International Microbiology (2020) 23:625–640
https://doi.org/10.1007/s10123-020-00133-1

ORIGINAL ARTICLE

Lead and cadmium-resistant bacterial species isolated from heavy metal-contaminated soils show plant growth-promoting traits

Samaneh Abdollahi[1] · Ahmad Golchin[1] · Fatemeh Shahryari[2]

Received: 26 February 2020 / Revised: 27 May 2020 / Accepted: 2 June 2020 / Published online: 13 June 2020

Abstract

Application of metal-resistant rhizobacteria is a promising approach for detoxification and bioremediation of contaminated soils. In order to isolate, identify, and characterize lead and cadmium-resistant bacteria, nearly 30 soil samples were collected from heavy metal-contaminated sites, and five resistant bacterial strains were isolated and identified based on their cultural, physiological, biochemical, and molecular characteristics as *Enterobacter cloacae*, *Enterobacter kobei*, *Bacillus cereus*, *Rhizobium pusense*, and *Agrobacterium tumefaciens*. The nucleotide information of these strains is available in GenBank under the accession numbers of MH327251, MH327252, MH327253, MH327254, and MK123361, respectively. The minimum inhibitory concentrations (MICs) against lead and cadmium differed for each isolate and the isolates showed higher MIC against lead (3500 μg ml^{-1}) than cadmium (100 μg ml^{-1}). Assessment of the heavy metal degradation capacity of the species showed 10–60% and 5–40% reduction in concentrations of lead and cadmium, respectively. The highest ability for P-solubilization was measured for the *R. pusense*, *A. tumefaciens*, and *B. cereus* species, while the *R. pusense* and *B. cereus* species had the capability to solubilize potassium. The studied species also had the ability to produce indole acetic acid (IAA) and/or hydrogen cyanide production (HCN). Inoculation of ornamental cabbage cultivated in a heavy metal-contaminated soil with the isolated species significantly increased biomass and Pb and Cd uptake of the plant. With respect to plant growth promoting and heavy metal-resistant traits of the studied species, it is concluded that these species can have great significance in bioremediation and management of environmental pollution.

Keywords Antibiotic · *Bacillus* · *Enterobacter* · HCN · IAA · *Rhizobium*

Introduction

The essential elements of life, such as air, water, and land, are contaminated constantly with different pollutants (Chhikara and Dhankhar 2008). The main group of inorganic pollutants is heavy metals which can accumulate in soils, plants, animals, aquatic organisms, and humans at toxic levels (Muduli et al. 2012). Therefore, the biomagnification of heavy metals in the environment is a serious threat to human health (Hooda 2007; Yigit and Altindag 2006).

Heavy metals like mercury (Hg), lead (Pb), arsenic (As), and cadmium (Cd) have no beneficial effect on organisms and are even toxic to human(s) and other living systems (Adriano 2001). Metal toxicity occurs when essential elements are replaced from their native binding sites, and the structure of DNA and proteins is changing, and by interference in enzymatic ATP formation and osmoregulation (Poole and Gadd 1989). Pb and Cd which are major pollutants found in the environment cause damage to cell membranes, changes in the particularity of enzymes, and carcinogenesis (Olaniran et al. 2013). Chronic toxicity of Cd results in proteinuria and lung emphysema, and its acute toxicity causes headaches, nausea, and diarrhea. Toxicity of Pb also creates various symptoms in the hematopoietic, hepatic, and renal system even in the nervous system, and chronic toxicity with blood

✉ Samaneh Abdollahi
samaneh.abdollahi87@yahoo.com

Ahmad Golchin
agolchin2011@yahoo.com

Fatemeh Shahryari
shahryari14@yahoo.com

[1] Soil Science Department, Faculty of Agriculture, University of Zanjan, Zanjan, Iran

[2] Plant Protection Department, Faculty of Agriculture, University of Zanjan, Zanjan, Iran

concentrations of about 400–600 mg L^{-1} can lead to persistent vomiting, lethargy, delirium, convulsions, and coma if not attended timely (Flora et al. 2012).

Utilization of bacteria possessing metal-detoxifying traits and plant growth-promoting attributes is an efficient and environmentally friendly treatment approach and when these bacteria are used as bioinoculants and biofertilizers in heavy metal-polluted soils significantly improve the growth of plants and enhance the phytoremediation process (Khan et al. 2009; Pilon-Smits 2005). These bacteria increase metal mobility and bioavailability in soils and thus increase uptake of metals by plants. So, growth and metal accumulation are stimulated via reducing soil pH, producing indole acetic acid (IAA), monocyclopropane-1-carboxylate (ACC) deaminase, siderophores (metal-chelating compounds), and organic acids (Ahemad and Kibret 2014; Rajkumar et al. 2010). It has been reported that under conditions of heavy metal stress, the growth of plants is enhanced by auxin and gibberellin which are synthesized by rhizobacteria and are called plant growth-promoting hormones (Sharp et al. 2011). Heavy metal remediation is performed by microorganisms via different methods, including biosorption, intracellular accumulation, enzyme-catalyzed transformation, bioleaching (the extraction of metals through the use of living organisms), biomineralization (minerals formed by living organisms), and redox reactions (Lloyd et al. 2002). Furthermore, rhizobacteria augment plant tolerance to metals by inducing thiol compounds, superoxide dismutase, or metallothionein (Khalid et al. 2017). (Ahmad et al. 2016; Nath et al. 2012)

Conventional remediation approaches for heavy metal-polluted soils and water are generally physical, chemical, and biological techniques, which can be used in association with each other. Compared to physical-chemical methods, biological techniques show the great advantage with respect to economical, eco-friendly, less disruptive, field-scale application, high public acceptability, low time of remediation, and cost involved (Khalid et al. 2017). One of the most promising methods of bioremediation is microbial remediation that makes use of microorganisms to promote absorption, precipitation, oxidation, and reduction of heavy metals in the soil.

In this research, ornamental cabbage was selected due to its ability to tolerate and absorb high concentrations of heavy metals (Boyd and Barbour 1986). This cabbage variety is usually planted in autumn and winter, the seasons that are not suitable for growth of most plants used in the phytoremediation process. Thus, in cool seasons, this plant could be a good choice for the phytoremediation of heavy metal-polluted sites in urban areas and around smelter plants, where the soils are contaminated with high levels of Cd and Pb. Inoculation of plants with heavy metal-resistant plant growth-promoting rhizobacteria (PGPR) has been found as an interesting option to improve plant performance under stressed conditions. The aims of this study were to separate, identify, and describe the features of Cd- and Pb-resistant bacteria from heavy metal-polluted soils to acquire strains that might be suitable for inoculation of Pb- and Zn-contaminated soils under unfavorable ecological conditions. These strains also could be utilized for immobilization and detoxification of heavy metals in contaminated soils and for intensification of the phytoremediation process.

Material and methods

Study area and sample collection

Nearly 30 soil samples were collected from six different sites nearby a lead-zinc factory located in Dandi city, Zanjan province (between 36° 32′ and 36° 35′ N and 47° 36′ and 47° 40′ E), Northwest of Iran. The collected samples in labeled presterilized bottles were moved to the lab and kept at 4 °C during experiments.

Measurement of physicochemical parameters of soil samples

The physicochemical parameters of soil samples, including available concentrations of Pb and Cd (Lindsay and Norvell 1978), total concentrations of Pb and Cd (Hseu 2004), available concentrations of potassium and phosphorus (Helmke and Spark 1996; Olsen 1954), and pH (Thomas 1996), were measured.

Isolation of bacterial strains

For isolation of bacterial strains, 1 g of each soil sample was added to 9 ml of sterile water to prepare a suspension. Then, a serial dilution (10^{-1} to 10^{-6}) was prepared from the suspension with sterile nutrient broth (NB) medium and incubated at 36–37 °C for 24 h. Then, 100 μl of each dilution was spread on nutrient agar (NA) medium and incubated at 37 °C for 24 h. Depending on differences in color, morphology, and shape, each microbial colony was selected and separately streaked on NA medium and incubated overnight at 37 °C. Then, pure strains grown in this medium were stored in nutrient broth medium containing 25% (*v*/*v*) glycerol at − 20 °C or − 80 °C for further studies (Jamaluddin et al. 2012).

Determination of minimum inhibitory concentration

The minimum inhibitory concentrations (MICs) of Pb and Cd for isolated strains were determined by agar dilution technique (Chen et al. 2006a). NB media with different concentrations of Cd and Pb were prepared. The concentrations of Pb were 50, 250, 500, 1000, 1250, 1500, 2000, 2500, 3000, and 3500 μg ml^{-1}, and the concentrations of Cd were 5, 10, 20,

25, 35, 50, 75, 100, and 150 μg ml^{-1} using lead nitrate [$Pb(NO_3)_2$] and cadmium sulfate [$3Cd(SO_4) \times 8H_2O$] as the sources, respectively.

Heavy metal accumulation assay

Bacterial strains were transferred to LB medium and cultivated for 1 h at 37 °C with shaking at 150 rpm to the optical density ($OD_{600\ nm}$) of 0.6. Then, 2 ml of sterilized Pb or Cd solution with a concentration of 100 ppm was added separately to each culture flask and again incubated for 24 h, at the same condition. After incubation, the whole bacterial cells were removed by centrifugation at 5000 rpm for 15 min using a mini spin rotor and supernatants were mixed with two volumes of concentrated HNO_3 (70%). The HNO_3-treated samples were heated on a hotplate stirrer (IKA, RTC basic) to 100 °C until the sample volumes reduced to initial supernatant volume for acid digestion. The extract was clarified by removing insoluble material using filter paper (Whatman 42). This extract was analyzed by atomic absorption spectrophotometer (Varian Specter. AA20) for the concentration of heavy metals, and the results were compared with control to calculate the reduction in the heavy metal concentration and thus, accumulation capacity (%) as follows (Marzan et al. 2017):

$$\text{Heavy metal accumulation capacity (\%)} = \frac{\text{Heavy metal utilized (ppm) by a microbial strain}}{\text{Heavy metal added to the LB medium (ppm)}} \times 100 \quad (1)$$

Heavy metal utilized (ppm) by a microbial strain = Heavy metal added to the LB medium (ppm)–Heavy metal at the end of culture (ppm)

Identification of phenotypic and biochemical traits of bacterial strains

The cultural and biochemical traits were used to identify the bacterial strains, including Gram reaction, potato soft rot, oxidation/fermentation of glucose, oxidase and catalase reactions, production of fluorescent pigment on King's medium B, levan production, and hypersensitive reaction (HR) on tobacco plants (Schaad et al. 2001).

Resistance to antibiotics was determined on Mueller Hinton agar plates (MHA) by disk diffusion method (Oyetibo et al. 2010). Tests were conducted in triplicate with (Raja et al. 2009) the following antibiotics: ampicillin (100 μg ml^{-1}), amoxicillin (50 μg ml^{-1}), tetracycline (20 μg ml^{-1}), kanamycin (30 μg ml^{-1}), erythromycin (50 μg ml^{-1}), and nalidixic acid (10 μg ml^{-1}).

