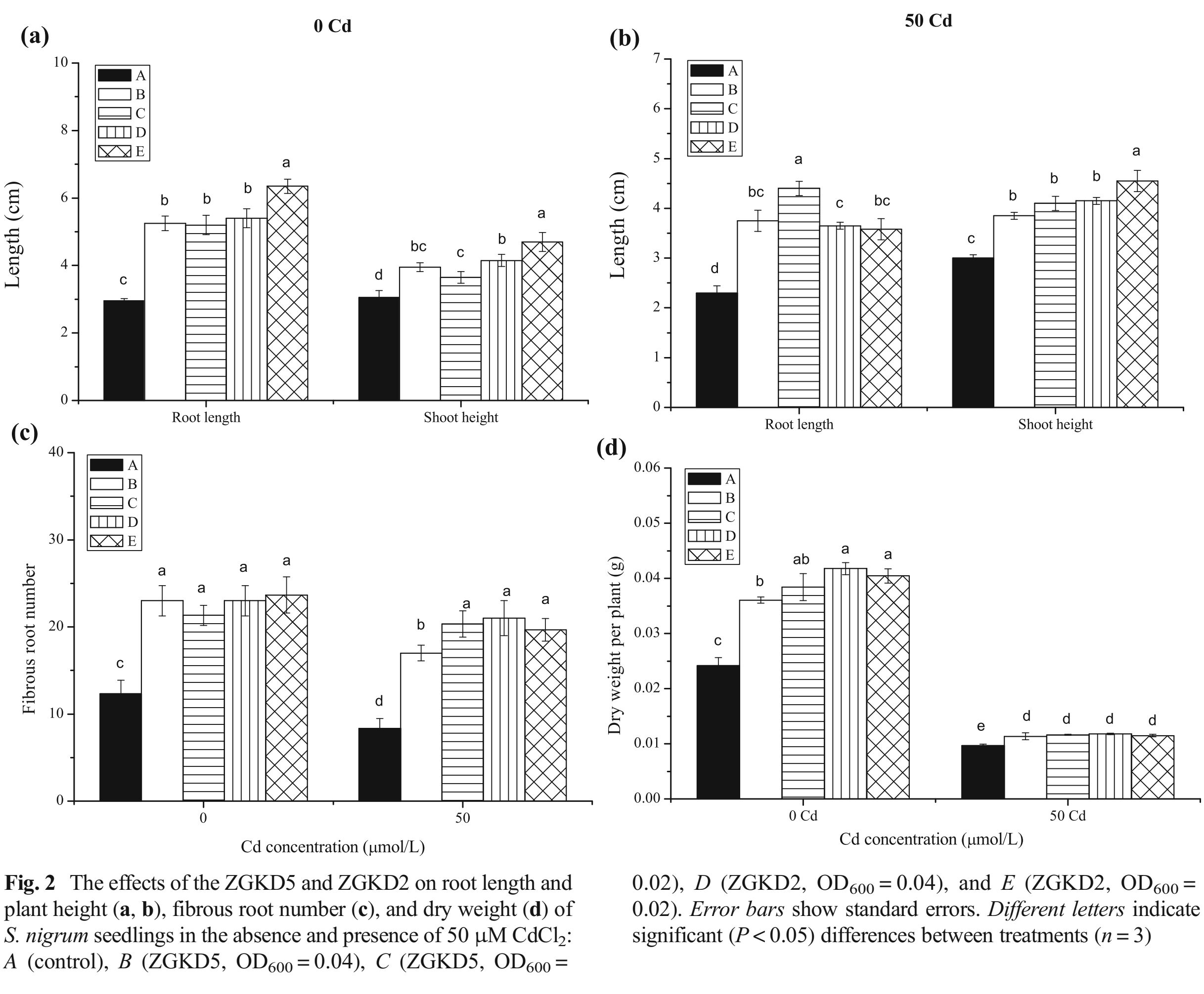

Fig. 2 The effects of the ZGKD5 and ZGKD2 on root length and plant height (**a**, **b**), fibrous root number (**c**), and dry weight (**d**) of *S. nigrum* seedlings in the absence and presence of 50 μM $CdCl_2$: *A* (control), *B* (ZGKD5, $OD_{600} = 0.04$), *C* (ZGKD5, $OD_{600} = 0.02$), *D* (ZGKD2, $OD_{600} = 0.04$), and *E* (ZGKD2, $OD_{600} = 0.02$). *Error bars* show standard errors. *Different letters* indicate significant ($P < 0.05$) differences between treatments ($n = 3$)

