



Isolation and characterization of a heavy metal-resistant *Burkholderia* sp. from heavy metal-contaminated paddy field soil and its potential in promoting plant growth and heavy metal accumulation in metal-polluted soil

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Abstract

A heavy metal-resistant bacterial strain was isolated from heavy metal-contaminated soils and identified as *Burkholderia* sp. J62 based on the 16S rDNA gene sequence analysis. The heavy metal- and antibiotic resistance, heavy metal solubilization of the isolate were investigated. The isolate was also evaluated for promoting plant growth and Pb and Cd uptakes of the plants from heavy metal-contaminated soils in pot experiments. The isolate was found to exhibit different multiple heavy metal and antibiotic resistance characteristics. Atomic absorption spectrometer analysis showed increased bacterial solubilization of lead and cadmium in solution culture and in soils. The isolate produced indole acetic acid, siderophore and 1-aminocyclopropane-1-carboxylate deaminase. The isolate also solubilized inorganic phosphate. Inoculation with the isolate was found to significantly ($p < 0.05$) increase the biomass of maize and tomato plants. Increase in tissue Pb and Cd contents varied from 38% to 192% and from 5% to 191% in inoculated plants growing in heavy metal-contaminated soils compared to the uninoculated control, respectively. These results show that heavy metal-solubilizing and plant growth promoting bacteria are important for plant growth and heavy metal uptake which may provide a new microbial enhanced-phytoremediation of metal-polluted soils.

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Keywords: *Burkholderia* sp. J62; Heavy metals; Plant growth promoting rhizobacteria; Bioavailability; Phytoextraction

1. Introduction

Pollution of soils with heavy metals is becoming one of the most severe environmental and human health hazards. Elevated levels of heavy metals not only decrease soil microbial activity and crop production, but also threaten human health through the food chain (McLaughlin et al., 1999). Phytoextraction is emerging as a potential cost effective solution for the remediation of heavy metal-contaminated soils in opposition to the conventional chemical and physical remediation technologies that are generally

too costly and often harmful to soil characteristics (Blaylock et al., 1997; Quartacci et al., 2006). In recent years, some plant species (identified as hyperaccumulators) growing in heavy metal-contaminated sites have been found with the ability to accumulate unusually high concentrations of heavy metals without impacting on their growth and development (Baker and Brooks, 1989). However, most hyperaccumulators identified so far are not suitable for field phytoremediation applications due to their small biomass and slow growth (Shen and Liu, 1998). In addition, a large proportion of many metals is adsorbed or occluded by carbonates, organic matters, Fe–Mn oxides and primary or secondary minerals (Garbisu and Alkorta, 2001). Low bioavailability of heavy metals in soils may also

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limit the efficiency of phytoremediation (Kayser et al., 2000; Chen et al., 2004; Sheng and Xia, 2006).

Soil microorganisms can affect trace metal mobility and availability to the plants (Abou-Shanab et al., 2003; Idris et al., 2004). For example, the presence of rhizosphere (defined as the volume of soil adjacent to and influenced by the plant root) (Smalla et al., 2001) bacteria increased the uptake of Cd in *Brassica napus* (Sheng and Xia, 2006) and Ni in *Alyssum murale* (Abou-Shanab et al., 2006). Arbuscular mycorrhizal (AM) fungi are ubiquitous symbiotic associations found in both natural and heavy metal-contaminated sites (Wang et al., 2007). AM fungi may stimulate phytoextraction by essentially improving plant growth and increasing the total metal uptake (Wang et al., 2007). Mycorrhizal fungi associated with these plants play a great role in the establishment of these plants on the contaminated soils. Therefore, the application of heavy metal-solubilizing microorganisms is a promising approach for increasing heavy metal bioavailability in heavy metal amended soils. In addition, bacteria producing indole acetic acid, siderophores and l-aminocyclopropane-l-carboxylate deaminase and phosphate-solubilizing bacteria are capable of stimulating plant growth (Glick et al., 1995; Chabot et al., 1996; Rajkumar et al., 2006). Plant growth promoting bacteria (PGPB) could improve plant competitiveness and responses to external stress factors (Egamberdiyeva and Hflich, 2004). However, little is known about the potential of heavy metal-resistant and heavy metal-solubilizing bacteria on the phytoextraction of Pb and Cd from heavy metal-contaminated soils. The objectives of this study were to isolate and characterize heavy metal-resistant and heavy metal-solubilizing bacteria from heavy metal-contaminated soils, and to select PGPB strains which might be useful to increase plant biomass production and Pb and Cd uptakes by plants under unfavorable environmental conditions for improving the efficiency of phytoextraction of Pb- and Cd-polluted soils.

