

Cadmium-tolerant bacteria induce metal stress tolerance in cereals

Iftikhar Ahmad · Muhammad Javed Akhtar ·
Zahir Ahmad Zahir · Muhammad Naveed ·
Birgit Mitter · Angela Sessitsch

Received: 4 March 2014 / Accepted: 6 May 2014
© Springer-Verlag Berlin Heidelberg 2014

Abstract Cadmium usually hampers plant growth, but bacterial inoculation may improve stress tolerance in plants to Cd by involving various mechanisms. The objective was to characterize and identify bacteria that improve plant growth under Cd stress and reduce Cd uptake. Cadmium-tolerant bacteria were isolated from rhizosphere soil, which was irrigated with tannery effluent, and six strains were selected as highly tolerant to Cd, showing minimum inhibitory concentration as 500 mg L^{-1} or 4.45 mmol L^{-1} . These strains were identified by 16S rRNA gene analysis and functional analysis in regard to plant growth promotion characteristics. To determine their effect on cereal growth under Cd stress, seeds were inoculated with these strains individually and grown in soil contaminated with three Cd levels (0, 40 and 80 mg kg^{-1}). Biomass production, relative water content (RWC), electrolyte leakage (ELL) and tissue Cd concentration were measured. Biomass of both cereals was inhibited strongly when exposed to Cd; however, bacterial inoculation significantly reduced the suppressive effect of Cd on cereal growth and physiology. The bacterial isolates belonged to the genera *Klebsiella*, *Stenotrophomonas*, *Bacillus* and *Serratia*. Maize was more sensitive than wheat to Cd. *Klebsiella* sp. strain CIK-502 had the most pronounced effects in promoting maize and wheat growth and lowering Cd uptake under Cd stress.

Keywords Metal stress · Cereal growth · Physiology · Bioremediation

Responsible editor: Elena Maestri

I. Ahmad · M. J. Akhtar · Z. A. Zahir
Institute of Soil and Environmental Sciences, University of
Agriculture, Faisalabad 38040, Pakistan

I. Ahmad (✉) · M. Naveed · B. Mitter · A. Sessitsch
Bioresources Unit, AIT Austrian Institute of Technology GmbH,
3430 Tulln, Austria
e-mail: iffises@yahoo.com

Introduction

Land degradation, salinity, waterlogging and pollution contribute to the deterioration of agricultural soils. In Pakistan textile, tannery, paper, oil and ghee industries produce high amounts of industrial effluents, which are discarded in the environment. The untreated industrial effluent causes pollution in urban areas and frequent usage of this effluent for irrigation of growing crops poses a threat to the local communities. About 20 million hectares of agricultural land worldwide is irrigated with industrial effluent while in Pakistan, it is about 0.03 million hectares (Iram et al. 2012). These effluents contain trace elements, especially Ni, Cd, Cr, As and Pb, which are deposited in soil and reach concentrations that are toxic to a variety of organisms. Trace elements are very mobile and therefore easily enter the food chain and cause harmful effects in humans (Malik 2004). Natural occurrence of Cd is $<1 \text{ mg kg}^{-1}$ in most of the soils (Alloway 1995), but the continuous application of industrial wastewater increased its value more than the permissible limits (Murtaza et al. 2008). It usually affects plant growth owing to the accumulation of the contaminant in the edible parts of crops. Its toxicity can cause chlorosis and instability to lipid membrane thus inhibits plant growth (Khan and Lee 2013).

Abiotic stresses (salinity, drought and metal) are the most devastating factors that hamper crop growth and yield (Naveed et al. 2014; Ahmad et al. 2012; Nadeem et al. 2010). However, plant-associated microorganisms may be applied to support plant growth under adverse conditions as they may fix atmospheric nitrogen, solubilize phosphate, produce various growth-promoting substances like phytohormones, aminocyclopropane-1-carboxylate deaminase (ACCD) or siderophores. These traits are particularly important for supporting plant growth under stress conditions. In particular, ACC deaminase ameliorates stress as this bacterial enzyme modulates ethylene production in plants (Belimov et al. 2005;

Glick 2003). Soil microorganisms may release chelating substances, which may acidify the soil by releasing protons and thus alter metal availability in soil solution (Smith and Read 1997; Lasat 2002). The siderophores are excreted by many plants and microbes and scavenge iron, which is then better available to the plant (Dimkpa et al. 2009; Reichman and Parker 2005). Karimzadeh et al. (2012) reported increased accumulation of Cd in *Thlaspi caerulescens* due to the application of DFO-B, a naturally produced siderophore by rhizosphere bacteria. Microbial siderophores were also shown to decrease the synthesis of free radicals, thus protecting microbial auxin from degradation resulting in increased plant growth and metal uptake (Dimkpa et al. 2009).

The common methods used for remediation of trace element-contaminated soil are either physical, chemical or biological. The latter treatment is termed phytoextraction and typically involves trace element-accumulating plants (Lasat 2002). The limiting factor in phytoextraction applications is the bioavailability of the contaminants, which is affected by microbial activities. Several reports have demonstrated that bacteria can either increase or decrease heavy metal bioavailability resulting in higher or lower trace element uptake in plants (Chen et al. 2013; Kuffner et al. 2008, 2010). Furthermore, plant growth-promoting rhizobacteria (PGPR) can enhance phytoextraction of heavy metals by supporting plant growth under stress conditions due to the mechanisms mentioned above (Sessitsch et al. 2013; Glick 2010; Vangronsveld et al. 2009).

In contrast to the above-mentioned process of phytoextraction, trace elements can be immobilized in soils leading to reduced uptake of the contaminants, which is termed as phytostabilization (Vangronsveld and Cunningham 1998). Plants having high tolerance to a trace element and high retention capacity in roots are essential for phytostabilization applications (Zhang et al. 2012). The tolerance of plants to metal stress can be increased by rigorous selection of the crop species; however, in addition, bacteria with immobilizing capacities may be applied to reduce contaminant uptake (Kuffner et al. 2010).

The objective of the present study was to select rhizosphere bacteria, which can tolerate high Cd concentrations and are able to improve the growth of wheat and maize under Cd stress conditions. The ability of the selected strains to reduce Cd uptake by plants was also determined.

Materials and methods

Isolation of bacteria

Bacteria were isolated from the rhizosphere of maize at cob formation irrigated with industrial effluent containing toxic metals, grown around a tannery in Kasur (31° 7' N, 74° 27' E),

Punjab, Pakistan. Total metal concentrations of tannery effluent and soil are listed in Table 1. Bacteria were plated in different dilutions on Luria Bertani (LB) agar plates having composition (g L⁻¹) as follows: bacterial tryptone 10, NaCl 10, yeast extract 5 and agar 15. Series of dilutions were made (10⁻¹ to 10⁻⁷). Plates were incubated at 28±1 °C for 72 h. Isolates representing different colony morphologies were purified, coded and stored at -20 °C for subsequent use.

Minimum inhibitory concentration (MIC)

The selected bacterial strains were grown on LB agar plates for 48 h at 28±1 °C. The colonies were picked from the formerly grown bacteria and streaked on the freshly LB agar plates supplemented with 20 mg Cd L⁻¹ or 0.178 mmol L⁻¹ as CdCl₂·H₂O. Successfully grown bacteria were further tested under various concentrations of Cd (50, 100, 200, 300, 400, 500 mg L⁻¹ or 0.445, 0.89, 1.78, 2.67, 3.56, 4.45 mmol L⁻¹) until bacterial proliferation was completely inhibited by the Cd. The Cd level that completely inhibited the growth of organisms was designated as MIC. The bacterial strains that were unable to grow at any Cd levels were discarded; only Cd-tolerating strains were further exposed to higher Cd concentrations. Strains having a MIC of 500 mg Cd L⁻¹ or 4.45 mmol L⁻¹ (in LB medium) were further selected.

