International Journal of Phytoremediation

ISSN: 1522-6514 (Print) 1549-7879 (Online) Journal homepage: http://www.tandfonline.com/loi/bijp20

The effect of plant growth-promoting rhizobacteria on the growth, physiology, and Cd uptake of Arundo donax L.

Chinnathambi Sarathambal, Premraj Jagoji Khankhane, Yogita Gharde, Bhumesh Kumar, Mayank Varun & Sellappan Arun

To cite this article: Chinnathambi Sarathambal, Premraj Jagoji Khankhane, Yogita Gharde, Bhumesh Kumar, Mayank Varun & Sellappan Arun (2017) The effect of plant growth-promoting rhizobacteria on the growth, physiology, and Cd uptake of Arundo donax L., International Journal of Phytoremediation, 19:4, 360-370, DOI: 10.1080/15226514.2016.1225289

To link to this article: http://dx.doi.org/10.1080/15226514.2016.1225289

Accepted author version posted online: 03 Sep 2016.
Published online: 03 Sep 2016.

INTERNATIONAL JOURNAL OF PHYTOREMEDIATION
2017, VOL. 19, NO. 4, 360–370
http://dx.doi.org/10.1080/15226514.2016.1225289

Taylor & Francis
Taylor & Francis Group

The effect of plant growth-promoting rhizobacteria on the growth, physiology, and Cd uptake of *Arundo donax* L.

Chinnathambi Sarathambal[a], Premraj Jagoji Khankhane[a], Yogita Gharde[a], Bhumesh Kumar[a], Mayank Varun[b], and Sellappan Arun[c]

[a]ICAR—Directorate of Weed Research, Jabalpur, Madhya Pradesh, India; [b]Department of Botany, St. John's College, Agra, Uttar Pradesh, India; [c]Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

ABSTRACT

In this study, plant growth-promoting potential isolates from rhizosphere of 10 weed species grown in heavy metal-contaminated areas were identified and their effect on growth, antioxidant enzymes, and cadmium (Cd) uptake in Arundo donax L. was explored. Plant growth-promoting traits of isolates were also analyzed. These isolates were found to produce siderophores and enzymes such as 1-aminocyclopropane-1-carboxylate (ACC) deaminase, and aid in solubilization of mineral nutrients and modulate plant growth and development. Based on the presence of multiple plant growth-promoting traits, isolates were selected for molecular characterization and inoculation studies. Altogether, 58 isolates were obtained and 20% of them were able to tolerate Cd up to 400 ppm. The sequence analysis of the 16S rRNA genes indicates that the isolates belong to the phylum Firmicutes. Bacillus sp. along with mycorrhizae inoculation significantly improves the growth, the activity of antioxidants enzymes, and the Cd uptake in A. donax than Bacillus alone. Highly significant correlations were observed between Cd uptake, enzymatic activities, and plant growth characteristics at 1% level of significance. The synergistic interaction effect between these organisms helps to alleviate Cd effects on soil. Heavy metal-tolerant isolate along with arbuscular mycorrhizae (AM) could be used to improve the phytoremedial potential of plants.

KEYWORDS

16S rRNA sequencing; antioxidant enzymes; *Arundo donax*, cadmium; plant growth-promoting rhizobacteria (PGPR)

Introduction

One of the major threats facing the industrialized world today is the contamination of soils, groundwater, sediments, surface water, and air with heavy metals (Cheng 2003; Li *et al.* 2014). Heavy metals are phytotoxic at elevated concentrations, affects soil fertility, growth reduction, yield depression and disorders, alter metabolism and physiology in plants as well as in animals (Dahshan *et al.* 2013; Figueiredo *et al.* 2014a, b). Among various heavy metals, cadmium (Cd) is a cumulative toxic element. Industries, municipal sewage, fuels, and chemical fertilizers, especially phosphate ores are among the major sources of this contaminant (Gadd 1990). The accumulation of Cd in agricultural soils is derived primarily from industrial waste disposals, the application of phosphate fertilizers, and biosolids added to soil as amendments and a source of plant nutrients and soil organic matter (SOM), especially sewage sludge (Smith 2009). A high concentration of Cd in the soil is a serious threat to both the environment and human health over the long term (Abbas *et al.* 2014; Kirkham 2006; Varghese 2012). Cd is well known to inflict a negative influence on soil biota (*i.e.*, plants, microorganisms, and fauna) in Cd-polluted environments. Metals and other inorganic contaminants are the most prevalent form of contamination found at the waste site and their remediation in soil and sediment is technically very difficult as they cannot be destroyed by degradation. Although conventional physicochemical technologies used for remediation of metal-polluted soils have been well developed, these approaches have many limitations (Congeevaram *et al.* 2007).