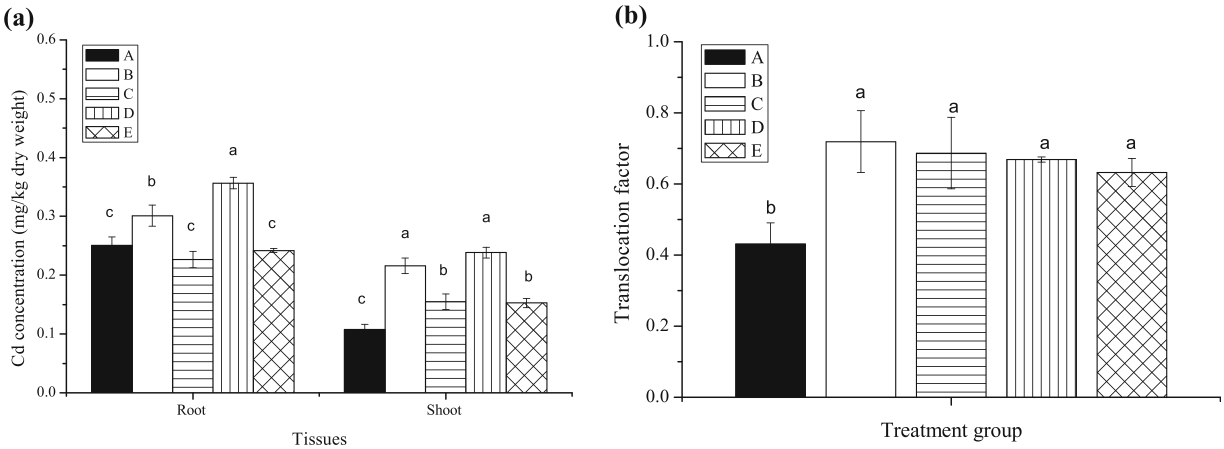

Fig. 3 The Cd concentration of shoot and root in *S. nigrum* (**a**) and translocation factor (TF) from root to shoot of *S. nigrum* (**b**) under 50 μM Cd stress: *A* (control), *B* (ZGKD5, $OD_{600} = 0.04$), *C* (ZGKD5, $OD_{600} = 0.02$), *D* (ZGKD2, $OD_{600} = 0.04$), and *E* (ZGKD2, $OD_{600} = 0.02$). *Error bars* show standard errors. *Different letters* indicate significant ($P < 0.05$) differences between treatments ($n = 3$)

3.5 Effects of ZGKD5 and ZGKD2 on the Antioxidant Response of *S. nigrum* Seedlings

Cd accumulated in plant cells induced excessive reactive oxygen species (ROS) production. Antioxidant enzymes synthesized by plant cells could alleviate the toxicity of ROS. In this study, the response of antioxidant enzymes in leaves and roots of *S. nigrum* seedling inoculated with ZGKD5 and ZGKD2 were assessed with or without Cd stress (Fig. 4). Results showed that the activities of SOD, POD, CAT, and APX in both leaves and roots of the inoculated and uninoculated plant were significantly increased under Cd treatment compared to the control plants ($P < 0.05$), indicating that Cd stress could induce the increase of antioxidant enzyme activities. In comparison with the uninoculated plant under Cd stress, the activities of SOD, POD, CAT, and APX in plants inoculated with ZGKD5 were decreased: activities of SOD, POD, and APX in shoots decreased by 15.9–17.6%, 49.1–52.9%, and 28.3–39.6%, respectively, and activities of SOD, POD, and CAT in roots decreased by 18.6–22.9%, 37.8–50.8%, and 39.5–50.8%, respectively. Similarly, the response of antioxidant enzymes in the inoculation of ZGKD2 plant was consistent with that of ZGKD5 under Cd stress (Fig. 4). The reduction of antioxidant enzyme activities in *S. nigrum* inoculated with strains in response to Cd stress might be related to the high level of Cd accumulation in plant tissues.