Identification of bacteria by 16S rRNA gene-based analysis

Bacterial cultures grown on freshly prepared LB media and log phase bacterial cultures were used to isolate genomic DNA by bead beating using the UltraClean® Microbial DNA Isolation Kit (MOBIO Lab., USA) according to the manufacturer's instructions. Before PCR amplification, purity

Table 1 pH, EC and total metal concentration (aqua regia extractable) of soil and industrial effluent

	Units	Soil ^a	Effluent	Permissible limits	
				Soil ^b	Effluent ^c
pH	–	7.6±1.2	7.1±1.62	–	6–10
EC	dS m ⁻¹	1.98±0.32	5.98±1.23	–	–
Total Cd	mg kg ⁻¹	ND	0.34±0.01	0.8	0.1
Total Pb	mg kg ⁻¹	ND	3.61±0.37	85	0.5
Total Ni	mg kg ⁻¹	9.79±2.97	1.99±0.12	35	1.0
Total Cr	mg kg ⁻¹	ND	248±5.46	100	1.0
Total Zn	mg kg ⁻¹	36±1.04	1.58±0.05	140	5.0
Total Fe	mg kg ⁻¹	44±2.76	3.59±0.10	–	2.0

^a Soil sample collected from contaminated site Kasur, values are means±SE, ND not detected

^b Heavy metal permissible limits (Dutch standard, Iram et al. 2012)

^c The permissible limits according to National Environment Quality Standard of Pakistan for industrial effluent

and concentration of DNA were determined using Nanodrop (Thermo Scientific ND-1000, USA). A total of 25 μL PCR mixture contained 2 μL DNA template, 2.5 μL each of $10\times$ buffer, 2 mM dNTPs, 25 mM MgCl_2 , 1.5 μM primer 8f (5'-AGAGTTTGATCCTGGCTCAG-3') (Weisburg et al. 1991), 1.5 μM primer 1520r (5'-AAGGAGGTGATCCAGCCGCA-3') (Edwards et al. 1989) and 1 U of Taq Polymerase (Invitrogen, USA) and 10.3 μL sterile Milli-Q water. A thermocycler (BIORAD, USA) by setting the following program was used: 5 min of pre-heating at 95 $^\circ\text{C}$, 30 cycles of 30 sec of denaturation at 95 $^\circ\text{C}$, 1 min of primer annealing at 55 $^\circ\text{C}$, 2 min of elongation at 72 $^\circ\text{C}$ and 10 min of extension step at 72 $^\circ\text{C}$. To confirm the fragment size of 16S ribosomal RNA (rRNA) gene amplicons, PCR products were loaded on 1 % *w/v* agarose (Sigma, USA) gel in Tris-borate-EDTA (TBE) buffer (Sigma-Aldrich, USA) containing 0.125 $\mu\text{g mL}^{-1}$ ethidium bromide (Sigma, USA) for staining. Sequencing was performed with primer 1520r making use of the Sanger sequencing service of the company AGOWA (Berlin, Germany). Retrieved sequences were aligned using the *BioEdit* software and matched against nucleotide sequences present in GenBank using the BLASTn program on National Center for Biotechnology Information (NCBI) database. The nucleotide sequences were submitted to NCBI under the accession numbers KJ471474 to KJ471479.

Functional testing of the selected bacterial strains

The selected bacteria were characterized *in vitro* for various growth-promoting traits such as indole acetic acid (IAA), exopolysaccharide (EPS), siderophore, oxidase, catalase and ACC deaminase production.

IAA, EPS and siderophore production assays

IAA production was determined by colorimetric assay following the method of Sarwar et al. (1992). LB broth with and without L-tryptophane (1 g L^{-1}) was inoculated with the selected strains and incubated for 3 days at 28 ± 2 $^\circ\text{C}$ and shaking at 100 rpm. Three-day-old cultures were filtered through Whatman No. 2, and filtrates were used to determine IAA production. Standard calibration curve was established using pure IAA (Merck, Germany); intensities of standards and filtrates were determined by measuring absorption at a wavelength of 535 nm. EPS production was determined according to the method described by Nicolaus et al. (1999). One thousand-milliliter broth with the following composition was prepared: 10 g L^{-1} yeast extract, 3 g L^{-1} trisodium citrate, 7.5 g L^{-1} casamino acid, 2 g L^{-1} KCl, 20 g L^{-1} $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$, 0.36 mg L^{-1} $\text{MnCl}_2\cdot 4\text{H}_2\text{O}$, 50 mg L^{-1} $\text{FeSO}_4\cdot 7\text{H}_2\text{O}$ and 5 % NaCl (Nicolaus et al. 1999). Strains were cultured for 5 days at 150 rpm at room temperature and then centrifuged at 14,000 rpm for 10 min at 4 $^\circ\text{C}$ to collect supernatants. Then,

cold absolute ethanol (3-fold to bacterial culture) was added to the supernatants under continuous stirring. Strains were considered positive for EPS production if precipitates were formed. Siderophore production was analysed following chrome azurol sulphonate (CAS) assay as described by Schwyn and Neilands (1987). Bacteria were cultured on these plates, and the appearance of orange depletion zone around the bacterial colonies indicated siderophore production. The assays were repeated three times.

ACC deaminase, oxidase and catalase assays

ACC deaminase activity was tested on the minimal medium described by Brown and Dilworth (1975) containing 0.7 g ACC L^{-1} as sole nitrogen source. To avoid potential contamination with ammonium during autoclaving, nutrient stock solutions were sterile-filtered and agarose was sterilized by 20 min of boiling. Minimal medium without nitrogen was used as negative control; positive control contained 0.7 g $\text{NH}_4\text{Cl L}^{-1}$. Plates were incubated for 2 weeks at room temperature. Oxidase assay was performed using the Kovács oxidase reagent (Kovács 1956), i.e. 1 % tetra-methyl-p-phenylenediamine dihydrochloride (TMPD). Freshly prepared bacterial culture was rubbed on filter paper already dipped in TMPD. If a bacterial colony turned to dark blue color, then it was considered positive for oxidase activity (Kovács 1956). For catalase assay, overnight-proliferated bacterial cultures were spread on a glass slide and one drop of 3 % H_2O_2 was added. The formation of bubbles within 5 to 10 s indicated the catalase activity. The assays were repeated three times.

Plant experiment

Based on the screenings described above, six bacterial strains were selected and used in tests to further screen them for their plant growth-promotion potential. Cadmium levels (40 and 80 mg kg^{-1}) were developed in sand by dissolving required quantity of salt ($\text{CdCl}_2\cdot\text{H}_2\text{O}$) in deionized water and left for 2 weeks. In the case of non-contaminated control, sand was flooded with deionized water and left for same period of time. Then, these were sterilized at 121 $^\circ\text{C}$ for 20 min and used to grow plants. Cd-sensitive cultivars of wheat (Inqlab-91 obtained from Directorate of Wheat, Ayub Agriculture Research Institute, Faisalabad, Pakistan) and maize (Pioneer hybrid 3068 supplied by the Pioneer Seed Company, Sahiwal, Pakistan) were used for the screening. Seeds were surface sterilized with 5 % sodium hypochlorite (Sigma-Aldrich, Germany) for 5–10 min, and three washings with 95 % ethanol followed by five washings with sterilized deionized water. For each bacterial strain, inoculum was prepared in flasks using LB broth. Each flask containing 50 mL of broth was inoculated with a selected bacterial strain and incubated for

72 h under shaking (100 rpm) conditions at 28 ± 1 °C in a shaking incubator. Surface disinfected seeds were inoculated with sterilized peat mixed with 15 % sterilized sugar solution [1:1 w/w inoculum (10^8 – 10^9 cfu mL⁻¹) to peat ratio]. For the uninoculated control treatment, seeds were coated with sterilized peat treated with sterilized broth containing no bacterial culture. Seeds were sown in jars containing sterilized sand, and 50 mL of sterilized Hoagland solution (half strength) to each jar was provided as and when necessary (Nadeem et al. 2010). Light and dark period was adjusted at 10 and 14 h, respectively, a temperature of 25 ± 2 °C and light intensity of $275 \mu\text{mol m}^{-2} \text{s}^{-1}$ was maintained during the whole growth period. In total, six selected strains along with the uninoculated control and three Cd levels were allocated in a completely randomized design in two factor factorial arrangement (CRD-factorial), and each treatment was replicated three times. Crops were harvested after 25 days, and root and shoot biomass was determined. Furthermore, the parameters described below were determined.