In the last two decades, phytoremediation has evolved as an *in situ*, cost-effective, high-energy, non-conventional, non-intrusive, esthetically pleasing, ecologically benign method to remediate contaminated soils, sediments, and water resources (Abhilash *et al.* 2009; Blaylock *et al.* 2000). Use of plants that have constitutive and adaptive mechanisms for tolerating or accumulating high metal contents in their tissues is the emerging *in situ* remediation technology employed in the cleanup of soils, sediments, and water resources that have been polluted by organic and inorganic contaminants (Glick 2010). To date, approximately 450 plants have been identified as known hyperaccumulators, but most of them are not appropriate for phytoextraction because of their small biomass and slow growth (Reeves and Baker 2000). Many phytoremediation studies have shown the potential of weed species to remove heavy metals from contaminated environment (Girdhar *et al.* 2014; Khankhane and Varsheny 2008; Lum *et al.* 2014; Varun *et al.* 2015). Regardless of the plant used, an efficient metal phytoextraction is often limited by the availability of metals in soil for root uptake. One promising strategy is to use microorganism-

CONTACT Mayank Varun 30mayank@gmail.com Department of Botany, St. John's College, Agra-282002, Uttar Pradesh, India.
Color versions of one or more of the figures in this article can be found online at www.tandfonline.com/bijp.

assisted phytoextraction, which rapidly ensures availability of metals by releasing compounds that can desorb metals from the soil matrix to form water-soluble metal complexes into the soil solution for plant (Ma *et al.* 2015b). Hence, understanding the complex interactions in the rhizosphere and plant-based mechanisms is essential for effective phytoremediation.

Rhizoremediation is one of the phytoremediation methods and depends upon interactions between plants, microbes, and pollutants, where exudates released by plant roots help to stimulate survival and activity of bacteria, which improve soil chemical and physical properties, and enhance nutrient acquisition, metal detoxification, and alleviation of abiotic stress in plants (Kamaludeen and Ramasamy 2008). Soil microorganisms and fauna are the factors that may have an influence on the mobility and bioavailability of Cd in soil–plant systems (Ma *et al.* 2006). The metal-resistant bacteria harboring plant growth-promoting traits frequently increase plant growth, nutrient uptake, metal tolerance, and rhizoremediation process in soils polluted with heavy metals (Ma *et al.* 2011b, 2015a; Rajkumar *et al.* 2008; Zhang *et al.* 2011; Zhu *et al.* 2014).

Many soil bacteria tolerate heavy metals and play important roles in mobilization of heavy metals (Wenzel 2009). Some rhizobacteria containing the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase decrease the plant ethylene levels and enhance plant growth (Ahmad and Kibret 2014). Rhizobacteria have been shown to possess several traits that can alter heavy metals bioavailability through the release of chelating substances, acidification of the microenvironment, and by influencing changes in redox potential (Ma *et al.* 2015b).

According to Dahmani-Muller *et al.* (2000), a few of the higher plant species have adaptations that enable them to survive and to reproduce in soils heavily contaminated with zinc (Zn), copper (Cu), lead (Pb), Cd, nickel (Ni), and arsenic (As). Such species are divided into two main groups: the so-called pseudometallophytes that grow on both contaminated and non-contaminated soils and the absolute metallophytes that grow only on metal-contaminated and naturally metal-rich soils.