2. Materials and methods

2.1. Isolation of heavy metal-resistant bacteria

The non-rhizospheric soil samples were collected from a heavy metal-polluted paddy field in Zhejiang, China. The basic properties and the heavy metal contents of the soil samples were pH (1:2 w/v water) 7.85; organic matter, 32.4 g kg⁻¹; total N, 3.2 g kg⁻¹; cation exchange capacity (CEC), 13.1 cmol kg⁻¹; total Pb, 245 mg kg⁻¹; total Cd, 2.8 mg kg⁻¹. Organic matter content, total N and CEC were determined following the methods described in the Physical Chemical Analysis of soils (SSICA, 1980). Soil total Pb and Cd were extracted with HF–HClO₄ (SSICA, 1980). The above Pb and Cd concentrations in the extracts were determined with atomic absorption spectrometer (AAS) (TAS-986, Beijing, China). The medium for isolating heavy metal-resistant bacteria was the sucrose-minimal salts low-phosphate (SLP) medium (sucrose, 1%; (NH₄)₂SO₄, 0.1%;

K₂HPO₄, 0.05%; MgSO₄, 0.05%; NaCl, 0.01%; yeast extract, 0.05%; pH 7.2) supplemented with 50 mg l⁻¹ of Cd as 3CdSO₄ · 8H₂O. To prevent the growth of soil fungi, the media were supplemented with 10 mg l⁻¹ fungicidin (USP, Amresco, USA) after autoclaving. Cd-resistant bacteria were isolated according to the method described by Sheng and Xia (2006). Among the 21 isolates selected, seven (J1, J2, J5, J6, J41, J62 and J80) could be resistant to 200 mg l⁻¹ of Cd and stored on slants for studying the solubilization of poorly soluble heavy metals.

2.2. Solubilization of heavy metals

Inocula of the heavy metal-resistant bacterial isolates were prepared by using 20 h logarithmic phase cells. The composition of the incubation medium was the same as the above selection medium supplemented with 500 mg l⁻¹ of Pb as PbCO₃ or Cd as CdCO₃. Triplicate test tubes (18 mm × 180 mm) containing 10 ml of sterile medium were inoculated with 0.1 ml (10⁸ cells ml⁻¹) of the inoculum. Uninoculated Pb- or Cd-amended medium or inoculated Pb- or Cd-free medium was made as the controls to determine the abiotic influences on Pb and Cd solubility. The test tubes were incubated at 28 °C on the rotary shaker at 150 rpm. After 0, 12, 22, 36, 48, 72 and 96 h, the sizes of the viable bacterial populations in the test tubes were estimated by plate counts. One half of the spent culture was filtered through a 0.22-μm Millipore filter for pH determinations. The other half of the spent culture was used for water-soluble Pb and Cd determination. The harvested spent culture medium was centrifuged at 925 g for 20 min at 6 °C and filtered through a 0.22-μm Millipore filter. The Pb and Cd concentrations in the supernatant were determined with AAS (TAS-986, Beijing, China).

2.3. Identification of the J62 bacterium

J62 was finally selected as the most active strain for the experiments of the plant growth promotion and bacterial-enhanced phytoextraction based on the relative ability of the heavy metal solubilization of the tested strains (Table 1). For the 16S rDNA analysis, genomic DNA was

Table 1
Effects of the isolates on water-soluble Pb and Cd (μg l⁻¹) and pH in the solutions

Isolates	Pb	Cd	pH	
			Pb	Cd
Uninoculation (CK)	156 ± 19	2670 ± 169	7.44	7.23
J1	207 ± 28*	2013 ± 204	7.66	7.44
J2	158 ± 20	2113 ± 233	7.88	7.53
J5	289 ± 12*	2965 ± 81*	7.43	7.31
J6	1348 ± 96*	4977 ± 344*	6.54	6.78
J41	3626 ± 87*	15917 ± 1546*	5.62	5.34
J62	6483 ± 410*	25300 ± 832*	3.33	4.42
J80	4513 ± 260*	21050 ± 1308*	4.48	4.87

An asterisk (*) denotes a value significantly greater than the control value ($p < 0.05$).