Relative water content (RWC)

Relative water content of maize and wheat leaves was determined according to the method of Mayak et al. (2004). After weighing, fresh leaves were dipped overnight in water. Then, leaves were weighed again to get fully turgid weight, and afterwards, they were placed in an oven at 70 ± 2 °C to measure the oven dry weight.

$$\text{Relative water content(RWC)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fully turgid weight} - \text{Dry weight}} \times 100$$

Electrolyte leakage (ELL)

Maize and wheat leaves were cut into uniform discs with the help of a sharp cork borer. Discs were placed in test tubes having 10-mL distilled water and shaken in a shaking incubator for 4 h at 150 rpm. The electrical conductivity of their leaf water was measured before and after autoclaving at 121 °C for 20 min. Electrolyte leakage was determined by using the following formula:

$$\text{Electrolyte leakage(ELL)} = \frac{\text{EC before autoclavation}}{\text{EC after autoclavation}} \times 100$$

Cd concentration in plant tissues

Cd concentration of wheat and maize leaf tissues was determined by first drying all samples in an oven at 70 °C for 24 h. Then, plant samples were grinded, weighed and digested with 3 mL nitric (HNO₃) and 1 mL perchloric (HClO₄) acid (3:1 ratio v/v). Afterwards, samples were heated on a hot plate at

350 °C until dense white fumes appeared. The contents of these flasks were cooled, filtered and stored in plastic bottles for further determinations on atomic absorption spectrophotometer (PerkinElmer, 100 Analyst, Waltham, USA) (Yang et al. 2009). Translocation and bioaccumulation of Cd were measured by using the following formulas:

$$\text{Translocation factor(TF)} = \frac{\text{Cd concentration in shoot}}{\text{Cd concentration in root}}$$

$$\text{Bioaccumulation factor(BAF)} = \frac{\text{Cd concentration in shoot}}{\text{Cd concentration in soil}}$$

Statistical analysis

Wheat and maize experiments were designed in completely randomized design in factorial arrangement. Each experiment was performed with two factors; six strains along with uninoculated control and Cd 0, 40 and 80 (7×3). Analysis of variance (ANOVA) was carried out to analyze data using the software STATISTIX v8.1. Treatment means were compared by using post hoc HSD Tukey test at a 5 % significance level.

Results

Isolation of rhizosphere bacteria, identification and MIC to Cd

Thirty bacterial isolates showing tolerance to Cd concentrations ranging from 20 to 400 mg L⁻¹ or 0.178 to 3.56 mmol L⁻¹ were isolated from the rhizosphere of maize. Six strains (CIK-502, CIK-512, CIK-515, CIK-516, CIK-517Y and CIK-524) which showed a MIC of at least 500 mg Cd L⁻¹ or 4.45 mmol Cd L⁻¹ were selected for further testing.

Bacterial isolates were identified based on the comparison of partial and nearly full length 16S rRNA genes (700 to 1,500 bp, Table 2) with those of known bacteria. The strains were assigned to the *Bacillaceae* (CIK-512, CIK-515 and CIK-516), *Xanthomonadaceae* (CIK-517Y) and *Enterobacteriaceae* (CIK-502, CIK-524) (Table 2).

Characterization of bacterial isolates

The six selected strains were assessed for their plant growth-promotion potential (Table 2). All strains were able to produce IAA (although in different amounts) and EPS, whereas CIK-502, CIK-512, CIK-515, CIK-517Y and CIK-524 showed catalase activity. Three isolates CIK-502, CIK-512 and CIK-524 showed oxidase activity, whereas CIK-502, CIK-512, CIK-516, CIK-517Y and CIK-524 exhibited ACC deaminase activity. Siderophore activity was observed only in two isolates CIK-502 and CIK-524. Bacterial strain CIK-502 showed

Table 2 Taxonomy and plant growth-promoting characteristics of selected bacterial strains

Strain name	Closest match on the basis of 16S rRNA [accession number]	Bp length	Max score	Identity (%)	Tentative phylogenetic group	EPS	ACCD	SID	CAT	OXI	IAA ($\mu\text{g mL}^{-1}$)	
											Without L-tryp.	With L-tryp.
CIK-502	<i>Klebsiella</i> sp. [JQ838151]	1,419	1,164	100	<i>Enterobacteriaceae</i>	++	++	+	++	++	14.3±1.09	89.5±10.64
CIK-512	<i>Bacillus</i> sp. [HF584925]	1,095	1,295	99	<i>Bacillaceae</i>	+	+	-	++	++	16.9±1.04	26.5±1.58
CIK-515	<i>Bacillus</i> sp. [JX517219]	1,417	1,103	100	<i>Bacillaceae</i>	+	-	-	++	-	16±0.99	156.8±4.69
CIK-516	<i>Bacillus</i> sp. [FJ827134]	789	736	97	<i>Bacillaceae</i>	++	+	-	-	-	21.7±0.83	11.8±0.87
CIK-517Y	<i>Stenotrophomonas</i> sp. [JX505430]	1,363	1,072	99	<i>Xanthomonadaceae</i>	++	+	-	++	-	19.1±0.89	17±0.71
CIK-524	<i>Serratia</i> sp. [JF494817]	1,398	1,214	100	<i>Enterobacteriaceae</i>	++	++	+	++	++	18.6±1.82	48.6±1.35

EPS exopolysaccharide, ACCD aminocyclopropane-1-carboxylate deaminase, SID siderophore, CAT catalase, OXI oxidase, IAA indole acetic acid (values presented as means of three replicates±SD), L-tryp L-tryptophan

+ weak, ++ strong activity

multiple characters; it strongly produced EPS, showed siderophore, oxidase and catalase activity and was also able to produce ample amounts of IAA in the absence and presence of L-tryptophan. Strain CIK-515 showed maximum IAA production ($156.8 \mu\text{g mL}^{-1}$) in the presence of the auxin precursor L-tryptophan. Some bacterial strains (CIK-502, CIK-512, CIK-515 and CIK-524) showed enhanced IAA production in the presence of L-tryptophan, whereas others (CIK-516 and CIK-517Y) reduced IAA production under these conditions.

Effect on growth, physiology and Cd concentration of wheat

Biomass production

The exogenous application of Cd decreased shoot and root dry biomass of wheat (by 41 and 14 %, respectively, as compared to control) in soil contaminated with 80 mg Cd kg^{-1} soil. However, inoculated plants showed an increase in plant dry biomass. Only strain CIK-524 showed significantly increased shoot biomass production, whereas inoculation with strain CIK-502 led to significantly higher root biomass production under non-stressed conditions (Table 3). Under Cd exposure, only strain CIK-502 (Cd-80) resulted in significantly higher shoot biomass production. Treatments with application of strains CIK-502 and CIK-512 showed significantly increased root biomass production but only at the lower Cd exposure level (Cd-40) (Table 3). The significant interaction between Cd and bacterial strains for shoot and root biomass of wheat (Table 5) indicates that both, Cd and bacterial inoculation, influenced plant biomass.

Relative water content and electrolyte leakage

The maximum decrease in RWC of wheat (15 % as compared to control) was found in the treatment, which received no inoculation treatment and 80 mg Cd kg^{-1} soil (Fig. 1a). The significant effect of Cd application on RWC indicates the negative impact of Cd on RWC (Table 5). Although bacterial inoculation increased RWC of wheat in non-stressed and Cd-stressed conditions, no significant effects were found. Cadmium stress caused membrane permeability in wheat plants recorded in terms of ELL; thereby, a maximum increase (75 % as compared to respective control) was found in the treatment, which received no inoculation and 80 mg Cd kg^{-1} soil (Fig. 1c). Plants inoculated with bacterial strains CIK-502, CIK-512, CIK-515 and CIK-517Y showed a significantly lower decrease in ELL under higher Cd exposure (Cd-80) as compared to the respective control. Table 5 shows that the significant interactive effects of Cd and bacterial strains for ELL elucidates the positive influence of bacterial strains in decreasing ELL and increasing membrane stability.

Cd accumulation

Cd concentration in wheat shoot and root was significantly affected by exogenous application of Cd; however, inoculated bacterial strains significantly influenced Cd uptake in wheat (Table 5, Fig. 2a, c). Plants grown on Cd-contaminated soil showed significantly less Cd uptake in root upon inoculation of bacterial strains CIK-502 and CIK-512, whereas Cd uptake in shoots was only reduced by strain CIK-502.