The Pikovskaya medium was used to determine the qualitative activity of a selected strain for mineral phosphate solubilization (Subba Rao 2016). The colony and halo zone diameters were determined and used to calculate the index of solubilization by the following formula (Premono et al. 1996):

$$SI = \frac{\text{colony diameter} + \text{halo zone diameter}}{\text{colony diameter}} \quad (2)$$

Quantitative analysis of mineral phosphate solubilization, in Pikovskaya broth medium containing tricalcium phosphate (5 g L^{-1}), was performed as described by Subba Rao (2016). A standard curve was prepared using KH_2PO_4 and the amount of soluble phosphate was measured from the standard curve (Olsen and Sommers 1982). The method of Aleksandrov medium (Jones Jr 2001) was used to determine potassium solubilization, the method of Patten and Glick (2002) to determine IAA production, and the method of Alstrom and Burns (Alström and Burns 1989) to determine HCN production..(Alström and Burns 1989; Patten and Glick 2002; Subba Rao 2016).

DNA extraction

The following method was used to extract DNA from heavy metal-resistant bacteria:

Bacterial strains were grown in 3 ml of LB medium at 28 °C for 48 h, and then, the bacterial suspension was centrifuged at 3000 rpm for 3 min and the supernatant was removed and the pellet was washed twice in 400 μl of sterile-deionized H_2O and solved in 200 μl of sterile-deionized H_2O. After adding 400 μl of 2X buffer (SDS 1%, 25 mM EDTA, 50 mM Tris-HCl, pH = 8) and 2 μl of proteinase K (10 μg ml^{-1}; Sigma), the bacterial suspension was further incubated at 55 °C for 3 h. After lacing the cell walls of the bacteria, 400 μl of 7.5 M ammonium acetate was added and mixed gently but completely, and the mixture was centrifuged (Hettich Mikro 220R) at 12,500 rpm for 20 min at 4 °C. The aqueous phase was transferred to a clean polypropylene tube and 750 μl of cool isopropanol was added and kept overnight at − 20 °C. In the next step, tubes were centrifuged (Hettich Mikro 220R) at 12,500 rpm for 30 min at 4 °C. Then, pellet was rinsed twice with 70% ethanol, and finally, the DNA dissolved in 20 μl of sterile-deionized H_2O and kept at − 20 °C.

Molecular identification

The polymerase chain reaction (PCR) was used to amplify the 16S rRNA gene of the extracted DNA using a universal primer pair 63f (5′-CAG GCC TAA CAC ATG CAA GTC-3′) and

1387r (5′-GGG CGG WGT GTA CAA GGC-3′) in 30 μl of a reaction mixture possessing 4 μl of DNA template, 1.5 μl of each primer at a concentration of 5 mM, and 16 μl of master mix at a concentration of 10 mM. To perform PCR, a thermocycler (Astec PC320, JAPAN) with the following program was used. The first denaturation was carried out at 95 °C for 5 min followed by 40 cycles of denaturation at 94 °C for 30 s; annealing was carried out at 55 °C for 30 s followed by the first extension at 72 °C for 1.5 min and the final extension at 72 °C for 10 min. The PCR product was sequenced bi-directionally by Bioneer company (Daejeon, South Korea). The obtained nucleotide sequences (1018–1470 bp) were edited and compared with other relevant sequences available in the GenBank database using the BLAST homology search program. Also, phylogenetic analyses were conducted with MEGA 6 software (Molecular Evolutionary Genetics Analysis, version 6.0) (Tamura et al. 2013) and the phylogenetic trees were constructed using the neighbor-joining method with 1000 bootstrap replications.

Pot trial

The effects of soil inoculation with five selected strains on the growth and accumulation ability of heavy metal of Pb and Cd of the ornamental cabbage plant were evaluated in pot trial. A soil with medium pollution for heavy metals (lead and cadmium) was used for the pot experiment and each pot was filled with 4 kg of this soil ($X = 737{,}500$, $Y = 4{,}048{,}000$ (UTM), total concentrations of Pb = 560 mg kg^{-1}, available concentrations of Pb = 54 mg kg^{-1}, total concentrations of Cd = 7 mg kg^{-1}, available concentrations of Cd = 0.62 mg kg^{-1}, pH = 7.69, available phosphorus = 12.5 mg kg^{-1}, available potassium = 422 mg kg^{-1}), and then, three seedlings of ornamental cabbage (*Brassica oleracea var. acephala* L. Pigeon Victoria F1) were planted in each pot. The rhizosphere soils of ornamental cabbage were inoculated in triplicate with 2 ml of bacterial suspension from each strain with 10^7–10^8 cfu ml^{-1} and the plants were placed in an experimental greenhouse with a photoperiod of 12 h and night and a day temperature of 15 °C and 20 °C, respectively. A control treatment containing uninoculated rhizosphere soil was also used. Three months after sowing, fresh and dry weights of plant biomass, dry weights of root and shoot, concentrations of Pb and Cd in the root and shoot as well as plant uptakes of Pb and Cd were measured. Thus, plants were harvested and then the harvested plant materials divided into root and aerial parts and washed with tap and distilled water, respectively. The plant samples were oven-dried at 60 °C for 72 h before determination of dry matter (DM). Samples of the root and aerial parts were digested by three-acid mixture [H_2SO_4 (65%), $HClO_4$ (65%), and HNO_3 (70%)] at the ratio of 1:1:5 (Allen et al. 1986), and then, the concentrations of Pb and Cd were measured in the extract of digestion using Atomic Absorption Spectrometer (Varian Spectra. AA20).

The bioaccumulation factor (BAF) determines the rate of transfer of lead and cadmium contaminants from soil to plant and is calculated by dividing the concentration (mg kg^{-1}) of an element in the root to the total concentration (mg kg^{-1}) of the same element in the soil. The translocation factor (TF) determines the mobility and distribution of heavy metals in plants and is calculated by dividing the concentration (mg kg^{-1}) of an element in the shoot to the concentration (mg kg^{-1}) of the same element in the root (Li et al. 2007).

Statistical analysis

For assessment of microbial traits, all tests were carried out in triplicate and the means of replicates analyzed statistically and where significant differences observed between the means, standard deviation, and student test were used to differentiate the means. To study the effects of microbial strains on plant growth, the analysis of variance (ANOVA) of data was carried out by SAS software (version 9.4), and Duncan's multiple range test at 1 and 5% probability levels was used for mean comparison.

Results

Isolation of bacteria from soils

In the current study, 83 bacterial single colonies with differences in apparent characteristics and shape of colony were isolated from six soil samples. From the bacterial strains isolated, only 24 strains could tolerate Cd and Pb in nutrient agar (NA) with the concentrations of 5 and 50 μg ml^{-1}, respectively.

MIC each metal

MIC for Pb ranged from 50 to 3500 μg ml^{-1} and for Cd from 5 to 150 μg ml^{-1}, respectively. In this experiment, the number of resistant strains decreased with an increase in the heavy metal concentration of media. As summarized in Table 1, 12 strains were resistant to a wide range of concentrations of Pb and Cd. According to the results, five strains (52, 56, 57, 59, and 60) which showed good tolerance capacity against Pb and Cd were selected (Table 1). These five strains showed better tolerance to Pb so that the strains 52 and 56 were able to tolerate 3000 μg Pb/ml and 50 μg Cd/ml, and the strains 57, 59, and 60 were able to tolerate 3500 μg Pb/ml and 100 μg Cd/ml. These five potential strains (52, 56, 57, 59, and 60) were also selected for conducting further bioremediation tests.

Assessment of heavy metal accumulation capacity of bacterial strains

Heavy metal accumulation capacity was measured by analyzing the heavy metal concentration of the treated samples by atomic absorption spectrophotometer (Varian Specter. AA20) and comparing it with that of control. Strain 57 showed the highest Pb accumulation ability and strains 52 and 60 stood in second and third places. Strain 57 was able to reduce 60% of Pb and 40% of Cd at 24 h (Table 1).

Phenotypic and molecular identification of selected strains

Several attributes (cultural, morphological, and biochemical) of five potential heavy metal degrading strains were measured based on Schaad et al. (2001) methods and the results are shown in Table 2. (Schaad et al. 2001).

The sensitivity of selected bacterial strains to six antibiotics was determined. Those strains were considered susceptible to antibiotics when the inhibition zone was 12 mm or more in diameter. In the present study, the strain 52 exhibited a high resistance pattern towards all antibiotics used. All strains were resistant to the nalidixic acid antibiotic (Table 2) and the strains 57 and 60 had a very high sensitivity to erythromycin antibiotic. The strain 59 also showed a medium sensitivity to ampicillin, amoxicillin, erythromycin, and kanamycin.

The results of this study showed that strains 57, 59, and 60 were able to solubilize tricalcium phosphate ($Ca_3(PO_4)_2$ in broth medium and the highest solubilizing ability belonged to strain 59 with 145.83 μg ml^{-1}. In general, the pH of culture medium was regarded as an index for phosphate availability and the phosphate availability increased as the pH of the culture medium decreased. Since strain 59 decreased the pH value of the culture medium from 7.7 to 4.7, it was regarded as the most effective strain in this way (Table 2). Equation 3 which shows P solubilization from tricalcium phosphate as a result of the decrease in pH is given as follows (Bolan et al. 2003):

$$2Ca_3(PO_4)_2 + 8\ H^+ \rightarrow 3Ca^{2+} + 4H_2PO_4^-$$

The results showed that strains 57 and 59 were capable of solubilizing potassium in broth medium containing 2 g L^{-1} muscovite mineral. The highest solubilizing ability belonged to strain 59 with 55.33 μg ml^{-1}, and strain 57 with 42.67 μg ml^{-1} stood in second place (Table 2).

Development of pink color in flasks indicated IAA production by strains 52, 56, and 59. The amounts of IAA produced by these strains ranged from 3.77 to 31.94 μg ml^{-1} and the best IAA producer was strain 52 (Table 2).

The bacterial strains possessing HCN production ability were classified into four groups with very high, high, medium, and low ability. The results showed that strains 57, 59, and 60 were capable to produce HCN. Also, it was observed that HCN production ability in strains 57 and 60 was weak and in strain 59 was high and the color of filter paper changed to light brown (Table 2).