Arundo donax L. (giant reed) is a fast-growing wild perennial grass belonging to the family Poaceae. It is a hydrophyte, growing along lakes, streams, drains, and other wet sites; it possesses hollow stem 2–3 cm in diameter, with alternate leaves, 30–60 cm long and 2–6 cm broad, tapered tips and hairy tuft, at the base. It is well documented that its growth and photosynthesis is not affected by the presence of high levels of Cd, Ni, Se, and As in the soil (Hassan *et al.* 2015; Mirza *et al.* 2011; Papazoglou *et al.* 2005). It is an energy crop, which displays many attractive characteristics such as the production of biomass (Angelini *et al.* 2005), pulp and paper (Shatalov and Pereira 2005), and activated carbons (Mavrogianopoulos *et al.* 2002). Also, this species could be used as biofiltering material for sewage effluent treatment (Vernersson *et al.* 2002). It is one of the most used plants because of its capacity to absorb contaminants such as trace elements, especially via phytoremediation process, which cannot be easily biodegraded (Mirza *et al.* 2011). Many studies have revealed that rhizospheric microorganisms are efficient in enhancing metal phytoremediation (Carlot *et al.* 2002), as they are able to tolerate, survive, and improve soil structure for efficient plant–microbe interaction.

The aim of this study was to (1) identify plant growth-promoting potential isolates from rhizosphere of selected weed species grown in heavy metal-contaminated areas; (2) determine the plant growth-promoting traits of selected isolates; (3) carry out molecular characterization of isolates; and (4) explore the effect of isolates on growth, antioxidant enzymes, and Cd uptake enhancement in *A. donax*.

Materials and methods

Rhizosphere soil sampling and isolation of Cd-tolerant bacteria

Plants from different heavy metal-contaminated areas were uprooted carefully and the soil adhering to the root was separated in a sterile Petri dish and mixed thoroughly so as to make a composite sample for microbial analysis. Soil samples collected were transported to laboratory in icebox for further analysis. Microorganisms were isolated using serial dilution technique on Kings' B media. Aliquots (0.1 mL) from the serially diluted samples (10^{-3} to 10^{-6}) were added to four different media in Petri plates and kept in an incubator at 30°C. Three days after incubation, colonies growing on media were counted and grouped according to their morphological characteristics.

The isolated bacteria strains were tested for Cd resistance by dissolving salt of $Cd(NO_3)_2$ in distilled water so as to obtain 400 μg/mL concentration. The cultures were streaked on the surface of the King's B medium and incubated at 30°C for 3 days and growth was observed. Single colonies were picked from the Petri dishes and subcultured to obtain pure cultures. Stock cultures were made in nutrient broth containing 50% (wt/vol) glycerol and stored at −80°C.

Determination of plant growth-promoting traits

The ACC deaminase activity was measured by growing cells in minimal medium with 3 mM ACC as the sole nitrogen (N) source. Production of α-ketobutyrate as a product of enzymatic cleavage of ACC by ACC deaminase was measured at 540 nm, as per the method by Penrose and Glick (2003). Production of siderophores by bacterial isolates were assayed on Chrome Azurol S (CAS) agar plates and incubated for 24 hours at room temperature (Schwyn and Neilands 1987). The formation of bright zone with yellowish fluorescent color by the culture in the medium indicates siderophore production. The presence of catechol-type siderophore was determined by Arnow's assay (1937).

Phosphate solubilizing bacteria were identified by the appearance of a clear halo around colonies against an opaque background (Katznelson and Bose 1959). Quantitative estimation of phosphate solubilization was carried out in Erlenmeyer flasks containing 20 mL of Sperberg's hydroxyapatite broth, and inoculated with tested isolates (500 μL inoculum with $\sim 2 \times 10^8$ cfu/mL). Sterilized, uninoculated medium served as control. The flasks were incubated for 5 days at 28 ± 2°C on an incubator. The cells were harvested by centrifugation at 8000*g* for 10 minutes and the supernatants were used to assay the available phosphorus (P) (Olsen and Sommers 1982). A standard curve was prepared with orthophosphate and amount of P solubilized was calculated by using

the standard graph. The available P content was expressed in μg/mL.

To measure indoleacetic acid (IAA), 1 mL of the culture at exponential stage was inoculated in 100 mL lysogeny broth (LB) containing filter-sterilized L-tryptophan (0.01% wt/vol). All the flasks were enclosed with black paper to avoid photo inactivation. The flasks were incubated at room temperature for 7 days. The cell-free extracts were assayed according to the method by Gorden and Paleg (1957). A standard curve was prepared from a series of IAA solutions of known concentrations and amount of IAA was calculated by using the standard graph. The quantity of IAA was expressed in μg/mL.