Root to shoot translocation of Cd was increased with the inoculation of bacterial strains, but effects were not

Table 3 Effect of selected strains on shoot and root dry biomass (mg) of wheat under Cd stress

	Cd-0	% [†]	Cd-40	%	Cd-80	%
Shoot dry biomass						
Control	41±5.51 ^{b-d‡}	–	32±4 ^{d-g}	–	24±5.51 ^g	–
CIK-502	38±1.53 ^{b-e}	–5.7	34±5.29 ^{c-f}	6.3	38±0.58 ^{b-e}	57.6
CIK-512	43±0.58 ^{bc}	4.9	34±2.65 ^{c-f}	6.3	30±1 ^{c-g}	23.3
CIK-515	41±1.53 ^{b-d}	0.0	34±0.58 ^{c-g}	5.2	31±0.58 ^{c-g}	26.0
CIK-516	41±4.36 ^{b-d}	0.8	40±2.89 ^{b-d}	26.0	30±3.51 ^{e-g}	24.7
CIK-517Y	47±2.08 ^{ab}	16.4	35±1.53 ^{c-f}	10.4	34±5.69 ^{c-g}	38.4
CIK-524	54±0.58 ^a	32.0	32±2 ^{d-g}	0.0	26±1.15 ^{fg}	8.2
Root dry biomass						
Control	14±1 ^{de‡}	–	14±2 ^{de}	–	12±0.58 ^e	–
CIK-502	24±4 ^a	71.4	22±3.46 ^{a-c}	57.1	16±1.73 ^{c-e}	29.8
CIK-512	19±2.08 ^{a-d}	38.1	23±3.06 ^{ab}	66.7	13±1.53 ^e	2.7
CIK-515	17±2.08 ^{c-e}	19.0	17±2.65 ^{c-e}	21.4	17±1.53 ^{c-e}	35.2
CIK-516	14±1 ^{de}	0.0	15±1 ^{de}	7.1	16±0.58 ^{de}	27.1
CIK-517Y	17±2.08 ^{b-e}	23.8	19±3.46 ^{a-d}	35.7	15±2.52 ^{de}	24.4
CIK-524	13±1.15 ^e	–9.5	16±0.58 ^{de}	11.9	16±2 ^{c-e}	29.8

[†] Percent increase or decrease compared to their respective controls

[‡] Values are means of three replicates±SD, means sharing similar letters don't differ significantly

significant (Fig. 3a). Similarly, no significant effects were found due to inoculation on bioaccumulation of Cd (Fig. 3a). Table 5 summarizes significant effects

(Cd, bacterial strains) and shows that bacterial strains influenced Cd bioaccumulation to a greater extent than its translocation from root to shoot.

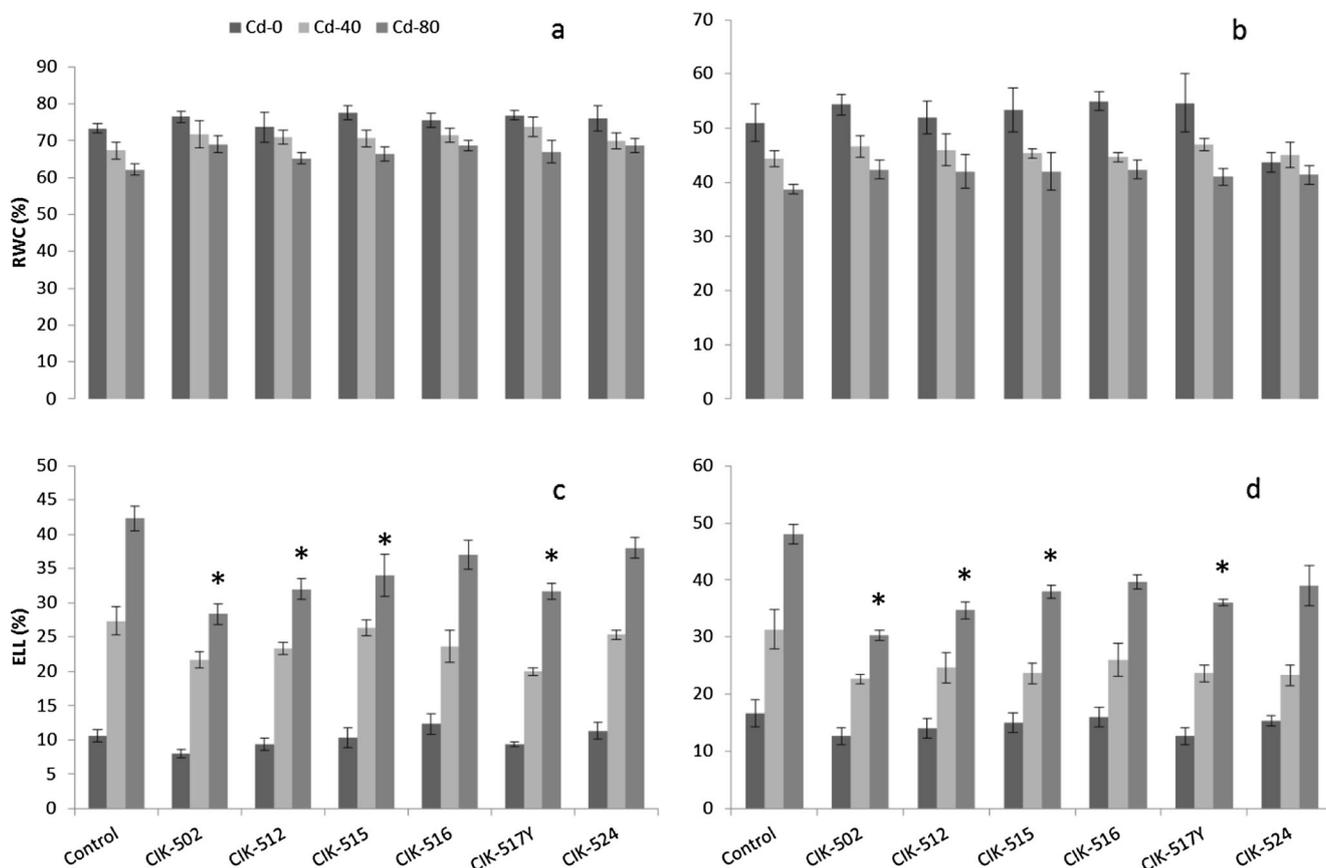


Fig. 1 Effect of bacterial inoculation on relative water content (RWC) and electrolyte leakage (ELL) of wheat (a, c) and maize (b, d) under Cd stress. Values are means of three replicates±SE. The asterisk showed

significant difference ($p < 0.05$) of treatment means as compared to their respective controls according to post hoc HSD Tukey test