Table 1 Minimum inhibitory concentration (MIC) and accumulation capacity of bacterial strains to heavy metals of Pb and Cd

Strains	Minimum inhibitory concentration (MIC) against Pb (μg ml^{-1})	Minimum inhibitory concentration (MIC) against Cd	Accumulation capacity of Pb (μg ml^{-1})	Accumulation capacity of Cd
51	2500	25	10	5
52	3000	50	45	30
53	2500	25	15	5
54	2500	25	10	5
55	2500	25	15	5
56	3000	50	25	20
57	3500	100	60	40
58	3000	35	20	10
59	3500	100	50	30
60	3500	100	20	20
61	2500	25	15	5
62	2500	25	10	5

Molecular identification and phylogenetic analysis

Nucleotide BLAST search in the GenBank database with the partial sequences of 16S rRNA gene and phylogenetic analysis showed that the strains 56, 57, and 59 had a homology of 100% with *Enterobacter kobei* DSM 13645T, clone 3 (Accession No. LT547822.1), *Bacillus cereus* isolate BCsn (Accession No. HE660034.1), and *Rhizobium pusense* strain: Naga 0113 (Accession No. LC208007.1), respectively. Also, the strains 52 and 60 clustered phylogenetically with *Enterobacter cloacae* isolate L2 (Accession No. LK391629.1) and *Agrobacterium tumefaciens* strain A78 (Accession No. KC196487.1) with 99.66% and 98.54% sequence similarity, respectively. Figure 1 shows the phylogenetic relationship between strains. The sequences acquired in this study were stored in the GenBank database under the accession numbers of MH327251, MH327252, MH327253, MH327254, and MK123361, respectively.

Pot trial

According to the analyses of variance of data, the effects of soil inoculation with bacterial species on fresh and dry weights of plant biomass, dry weights of root and shoot, Pb accumulation in the root, Cd accumulation in the root and

Table 2 Characteristics of the five strains of heavy metal-resistant bacteria

Character	Strains				
	52	56	57	59	60
Morphological					
Colony color on NA	Pink Viscous	White Cloudy	White Cloudy	White Viscous	Pink Cloudy
Colony color on medium contain Pb	Brown	Brown	White	Brown	Brown
Gram reaction	–	–	+	–	–
Biochemical					
Catalase	+	+	+	+	+
Potato soft rot	–	–	–	–	–
Hypersensitive reaction (HR)	–	–	–	–	–
Fluorescent pigment on King's medium B	–	+	–	–	–
Levan production	–	–	–	+	–
Oxidation/fermentation of glucose	+/+	+/+	+/+	+/–	+/–
Production of callus-like swellings on carrot disk	–	–	–	–	–

Strains	Antibiotics					
	Ampicillin	Amoxicillin	Erythromycin	Kanamycin	Nalidixic acid	Tetracycline
52	–	–	–	–	–	–
56	–	–	**	–	–	–
57	–	–	****	**	–	***
59	**	**	**	**	–	–
60	**	***	****	**	–	**

Strains	Mineral phosphate			Potassium			Final pH
	colony diameter + halo zone (mm)	SI index	Soluble P concentration (μg ml^{-1})	colony diameter + halo zone (mm)	SI index	Soluble K concentration (μg ml^{-1})	
52	2.0	1.00	1.94	2.0	1.00	3.00	6.6
56	3.1	1.00	1.30	2.3	1.00	2.75	6.2
57	4.2	1.30	32.23	5.97	2.23	42.67	5.5
59	16.3	3.56	145.83	12.7	2.89	55.33	4.7
60	8.07	2.42	136.83		2.0	1.00	2.16 5.3

Strains	IAA		HCN	
	pink color production	IAA production (μg ml^{-1})	HCN production	Numeral
Control	–	–	–	0
52	+	31.94	+	1
56	+	24.11	–	0
57	–	3.89	+	1
59	+	26.78	+++	3
60	–	3.77	+	1

Note: "+" positive; "–" negative

"–" Resistant, *low, **medium, ***high, and ****very high sensitivity to antibiotics, respectively

SI (solubilization index) = (colony diameter + halo zone)/colony diameter, initial pH = 7.7

The values are the average of three replicates; The color of filter paper in the test of HCN for 1, 2, 3, and 4 was yellow, cream, light brown, and dark brown, respectively

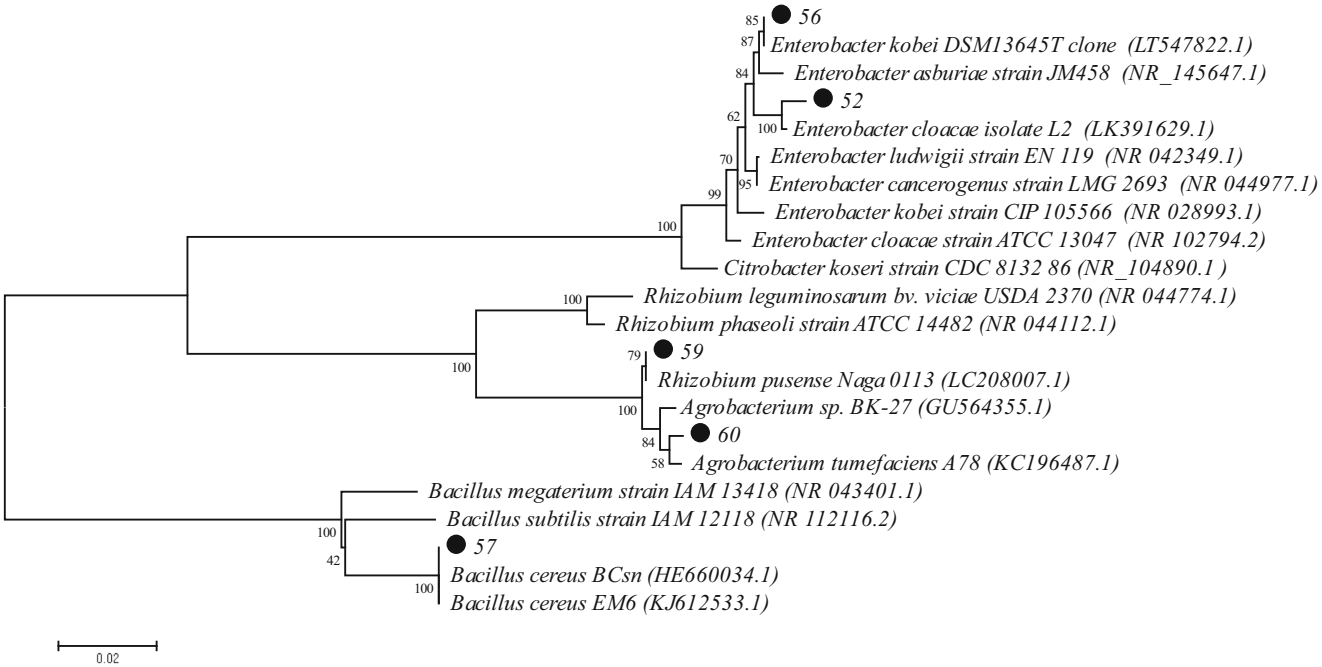

Fig. 1 The phylogenetic tree constructed using neighbor-joining method based on partial 16S rRNA gene sequences of the strains obtained in this study and selected relevant strains from GenBank. The numbers next to nodes are confidence values of bootstrap (1000 replicates). The scale at the bottom represents genetic distance in nucleotide substitutions per site

shoot, as well as Pb and Cd uptake of the plant, were significant at the probability level of 1% ($p < 0.01$) (Table 3).

Inoculation of the soil by bacterial species caused fresh and dry weights of the biomass of ornamental cabbage and dry weights of root and shoot to increase significantly when compared with control treatment (Fig. 2). The largest increase in fresh and dry weights of plant biomass and dry weights of root and shoot was observed when the rhizosphere soils were inoculated by *R. pusens*e (strain 59) and *E. cloacae* (strain 52), respectively (Fig. 2).

Inoculation of rhizosphere soils with the bacterial species significantly increased Pb accumulation in the root and Cd accumulation in the root and shoot compared with uninoculated plants or control treatments (Fig. 3). The highest concentration of Pb in the root and the highest concentrations of Cd in the root and shoot were measured for treatments inoculated with *A. tumefaciens* (strain 60). But significant differences in Pb concentrations of shoots were not observed when inoculated treatments were compared to control treatment (Fig. 3).

The highest uptake performances of Pb and Cd in biomass of ornamental cabbage were measured in treatments inoculated with *A. tumefaciens* (strain 60) and *R. pusense* (strain 59) (Fig. 4). When *A. tumefaciens* (strain 60) used as a soil inoculant, the uptake performances of Pb and Cd in biomass of ornamental cabbage increased by 3 and 6 times, respectively, and by using *R. pusense* (strain 59), the uptake performances of Pb and Cd increased by 3 and 5 times, respectively (Fig. 4).

Table 3 The ANOVA results, indicating the effects of soil inoculation with bacterial species on fresh-dry biomass weight, root and shoot dry weight, concentrations of Pb and Cd in the root and shoot, and uptake performances in ornamental cabbage

Sources of variations	*df*	Mean square									
		Fresh biomass	Dry biomass	Dry weight		Concentration of Pb		Pb uptake	Concentration of Cd		Cd uptake
				Root	Shoot	Root	Shoot		Root	Shoot	
Bacterial species	5	939.80**	33.40**	0.60**	3.71**	19,629.96**	1.73^{ns}	0.04**	503.09**	0.13**	0.001**
Error	12	0.13	0.08	0.002	0.008	26.65	1.70	0.0001	0.31	0.003	0.00001
Coefficient of variation (%)	–	0.69	2.84	3.78	2.84	3.37	2.45	3.72	3.13	0.97	5.97

*, ** Significant, respectively, at 5% and 1%

^{ns} Differences not significant

Inoculation of ornamental cabbage with the bacterial species significantly increased the bioaccumulation factors of Pb and Cd. In all treatments, the calculated bioaccumulation factor of Pb was less than one, but that of the Cd was more than one (Fig. 5a). Also, the calculated translocation factors of Pb and Cd were less than one, suggesting the immobilization of Pb and Cd in the root (Fig. 5b).