Compatibility assessment and pathogenicity

The compatibility of multiple growth-promoting activities of *Azotobacter chroococcum, Azospirillum lipoferum, Bacillus megaterium*, and *Rhizobium leguminosarum* isolates were tested before applying them in plants. The nutrient agar medium was prepared and compatibility of isolates was tested among themselves by streaking one isolate on one side and the other isolate perpendicularly up to the test isolate (as cross streak). The growth of bacteria was observed after 48 hours and recorded as positive or negative. The pathogenicity experiment was conducted in polyhouse at 28 $\pm$ 2°C and 75 $\pm$ 5% relative humidity for a period of 24 days with tomato plants by pinprick method (Aneja 2003).

16S rRNA gene sequencing

Almost full-length of 16S rRNA gene was amplified from selected isolates using universal eubacterial primers, FD1 and RP2 (Weisburg *et al.* 1991) and the band of expected size was gel-purified using spin columns according to the manufacturer's instructions and cloned using pTZ57R/T vector supplied with TA cloning kit before sequencing. Sequencing reactions were performed and electrophoresis of the products were carried out on an Applied Biosystems (Model 3100) automated sequencer. The identity of 16S rRNA sequence was established by performing a resemblance against the GenBank database (http://www.ncbi.nih.gov/BLAST). The phylogenetic tree was constructed with existing 16S rRNA gene sequences from closely related bacteria, obtained from the National Center for Biotechnology Information (NCBI) GenBank database. The phylogenetic tree was constructed by neighbor-joining method of Saitou and Nei (1987) using MEGA7.0 software (Kumar *et al.* 2016).

Plant inoculation experiments

In greenhouse pot culture experiment, disease-free seedlings of *A. donax* were raised along with 3 and 5 ppm Cd concentrations in sterile sand culture medium. The three elite multifunctional strains were grown in LB broth till the population reached 10^{10} cells/mL. The cells were then harvested by centrifugation at 6000*g* for 5 minutes at room temperature. The cell pellets were washed twice with 20 mL of phosphate buffer and resuspended in 1.5 mL of phosphate buffer. Seedlings were treated with the selected bacterial inoculants for 15 minutes and were grown in a polyhouse. Inoculation of arbuscular mycorrhizae (AM) was done at the rate of 50 g/pot (containing 8–10 spores/g inoculum) while planting as per the treatment. To assess the performance of plant growth-promoting rhizobacteria (PGPR) and AM on test plant, parameters such as plant height, enzyme activity, Cd uptake, and dry biomass were recorded at the time of harvest, that is, 180 days after planting. Each treatment was performed in triplicate. The pots were arranged in a completely randomized factorial design and placed on a platform in the polyhouse.

Determination of antioxidant enzymes and Cd uptake

Crushed plant parts were homogenized in a 100 mM phosphate buffer (pH 6.8) for the analysis of peroxidase activity (1:7 ratio) and in 50 mM phosphate buffer before catalase activity (1:5 ratio) and centrifuged at 12,000 *g* for 20 minutes. The supernatants were used to determine the enzyme activity levels. The whole procedure was carried out at 4°C. The guaiacol peroxidase activity was measured at 470 nm according to the method by Fang and Kao (2000), with guaiacol as the substrate. The peroxidase activity was measured in a reaction mixture (3 mL) containing 50 mM phosphate buffer (pH 5.8), 1.6 μL H_2O_2, 1.5 μL guaiacol, and 0.2 mL enzyme extract. The activity was calculated using the extinction coefficient (26 mM/cm) for tetraguaiacol and was expressed in μmol tetraguaiacol consumed/min/mg protein.

The catalase activity was determined by following the consumption of H_2O_2 (an extinction coefficient of 39.4 mM/cm) at 240 nm for 30 seconds (Aebi 1984) and was expressed in μmol H_2O_2 consumed/min/mg protein. The reaction mixture (3 mL) contained 50 mM potassium phosphate buffer (pH 7.0), 15 mM H_2O_2, and 0.2 mL enzyme extract. To measure the contents of non-protein thiols, the plant material was homogenized in 5 vol/g mixture containing 5-sulfosalicylic acid (2 g/100 mL) and 1 mM ethylenediaminetetraacetic acid (EDTA) and sodium ascorbate (0.15 g/100 mL). The samples were centrifuged at 20,000*g* for 10 minutes at 4°C. Then 0.5 mL liquid supernatant, 0.5 mL of a 1 M sodium phosphate buffer (pH 8.0), and 100 μL of 10 mM 5,5′-dithio-*bis*(2-nitrobenzoic acid) (DTNB) were put into test tubes. The absorbance at 415 nm was read 1 minute after the addition of DTNB. The number of non-protein SH groups was established based on a curve prepared using L-cysteine and expressed as nanomoles of SH per gram fresh weight (Mass 1987).