4 Discussion

4.1 ZGKD5 and ZGKD2 Could Improve the Growth of *S. nigrum*

Cd contamination can cause serious oxidative stress and significant reduction in plant biomass. PGPB could alleviate the oxidative stress and enhance the growth of plant through plant growth-promoting traits. When metal-accumulators (*Thlaspi caerulescens* and *Brassica juncea*) and non-accumulator plants (*Nicotiana tabacum*) were exposed to 400 μM Cd for 5 days, accumulation of hydrogen peroxide (H_2O_2) and MDA contents in leaves increased, while photosynthetic rate and plant growth decreased (Wang et al. 2008). The previous work in our lab also showed increase of H_2O_2 accumulation and reduction of plant biomass in *S. nigrum* in response to 100 μM Cd (Liu et al. 2013). Khan et al. (2015) reported that Cd caused significant cessation in plant growth and chlorophyll content in *S. nigrum*; the negative effects were partly due to inhibition of photosynthesis and competition of metals with other essential macro-elements for the uptake. This study showed that the growth of both inoculated and uninoculated plants under Cd stress was obviously inhibited, suggesting that Cd in *S. nigrum* seedling might exert toxic effects through the excessive production of ROS.

PGPB strains that produce IAA, siderophores, ammonia, and ACC deaminase and solubilize inorganic