extracted and 16S rDNA was amplified in polymerase chain reaction (PCR) using the genomic DNA as template and bacterial universal primers, 27 f (5'-GAGTTTGA-TCACTGGCTCAG-3') and 1492 r (5'-TACGGCTACC-TTGTTACGACTT-3') (Byers et al., 1998). The PCR mixture (25 μ l) contained 1 μ l template, 2.5 μ l of 10 \times Taq DNA polymerase buffer, 5 mM MgCl₂, 1 μ l of dNTP at 2.5 mM, 0.5 μ l of 2.5 unite Taq DNA polymerase, 3.75 pmol primers (each), and 0.5 μ l of 2.5 unite Taq polymerase. The PCR was performed in a DNA Engine Thermal Cycler (PTC-200, BIO-RAD, USA) with a hot starting performed at 94 °C for 3 min, followed by 30 cycles of 94 °C for 0.5 min, 54 °C for 0.5 min, and 72 °C for 1.5 min, followed by a final extension performed at 72 °C for 5 min. The amplification products were purified using a DNA purification kit (Beyotime, China) and sequencing was performed at Nanjing Boya Biotechnology Company, Limited (Nanjing, China). The 16S rDNA sequence was compared against the GenBank database using the NCBI Blast program (Altschul et al., 1997). The 16S rDNA sequence of strain J62 has been deposited in GenBank under accession No. EF555575.

2.4. Minimum inhibitory concentration (MIC) of heavy metals and antibiotic resistance of the isolate

The MIC of the metals (Pb, Cd, Cu, Ni, Zn and Cr) for strain J62 was determined by the plate dilution method as adopted by Summers and Silver (1972) and Aleem et al. (2003). The lowest concentration that prevented bacterial growth was considered the MIC. Antibiotic resistance was tested using SLP agar containing kanamycin (100 μ g ml⁻¹), streptomycin (200 μ g ml⁻¹), ampicillin (500 μ g ml⁻¹), tetracycline (50 μ g ml⁻¹), or rifampicin (50 μ g ml⁻¹), which were added aseptically to the medium after autoclaving. The SLP agar plates without antibiotics were used as controls. The experiments were carried out in triplicate. Cultures were incubated at 30 °C for 7 d.

2.5. Plant growth promoting characteristics of the isolate

The bacterial strain J62 was cultured for 4 d in flasks containing 20 ml of sucrose-minimal salts (SMS) medium (sucrose, 1%; (NH₄)₂SO₄, 0.1%; K₂HPO₄, 0.2%; MgSO₄, 0.05%; NaCl, 0.01%; yeast extract, 0.05%; CaCO₃, 0.05%; pH 7.2) supplemented with 0.5 mg ml⁻¹ of tryptophane. After incubation, a 1 ml cell suspension was transferred into a tube and mixed vigorously with 2 ml of Salkowski's reagent (Gordon and Weber, 1951) and allowed to stand at room temperature for 20 min, after which time a pink color developed in the cell suspensions. The absorbance of pink color developed after 25 min incubation was read at 530 nm. The indole acetic acid (IAA) concentration in the culture was determined using a calibration curve of pure IAA as a standard following the linear regression analysis.

Siderophores secreted to the growth medium by strain J62 were detected and quantified according to the method

described by Schwyn and Neilands (1987). The siderophore 2,3-dihydroxybenzoic acid was used to produce a standard curve from 0.5 to 25 mg l⁻¹. The incubation for the standard was 24 h at room temperature (Burd et al., 2000).

The 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity of cell-free extracts was determined by estimating the amount of α -ketobutyrate (α -KB) generated by the enzymatic hydrolysis of ACC (Saleh and Glick, 2001) according to the procedure of Honma and Shimomura (1978).

The phosphate solubilization ability of strain J62 was determined in Pikovskays's medium (Zaidi et al., 2006) with 0.5% of tricalcium phosphate. Triplicate 50-ml Erlenmeyer flasks containing 20 ml medium were inoculated with 0.2 ml (3 \times 10⁸ cfu ml⁻¹) of the bacterial suspension. Dead bacterial (autoclaved at 121 °C for 30 min) inoculated media were made as the controls to determine the abiotic influences on phosphate solubility. The flasks were incubated at 28 °C for 3 d on the rotary shaker at 150 rpm. The culture supernatants were collected by centrifugation at 12000 rpm for 10 min. Soluble phosphate in the culture supernatant was estimated by Mo-blue method (Watanabe and Olsen, 1965).