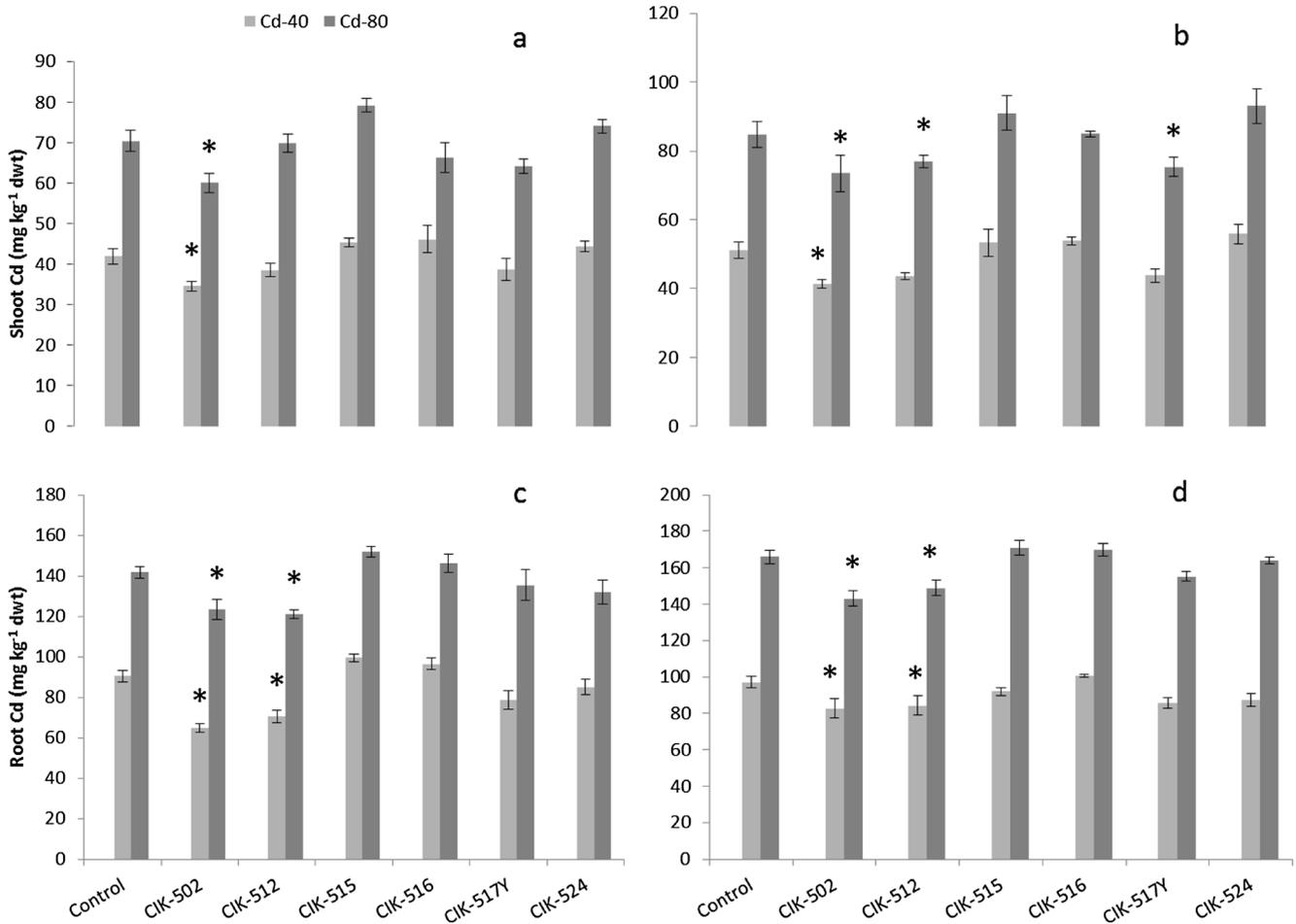


Fig. 2 Effect of bacterial inoculation on shoot and root Cd concentration of wheat (a, c) and maize (b, d) under Cd stress. Values are means of three replicates ± SE. The asterisk showed significant difference ($p < 0.05$) of

treatment means as compared to their respective controls according to post hoc HSD Tukey test

Effect on growth, physiology and Cd concentration of maize

Biomass production

Cd stress decreased shoot and root dry biomass of maize by 36 and 17 %, respectively, in soil contaminated with 80 mg Cd kg⁻¹ soil as compared to control (Table 4). Under non-stressed conditions, strains CIK-502 and CIK-517Y promoted shoot and root biomass production, whereas strain CIK-512 promoted only root growth. All strains, except strain CIK-524, led to significantly higher shoot biomass production at the higher level of Cd exposure, whereas only inoculation with strain CIK-517Y showed increased shoot biomass production at the application of 40 mg Cd kg⁻¹ soil (Table 4). Under the same Cd exposure, most strains (CIK-502, CIK-512, CIK-517Y and CIK-524) led to significantly higher root biomass production; however, at the application of 80 mg Cd kg⁻¹ soil, only the strain CIK-502 showed significant effects (Table 4). The positive effect of the interaction between Cd and bacterial inoculation was

significant, whereas no significant interactive effect was found on root biomass (Table 5).

Relative water content and electrolyte leakage

RWC of maize was significantly decreased under Cd-stressed conditions, but the inoculation with bacterial strains did not have a significant effect (Table 5, Fig. 1b). Cadmium stress caused almost 3-fold increase in ELL of stressed maize (80 mg Cd kg⁻¹ soil) as compared to the non-stressed control. Table 5 shows significantly increased ELL in maize leaves in response to exogenous application of Cd, whereas bacterial inoculation significantly decreased ELL. All strains except strains CIK-516 and CIK-524 significantly decreased ELL under high Cd exposure (80 mg Cd kg⁻¹ soil) (Fig. 1d).

Cd accumulation

Cd uptake in both maize tissues significantly decreased upon inoculation with strains CIK-502 and CIK-512 at least at one

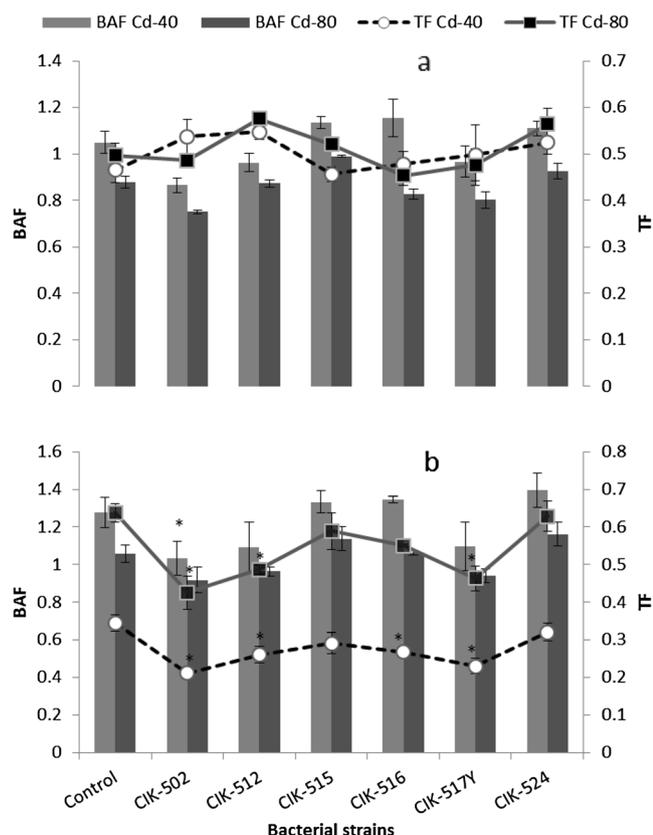


Fig. 3 Effect of bacterial inoculation on Cd translocation (TF) and bioaccumulation factor (BAF) of wheat (a) and maize (b) under Cd stress. Values are means of three replicates \pm SE. The asterisk showed significant difference ($p < 0.05$) of treatment means as compared to their respective controls according to post hoc HSD Tukey test

level of Cd exposure (Fig. 2b, d). Strain CIK-517Y only led to decreased Cd accumulation in shoot tissues upon the application of 80 mg Cd kg⁻¹ soil. The significant interaction between Cd and bacterial strains for Cd uptake in root and shoot (Table 5) shows that the bacterial inoculation influenced Cd uptake.

The translocation of Cd from root to shoot decreased due to the inoculation of most bacterial strains, under at least at one level of Cd exposure (Table 5, Fig. 3b), with strain CIK-502 having the most pronounced effect. Bioaccumulation was only significantly affected by strain CIK-502 (Table 5, Fig. 3b).

Discussion

Plants and plant-associated bacteria inhabiting extreme environments (such as Cd-contaminated soils) are likely to be more tolerant to high level of metals. Despite the fact that many bacteria are not accessible by cultivation on standard nutrient media, our intention was to get access to rhizosphere bacterial strains, which are able to tolerate high Cd

concentrations and potentially can be applied to reduce Cd accumulation in plants. In the present study, bacteria isolated from rhizosphere of maize were enriched on media supplemented with Cd. Out of 30 isolates, 73 % were grown on LB media supplemented with 50 mg Cd L⁻¹ or 0.445 mmol Cd L⁻¹ whereas 20 % showed a MIC of 500 mg Cd L⁻¹ or 4.45 mmol Cd L⁻¹. Cd inhibited bacterial growth at first exposure, but the successive Cd levels did not affect bacterial proliferation. Similar results were reported earlier by Prapagdee and Watcharamusik (2009), who showed ten times higher tolerance to CdCl₂ in Cd-induced cells than in non-induced cells. The tolerance to higher Cd concentration achieved by these strains may be due to various tolerance mechanisms including intracellular/extracellular sequestration, exclusion, detoxification and ATP-mediated efflux (Gadd 2004; Schwager et al. 2012).