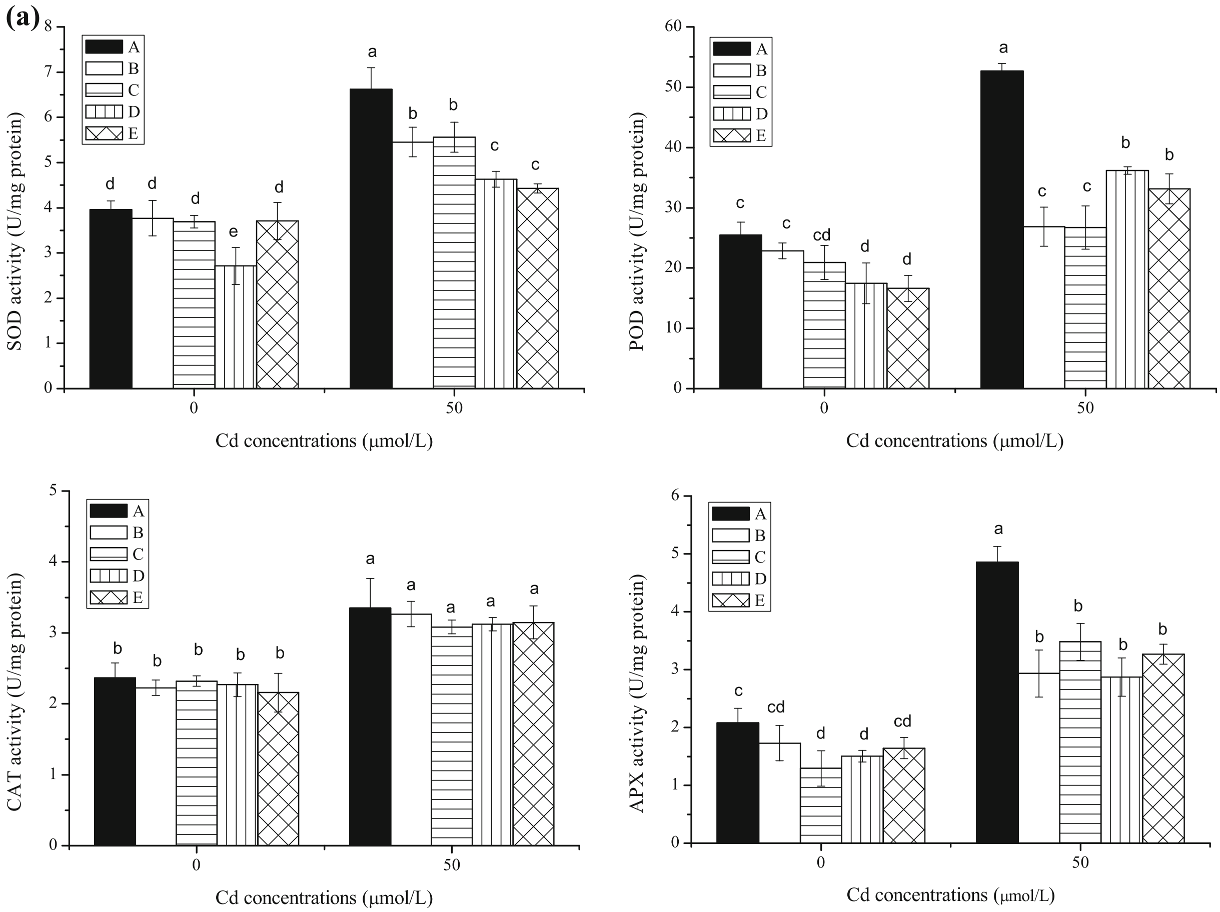

Fig. 4 Changes of SOD, POD, CAT, and APX in leaves (**a**) and roots (**b**) of *S. nigrum*: *A* (control), *B* (ZGKD5, $OD_{600} = 0.04$), *C* (ZGKD5, $OD_{600} = 0.02$), *D* (ZGKD2, $OD_{600} = 0.04$), and *E* (ZGKD2, $OD_{600} = 0.02$). *Error bars* show standard errors. *Different letters* indicate significant ($P < 0.05$) differences between treatments ($n = 3$)

phosphate, were capable of stimulating plant growth and improving responses to external stress factors (Jiang et al. 2008). IAA produced by bacteria could enhance root length and root surface, leading to more nutrients being uptaked by the roots. Siderophores have high affinity for iron and could enhance iron uptake by plants. Ammonia is taken up by plants as nitrogen source for the nitrogen containing bio-molecules (Orhan. 2016). ACC deaminase could alleviate heavy metal toxicity by reducing stress-induced ethylene production and thus facilitate the growth of plants. Phosphate solubilization and nitrogen fixation could enhance the availability of phosphorous and nitrogen in the soil for plants (Ghosh et al. 2015). Biosurfactants could enhance emulsification and dispersal water-insoluble compounds (Plaza et al. 2006). Heavy metal-tolerant *Burkholderia* sp. J62 produced IAA, siderophore, and ACC deaminase, and also solubilized inorganic phosphate. Inoculation with J62 significantly increased the biomass of maize and tomato plants in Pb- and Cd-contaminated soil (Jiang et al. 2008). Cu-tolerant *Burkholderia* sp. GL12 showed a high level of ACC deaminase activity and capacities of producing IAA and siderophores. Inoculation with GL12 significantly increased root and aboveground tissue dry weights of their host *E. splendens* and non-host *Brassica napus* plants in pot experiments (Sun et al. 2016). Pb-resistant *Acinetobacter* sp. Q2BJ2 and *Bacillus* sp. Q2BG1 isolated from *Commelina communis* plants grown on lead and zinc mine tailing had the characteristics of producing ACC deaminase, IAA, and siderophores, and effectively increased the dry weight of aboveground tissues and roots of rape in quartz sand containing 100 mg/kg Pb (Zhang et al. 2011). Our results showed that ZGKD5 and ZGKD2 had the capacity to produce IAA, siderophores, ammonia, and biosurfactants, and to fix nitrogen and solubilize inorganic phosphate. Inoculation with ZGKD5 and