2.6. Pot experiment

The experiment for studying the effect of strain J62 on plant growth and heavy metal uptake of plants was conducted in pots (10 cm diameter) containing 300 g of heavy metal-contaminated yellow brown soil (Alfisols) from Nanjing (China). The basic properties of the soil sample were organic matter, 22.4 g kg⁻¹; CEC, 16.8 cmol kg⁻¹; total N, 1.58 g kg⁻¹; total P, 1.04 g kg⁻¹; available P 5.68 mg kg⁻¹; pH 6.71; total bacterial count (dilution-plate method), 3.6 \times 10⁷ cfu g⁻¹; heavy metal-resistant bacterial count (dilution-plate method) 1.5 \times 10³ cfu g⁻¹; total Pb, 150.1 mg kg⁻¹, total Cd, 37.3 mg kg⁻¹. The soil was thoroughly mixed with fertilizers before adding to the pots. Each kilogram of the potted soil received 0.44 g urea and 0.88 g KH₂PO₄. Seeds of *Brassica juncea* L. Czern, *Zea mays* L. variety Denhai-11 and *Lycopersicon esculentum* variety Qin-hong-3 were surface-sterilized with a mixture of ethanol and 30% H₂O₂ (1:1) for 10 min and washed with sterile water. Three pots were used for each treatment. Five surface-sterilized seeds were placed in each pot at a 1.0 cm depth. After germination, plants were thinned to two plants per pot.

For inoculation, strain J62 was grown in SMS medium. Cells in the exponential phase were collected by centrifugation at 12,000 rpm for 10 min, washed with sterile distilled water, and centrifuged. Bacterial inoculum was prepared by resuspending pelleted cells in sterile distilled water to get an inoculum density of ca. 10⁸ cfu ml⁻¹. Bacterial suspensions (10 ml pot⁻¹) were sprayed on the soil surface four weeks after seedling emergence. Non-inoculation was made as a control. Pots were placed outdoors and were moved to indoors in order to protect them from the rainfall. During cultivation, minimal and maximal temperature ranged

from 13 to 17 °C and from 22 to 32 °C, respectively. The soil was moistened with water and maintained at 60% of its holding capacity. The plants were harvested two weeks after the inoculation treatments. Plants were removed from their pots and the soil was removed from the roots. Shoots and roots were separated and washed extensively, first in several changes of 0.01 M EDTA and then in distilled water to remove any nonspecifically bound Pb and Cd and were dried at 105 °C before determining the shoot and root dry weight. The oven-dried samples (shoot and root) were ground using a stainless steel mill (FZ102, Tianjing, China) to 0.5 mm for analysis. Then the subsamples of ground shoot samples (200 mg) and root samples (100 mg) were digested in a mixture of concentrated HNO₃ and HClO₄ (4:1, v/v) (Chen et al., 2004). The volume of each sample was adjusted to 10 ml using double deionized water. The contents of Pb and Cd in the samples were determined by AAS (TAS-986, Beijing, China). Reagent blank and analytical duplicates were used where appropriate to ensure accuracy and precision in the analysis. The NH₄OAc-extractable Pb and Cd concentrations in rhizosphere soils of the plants were determined by AAS.

2.7. Statistical analysis

Analysis of variance and the Student–Newman–Keuls test ($p < 0.05$) were used to compare treatment means. All the statistical analyses were carried out using SPSS 10.0.

3. Results

3.1. Isolation and identification of heavy metal-resistant bacteria

We have isolated metal-resistant bacterial isolates from heavy metal-contaminated field soils by using a spread

plate procedure with pH-neutral SLP medium. This low-phosphate medium is designed to avoid the precipitation of heavy metal salts at 50 mg l⁻¹. The bacterial strain J62 was the best at Pb and Cd solubilizing in solution culture and was identified as *Burkholderia* sp. J62 based on the 16 S rDNA gene sequence analysis.

3.2. Bacterial heavy metal mobilization

The time courses of growth of *Burkholderia* sp. J62, Pb and Cd release, pH, under PbCO₃ and CdCO₃ condition are shown in Fig. 1. There was no significant change in the water-soluble Pb and Cd concentrations and in the pH value of the medium with the uninoculated control. By contrast, the concentrations of water-soluble Pb and Cd were significantly ($p < 0.05$) increased after 48 h from the inoculation with the strain J62. This was associated with a significant pH decrease. The peak values of water-soluble Pb and Cd in solution with strain J62 were 5.3 and 25.8 mg l⁻¹, respectively.