Discussion

The five bacterial species identified in this study showed a high resistance pattern against Pb and Cd. MICs of Pb and Cd for *E. cloacae* (strain 52) and *E. kobei* (strain 56) were 3000 μg Pb/ml and 50 μg Cd/ml, respectively. Also, *B. cereus* (strain 57), *R. pusense* (strain 59), and *A. tumefaciens* (strain 60) showed simultaneous resistance to Pb and Cd with MICs of 3500 μg Pb/ml and 100 μg Cd/ml (Table 1). Sevim and Sevim (2015) isolated 15 *Bacillus* strains from soil samples and one of them, which belonged to the *B. cereus*, was resistant to heavy metals. The MICs of heavy metals for this strain were 2500 μg ml^{-1} for Pb and 250 μg ml^{-1} for Cd. Rohini and Jayalakshmi (2015) isolated a *B. cereus* strain from the copper-polluted area and the strain was considered as a highly potential strain for bacterial bioremediation of contaminated area since it had a maximum tolerable capacity of 600 ppm which was significantly higher than most reported tolerance

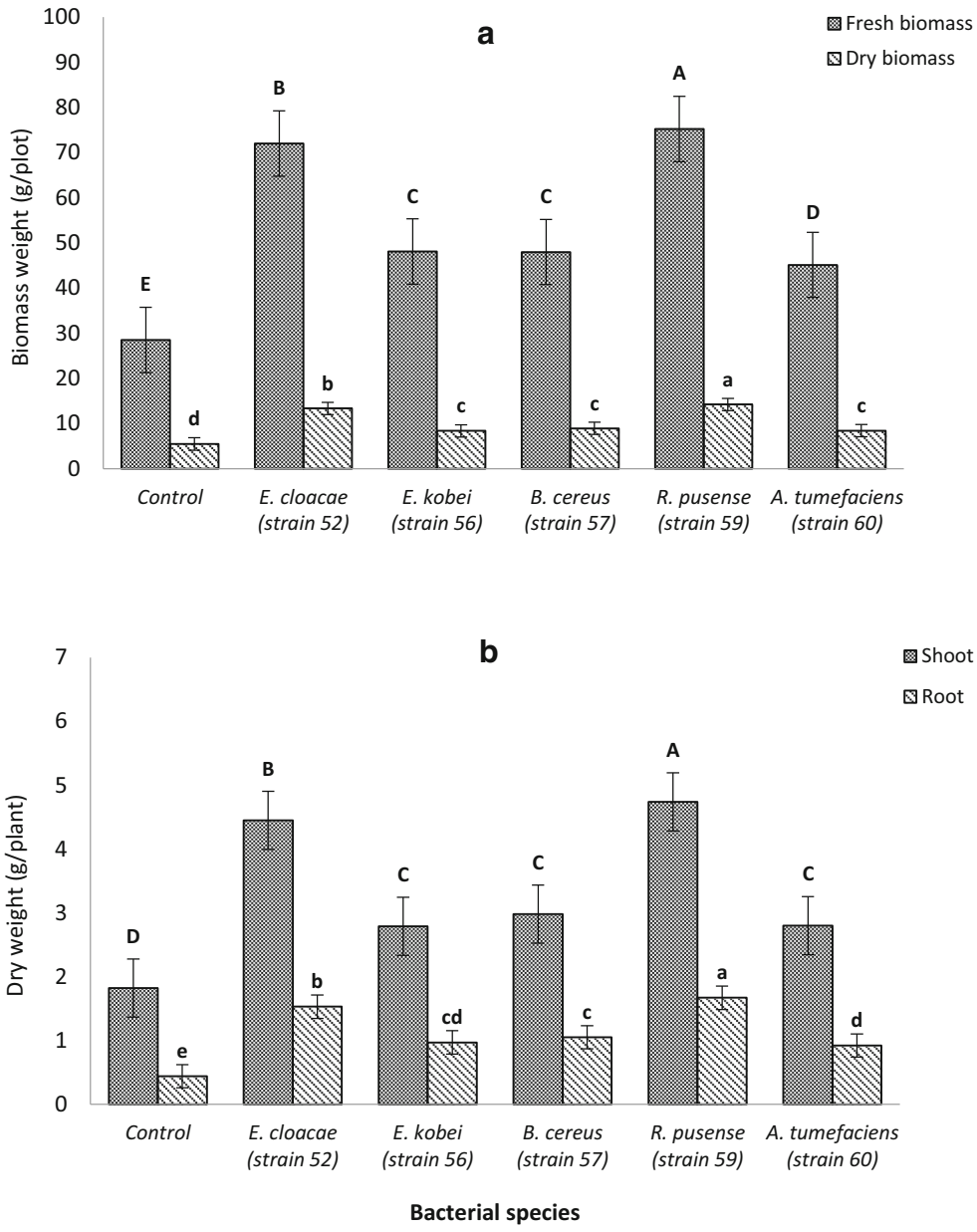

Fig. 2 The effect of soil inoculation with bacterial species on fresh and dry weights of biomass (**a**) and dry weights of root and shoot of ornamental cabbage (**b**) after 3 months of transplanting in soil with medium pollution of lead and cadmium

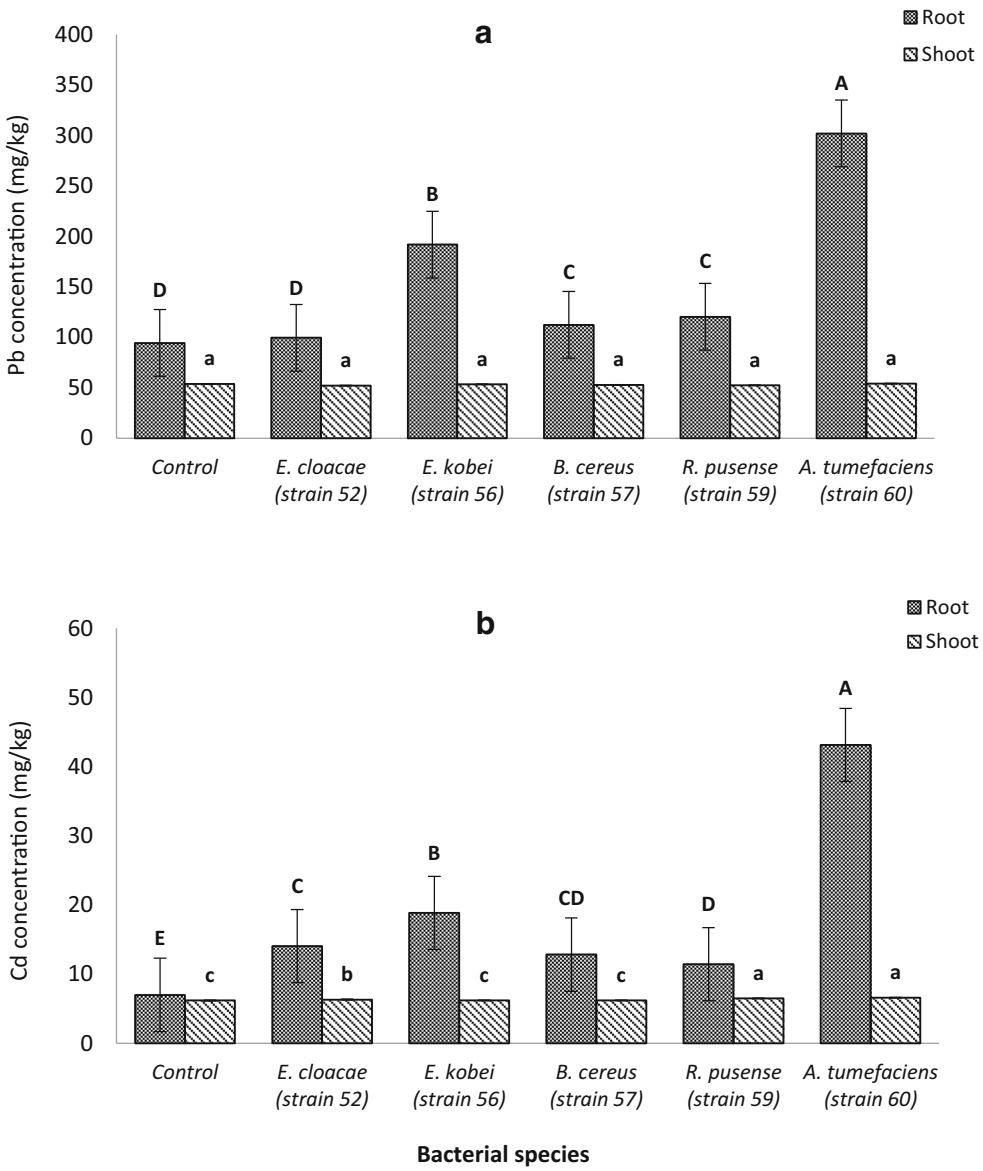

Fig. 3 The effect of soil inoculation with bacterial species on concentrations of Pb (**a**) and Cd (**b**) in the root and shoot in ornamental cabbage after 3 months of transplanting in soil with medium pollution for lead and cadmium

level. A bacterium was separated from disposing yard of municipal solid waste and characterized by Khatun et al. (2012). According to biochemical and 16S rDNA sequence profile, it was identified as *B. cereus*. The tolerance of this bacterium against heavy metals like Cd^{2+} (1.25 mg ml^{-1}) and Pb^{2+} (0.75 mg ml^{-1}) was observed and it was noted that the bacterium had the most resistance to Cd^{2+} compared to other metals. Naik et al. (2012) separated a lead-resistant bacterium from industrial effluent and it was recognized as *E. cloacae* based on its morphological and biochemical traits and 16S rDNA sequence data. This bacterium resisted lead nitrate up to 1.6 mM. Singh et al. (2010) reported that the *B. cereus* (SIU1) indicated a high level of resistance to elevated concentration of lead (600 μg ml^{-1}). A potent heavy metal accumulating microbial strain was isolated from a polluted soil by Banerjee et al. (2015) and characterized as *E. cloacae*. The MICs of lead and cadmium for this strain were 1100 and 900 ppm, respectively. This bacterial strain had a high potential for the lead bioaccumulation (95.25%) and followed by cadmium (64.17%). (Banerjee et al. 2015; Khatun et al. 2012; Naik et al. 2012; Rohini and Jayalakshmi 2015; Sevim and Sevim 2015; Singh et al. 2010; Yang et al. 2007).

Other researchers have also reported Pb- and Cd-resistant species, including *Pseudomonas aeruginosa* (BC2), *Pseudomonas aeruginosa* (BC5) (Raja et al. 2009), *Pseudomonas* sp., *Bacillus* sp. (Nath et al. 2012), *Enterobacter* sp. (EG16) (Chen et al. 2016), *Rhizobium halophytocola* (RT7) (Gupta et al. 2016), *Pseudomonas* (RS-1), *Bacillus* (RS-2), *Bacillus* (RS-3) (Kumar et al. 2016), *Bacillus* (CIK-517), *Bacillus* (CIK-519), and *Enterobacter* (CIK-521R) (Ahmad et al. 2016).