The superoxide dismutase (SOD) activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) and the change in absorbance was measured at 560 nm (Beau-Champ and Fridovich 1971). The reaction mixture consisted of 25 mM phosphate buffer (pH 7.8), 65 M NBT, 2 M riboflavin, enzyme extract, and TEMED (Tetramethylethylenediamine) and the reaction mixture was exposed to light at 350 units/μmol/m^2/s for 15 minutes.

All plant samples were harvested and analyzed to determine available Cd concentrations at the end of the experiment. Plant shoots and roots were oven-dried at 75°C for 48 hours and were ground to a fine powder. Plant samples (0.2 g) were digested with a mixture of H_2SO_4/H_2O_2 method (Harmon and Lajtha 1999). The concentration of exchangeable Cd extracted by 0.5 M KNO_3 in soil samples was measured by atomic absorption spectrophotometer (model: Varian 220).

Statistical analysis

Before the analysis, data were checked for normality and other error assumptions (*e.g.*, randomness and homogeneity of the error variance) by analysis of variance (ANOVA). They were confirmed with studentized residuals and Shapiro–Wilk normality test (Onofri *et al.* 2010). Thereafter, non-normal data were transformed using suitable transformation. Data were subjected to ANOVA and statistical significant differences between the treatments were computed using Tukey's honest significant difference (HSD) test at 5% level of significance. Relationships between different parameters were studied using different models (linear and non-linear) fitted to the data. By using these models, relationship between different parameters were explained along with their correlation. Analysis was performed using SAS 9.3 (SAS Institute Inc., Cary, NC, USA) and Microsoft office Excel 2013 for Windows.

Results

Bacterial isolation and screening for Cd tolerance

Based on their predominance in each region, a total of 10 different weed species (8 herbs, 1 grass, and 1 hydrophyte), viz., *Ageratum conyzoides* L., *Ammannia auriculata* Willd., *Chenopodium album* L., *Cyperus rotundus* L., *Eichhornia crassipes* (Mart.) Solms, *Eryngium foetidum* L., *Panicum repens* L., *Rumex crispus* L., *Sida acuta* Burm.f., and *Typha latifolia* L. were sampled along with rhizosphere soil. The samples collected from different locations were analyzed for isolation of Cd-tolerant PGPR. Fifty-eight isolates were obtained by using King's B medium after 3 days of incubation (Table 1). Among the weeds, *A. conyzoides* (8) harbored more number of metal-tolerant bacteria followed by *T. latifolia* (7), *C. rotundus* (7), and *C. album* (7). Among 58 rhizosphere isolates, 12 isolates were able to grow in Cd-treated King's B medium plates and were selected for further study of plant growth-promoting characteristics.

In vitro screening for PGPR and molecular identification

Apart from metal tolerance, the bacterial isolates used in this study possess different plant growth-promoting traits, which are described in Table 2. All the isolates gave positive result with regard to siderophore and the maximum siderophore production was recorded in isolate TLP2 (66.30 ± 3.21 μg/mg protein) followed by isolate RCB4 (54.6 ± 2.14 μg/mg protein). Among the rhizosphere isolates, five were able to produce ACC deaminase where the isolate RCB4 registered the highest activity of 92.5 ± 8.12 nmoles α-ketobutyrate/mg/h. Out of 12 isolates, 11 isolates showed IAA production. The maximum amount of IAA was produced by RCB4 (42.4 ± 3.3 μg/mL) followed by RCB5 (34.5 ± 1.6 μg/mL).

In the qualitative assay of P solubilization, all the 12 rhizosphere isolates were found to be positive, where TLP2 and SAU6 exhibited maximum solubilization efficiency of 250% and 233%, respectively. The amount of available P was significantly higher in the isolate TLP2 (0.96 ± 0.09 μg/mL) compared with the other isolates. Based on siderophore production, ACC deaminase activity, and P solubilization traits, three elite rhizosphere isolates were selected for further molecular identification and plant inoculation studies. These selected strains were highly compatible with standard bioinoculants and did not show any disease symptoms in tomato plants.