The rhizosphere is a hot spot of bacterial abundance (Morgan et al. 2005), and many rhizosphere bacteria have beneficial activities for plant growth and health. In the present study, bacterial isolates showed in vitro ability to produce IAA, ACC deaminase and EPS; however, whether these traits are also active in the field depends on the survival competency of any inoculant strain in the environment and its ability to colonize roots of its host plant. The production of hormones by the inoculated microbial strains is one of the most important mechanisms to improve plant growth. It is well known that bacteria may synthesize auxin in the rhizosphere from its precursor tryptophan, which is responsible for the improvement of plant root growth (Hussain et al. 2014; Naveed et al. 2013). The positive changes in root growth induced by inoculated microorganisms may improve plant growth by increasing nutrient and water uptake. Recently, Dourado et al. (2013) demonstrated that *Burkholderia* sp. producing IAA promoted tomato growth in the presence of Cd. In addition to this, *Burkholderia* sp. showed high Cd tolerance and accumulation but decreased its uptake in tomato roots that may decrease Cd toxicity and attenuated tomato growth in Cd-contaminated soil. Similarly, in this study, all the bacterial strains showed in vitro tolerance to Cd but only three strains (CIK-502, CIK-512 and CIK-517Y) decreased Cd uptake in cereals and increased their biomass. Moreover, the bacterial strains having the ability to produce auxin were shown to increase dry biomass and yield of maize under normal and stress conditions (Naveed et al. 2014). Cassana et al. (2009) also reported some physiological changes in corn and soybean upon inoculation with the auxin producers *Azospirillum brasilense* strain Az39 and *Bradyrhizobium japonicum* strain E109, which were responsible for early growth promotion. However, as all strains showed similar in vitro IAA production without the amendment of tryptophan and as only few of them showed plant growth promotion with or without exposure to Cd, IAA production might not have contributed to the observed results.

Table 4 Effect of selected bacterial strains on shoot and root dry biomass (mg) of maize under Cd stress

	Cd-0	% [†]	Cd-40	%	Cd-80	%
Shoot dry biomass						
Control	91±3.51 ^{c-e‡}	–	70±2 ^{Fh}	–	58±3.79 ^h	–
CIK-502	112±8.19 ^{ab}	22.6	85±6.75 ^{d-g}	20.8	93±8.82 ^{b-e}	61.9
CIK-512	105±4.37 ^{a-d}	15.0	99±7.21 ^{a-f}	41.4	84±3 ^{e-g}	45.7
CIK-515	95±10.91 ^{b-e}	3.5	80±10 ^{e-g}	14.3	90±5 ^{c-f}	56.1
CIK-516	106±2.12 ^{a-c}	15.8	86±3.79 ^{c-g}	22.4	95±7.57 ^{b-c}	64.2
CIK-517Y	120±4.04 ^d	31.0	92±5.03 ^{b-c}	31.9	83±4.51 ^{c-g}	43.4
CIK-524	94±13.58 ^{b-e}	2.6	91±5.36 ^{gh}	30.2	66±5.13 ⁱ⁻¹	13.9
Root dry biomass						
Control	70±5 ^{b-e‡}	–	60±3.79 ^{de}	–	58±2.08 ^e	–
CIK-502	92±8.02 ^a	31.9	84±6 ^{a-c}	39.2	80±7.09 ^{a-d}	37.7
CIK-512	92±4.51 ^a	31.0	86±6.24 ^{a-c}	42.5	77±4.04 ^{a-e}	32.6
CIK-515	89±3.56 ^{ab}	27.0	68±10.24 ^{c-e}	12.0	72±8.33 ^{a-e}	24.0
CIK-516	85±8.23 ^{a-c}	20.8	59±3.51 ^e	–1.7	61±4.58 ^{de}	4.6
CIK-517Y	91±7.64 ^a	30.5	85±7.64 ^{a-c}	41.4	77±3.51 ^{a-e}	31.4
CIK-524	83±8.50 ^{a-c}	19.0	85±8.74 ^{a-c}	41.4	70±4.16 ^{b-e}	20.6

[†] Percent increase or decrease compared to their respective controls

[‡] Values are means of three replicates±SD, means sharing similar letters don't differ significantly

An additional important plant hormone, ethylene, which is produced under stress conditions usually leads to reduced root and shoot growth (Nadeem et al. 2010; Mayak et al. 2004). The degradation of ACC (i.e. the precursor of ethylene) by the bacterial ACC deaminase may rescue plant growth under stress conditions (Mayak et al. 2004). In this study, plant biomass decreased under Cd stress (Tables 3 and 4) probably due to production of ethylene. However, inoculation with bacterial strains with ACC deaminase activity may have resulted in reduced stress sensitivity due to inhibited ethylene synthesis. Almost all bacterial strains used in this study produced ACC deaminase except strain CIK-515 (Table 2), but only three strains CIK-502, CIK-512 and CIK-517Y had significant effects on studied parameters of both cereals (Tables 3 and 4). Particularly, under high Cd exposure, these strains performed very well. The rhizobacteria also produced EPS, which has been reported to improve soil aggregation and macropores resulting in an increased nutrient use efficiency and water uptake by inoculated plants (Alami et al. 2000). All strains tested in this study showed EPS production (Table 2), although only some promoted plant growth; however, it might be that under nutrient-poor conditions in natural soils, this trait is more relevant and inoculation of these strains increased root and shoot dry biomass of both cereals (maize and wheat, Tables 3 and 4). EPS-producing bacteria increased root and shoot growth of wheat under drought stress (Hussain et al. 2014); moreover, Vanderlinde et al. (2010) reported that rhizobia produce EPS which helps in the development of biofilm where they get protection from environmental anomalies and may help the plants by extracting more water and

nutrients. Exopolymer secreted by bacteria are also known to bind cations in soil (Gadd 2004); therefore, it might be involved in stabilization of Cd in soil.

Plants grown under stress conditions accumulate various molecules like glycine, betaine, proline and phenols thereby protect plants to metal stress (Kuffner et al. 2010; Farwell et al. 2007), salt stress (Rojas-Tapias et al. 2012; Nadeem et al. 2010), water and flooding stress (Naveed et al. 2014; Farwell et al. 2007). Plants produce many enzymatic and non-enzymatic antioxidants to scavenge reactive oxygen species (ROS) produced under stress. Many researchers have reported antioxidant production in response to Cd stress, for example, it increased peroxide contents in tomato (Dourado et al. 2013). ROS produced under metal stress are known to cause membrane damage and decrease water and chlorophyll contents in maize, but ROS are alleviated by enzymatic (catalase, ascorbate peroxidase, glutathione reductase and superoxide dismutase) and non-enzymatic (proline, glutathione, ascorbate and carotenoids) antioxidants (Ekmekci et al. 2009). In addition, membrane damage and water content have been reported to be good indicators of stress conditions (Mayak et al. 2004; Nadeem et al. 2010) which were chosen for this study. By using these parameters, it was observed that Cd stress decreased RWC of both cereals (maize relatively affected at greater extent, Fig. 1) as compared to the control treatment; however, inoculation with some strains improved, although not significantly, RWC in stressed and non-stressed conditions compared to the respective control. This is because plants may extract more water due to an increased plant root biomass and the development of numerous secondary roots (Dodd et al. 2010) upon bacterial inoculation.

Table 5 ANOVA shows the effect of cadmium-tolerant bacterial inoculation on growth, relative water content, electrolyte leakage and tissue Cd concentration of wheat and maize

	Wheat			Maize		
	Cd	Strains	Cd×S	Cd	Strains	Cd×S
Shoot dry biomass	96.66** ($p<0.01$)	4.11** ($p<0.01$)	6.91** ($p<0.01$)	64.4** ($p<0.01$)	17.65** ($p<0.01$)	5.02** ($p<0.01$)
Root dry biomass	12.09** ($p<0.01$)	13.32** ($p<0.01$)	4.64** ($p<0.01$)	29.85** ($p<0.01$)	16.78** ($p<0.01$)	1.8 ^{ns} ($p=0.08$)
Electrolyte leakage	461.32** ($p<0.01$)	9.25** ($p<0.01$)	2.06* ($p<0.05$)	281.43** ($p<0.01$)	8.90** ($p<0.01$)	1.57 ^{ns} ($p=0.14$)
Relative water content	26.20** ($p<0.01$)	1.75 ^{ns} ($p=0.13$)	0.31 ^{ns} ($p=0.98$)	30.43** ($p<0.01$)	1.27 ^{ns} ($p=0.29$)	0.76 ^{ns} ($p=0.69$)
Shoot Cd	2,519** ($p<0.01$)	9.66** ($p<0.01$)	3.94** ($p<0.01$)	2125** ($p<0.01$)	10.55** ($p<0.01$)	2.95** ($p<0.01$)
Root Cd	2,935** ($p<0.01$)	17.42** ($p<0.01$)	4.73** ($p<0.01$)	5300** ($p<0.01$)	11.15** ($p<0.01$)	4.03** ($p<0.01$)
Translocation factor	998** ($p<0.01$)	2.80** ($p<0.01$)	1.44 ^{ns} ($p=0.18$)	896** ($p<0.01$)	13.43** ($p<0.01$)	3.55** ($p<0.01$)
Bioaccumulation factor	1,825** ($p<0.01$)	8.43** ($p<0.01$)	3.34** ($p<0.01$)	1953** ($p<0.01$)	12.40** ($p<0.01$)	3.70** ($p<0.01$)