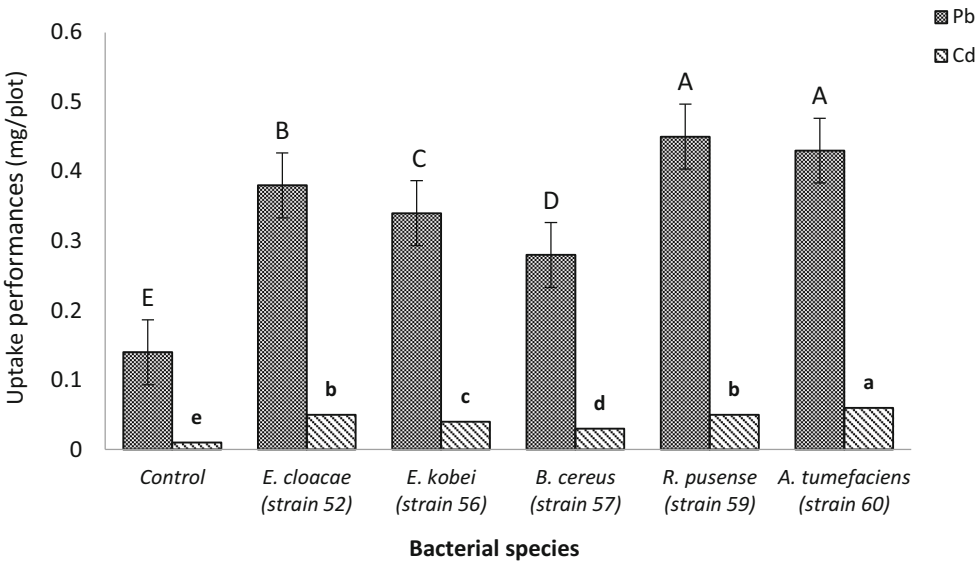

Fig. 4 The effect of bacterial species on uptake performances of the Pb and Cd in ornamental cabbage after 3 months of transplanting in soil with medium pollution for lead and cadmium

In accordance with the results of this study, *B. cereus* (strain 57) had the highest accumulation capacity for Pb and Cd which was 60% and 40%, respectively. Similarly, *E. cloacae* (strain 52) and *R. pusense* (strain 59) species showed, respectively, an

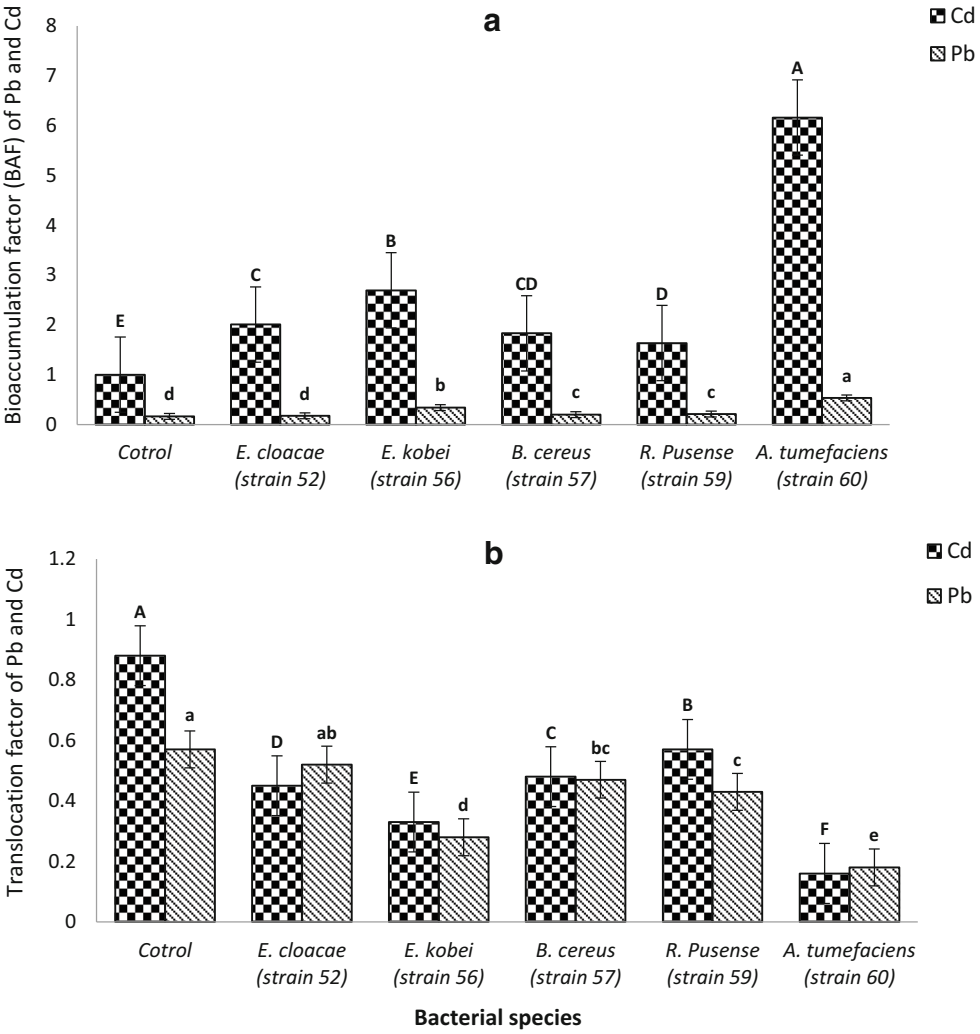

Fig. 5 The effect of bacterial species on the bioaccumulation factor (**a**) and the translocation factor (**b**) of the Pb and Cd in ornamental cabbage plant

accumulation capacity of 45% and 50% for Pb and 30% for Cd (Table 1). The *B. cereus* (S5 strain) was considered as a biological adsorbent for remediation of severe and trace cadmium pollution by Wu et al. (2016) since this bacterium was able to eliminate a high quantity (72.1–83.1%) of Cd^{2+} (mg l^{-1}) from the medium. Syed and Chinthala (2015) reported that *B. cereus* (NSPA8) showed a remarkable level of lead biosorption with a maximum of 87–90%. Three bacterial strains, two of *Klebsiella* sp. and one *E. cloacae*, were separated from wastewaters of chemical and textile industries. These strains showed high efficiency in removing cadmium from the medium, and when 100 μg ml^{-1} of Cd was added to the medium, the strains namely CMBL-Cd1, CMBL-Cd2, and CMBL-Cd3, respectively, removed or accumulated 86%, 87%, and 85% of Cd from the medium within 24 h (Haq et al. 1999). Kumar et al. (2015) reported that *Bacillus thuringiensis* (strain Simi) had an accumulation capacity of 54% for Pb.

In this study, all microbial species were tested for their response to several widely used antibiotics. The antibiotic resistance patterns of the strains were different and only *E. cloacae* (strain52) species showed resistance to all antibiotics (Table 2). Kim and Wei (2007) showed that *E. cloacae* were resistant to ampicillin, cephalothin, and amoxicillin antibiotics. Singh et al. (2010) reported that the *B. cereus* (SIU1) was resistant to antibiotics such as penicillin, lincomycin, cloxacillin, and pefloxacin. Sevim and Sevim (2015) isolated *B. cereus* from soil samples that were resistant to some antibiotics (ampicillin, methicillin, cephalothin, trimethoprim/sulfamethoxazole, and oxacillin). Pramanik et al. (2018b) reported that *E. kobei* was resistant to some antibiotics such as erythromycin, cephalexin, ampicillin, and lincomycin. From resistance to antibiotics that were observed in strains that had not been exposed to antibiotics so far, it can be concluded that in the lack of direct pressure, concurrent resistance to multiple antibiotics can happen in bacteria. A relationship between bacterial tolerances to heavy metals and antibiotics has been indicated in many studies (Verma et al. 2001). Tolerances to antibiotics and heavy metals may help bacteria to adapt themselves to conditions of heavy metal stress faster by the expansion of resistant factors than by mutation and natural selection (Silver and Misra 1988). (Kim and Wei 2007; Pramanik et al. 2018b).

A large portion of soil P is not available for plants and phosphate solubilizing bacterial (PSB) can convert it to available forms (Zaidi et al. 2009). Several studies have reported *Pseudomonas*, *Bacillus*, and *Rhizobium* genera as potent mineral phosphate solubilizers (Gandhi et al. 2014; Karpagam and Nagalakshmi 2014; Susilowati and Syekhfani 2014; Tripti 2012). Their study showed that *Pseudomonas* had the highest P-solubilizing ability which was 12.23 mg P/l from tricalcium phosphate. The highest amounts of P solubilized from tricalcium phosphate by *Bacillus* and *Rhizobium* were 0.32 mg l^{-1} and 0.28 mg l^{-1}, respectively. Other bacteria which mineralize and solubilize poorly available phosphorus included *Flavobacterium*, *Achromobacter*, *Agrobacterium* spp., *Aerobacter*, *Micrococcus*, *Pseudomonas* spp., *Rhizobium* spp. (Babalola and Glick 2012; Rodriguez and Fraga 1999), *Azotobacter* (Kumar et al. 2014), *Burkholderia* (Istina et al. 2015; Mamta et al. 2010; Rodriguez and Fraga 1999; Zhao et al. 2014), *Enterobacter* and *Erwinia* (Chakraborty et al. 2009; Rodriguez and Fraga 1999), and *Bacillus* spp. (Babalola and Glick 2012; Jahan et al. 2013; Raj et al. 2014). Among the studied strains, *R. pusense* (strain 59) (145.83 μg ml^{-1}) and *A. tumefaciens* (strain 60) (136.83 μg ml^{-1}) showed the highest P-solubilizing ability when compared to other strains (Table 2). Dhull et al. (2018) isolated several microbial strains from the root nodules of cluster bean that their efficiency for P solubilization varied from 36 to 79%. (Dhull et al. 2018).

Since a powerful correlation was established between the quantity of phosphorus solubilized and the pH of culture media, it is concluded that the main mechanism of phosphate solubilization is acidification of culture media (Chen et al. 2006b). A highly significant correlation was found between the amount of phosphate solubilized and the pH of the culture media. This observation strongly suggests that the main mechanism of phosphate solubilization is medium acidification (Castagno et al. 2011). The phosphate-solubilizing strains reduce the pH of the culture medium by the production of organic acids and *R. pusense* (strain 59) and *A. tumefaciens* (strain 60) reduced it from 7.7 to 4.7 and 5.3, respectively (Table 2).

The results of this study also showed that *B. cereus* (strain 57) and *R. pusense* (strain 59) species were capable of solubilizing potassium (42.67 and 55.33 μg ml^{-1}, respectively) (Table 2). Species such as *B. cereus* IARI-J-6 and *B. mycoides* have already been introduced as potassium solubilizer with a value of 72.8 and 66.4 mg K/l, respectively (Rajawat et al. 2014). Meena et al. (2015) studied the release of potassium from waste mica (muscovite and biotite) and found that *A. tumefaciens* and *R. pusense* were potassium-solubilizing rhizobacteria. Also, *R. pusense* strain KRB-2 (MF135560) could release 7.05 mg K/l from mica (muscovite) after 6 weeks of incubation (Hauka et al. 2017). (Meena et al. 2015).

Researchers have shown that the majority of microorganisms (80%) isolated from the rhizosphere of different crops has the ability to synthesize and release auxins as secondary metabolites (Loper and Schroth 1986). It has been shown that from different PGPR strains, genera *Azospirillum*, *Pseudomonas*, *Xanthomonas*, and *Rhizobium* together with *Agrobacterium*, *Alcaligenes*, *Enterobacter*, *Acetobacter*, and *Bradyrhizobium* have the ability to synthesize auxins which increase plant growth (Egamberdieva et al. 2008; Khan et al. 2014; Kumar et al. 2008; Poonguzhali et al. 2008; Wani et al. 2007). Indole-3-acetic acid (IAA) is one of those important

hormones provided by microbe to plant that enhances the growth of root and stem and thus the length of the plant (Aloni et al. 2006). Comparatively, some strains used in this study presented IAA production, including, *E. cloacae* strain 52, *R. pusense* strain 59, and *E. kobei* strain 56 (31.94, 26.78, and 24.11 μg ml^{-1}, respectively) (Table 2). The *E. cloacae* UW5, a plant growth-promoting rhizobacterium, was able to produce high levels of IAA through the indolepyruvate pathway (Ryu and Patten 2008) by utilizing indole-3-pyruvate decarboxylase enzyme (Koga et al. 1994). Strains of *Enterobacter* sp. separated from the rhizosphere of sugar cane produced about 2.21 μg IAA/ml in vitro (Mantelin and Touraine 2004). IAA production by PGPR could be varied among different strains and is also affected by culture and medium conditions, growth stage, and substrate availability (Kumar et al. 2012). Goswamia et al. (Goswami et al. 2013) reported strains of *Pseudomonas* spp. that had the ability to produce IAA (29 mg ml^{-1}) and solubilize phosphorus (34 mg ml^{-1}). Pramanik et al. (2018b), after screening rhizosphere soil of rice supplemented with Cd, isolated *E. kobei* which grew profusely in high concentration (1000 mg l^{-1}) of Cd and had PGP traits (P solubilization, IAA production, NH3 production, HCN activity, etc) which were essential for plant growth promotion. The production of IAA also increases root growth, which is needed for obtaining nutrients under Cd stress conditions (Mitra et al. 2018a; Mitra et al. 2018b; Pramanik et al. 2018a; Pramanik et al. 2017). (Goswami et al. 2013; Pramanik et al. 2018b).