The selected three bacterial colonies were characterized by morphology and Gram's reaction. All the authenticated isolates were further phylogenetically identified using 16S rRNA gene sequence homology, which revealed the presence of diversity of Firmicutes. The isolate RCB5 showed close similarity to *Bacillus cereus* and the isolates RCB4 found close similarity to *Bacillus* sp. The isolates TLP2 were found to be closely similar to *B. megaterium* (Table 3). The 16S rRNA gene sequences from the plant growth-promoting isolates and the available 16S rRNA gene sequences from NCBI were aligned and used for construction of phylogenetic tree (Figure 1).

Plant inoculation experiments

Seedling inoculation with the test strains enhanced the growth and biomass in almost all the treated plants in comparison to uninoculated control (Table 4). The maximum plant height was observed for the plant treated with Cd 5 ppm + *B. cereus* + AM (89.4 ± 2.1 cm), while the number of tillers and dry weight were recorded in Cd 3 ppm + *B. cereus* + AM (19.0 ± 3 and 25.4 ± 2.5 g).

Application of plant growth-promoting isolates along with AM made significant impact on uptake of Cd that was more in Cd 3 ppm + *B. cereus* + AM (2.3 ± 0.5 mg/g), while the next best treatment was Cd 5 ppm + *B. cereus* + AM (1.9 ± 0.9 mg/kg).

Table 1. Selected PGPR isolates from rhizosphere of weed species collected from heavy metal-contaminated areas.

Botanical name	Common name	Family	Description	Isolate code	No. of isolates
Ageratum conyzoides[a]	Billygoat-weed	Asteraceae	Drainage	ACR	8
Ammannia auriculata[a]	Monarch redstem	Lythraceae	River	AAP	4
Chenopodium album[a]	Lamb's quarters	Amaranthaceae	Drainage	CAP	7
Cyperus rotundus[a]	Nut grass	Cyperaceae	Drainage	CRP	7
Eichhornia crassipes[c]	Water hyacinth	Pontederiaceae	Drainage	ECU	6
Eryngium foetidum[a]	Wild coriander	Apiaceae	River	EFP	2
Panicum repens[b]	Torpedo grass	Poaceae	Gelatin factory	PRB	5
Rumex crispus[a]	Yellow dock	Polygonaceae	Gelatin factory	RCB	6
Sida acuta[a]	Baraira	Malvaceae	Drainage	SAU	6
Typha latifolia [a]	Common cattail	Typhaceae	Drainage	TLP	7

[a]Herb; [b]grass; [c]hydrophyte

Table 2. Plant growth-promoting characteristics of bacteria associated with rhizosphere of weed species.

Isolate	Siderophore (catechol type) (μg/mg protein)	ACC deaminase (nmoles of α-ketobutyrate/mg/protein/h)	P solubilization efficiency (%)	Available P (μg/mL)	IAA (μg/mL)
RCB1	22.4 ($\pm$1.10)^{c}	ND	60 ($\pm$1.14)^{e}	0.34 ($\pm$0.03)^{f}	17.7 ($\pm$0.5)^{e}
RCB4	54.6 ($\pm$2.14)^{b}	92.5 ($\pm$8.12)^{a}	225 ($\pm$12.16)^{b}	0.67 ($\pm$0.02)^{c}	42.4 ($\pm$3.3)^{a}
RCB5	38.4 ($\pm$1.19)^{b}	74.3 ($\pm$7.98)^{b}	200 ($\pm$2.64)^{bc}	0.55 ($\pm$0.06)^{d}	34.5 ($\pm$1.6)^{b}
AAP4	18.9 ($\pm$1.24)^{cde}	ND	150 ($\pm$11.19)^{cd}	0.68 ($\pm$0.03)^{c}	18.4 ($\pm$1.6)^{e}
TLP1	20.9 ($\pm$1.15)^{cd}	ND	140 ($\pm$11.64)^{d}	0.46 ($\pm$0.04)^{e}	12.3 ($\pm$1.2)^{g}
TLP2	66.3 ($\pm$3.21)^{a}	76.8 ($\pm$2.98)^{b}	233 ($\pm$1 3.10)^{a}	0.96 ($\pm$0.01)^{a}	30.8 ($\pm$2.1)c
TLP 7	13.5 ($\pm$1.18)^{e}	87.9 ($\pm$3.29)^{ab}	50 ($\pm$2.17)^{e}	0.81 ($\pm$0.01)^{b}	23.4 ($\pm$2.1)^{d}
SAU6	13.5 ($\pm$1.13)^{e}	ND	250 ($\pm$13.53)^{a}	0.67 ($\pm$0.04)^{c}	17.5 ($\pm$1.5)^{e}
RCP4	23.5 ($\pm$2.11)^{cd}	83.9 ($\pm$4.96)^{ab}	125 ($\pm$11.29)^{d}	0.55 ($\pm$0.01)^{d}	10.8 ($\pm$1.2)^{h}
ACR3	18.4 ($\pm$1.70)^{de}	ND	150 ($\pm$12.10)^{cd}	0.29 ($\pm$0.01)^{g}	ND
ECU6	20.4 ($\pm$2.24)^{cde}	ND	33 ($\pm$0.98)^{e}	0.26 ($\pm$0.01)^{g}	13.9 ($\pm$1.2)^{f}
CRP1	20.9 ($\pm$2.26)^{cd}	ND	167 ($\pm$11.54)^{cd}	0.32 ($\pm$0.03)^{h}	17.3 ($\pm$1.1)^{e}