Asterisks show significant difference at ** 1 and * 5 % according to Tukey HSD comparison test

^{ns} non-significant at 1 and 5 % according to Tukey HSD comparison test

Cadmium stress induced ELL, which damaged plant, whether these were inoculated or uninoculated, and its severity increased with increased level of stress. The positive correlation between ELL and stress sensitivity has been reported earlier by Vardharajula et al. (2011), and bacterial inoculation has been shown to damage membranes in stressed plants (Naveed et al. 2014). Moreover, plant-associated bacteria may exudate osmolytes such as proline, glycine betaine and trehalose in response to stress, which along with other PGP activities, can possibly act synergistically with plant-produced osmolytes (Paul and Nair 2008). In this study, inoculated bacteria had catalase and oxidase activities, which may induce/activate plant defense enzymes such as catalase, peroxidase or phenolic compounds and may alleviate the oxidative damage elicited by metal stress. Hussain et al. (2014) reported that bacterial catalases and oxidases are important to protect plants from cellular damage under water stress conditions. Moreover, Guo et al. (2012) reported that bacterial catalases are required for plant pathogenesis. They confirmed that bacterial inoculation induced catalases in plants and helped the pathogen *Pseudomonas syringae* to survive in host plant by scavenging H₂O₂.

When both cereals were grown on sterilized Cd-contaminated soil and inoculated with the selected bacteria, strain CIK-502 (*Klebsiella* sp.) decreased root and shoot Cd uptake compared to uninoculated control (Fig. 2). This strain showed high tolerance to Cd (MIC value is 500 mg L⁻¹ or 4.45 mmol L⁻¹) and showed various PGP characteristics such as ACC deaminase, siderophore, IAA and EPS production. Our results revealed that *Klebsiella* sp. is more tolerant and able to decrease Cd uptake as compared to other bacteria suggesting that the inoculation of this strain would be beneficial particularly for Cd-sensitive plants. This study confirmed that Cd-tolerant *Klebsiella* sp. performed better when associated with Cd sensitive wheat and maize cultivars

Inqlab-91 and Pioneer hybrid 3068, respectively. The inoculation of high metal tolerant and accumulating bacteria is a promising approach to improve plant tolerance to metal stress (Dourado et al. 2013). Gadd (2004) reported that bacterial products especially polymer (EPS) can bind metal and decrease metal availability in the rhizosphere. Moreover, it has been shown that rhizosphere bacteria may mobilize or immobilize trace elements (Kuffner et al. 2010). Immobilization of Cd in the rhizosphere may lead to reduced uptake by plants (Ajaz haja mohideen et al. 2010; Kuffner et al. 2008, 2010; Madhaiyan et al. 2007). Mechanisms employed by strains leading to a lower Cd uptake in wheat or maize might include the production of EPS or siderophores leading to immobilization effects. However, we have to take into consideration that immobilization effects might be more relevant for plants grown under natural soils containing Cd contaminants for a long time. Alternatively, strains might have induced a plant response leading to reduced Cd uptake. Siderophore production by the *Klebsiella* sp. (CIK-502) in the present study seems to be involved in Cd immobilization in soil which resulted to an increased plant growth. Immobilization of Cd in soil upon bacterial inoculation is a very important property to increase plant growth particularly in Cd stress conditions as it was demonstrated by Dourado et al. (2013). They further claimed that Cd immobilization by bacterial inoculation leads to reduced toxicity, and thereby, the plant experiences better nutrient and water uptake resulting in healthy growth. This study shows stabilization of Cd resulting in growth improvement of cereals upon bacterial inoculation; however, a better mechanism is needed to document bacterial effects on plant growth keeping in view their mobilizing or stabilizing effects.

Bacterial isolates having PGP traits are useful in improving plant growth not only in normal soil but also in Cd-contaminated soil. These traits may also enhance metal extraction or stabilization in soil. The increased plant biomass,

RWC whereas lowered ELL conferred bacterial ability to alleviate Cd stress. The maize plant whether inoculated or uninoculated showed higher Cd accumulation as compared to wheat. Exploitation of the efficient isolates CIK-502 (*Klebsiella* sp.), CIK-512 (*Bacillus* sp.) and CIK-517Y (*Stenotrophomonas* sp.) for improving growth, yield and physiology of cereals in metal-contaminated soil may be beneficial to check their competency to combat metal stress in natural conditions.

Acknowledgments The first author acknowledges the “Higher Education Commission (HEC) of Pakistan” to provide financial support for this project under the Indigenous 5000 Fellowship Scheme Batch IV.