To obtain a higher crop yield and for phytoremediation of heavy metal-polluted soils, rhizobacteria possessing PGP traits can be used (Kumar et al. 2016). The association of heavy metal-tolerant plants and useful rhizospheric microorganisms is one of the most important steps in developing an effective phytoremediation system with the use of microorganisms. In this research, ornamental cabbage was selected due to the ability to tolerate and absorb high concentrations of heavy metals (Boyd and Barbour 1986). Our investigation clearly demonstrated that five selected Pb- and Cd-resistant bacterial species promoted fresh and dry weights of plant biomass and also, dry weights of root and shoot in the contaminated soil significantly (Fig. 2). Under abiotic stress conditions, beneficial rhizospheric soil-borne microbes may enhance plant growth by different mechanisms, including optimization of growth by the supply of nutrients, synthesis of phytohormones such as IAA and 1-aminocyclopropane-1-carboxylate (ACC) deaminase, phosphate solubilization, and bioaccumulation or leaching of metals (Yang et al. 2009).

The bacterial strains separated in this study had the ability to produce IAA and solubilize phosphate. The principal consequence of IAA production is the growth stimulation of lateral and adventitious roots which enhances the uptake of nutrients (Golubev et al. 2011). When the ornamental cabbage was inoculated by the bacterial species, significant increases in Pb concentration of root, Cd concentrations of root and shoot, and uptake performances of Pb and Cd in plant biomass were observed (Figs. 3 and 4). Phosphate solubilization, potassium solubilization, IAA and HCN production, and pH reduction traits of the isolated strains lead to mobilization of metals in the soil and increases in concentrations of Pb and Cd in the root and shoot and uptake performances of Pb and Cd in plant biomass. Uptake and translocation of heavy metals may differ significantly and rely upon bacterial species and kind of heavy metals. Different metals to varying degrees show different mobility rates and the mobilization rate of a particular metal could be higher than the other metals within a plant. Previous findings indicate that different groups of rhizospheric microorganisms can increase the Cd uptake of plants (Kartik et al. 2016; Prapagdee and Khonsue 2015; Sangthong et al. 2016; Sheng and Xia 2006; Wu et al. 2006) which was attributed to increased Cd bioavailability in soils and the protection of plants against the inhibitory effects of Cd (Kartik et al. 2016; Sangthong et al. 2016). A similar result was indicated that *A. tumefaciens* CCNWGS0286, plant growth-promoting bacterium, separated from the nodules of *Robinia pseudoacacia* growing in zinc-lead mine tailings in Gansu province, China, displayed high resistance to heavy metals and enhanced significantly the dry weight of *Robinia* plant stems by 14.63%, 23.56%, and 28.07% in the presence of 0, 300, and 600 mg kg^{-1} zinc, respectively, compared with uninoculated plants (Hao et al. 2012). Enhancement of the root length, shoot length, and root shoot biomass was also shown by Romam-Ponce et al. (Roman-Ponce et al. 2017) while experimenting with the effect of seven rhizobacterial strains (as members of *Alcaligenes*, *Bacillus*, *Curtobacterium*, and *Microbacterium*) on *Brassica nigra* seedling growth (Roman-Ponce et al. 2017). A similar type of PGP study was also performed by Lal et al. (2019) working on two rhizobacterial strains *Pantoea agglomerance* (PC1) and *Pseudomonas aeruginosa* (SA) and its effect on *Zea mays* L. that showed a significant increase in seed germination in the presence of Cd^{2+} and Pb^{2+} ions (Lal et al. 2019). Wang et al. (2020) reported that *Enterobacter* TJ6 had a high ability to reduce Cd and Pb uptake of lettuce and concentrations of water-soluble Cd and Pb in soil solution. This bacterium protected lettuce against Cd and Pb toxicity by extracellular adsorption, Cd and Pb immobilization, and increased pH. The effects of heavy metal immobilization by the strain of *Enterobacter* TJ6 can guarantee vegetable safety in situ for the bioremediation of heavy metal-polluted farmland. Sharma et al. (2020) reported that inoculation of *Cajanus cajan* plant by *Enterobacter* sp. C1D reduced the adverse effect of Cd, and various plant growth parameters were significantly increased by bacterial treatment. Al Azad et al. (2020) reported that *B. cereus* had phenomenal bioaccumulation and metal-tolerant properties and it can clearly be manipulated regarding bioremediation purposes. Jan et al. (2019) reported that

inoculation of rice by *B. cereus* under Cd^{2+} treatments enhanced plant growth, biomass production, and uptake of micronutrients. They said that *B. cereus* has the ability to alleviate Cd toxicity and increased phytoremediation efficiency of rice seedling under Cd stress. Li et al. (2019) reported that *Rhizobium pusense* KG2 had a minimal lethal concentration of 120 mg L^{-1} for Cd^{2+}. In pot soils containing 50 and 100 mg kg^{-1} of Cd^{2+}, strain KG2 caused a 45.9 and 35.3% decrease in soybean root Cd content, respectively. Meanwhile, KG2 improved the root and shoot length, nitrogen content, and biomass of soybean plants. Thus, it is concluded that plant inoculation by bacterial species can promote the effectiveness and efficiency of phytoremediation through growth enhancement and protection of plants against heavy metals.

In all treatments, the bioaccumulation factor of Pb was less than one, but that of the Cd was more than one (Fig. 5a). The translocation factors of Pb and Cd were also less than one (Fig. 5b). Ndeda and Manohar (2014) and Balabanova et al. (2015) reported that cadmium has the highest bioaccumulation factor among heavy metals. Similar results for translocation factors of Cd and Pb in cabbage grown in contaminated soils have been reported by other researchers (Hara and Sonoda 1979; Pandey and Sharma 2002; Xian 1989). It has been shown that the concentration of Cd in plants generally decreases in the order: root > leaves > fruits > seeds (Sarma et al. 2006), indicating more accumulation of this metal in the root than in the aerial parts. TF, also called shoot-root quotient, explains the ability of a plant species to translocate heavy metals from roots to shoots and leaves and plants with the TF values > 1 are considered suitable for phytoextraction whereas those with the TF values < 1 are appropriate for the phytostabilization programs (Shi et al. 2011; Wu et al. 2011). Based on the results of this study, the translocation factor of Cd in ornamental cabbage was less than one, but the bioaccumulation factor of Cd was more than one. Considering the higher concentration of the Cd in the roots than in the aerial parts, this plant can be classified as cadmium excluder plant. (Al Azad et al. 2020; Balabanova et al. 2015; Jan et al. 2019; Li et al. 2019; Ndeda and Manohar 2014; Sharma et al. 2020; Wang et al. 2020)

Conclusions

In heavy metal-polluted soils, microbial species have developed different resistance mechanisms to adapt themselves to the stress conditions caused by these metals. According to the results of this study, the long-term effects of pollutants have led to the emergence of resistant bacteria to heavy metals (Pb and Cd) in the study areas. Regarding the results of this study, very distinct behaviors were observed among microbial strains isolated from heavy metal-contaminated soils. Heavy metal-resistant microbial strains which produce IAA and HCN and solubilize phosphate and potassium could be utilized for immobilization and detoxification of heavy metals in contaminated soils and to intensify the phytoremediation process. On the other hand, ornamental cabbage is usually planted from early autumn until late winter. Since most of the plants used for phytoremediation cannot be grown during this time, ornamental cabbage could be as new plant hyperaccumulator and has potential for phytoremediation and utilized during autumn and winter in urban areas, especially around the factories with high levels of Pb and Cd.

Acknowledgments The authors would like to thank the Soil Science Department and Plant Protection Department of the University of Zanjan for providing facilities to perform this research.

Funding information The Soil Science Department and Plant Protection Department of the University of Zanjan provided financial supports.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

Adriano DC (2001) Trace elements in terrestrial environments: biogeochemistry, bioavailability and risks of metals, 2nd edn. Springer, New York

Ahemad M, Kibret M (2014) Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. J King Saud Univ Sci 26:1–20

Ahmad I, Akhtar MJ, Asghar HN, Ghafoor U, Shahid M (2016) Differential effects of plant growth-promoting rhizobacteria on maize growth and cadmium uptake. J Plant Growth Regul 35:303–315

Al Azad S, Farjana M, Mazumder B, Abdullah-Al-Mamun M, Haque AI (2020) Molecular identification of a *Bacillus cereus* strain from Murrah buffalo milk showed *in vitro* bioremediation properties on selective heavy metals. J Adv Vet Anim Res 7:62–68

Allen SE, Grimshaw HM, Rowland AP (1986) Chemical analysis. In: Moore PD, Chapman SB (eds) Methods in plant ecology. Blackwell, Scientific Publication, Oxford, London, pp 285–344

Aloni R, Aloni E, Langhans M, Ullrich CI (2006) Role of cytokinin and auxin in shaping root architecture: regulating vascular differentiation, lateral root initiation, root apical dominance and root gravitropism. Ann Bot 97:883–893

Alström S, Burns RG (1989) Cyanide production by rhizobacteria as a possible mechanism of plant growth inhibition. Biol Fertil Soils 7:232–238

Babalola OO, Glick BR (2012) Indigenous african agriculture and plant associated microbes: current practice and future transgenic prospects. Sci Res Essays 7:2431–2439

Balabanova B, Stafilov T, Bačeva K (2015) Bioavailability and bioaccumulation characterization of essential and heavy metals contents in R. *acetosa*, S. *oleracea* and U. *dioica* from copper polluted and referent areas. J Environ Health Sci Eng 13:1–13

Banerjee G, Pandey S, Ray AK, Kumar R (2015) Bioremediation of heavy metals by a novel bacterial strain *Enterobacter cloacae* and its antioxidant enzyme activity, flocculant production, and protein

expression in presence of lead, cadmium, and nickel. Water Air Soil Pollut 226:1–9