3.3. Plant growth promoting characteristics of the strain

The bacterial strain J62 had the capacity to produce IAA, siderophore and ACC deaminase and could solubilize inorganic phosphate. In the SMS medium supplemented with L-tryptophane, strain J62 produced $3.8 \pm 0.3 \mu\text{g ml}^{-1}$ of IAA and $46 \mu\text{g ml}^{-1}$ of 2,3-dihydroxybenzoic acid. Strain J62 utilized ACC as the sole nitrogen source and showed relatively high levels of ACC deaminase activity ($25.6 \pm 4 \mu\text{M } \alpha \text{ KB mg}^{-1} \text{ h}^{-1}$). In addition, significant increase ($p < 0.05$) in the soluble phosphate ($234 \pm 44 \mu\text{g ml}^{-1}$) released in the medium by strain J62 was observed compared to the control ($32.4 \pm 2 \mu\text{g ml}^{-1}$).

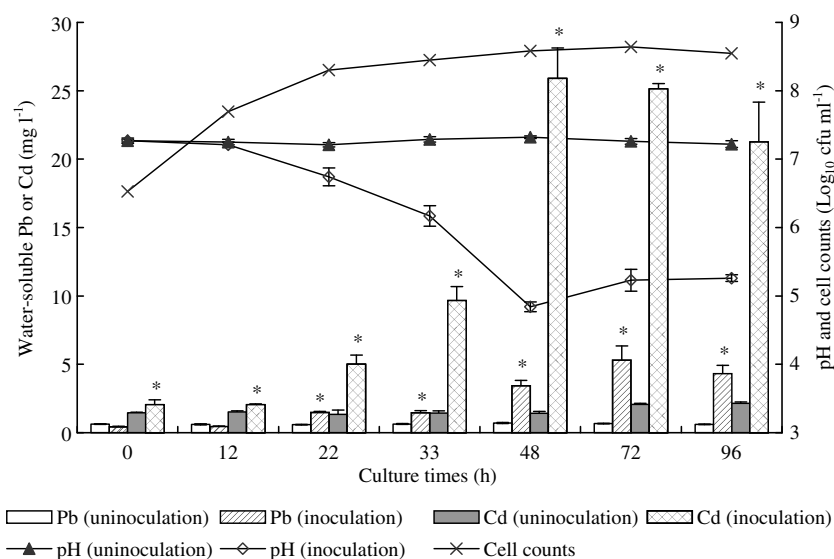


Fig. 1. Time courses of water-soluble Pb and Cd content, cell counts and pH under cadmium and lead carbonate condition. Error bars are \pm standard deviation ($n = 3$). An asterisk (*) denotes a value significantly greater than the corresponding control value ($p < 0.05$).

3.4. Heavy metal and antibiotic resistance of strain J62

The bacterial strain J62 showed a very high degree of resistance to heavy metals, especially to Pb and Cd. Strain J62 grew well in SLP medium containing Pb (1000 mg l^{-1}), Cd (2000 mg l^{-1}), Cu (100 mg l^{-1}), Zn (400 mg l^{-1}), Ni (50 mg l^{-1}) and Cr (20 mg l^{-1}), respectively. The order of the toxicity of the metals to strain J62 was found to be $\text{Cr} > \text{Ni} > \text{Cu} > \text{Zn} > \text{Pb} > \text{Cd}$. Strain J62 also exhibited antibiotic resistance characteristics to kanamycin ($100 \text{ } \mu\text{g ml}^{-1}$), streptomycin ($200 \text{ } \mu\text{g ml}^{-1}$), ampicillin ($500 \text{ } \mu\text{g ml}^{-1}$), tetracycline ($50 \text{ } \mu\text{g ml}^{-1}$), and rifampicin ($50 \text{ } \mu\text{g ml}^{-1}$), respectively.

3.5. Plant growth promotion

Significant increases ($p < 0.05$) of root dry weight of maize plant and shoot dry weight of maize and tomato plants were observed when the soil was inoculated with strain J62, compared to the uninoculated soil (Fig. 2). Root dry weight of maize plants was increased 75% ($p < 0.05$) and shoot dry weights of maize and tomato plants were increased from 30% to 54% ($p < 0.05$). There was no significant difference in shoot and root dry weights of Indian mustard plant grown in inoculated and uninoculated soils (Fig. 2). In addition, shoot dry weight of tomato plants grown in inoculated soil was increased by 82% and 194%, compared to maize and Indian mustard plants, respectively.