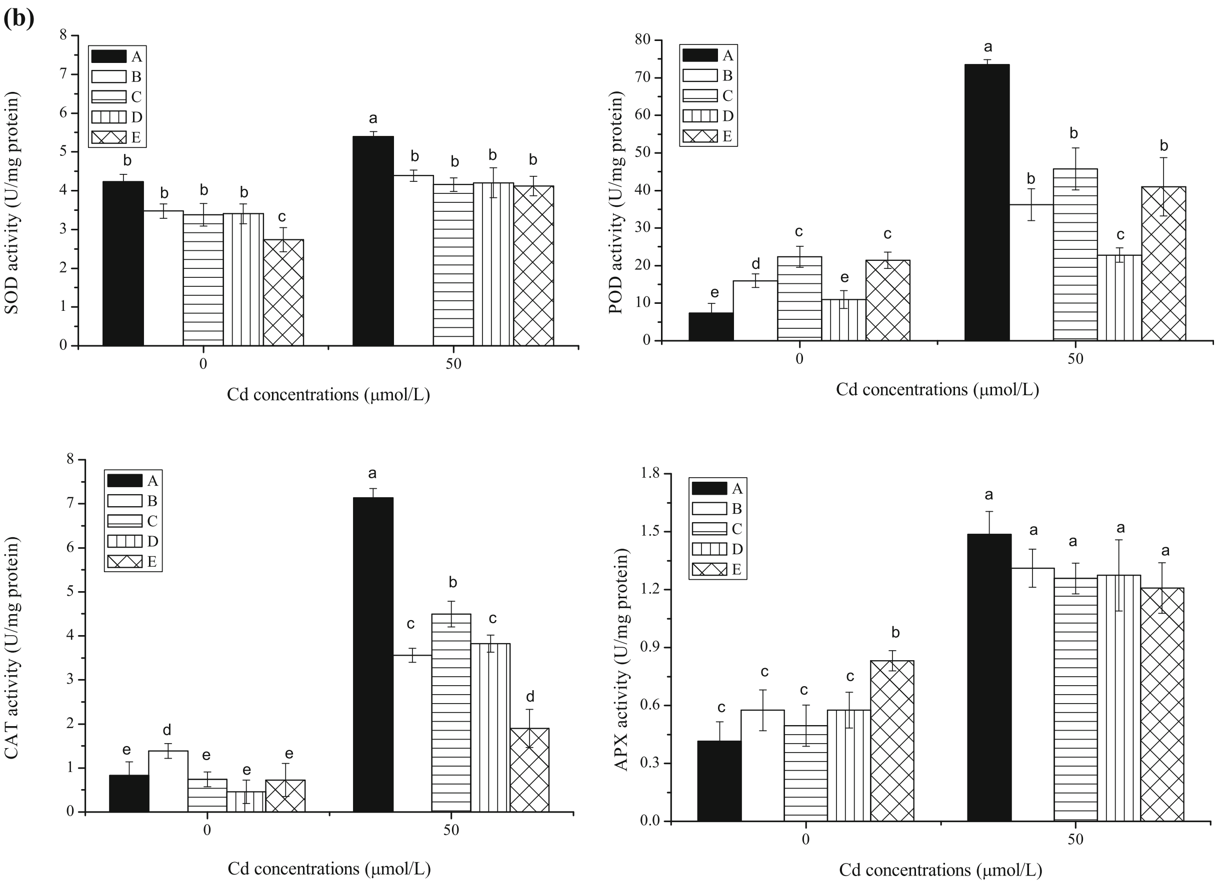

Fig. 4 continued.

ZGKD2 significantly enhanced the growth of *S. nigrum* in the presence or absence of Cd. This was confirmed by increased shoot height, root length, fibrous root number, and dry weight (Fig. 2), suggesting that inoculation of ZGKD5 and ZGKD2 helped ameliorate the metal stress and promote plant growth by plant growth-promoting properties. Therefore, both ZGKD5 and ZGKD2 are plant growth-promoting bacteria and have the potential of promoting the growth of *S. nigrum* under Cd stress. To our knowledge, this is the first report on the effect of *P. aeruginosa* on the growth and Cd accumulation of the Cd-hyperaccumulator *S. nigrum*.