Bolan NS, Adriano DC, Duraisamy P, Mani A (2003) Immobilization and phytoavailability of cadmium in variable charge soils. II. Effect of lime addition. Plant Soil 251:187–198

Boyd RS, Barbour MG (1986) Relative salt tolerance of Cakile edentula (*Brassicaceae*) from lacustrine and marine beaches. Am J Bot 73: 236–241

Castagno LN, Estrella MJ, Sannazzaro AI, Grassano AE, Ruiz OA (2011) Phosphate- solubilization mechanism and *in vitro* plant growth promotion activity mediated by *Pantoea eucalypti* isolated from *Lotus tenuis* rhizosphere in the Salado River Basin (Argentina). J Appl Microbiol 110:1151–1165

Chakraborty U, Chakraborty BN, Basnet M, Chakraborty AP (2009) Evaluation of *Ochrobactrum anthropi* TRS-2 and its talc based formulation for enhancement of growth of tea plants and management of brown root rot disease. J Appl Microbiol 107:625–634

Chen X, Shi J, Chen Y, Xu X, Xu S, Wang Y (2006a) Tolerance and biosorption of copper and zinc by *Pseudomonas putida* CZ1 isolated from metal-polluted soil. Can J Microbiol 52:308–316

Chen YP, Rekha PD, Arun AB, Shen FT, Lai WA, Young CC (2006b) Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Appl Soil Ecol 34:33–41

Chen Y, Chao Y, Li Y, Lin Q, Bai J, Tang L, Wang S, Ying R, Qiu R (2016) Survival strategies of the plant-associated bacterium *Enterobacter* sp. strain EG16 under cadmium stress. Appl Environ Microbiol 82:1734–1744

Chhikara S, Dhankhar R (2008) Biosorption of Cr (VI) ions from electroplating industrial effluent using immobilized *Aspergillus niger* biomass. J Environ Biol 29:773–778

Dhull S, Gera R, Sheoran HS, Kakar R (2018) Phosphate solubilization activity of *Rhizobial* strains isolated from root nodule of *Cluster bean* plant native to Indian soils. Int J Curr Microbiol App Sci 7: 255–266

Egamberdieva D, Kamilova F, Validov S, Gafurova L, Kucharova Z, Lugtenberg B (2008) High incidence of plant growth-stimulating bacteria associated with the rhizosphere of wheat grown on salinated soil in Uzbekistan. Environ Microbiol 10:1–9

Flora G, Gupta D, Tiwari A (2012) Toxicity of lead: a review with recent updates. Interdiscip Toxicol 5:47–58

Gandhi A, Muralidharan G, Sudhakar E (2014) Isolation and identification of elite phosphate solubilizing bacteria from soil under paddy cultivation. Int Lett Nat Sci 16:62–68

Golubev SN, Muratova AY, Wittenmayer L, Bondarenkova AD, Hirche F, Matora LY, Merbach W, Turkovskaya OV (2011) Rhizosphere indole-3-acetic acid as a mediator in the Sorghum bicolor–phenanthrene–*Sinorhizobium meliloti* interactions. Plant Physiol Biochem 49:600–608

Goswami D, Vaghela H, Parmar S, Dhandhukia P, Thakker JN (2013) Plant growth promoting potentials of *Pseudomonas spp.* strain OG isolated from marine water. J Plant Interact 8:281–290

Gupta R, Kumar T, Mittal A (2016) Isolation, identification and characterization of heavy metal resistant from soil of an iron industry, Haryana (India). Int J Pharm Sci Res 7:1308

Hao X, Xie P, Johnstone L, Miller SJ, Rensing C, Wei G (2012) Genome sequence and mutational analysis of plant growth promoting bacterium *Agrobacterium tumefaciens* CCNWGS0286 isolated from a zinc-lead mine tailing. Appl Environ Microbiol 78:5384–5394

Haq R, Zaidi SK, Shakoori AR (1999) Cadmium resistant *Enterobacter cloacae and Klebsiella* sp. isolated from industrial effluents and their possible role in cadmium detoxification. World J Microbiol Biotechnol 15:283–290

Hara T, Sonoda Y (1979) Comparison of the toxicity of heavy metals to cabbage growth. Plant Soil 51:127–133

Hauka FIA, Afify AH, El-Sawah AM (2017) Efficiency evaluation of some rhizobacteria isolated from Egyptian soils, *in vitro* as biofertilizers. J Agric Chem Biotechnol 8:231–235

Helmke PA, Spark DL (1996) Potassium. In: Sparks D et al (eds) Method of soil analysis: chemical methods, Soil science Society of America, Inc, vol 3. American Society of Agronomy, Inc, Madison, Wisconsin, pp 551–574

Hooda V (2007) Phytoremediation of toxic metals from soil and waste water. J Environ Biol 28:367

Hseu ZY (2004) Evaluating heavy metal contents in nine composts using four digestion methods. Bioresour Technol 95:53–59

Istina IN, Widiastuti H, Joy B, Antralina M (2015) Phosphate-solubilizing microbe from Saprists peat soil and their potency to enhance oil palm growth and P uptake. Procedia Food Sci 3:426–435

Jahan M, Mahallati MN, Amiri MB, Ehyayi HR (2013) Radiation absorption and use efficiency of sesame as affected by biofertilizers inoculation in a low input cropping system. Ind Crop Prod 43:606–611

Jamaluddin H, Zaki DM, Ibrahim Z (2012) Isolation of metal tolerant bacteria from polluted wastewater. Pertanika J Trop Agric Sci 35: 647–662

Jan M, Shah G, Masood S, Iqbal Shinwari K, Hameed R, Rha E, Jamil M (2019) *Bacillus cereus* enhanced phytoremediation ability of rice seedlings under cadmium toxicity. BioMed Research International: 1–12

Jones JB Jr (2001) Laboratory guide for conducting soil tests and plant analysis. CRC press, Boca Raton, London, Washington

Karpagam T, Nagalakshmi PK (2014) Isolation and characterization of phosphate solubilizing microbes from agricultural soil. Int J Curr Microbiol App Sci 3:601–614

Kartik VP, Jinal HN, Amaresan N (2016) Characterization of cadmium-resistant bacteria for its potential in promoting plant growth and cadmium accumulation in *Sesbania bispinosa* root. Int J Phytorem 18:1061–1066

Khalid S, Shahid M, Niazi NK, Murtaza B, Bibi I, Dumat C (2017) A comparison of technologies for remediation of heavy metal contaminated soils. J Geochem Explor 182:247–268

Khan MS, Zaidi A, Wani PA, Oves M (2009) Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. Environ Chem Lett 7:1–19

Khan MS, Zaidi A, Ahmad E (2014) Mechanism of phosphate solubilization and physiological functions of phosphate-solubilizing microorganisms. In: Phosphate solubilizing microorganisms. Springer, Cham, pp 31–62

Khatun M, Bera P, Mitra D, Mandal A, Samanta A (2012) Estimation of heavy metal tolerance and antibiotic susceptibility of *Bacillus cereus* isolated from municipal solid waste. Int J Pharm Bio Sci 3:819–829

Kim SH, Wei CI (2007) Expression of AmpC β-lactamase in *Enterobacter cloacae* isolated from retail ground beef, cattle farm and processing facilities. J Appl Microbiol 103:400–408

Koga J, Syōono K, Ichikawa T, Adachi T (1994) Involvement of L-tryptophan aminotransferase in indole-3-acetic acid biosynthesis in *Enterobacter cloacae*. BBA Protein Struct Mol Enzymol 1209:241–247

Kumar KV, Singh N, Behl HM, Srivastava S (2008) Influence of plant growth promoting bacteria and its mutant on heavy metal toxicity in *Brassica juncea* grown in fly ash amended soil. Chemosphere 72: 678–683

Kumar A, Kumar A, Devi S, Patil S, Payal C, Negi S (2012) Isolation, screening and characterization of bacteria from Rhizospheric soils for different plant growth promotion (PGP) activities: an *in vitro* study. Recent Res Sci Technol 4

Kumar S, Bauddh K, Barman SC, Singh RP (2014) Amendments of microbial biofertilizers and organic substances reduces requirement

of urea and DAP with enhanced nutrient availability and productivity of wheat (*Triticum aestivum* L.). Ecol Eng 71:432–437

Kumar KV, Jaiprakash C, Chander MP, Reesu R, Maile A, Lall C (2016) Heavy metal tolerant nonendosymbiont plant growth promoting Rhizobacteria associated with the roots of evergreen shrubs *Casuarina equisetifolia*. Recent Res Sci Technol 8:30–35

Kumar V et al. (2015) Bioremediation of heavy metals by employing resistant microbial isolates from agricultural soil irrigated with Industrial Waste Water. Orient J Chem 31:357–361

Lal S, Kumar R, Ahmad S, Dixit VK, Berta G (2019) Exploring the survival tactics and plant growth promising traits of root-associated bacterial strains under Cd and Pb stress: a modelling based approach. Ecotoxicol Environ Saf 170:267–277

Li MS, Luo YP, Su ZY (2007) Heavy metal concentrations in soils and plant accumulation in a restored manganese mineland in Guangxi, South China. Environ Pollut Control 147:168–175

Li Y, Yu X, Cui Y, Tu W, Shen T, Yan M, Wei Y, Chen X, Wang Q, Chen Q (2019) The potential of cadmium ion-immobilized *Rhizobium pusense* KG 2 to prevent soybean root from absorbing cadmium in cadmium-contaminated soil. J Appl Microbiol 126: 919–930

Lindsay WL, Norvell WA (1978) Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil Sci Soc Am J 42:421–428

Loper J, Schroth M (1986) Influence of bacterial sources of indole-3-acetic acid on root elongation of sugar beet. Phytopathology 76: 386–389

Lloyd JR, Chesnes J, Glasauer S, Bunker DJ, Livens FR, Lovley DR (2002) Reduction of actinides and fission products by Fe (III)-reducing bacteria. Geomicrobiol J 19:103–120

Mamta RP, Pathania V, Gulati A, Singh B, Bhanwra RK, Tewari R (2010) Stimulatory effect of phosphate-solubilizing bacteria on plant growth, stevioside and rebaudioside-A contents of *Stevia rebaudiana* Bertoni. Appl Soil Ecol 46:222–229

Mantelin S, Touraine B (2004) Plant growth-promoting bacteria and nitrate availability: impacts on root development and nitrate uptake. J Exp Bot 55:27–34

Marzan LW, Hossain M, Mina SA, Akter Y, Chowdhury AMMZ (2017) Isolation and biochemical characterization of heavy-metal resistant bacteria from tannery effluent in Chittagong city, Bangladesh: bioremediation viewpoint. Egypt J Aquat Res 43:65–74

Meena VS, Maurya BR, Verma JP, Aeron A, Kumar A, Kim K, Bajpai VK (2015) Potassium solubilizing rhizobacteria (KSR): isolation, identification, and K-release dynamics from waste mica. Ecol Eng 81:340–347