3.6. Mobilization of Pb and Cd to plants

Based on the bacterial heavy metal mobilization, one heavy metal-resistant strain *Burkholderia* sp. J62 was selected for the mobilization of Pb and Cd to plants. There was no significant difference in shoot Pb and Cd concentra-

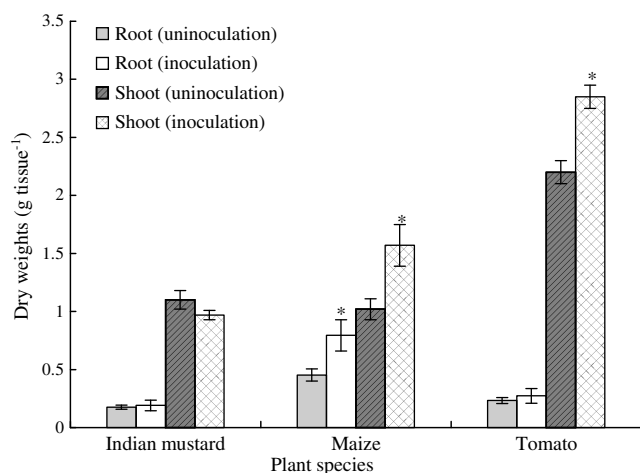


Fig. 2. The dry weights (g tissue^{-1}) of three plants with uninoculated and inoculated treatments. Error bars are \pm standard deviation ($n = 3$). An asterisk (*) denotes a value significantly greater than the corresponding control value ($p < 0.05$).

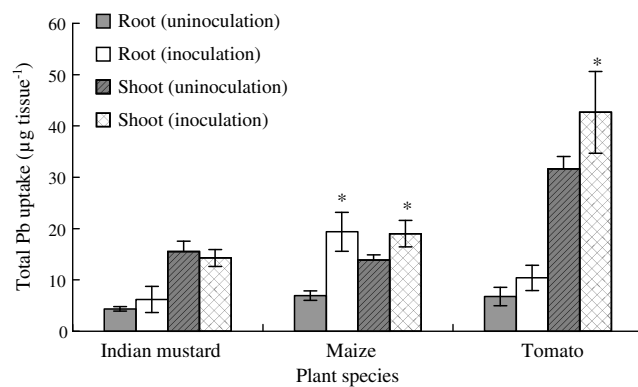


Fig. 3. The total Pb uptake ($\mu\text{g tissue}^{-1}$) by plants with uninoculated and inoculated treatments. Error bars are \pm standard deviation ($n = 3$). An asterisk (*) denotes a value significantly greater than the corresponding control value ($p < 0.05$).

tions of the Indian mustard and tomato plants grown in bacterial inoculated and uninoculated soils (data not shown). There was significant decrease ($p < 0.05$) in the shoot Cd concentration ($28 \pm 0.9 \text{ mg kg}^{-1}$) of the inoculated maize plants, compared to the shoot Cd concentration ($41 \pm 2.9 \text{ mg kg}^{-1}$) of the uninoculated maize plants. By contrast, significant increases ($p < 0.05$) in root Pb and Cd concentrations of the Indian mustard and maize plants were obtained in inoculated soil, compared to the uninoculated soil. For Indian mustard and maize plants growing in soil inoculated with J62, root Pb and Cd concentrations were increased from 28% to 67% and from 31% to 170% compared to the uninoculated control, respectively. Significant increases of the total shoot Pb and Cd uptakes of tomato were observed when the soil was inoculated with *Burkholderia* sp. J62, compared to the uninoculated soil (Figs. 3 and 4). In maize plant, significant increases of the total root Pb and Cd uptakes and the total shoot Pb uptake were obtained in inoculated soil

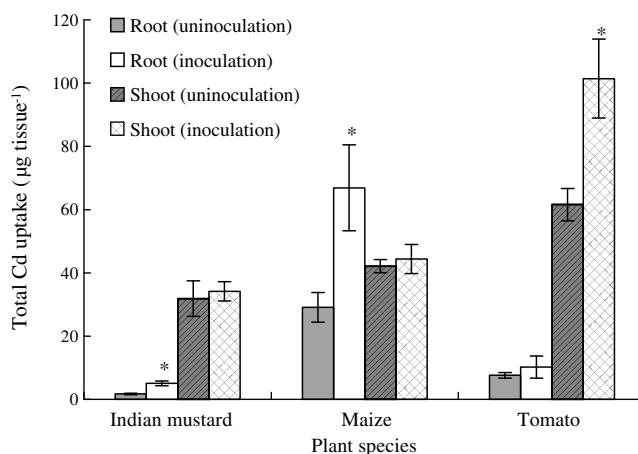


Fig. 4. The total Cd uptake ($\mu\text{g tissue}^{-1}$) by plants with uninoculated and inoculated treatments. Error bars are \pm standard deviation ($n = 3$). An asterisk (*) denotes a value significantly greater than the corresponding control value ($p < 0.05$).