References

- Ahmad I, Akhtar MJ, Zahir ZA, Jamil A (2012) Effect of cadmium on seed germination and seedling growth of four wheat (*Triticum aestivum* L.) cultivars. Pak J Bot 44:1569–1574
- Ajaz haja mohideen R, Arasu VT, Narayanan KR, Hussain MIZ (2010) Bioremediation of heavy metal contaminated soil by the *Exiguobacterium* and accumulation of Cd, Ni, Zn and Cu from soil environment. Int J Biol Technol 1:94–101
- Alami Y, Achouk W, Marol C, Heulin T (2000) Rhizosphere soil aggregation and plant growth promotion of sunflowers by an exopolysaccharide-producing *Rhizobium* sp. strain isolated from sunflower roots. Appl Environ Microbiol 66:3393–3398
- Alloway BJ (1995) Heavy metals in soil. Blackie Academic and Professional, London
- Belimov AA, Hontzeas N, Safronova VI, Demchinskaya SV, Piluzza G, Bullitta S, Glick BR (2005) Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (*Brassica juncea* L. Czern.). Soil Biol Biochem 37:241–250
- Brown CM, Dilworth MJ (1975) Ammonia assimilation by rhizobium cultures and bacteroids. J Gen Microbiol 122:61–67
- Cassana F, Perriga D, Sgroya V, Masciarellia O, Pennab C, Lunaa V (2009) *Azospirillum brasilense* Az39 and *Bradyrhizobium japonicum* E 109, inoculated singly or in combination, promote seed germination and early seedling growth in corn (*Zea mays* L.) and soybean (*Glycine max* L.). Eur J Soil Biol 45:28–35
- Chen S, Chao L, Sun L, Sun T (2013) Effects of bacteria on cadmium bioaccumulation in the cadmium hyperaccumulator plant *Beta vulgaris* var. Cicla L. Int J Phytorem 15:477–487
- Dimkpa CO, Merten D, Svatos A, Buchel G, Kothe E (2009) Metal-induced oxidative stress impacting plant growth in contaminated soil is alleviated by microbial siderophores. Soil Biol Biochem 41:154–162
- Dodd IC, Belimov AA, Sobeih WY, Safronova VI, Grierson D, Davies WJ (2010) Will modifying plant ethylene status improve plant productivity in water limited environments? In: New directions for a diverse planet: Proc. Int. Crop Sci. Congr., 4th, Brisbane, Australia, 26 September - 1 October 2004, Available at www.cropscience.org.au/icsc2004/poster/1/3/4/510doddicref.htm (verified 10 January 2010). Regional Inst., Gosford, NSW, Australia
- Dourado MN, Martins PF, Quecine MC, Piotto FA, Souza LA, Franco MR, Tezotto T, Azevedo RA (2013) *Burkholderia* sp. SCMS54 reduces cadmium toxicity and promotes growth in tomato. Ann Appl Biol 163:494–507
- Edwards U, Rogall T, Blocker H, Emde M, Bottger EC (1989) Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. Nucleic Acids Res 17:7843–7853
- Ekmekci Y, Tanyolac D, Ayhan B (2009) A crop tolerating oxidative stress induced by excess lead: maize. Acta Physiol Plant 31:319–330
- Farwell AJ, Vesely S, Nero V, Rodriguez H, McCormack K, Shah S, Dixon DG, Glick BR (2007) Tolerance of transgenic canola plants (*Brassica napus*) amended with plant growth-promoting bacteria to flooding stress at a metal-contaminated field site. Environ Pollut 147:540–545
- Gadd GM (2004) Microbial influence on metal mobility and application for bioremediation. Geoderma 122:109–119
- Glick BR (2003) Phytoremediation: synergistic use of plants and bacteria to clean up the environment. Biotechnol Adv 21:383–393
- Glick BR (2010) Using soil bacteria to facilitate phytoremediation. Biotechnol Adv 28:367–374
- Guo M, Block A, Bryan CD, Becker DF, Alfano JR (2012) *Pseudomonas syringae* catalases are collectively required for plant pathogenesis. J Bacteriol 194:5054–5064
- Hussain MB, Zahir ZA, Asghar HN, Asghar M (2014) Can catalase and exopolysaccharides producing rhizobia ameliorate drought stress in wheat? Int J Agric Biol 16:3–13
- Iram S, Ahmad I, Akhtar S (2012) Distribution of heavy metals in peri-urban agricultural areas soils. J Chem Soc Pak 34:861–869
- Karimzadeh L, Heilmeyer H, Merkel BJ (2012) Effect of microbial siderophore DFO-B on Cd accumulation by *Thlaspi caerulescens* hyperaccumulator in the presence of zeolite. Chemosphere 88:683–687
- Khan AL, Lee IJ (2013) Endophytic *Penicillium funiculosum* LHL06 secretes gibberellin that reprograms *Glycine max* L. growth during copper stress. BMC Plant Biol 13:86–100
- Kovács N (1956) Identification of *Pseudomonas pyocyanea* by the oxidase reaction. Nature (London) 178:703
- Kuffner M, Puschenreiter M, Wieshammer G, Gorfer M, Sessitsch A (2008) Rhizosphere bacteria affect growth and metal uptake of heavy metal accumulating willows. Plant Soil 304:35–44
- Kuffner M, De Maria S, Puschenreiter M, Fallmann K, Wieshammer G, Gorfer M, Strauss J, Rivelli AR, Sessitsch A (2010) Culturable bacteria from Zn- and Cd-accumulating *Salix caprea* with differential effects on plant growth and heavy metal availability. J Appl Microbiol 108:1471–1484
- Lasat HA (2002) Phytoextraction of toxic metals: a review of biological mechanisms. J Environ Qual 31(1):109–120
- Madhaiyan M, Poonguzhali S, Sa T (2007) Metal tolerating methylotrophic bacteria reduces nickel and cadmium toxicity and promotes plant growth of tomato (*Lycopersicon esculentum* L.). Chemosphere 69:220–228
- Malik A (2004) Metal bioremediation through growing cells. Environ Int 30:261–278
- Mayak S, Tirosh T, Glick BR (2004) Plant growth-promoting bacteria that confer resistance in tomato and pepper plants to salt stress. Plant Sci 166:525–530
- Morgan JAW, Bending GD, White PJ (2005) Biological costs and benefits to plant-microbe interactions in the rhizosphere. J Exp Bot 56:1729–1739
- Murtaza G, Ghafoor A, Qadir MJ (2008) Accumulation and implications of cadmium, cobalt and manganese in soils and vegetables irrigated with city effluent. J Sci Food Agric 88(1):100–107
- Nadeem SM, Zahir ZA, Naveed M, Asghar HN, Arshad M (2010) Rhizobacteria capable of producing ACC-deaminase may mitigate salt stress in wheat. Soil Sci Soc Am J 74:533–542
- Naveed M, Mitter B, Yousaf S, Pastar M, Afzal M, Sessitsch A (2013) The endophyte *Enterobacter* sp. FD17: a maize growth enhancer selected based on rigorous testing of plant beneficial traits and colonization characteristics. Biol Fertil Soils. doi:10.1007/s00374-013-0854-y

- Naveed M, Mitter B, Reichenauer TG, Wieczorek K, Sessitsch A (2014) Increased drought stress resilience of maize through endophytic colonization by *Burkholderia phytofirmans* PsJN and *Enterobacter* sp. FD17. *Environ Exp Bot* 97:30–39
- Nicolaus B, Lama L, Esposito E, Manca MC, Improta R, Bellitti MR, Duckworth AW, Grant WD, Gambacorta A (1999) *Haloarcula* spp. able to biosynthesize exo- and endopolymers. *J Ind Microbiol Biotechnol* 23:489–496
- Paul D, Nair S (2008) Stress adaptations in a plant growth promoting rhizobacterium (PGPR) with increasing salinity in the coastal agricultural soils. *J Basic Microbiol* 48:378–384
- Prapagdee B, Watcharamusik A (2009) Adaptive and cross-protective responses against cadmium and zinc toxicity in cadmium-resistant bacterium isolated from a zinc mine. *Braz J Microbiol* 40:838–845
- Reichman SM, Parker DR (2005) Metal complexation by phytosiderophores in the rhizosphere. *Biogeochemistry of trace elements in the rhizosphere*. Elsevier, New Jersey
- Rojas-Tapias D, Moreno-Galván A, Pardo-Díaz S, Obando M, Rivera D, Bonilla R (2012) Effect of inoculation with plant growth-promoting bacteria (PGPB) on amelioration of saline stress in maize (*Zea mays*). *Appl Soil Ecol* 61:264–272
- Sarwar M, Arshad M, Martens DA, Frankenberger WT Jr (1992) Tryptophan dependent biosynthesis of auxins in soil. *Plant Soil* 147:207–215
- Schwager S, Lumjaktase P, Stockli M, Weisskopf L, Eberl L (2012) The genetic basis of cadmium resistance of *Burkholderia cenocepacia*. *Environ Microbiol Rep* 4:562–568
- Schwyn B, Neilands JB (1987) Universal chemical assay for the detection and determination of siderophores. *Anal Biochem* 160:47–56
- Sessitsch A, Kuffner M, Kidd P, Vangronsveld J, Wenzel WW, Fallmann K, Puschenreiter M (2013) The role of plant-associated bacteria in the mobilization and phytoextraction of trace elements in contaminated soils. *Soil Biol Biochem* 60:182–194
- Smith SE, Read DJ (1997) *Mycorrhizal symbiosis*. Academic Press Inc., San Diego
- Vanderlinde EM, Harrison JJ, Muszynski A, Carlson RW, Turner RJ, Yost CK (2010) Identification of a novel ABC-transporter required for desiccation tolerance, and biofilm formation in *Rhizobium leguminosarum* bv. *viciae* 3841. *FEMS Microbiol Ecol* 71:327–340
- Vangronsveld J, Cunningham SD (1998) Introduction to the concepts. In: Vangronsveld J (ed) *Metal-contaminated soils: in-situ inactivation and phytoremediation*. Springer, Berlin, pp 1–15
- Vangronsveld J, Herzog R, Weyens N, Boulet J, Adriaensen K, Ruttens A, Thewys T, Vassilev A, Meers E, Nehnelajova E, van der Lelie D, Mench M (2009) Phytoremediation of contaminated soils and groundwater: lessons from the field. *Environ Sci Pollut Res* 16:765–794
- Vardharajula S, Ali SZ, Grover M, Reddy G, Bandi V (2011) Drought-tolerant plant growth promoting *Bacillus* spp.: effect on growth, osmolytes and antioxidant status of maize under drought stress. *J Plant Interact* 6:1–14
- Weisburg WG, Barns SM, Pelletier DA, Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* 43:1691–1705
- Yang Y, Zhang F, Li H, Jiang R (2009) Accumulation of cadmium in the edible parts of six vegetable species grown in Cd-contaminated soils. *J Environ Manag* 90:1117–1122
- Zhang S, Li T, Huang H, Zou T, Zhang X, Yu H, Zheng Z, Wang Y (2012) Cd accumulation and phytostabilization potential of dominant plants surrounding mining tailings. *Environ Sci Pollut Res Int* 9:3879–3888