4.2 Cd Accumulation and Translocation from Roots to Shoots in *S. nigrum* were Enhanced by ZGKD5 and ZGKD2

Heavy metal-tolerant and plant growth-promoting bacteria could not only improve biomass of plants, but also increase heavy metal accumulation and uptake in plants. Possible mechanisms include the improvement of biomass production and metal tolerance of plants, mobilization of heavy metals, and chelation of heavy metals by high-affinity ligands in soils. Heavy metal-resistant endophytic *Serratia nematodiphila* LRE07 isolated from Cd-hyperaccumulator *Solanum nigrum* showed plant growth-promoting properties, such as production of ACC deaminase, IAA, and siderophores and phosphate solubilizing activity; LRE07 could colonize the rhizosphere soil and facilitate the release of Cd from the non-soluble phases in the soil. Inoculation with LRE07 increased Cd concentration in *S. nigrum* tissues and aboveground biomass in moderately and highly Cd-contaminated soils, thus resulting in higher metal removal from soils (Chen et al. 2010). A multiple heavy metal resistance and plant growth-promoting endophyte *Bacillus* sp. SLS18 isolated from Mn-hyperaccumulator *Phytolacca acinosa* Roxb exhibited the capacity of producing IAA, siderophores, and ACC deaminase; inoculation with SLS18 significantly increased biomass production and Mn/Cd concentrations of sweet sorghum (*Sorghum bicolor*), suggesting that SLS18 has the potential of improving total metal uptake on heavy metal-polluted marginal land (Luo et al. 2012). Inoculation with the PGPB strain *Bacillus megaterium* JL35 significantly increased plant biomass, Cu concentration, translocation factors, and bioaccumulation factors in *E. splendens*, suggesting that JL35 has the potential in improving the efficiency of Cu phytoextraction by *E. splendens* in Cu-contaminated soil (Sun et al. 2016). Heavy metal-tolerant *Bacillus* sp. SC2b isolated from the rhizosphere of *S. plumbizincicola* showed the ability of solubilizing phosphate and producing ACC deaminase, IAA, and siderophores; inoculation of strain SC2b in a multiple metal-contaminated soil for 10 days increased concentrations of water-extractable Cd, Pb, and Zn. Furthermore, plant inoculated with SC2b markedly enhanced the dry weight and Cd and Zn accumulation in shoots and roots of *S. plumbizincicola*, indicating that SC2b can enhance Cd and Zn uptake by *S. plumbizincicola* through metal mobilization or plant-microbial mediated changes in chemical or biological soil properties (Ma et al. 2015). When siderophore-producing bacteria *Ralstonia metallidurans* and *P. aeruginosa* were inoculated in an agricultural soil containing Cr and Pb, only Cr accumulation in shoots and translocation in maize increased with *R. metallidurans*, while Cr and Pb accumulation in maize shoots increased with *P. aeruginosa* (Braud et al. 2009). Nickel-resistant *Streptomyces acidiscabies* E13 could produce siderophore; *Streptomyces*-derived cell-free supernatant containing siderophores and auxins could enhance the plant growth and amounts of metals in cowpea in soils containing elevated levels of metals, including Al, Cu, Fe, Mn, Ni, and U, suggesting that siderophores can lower the formation of free radicals, thereby protecting microbial auxins from degradation and enabling them to enhance plant growth which in turn resulted in augmented metal uptake (Dimkpa et al. 2009). In this study, both ZGKD5 and ZGKD2 could produce siderophores. Cd accumulation in inoculated plants was higher than that in uninoculated plant. In addition, siderophores produced by ZGKD2 had stronger affinity for Cd than that by ZGKD5. *S. nigrum* inoculated with ZGKD2 had higher Cd accumulation than that with ZGKD5, indicating that siderophores produced by ZGKD5 and ZGKD2 can chelate Cd and enhance Cd uptake in *S. nigrum*. These data suggested that ZGKD5 and ZGKD2 not only stimulated plant growth, but also enhanced Cd uptake and accumulation in *S. nigrum*.