Table 2
Influence of strain J62 on NH_4OAc -extractable Pb and Cd concentrations ($\mu\text{g kg}^{-1}$) in the rhizosphere soils

Plants	Pb		Cd	
	Inoculation	Uninoculation	Inoculation	Uninoculation
Indian Mustard	1140 ± 260	1030 ± 50	4070 ± 440	4060 ± 120
Maize	1210 ± 110*	980 ± 50	3790 ± 160*	3290 ± 160
Tomato	580 ± 90*	270 ± 60	5500 ± 400*	4620 ± 470

Average ± standard deviation from three separate experiments. An asterisk (*) denotes a value significantly greater than the corresponding control value ($p < 0.05$).

compared to the uninoculated soil. For tomato and maize plants growing in soil inoculated with J62, total root and shoot Pb uptakes were increased from 58% to 192% and from 39% to 42% compared to the uninoculated control, respectively, and the total root and shoot Cd uptakes were increased from 31% to 130% and from 5% to 25% compared to the uninoculated control, respectively. Total Pb and Cd uptakes in shoots of the inoculated tomato plants were increased from 127% to 194% and from 127% to 194% compared to the inoculated maize and Indian mustard plants, respectively. However, there were no significant difference in the total shoot Pb and Cd uptakes of Indian mustard between inoculated and uninoculated soils (Figs. 3 and 4). In the inoculated soil, NH_4OAc -extractable Pb and Cd concentrations in rhizosphere soils of maize and tomato plants were significantly increased compared to the uninoculated control. There was no significant difference in NH_4OAc -extractable Pb and Cd concentrations in bacterial inoculated and uninoculated rhizosphere soils of the Indian mustard plants (Table 2).

4. Discussion

Even metals exert their toxic effects on microorganisms through various mechanisms, and metal-tolerant bacteria could survive in these habitats and could be isolated and selected for their potential application in the bioremediation of contaminated sites (Piotrowska-Seget et al., 2005). In the present study, we have isolated twenty one bacterial isolates which could be resistant to Cd. Based on the relative ability of the Pb and Cd solubilization of the tested strains (Table 1), bacterial strain J62 was selected as the most active strain for the experiments.

Effective phytoextraction depends mainly on the plant itself and the interaction of plant roots with bacteria and on the bioavailability of heavy metals in the soils. Although the accumulation of metals by plants can be enhanced by chemical chelates (do Nascimento et al., 2006), these expensive compounds can increase the metal leaching risk and negative effects on soil fertility or soil structure (Blaylock et al., 1997; Khan et al., 2000; Kos and Lestan, 2003). The study showed that the insoluble Pb and Cd were gradually solubilized during the growth of strain J62 as shown

in Fig. 1, indicating that strain J62 has the potential of Pb- and Cd-resistance and Pb and Cd solubilization ability. Significant increases ($p < 0.05$) in water-soluble Pb and Cd in solution with strain J62 were obtained compared to the control. The high quantities of biomass produced by strain J62 were accompanied with high levels of soluble-Pb and Cd in the medium. Inverse relationship between pH and water-soluble Pb and Cd concentrations was observed in the bacterial Pb and Cd solubilization experiment.