Mitra S, Pramanik K, Ghosh PK, Soren T, Sarkar A, Dey RS, Pandey S, Maiti TK (2018a) Characterization of Cd-resistant *Klebsiella michiganensis* MCC3089 and its potential for rice seedling growth promotion under Cd stress. Microbiol Res 210:12–25

Mitra S, Pramanik K, Sarkar A, Ghosh PK, Soren T, Maiti TK (2018b) Bioaccumulation of cadmium by *Enterobacter* sp. and enhancement of rice seedling growth under cadmium stress. Ecotoxicol Environ Saf 156:183–196

Muduli PR, Vardhan KV, Ganguly D, Abhilash KR, Balasubramanian T (2012) Heavy metal contamination and risk assessment in the marine environment of Arabian Sea, along the southwest coast of India. Am J Chem 2:191–208

Naik MM, Pandey A, Dubey SK (2012) Biological characterization of lead-enhanced exopolysaccharide produced by a lead resistant *Enterobacter cloacae* strain P2B. Biodegradation 23:775–783

Nath S, Deb B, Sharma I (2012) Isolation and characterization of cadmium and lead resistant bacteria. Global Advanc Res J Microb 1:194–198

Ndeda LA, Manohar S (2014) Bioconcentration factor and translocation ability of heavy metals within different habitats of hydrophytes in Nairobi Dam, Kenya. J Environ Sci, Toxicol Food 8:42–45

Olaniran AO, Balgobind A, Pillay B (2013) Bioavailability of heavy metals in soil: impact on microbial biodegradation of organic compounds and possible improvement strategies. Int J Mol Sci 14: 10197–10228

Olsen SR (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. United States Department of Agriculture, Washington

Olsen SR, Sommers LE (1982) Phosphorus. In: Page AL (ed) Methods of soil analysis part 2 chemical and microbiological properties. American Society of Agronomy, Soil Science Society of America, Madison, pp 403–430

Oyetibo GO, Ilori MO, Adebusoye SA, Obayori OS, Amund OO (2010) Bacteria with dual resistance to elevated concentrations of heavy metals and antibiotics in Nigerian contaminated systems. Environ Monit Assess 168:305–314

Pandey N, Sharma CP (2002) Effect of heavy metals Co^{2+}, Ni^{2+} and Cd^{2+} on growth and metabolism of cabbage. Plant Sci 163:753–758

Patten CL, Glick BR (2002) Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. Appl Environ Microbiol 68:3795–3801

Pilon-Smits E (2005) Phytoremediation. Annu Rev Plant Biol 56:15–39

Poole RK, Gadd GM (1989) Metal-microbe interactions. vol 26. Special Published for the Society for General Microbiology by IRL Press Oxford University

Poonguzhali S, Madhaiyan M, Sa T (2008) Isolation and identification of phosphate solubilizing bacteria from chinese cabbage and their effect on growth and phosphorus utilization of plants. J Microbiol Biotechnol 18:773–777

Pramanik K, Mitra S, Sarkar A, Soren T, Maiti TK (2017) Characterization of cadmium-resistant *Klebsiella pneumoniae* MCC 3091 promoted rice seedling growth by alleviating phytotoxicity of cadmium. Environ Sci Pollut Res 24:24419–24437

Pramanik K, Mitra S, Sarkar A, Maiti TK (2018a) Alleviation of phytotoxic effects of cadmium on rice seedlings by cadmium resistant PGPR strain *Enterobacter aerogenes* MCC 3092. J Hazard Mater 351:317–329

Pramanik K, Mitra S, Sarkar A, Soren T, Kanti Maiti T (2018b) Characterization of a Cd^{2+}-resistant plant growth promoting rhizobacterium (*Enterobacter* sp.) and its effects on rice seedling growth promotion under Cd2þ-stress in vitro. Agric Nat Resour 52:215–221

Prapagdee B, Khonsue N (2015) Bacterial-assisted cadmium phytoremediation by *Ocimum gratissimum* L. in polluted agricultural soil: a field trial experiment. Int J Environ Sci Technol 12:3843–3852

Premono ME, Moawad AM, Vlek PLG (1996) Effect of phosphate-solubilizing *Pseudomonas putida* on the growth of maize and its survival in the rhizosphere. Indonesian J Crop Sci 11:13–23

Raj DPRS, Linda R, Babyson RS (2014) Molecular characterization of phosphate solubilizing bacteria (PSB) and plant growth promoting rhizobacteria (PGPR) from pristine soil. Int J Innov Sci Eng Technol 1:317–324

Raja CE, Selvam GS, Omine K(2009) Isolation, identification and characterization of heavy metal resistant bacteria from sewage. In: Int Joint Symp on Geodisaster Prevention and Geoenvironment in Asia, JS-Fukuoka, pp 205–211

Rajawat MVS, Singh S, Saxena AK (2014) A new spectrophotometric method for quantification of potassium solubilized by bacterial cultures. Indian J Exp Biol 52:261–266

Rajkumar M, Ae N, Prasad MNV, Freitas H (2010) Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. Trends Biotechnol 28:142–149

Rodriguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339

Rohini B, Jayalakshmi S (2015) Bioremediation potential of *Bacillus cereus* against copper and other heavy metals. Int J Advanc Res Biol Sci 2:200–209

Roman-Ponce B et al (2017) Plant growth-promoting traits in rhizobacteria of heavy metal-resistant plants and their effects on *Brassica nigra* seed germination. Pedosphere 27:511–526

Ryu RJ, Patten CL (2008) Aromatic amino acid-dependent expression of indole-3-pyruvate decarboxylase is regulated by TyrR in *Enterobacter cloacae* UW5. J Bacteriol 190:7200–7208

Sangthong C, Setkit K, Prapagdee B (2016) Improvement of cadmium phytoremediation after soil inoculation with a cadmium-resistant *Micrococcus* sp. Environ Sci Pollut Res 23:756–764

Sarma M, Handique GK, Handique AK (2006) Toxic heavy metal stress in paddy: metal accumulation profile and development of a novel stress protein in seed. Indian J Plant Physiol 11:227–233

Schaad NW, Jones JB, Chun W (2001) Laboratory guide for the identification of plant pathogenic Bacteria. APS Press, American Phytopathological Society

Sevim A, Sevim E (2015) Plasmid mediated antibiotic and heavy metal resistance in *Bacillus* strains isolated from soils in Rize, Turkey. SDÜ Fen Bil Enst Der 19:133–141

Sharma RK, Barot K, Archana G (2020) Root colonization by heavy metal resistant *Enterobacter* and its influence on metal induced oxidative stress on *Cajanus cajan*. J Sci Food Agric 100:1532–1540

Sharp RG, Chen L, Davies WJ (2011) Inoculation of growing media with the rhizobacterium *Variovorax paradoxus* 5C-2 reduces unwanted stress responses in hardy ornamental species. Sci Hortic 129:804–811

Sheng XF, Xia JJ (2006) Improvement of rape (*Brassica napus*) plant growth and cadmium uptake by cadmium-resistant bacteria. Chemosphere 64:1036–1042

Shi X, Zhang X, Chen G, Chen Y, Wang L, Shan X (2011) Seedling growth and metal accumulation of selected woody species in copper and lead/zinc mine tailings. J Environ Sci 23:266–274

Silver S, Misra TK (1988) Plasmid-mediated heavy metal resistances. Annu Rev Microbiol 42:717–743

Singh SK, Tripathi VR, Jain RK, Vikram S, Garg SK (2010) An antibiotic, heavy metal resistant and halotolerant *Bacillus cereus* SIU1 and its thermoalkaline protease. Microb Cell Factories 9:1–7

Subba Rao NS (2016) Phosphate solubilization by soil microorganisms. In: Advances in Agricultural Microbiology. Elsevier, pp 295–305

Susilowati LE, Syekhfani S (2014) Characterization of phosphate solubilizing bacteria isolated from Pb contaminated soils and their potential for dissolving tricalcium phosphate. J Degrade Min Land Manage 1:57–62

Syed S, Chinthala P (2015) Heavy metal detoxification by different *Bacillus* species isolated from solar salterns. Scientifica 2015:1–8

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729

Thomas GW (1996) Soil pH and soil acidity. In: Methods of soil analysis part 3-chemical methods. pp 475-490

Tripti, Kumar V, Anshumali (2012) Phosphate solubilizing activity of some bacterial strains isolated from chemical pesticide exposed agriculture soil. Int J Adv Eng Res Dev 3:1–6

Verma T, Srinath T, Gadpayle RU, Ramteke PW, Hans RK, Garg SK (2001) Chromate tolerant bacteria isolated from tannery effluent. Bioresour Technol 78:31–35

Wang T, Wang S, Tang X, Fan X, Yang S, Yao L, Li Y, Han H (2020) Isolation of urease-producing bacteria and their effects on reducing Cd and Pb accumulation in lettuce (*Lactuca sativa* L.). Environ Sci Pollut Res 27:8707–8718

Wani PA, Khan MS, Zaidi A (2007) Effect of metal tolerant plant growth promoting *Bradyrhizobium* sp.(vigna) on growth, symbiosis, seed yield and metal uptake by greengram plants. Chemosphere 70:36–45

Wu H, Wu Q, Wu G, Gu Q, Wei L (2016) Cd-resistant strains of B. cereus S5 with endurance capacity and their capacities for cadmium removal from cadmium-polluted water. PLoS ONE 11:1–25

Wu SC, Cheung KC, Luo YM, Wong MH (2006) Effects of inoculation of plant growth-promoting rhizobacteria on metal uptake by *Brassica juncea*. Environ Pollut 140:124–135

Wu Q, Wang S, Thangavel P, Li Q, Zheng H, Bai J, Qiu R (2011) Phytostabilization potential of *Jatropha curcas* L. in polymetallic acid mine tailings. Int J Phytorem 13:788–804

Xian X (1989) Effect of chemical forms of cadmium, zinc, and lead in polluted soils on their uptake by cabbage plants. Plant Soil 113:257–264

Yang C, Cheng Y, Ma X, Zhu Y, Holman HY, Lin Z, Wang C (2007) Surface-mediated chromate-resistant mechanism of *Enterobacter cloacae* bacteria investigated by atomic force microscopy. Langmuir 23:4480–4485

Yang YG, Jin ZS, Bi XY, Li FL, Sun L, Liu J, Fu ZY (2009) Atmospheric deposition-carried Pb, Zn, and cd from a zinc smelter and their effect on soil microorganisms. Pedosphere 19:422–433

Yigit S, Altindag A (2006) Concentration of heavy metals in the food web of Lake Egirdir, Turkey. J Environ Biol 27:475–478

Zaidi A, Khan M, Ahemad M, Oves M (2009) Plant growth promotion by phosphate solubilizing bacteria. Acta Microbiol Immunol Hung 56:263–284

Zhao K, Penttinen P, Zhang X, Ao X, Liu M, Yu X, Chen Q (2014) Maize rhizosphere in Sichuan, China, hosts plant growth promoting *Burkholderia cepacia* with phosphate solubilizing and antifungal abilities. Microbiol Res 169:76–82