TF is an important parameter for assessing the ability of a phytoremediator for the uptake of metals from soil and translocation of absorbed metals from roots to shoots. In this study, higher TF was observed in *S. nigrum* seedling inoculated with *P. aeruginosa* compared to the uninoculated seedling under Cd stress (Fig. 3), indicating both strains, ZGKD5 and ZGKD2, play an important role in Cd translocation from roots to shoots in *S. nigrum*. Zhu et al. (2015) also reported that the inoculation of *Phyllobacterium* sp. C65 enhanced Zn translocation in *Populus euphratica*. Enhancement of Cd translocation from roots to shoots might contribute to the reduction of Cd accumulation in roots and thus alleviate inhibition of root growth, allowing more Cd from the rhizosphere to be extracted by *S. nigrum*.

4.3 Antioxidant Enzyme Activities of *S. nigrum* Inoculated with ZGKD5 and ZGKD2 were Enhanced by Cd Stress

Oxidative stress caused by Cd stress was due to the excessive production of ROS molecules such as H_2O_2 and superoxide anion ($O_2^{\bullet -}$) (Pandey et al. 2012). Antioxidant enzymes including SOD, POD, CAT, and APX in plants could effectively scavenge overproduced ROS in cells and thus alleviate the toxic effects of unbalanced nutritional status, lipid peroxidation, and membrane damage (Anjum et al. 2016). Treatment with 100 μM Cd led to H_2O_2 accumulation and notable cell death in the primary root tips of *S. nigrum* at 24 h, while antioxidant activities of SOD, POD, CAT, and APX in seedlings were enhanced considerably to scavenge excess ROS induced by Cd stress (Liu et al. 2013). Exposure to 50 μM Cd significantly increased production of H_2O_2 and $O_2^{\bullet -}$in the roots of *S. nigrum*; meanwhile, activities of CAT, APX, SOD, and POD increased to protect plants from oxidative stress damage (Deng et al. 2010). In the present study, activities of CAT, APX, SOD, and POD increased in both leaves and roots in response to 50 μM Cd in comparison with control plants, indicating that the increase of antioxidant enzyme activity might be one of the mechanisms of Cd detoxification in *S. nigrum*. Moreover, the antioxidant enzyme activities in inoculated plants exposed to Cd were lower than that in uninoculated Cd treatment plants, but were still higher than that in the control group, suggesting that antioxidant enzymes in inoculated plant play important roles in resistance to Cd stress. The Cd concentration in inoculated plants was higher than that in uninoculated plants; the reduction of antioxidative enzyme activities might be attributed to the high level of Cd accumulation in inoculated plants. Cd accumulation caused the excessive accumulation of ROS, denaturing of proteins, and inactivation of enzymes, leading to decreasing of metabolism. The reduction of antioxidative enzyme activity in the inoculated plant might be one of the reasons for severe oxidative damage induced by Cd stress. Inoculation with ZGKD5 and ZGKD2 could enhance the Cd accumulation and translocation of *S. nigrum* seedling, whereas the Cd accumulation in seedling could induce serious damage to plants in response to prolonged Cd stress. Therefore, *P. aeruginosa* ZGKD5 and ZGKD2 have the potential to improve the efficiency of Cd phytoextraction by *S. nigrum* in farmland soil contaminated with low levels of Cd.

5 Conclusion

Cd-tolerant strains ZGKD5 and ZGKD2 showed many plant growth-promoting features such as production of IAA, siderophores, ammonia, and biosurfactant, and phosphate solubilization and nitrogen fixation activity. Inoculation of strains ZGKD5 or ZGKD2 could enhance plant growth, Cd accumulation, and translocation of Cd from roots to shoots in *S. nigrum*. Therefore, *S. nigrum* combined with ZGKD5 or ZGKD2 might have the potential to improve Cd phytoextraction efficiency in farmland soils contaminated with low levels of Cd.

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