Phytoremediation could clean up the heavy metal-contaminated soil (Sekhar et al., 2005; Fischerová et al., 2006), but slow growth and low biomass of hyperaccumulator plants in heavy metal-contaminated soil may limit the efficiency of Phytoremediation (Kumar et al., 1995; Burd et al., 2000). Studies have evidenced that heavy metal-resistant bacteria can promote plant growth and enhance metal uptake by hyperaccumulator or non-hyperaccumulator plants (de Souza et al., 1999; Whiting et al., 2001; Abou-Shanab et al., 2003; Zaidi et al., 2006) as has been shown in our experiment that the total Pb and Cd uptakes by maize and tomato plants were significantly enhanced by the heavy metal-resistant *Burkholderia* sp. J62. We choose the Indian mustard, maize and tomato as the tested plants for the bacterial assisted-phytoextraction due to their fast growth and high biomass. Pot experiment demonstrated that strain J62 facilitated maize and tomato growth, but could not increase the growth of the Indian mustard. As successful plant growth promoting inoculants, bacteria must be able to rapidly colonize the root system during the growing season (Defreitas and Germida, 1992). In the study, strain J62 was able to colonize and develop in the rhizosphere soil of maize and tomato after root inoculation (data not shown) and was able to promote the growth of maize and tomato. In addition, soil inoculation with strain J62 produced a larger above-ground biomass ($p < 0.05$). However, strain J62 could not colonize and develop in the rhizosphere soil of the Indian mustard (data not shown) and could not promote the growth of the Indian mustard. Although a number of studies have demonstrated the importance of bacterial inoculation for plant growth and heavy metal accumulation in heavy metal-polluted environments (Abou-Shanab et al., 2003; Idris et al., 2004; Khan, 2005; Sheng and Xia, 2006), to the best of our knowledge, this is the first research report elucidating the role of a heavy metal-resistant *Burkholderia* sp. in Pb and Cd solubilization in solution and Pb and Cd accumulation by maize and tomato with concurrent promotion of plant growth in a pot experiment. Strain J62 had the characteristics of producing IAA, siderophores, ACC deaminase and inorganic phosphate solubilization. Strain J62 also showed very high degree of resistance to heavy metals such as Cd, Pb, Cu, Zn, Ni and Cr and exhibited antibiotic resistance characteristics to kanamycin, streptomycin, ampicillin, tetracycline, and rifampicin.

Although strain J62 did not significantly influence the concentrations of Pb and Cd in shoot systems (data not

shown), the application of strain J62 effectively increased the bioavailability of Pb and Cd in the rhizosphere soils and promoted the growth of maize and tomato plants, consequently increasing the total Pb and Cd uptakes of the plants even under nonsterile conditions. Dell'Amico et al. (2008) also found that cadmium-resistant rhizobacteria did not influence the specific accumulation of cadmium in the root and shoot systems, but all increased the plant biomass and consequently the total cadmium accumulation of the *Brassica napus*. Strain J62 did not promote the total Pb and Cd uptakes of Indian mustard from the soil in our study. The tomato plant was the best in the total shoot Pb and Cd uptakes in the inoculated or uninoculated soils. The bacterial strains protected the plants against the inhibitory effects of cadmium probably due to the production of IAA, siderophores and ACC deaminase activity (Dell'Amico et al., 2008). Most likely, the heavy metal-resistant strain J62 producing IAA may indirectly promote metal accumulation by increasing the plant biomass (Zaidi et al., 2006). Halstead et al. (1969) also suggested that the process of solubilizing inorganic phosphates facilitates the uptake of the metals from soil. So, strain J62 having the characteristics of producing IAA, siderophores, ACC deaminase and inorganic phosphate solubilization may have the potential for the promotion of plant growth and Pb and Cd uptake (Glick et al., 1995; Chabot et al., 1996; Rajkumar et al., 2006; Zaidi et al., 2006). Thus, with the innate capability of expressing multiple traits, the strain J62 may serve as an effective metal sequestering and growth promoting bio-inoculant for plants in Pb- and Cd-stressed soil. Compared to the uninoculated maize plants, significant decrease in the shoot Cd concentration of the inoculated maize plants was observed, indicating that Cd was mainly accumulated in the root of the inoculated maize plants.

5. Conclusion

Our study demonstrated that the heavy metal-solubilizing and growth promoting bacterial strain J62 may increase the availability of the heavy metals in solution culture and the soils. Pot experiment demonstrated that the application of the bacterial strain J62 could significantly enhance maize and tomato plant biomass and heavy metal uptake. But neither plant growth nor heavy metal uptake of Indian mustard could be promoted by strain J62. The effective heavy metal-resistant and PGPB-plant systems must be tested and established in controlled vegetation experimental designs with consideration of the specific matching of plant and microbe. This promotion of plant biomass production and heavy metal uptake by strain J62 might have potential for the phytoextraction of the metals from soils. It may therefore provide a new microbe-assisted phytoremediation of metal-polluted soils. In addition, although strain J62 significantly increased the Pb and Cd uptakes by the maize and tomato, the total Pb and Cd accumulation of the plants is low. Further understanding of the basic mechanisms of plant–microbe interactions especially

hyperaccumulator-PGPB interactions is essential for Pb and Cd-contaminated soil phytoremediation.

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