Values are mean ($\pm$SE) (n = 3) and values followed by the same letter in each column are not significantly different from each other as detected by Tukey's HSD (p = 0.05).

ND, not detected; ACC deaminase, 1-aminocyclopropane-1-carboxylate deaminase; P solubilization efficiency, Phosphate solubilization efficiency; Available P, available phosphorus; IAA, indoleacetic acid.

Application of multiple growth-promoting isolates along with AM had a marked influence on antioxidants of *A. donax* (Table 5). From the results, higher amount of catalase was observed in Cd 5 ppm + *B. cereus* + AM inoculated pots (16.5 units/μg H_2O_2/g/min). We detected an increase of catalase concentration in *A. donax* in most cases of PGPR treatment. The concentration of peroxidase activity was higher in the plants that were exposed to Cd 3 ppm + *B. cereus* + AM (4.3 units/μmol/min/mg protein), whereas the Cd 5 ppm + *B. cereus* + AM-treated plants (3.9 units/μmol/min/mg protein) had comparable amounts of peroxidase activity. The SOD activity ranged from 2.1 to 5.1 (enzyme units/mg/protein/min) in plants. Interestingly, higher activities were found in Cd 5 ppm + *B. megaterium* (5.1 enzyme units/mg/protein/min). Inoculation of *B. cereus* and mycorrhizae in combination would stimulate plant defense and Cd uptake than alone.

The relationship between Cd uptake, enzymatic activities, and yield-attributing characteristics

Highly significant correlation was observed between Cd uptake, enzymatic activities, and yield-attributing characteristics at 1% level of significance (Table 6). Among these, Cd uptake showed strong correlation with catalase activity with correlation coefficient value to be 0.831. Plant and yield characteristics also showed significant correlation among them. Regression analysis revealed exponential relationship between Cd uptake and catalase activity with R^2 of 0.6995. Similarly, peroxidase activity and SOD activity also found significant relationship with Cd uptake with R^2 of 0.49 and 0.40, respectively. Cd uptake showed linear relationship with peroxidase activity, whereas it showed exponential relationship with SOD. Relationship between Cd uptake and plant characteristics was also studied. In general, Cd uptake inhibited the plant height and number of tillers as they were linearly related to R^2 values of 0.68 and 0.49, respectively (Figure 2).

Contrast analysis

In the pot experiment, plant growth-promoting isolates such as *Bacillus sp., B. megaterium, B. cereus*, and AM were used in the study to evaluate the antioxidant enzyme activity, Cd uptake, and plant growth. We were interested to know whether there is any significant difference in using these in consortium or they can give superior results when using single-handedly. Therefore, contrast analysis was performed on these data. A comparison was made between the treatments containing both *B. cereus* and AM and treatments where single organism was used. Results showed the considerable amount of additive effect on Cd uptake when *B. cereus* and AM were used in consortium.

Discussion

Contamination of soil with Cd can negatively affect biodiversity and the activity of soil microbial communities. Soils contaminated with metals often show plant stresses and nutrient deficiency (Ma *et al.* 2010; Wan *et al.* 2012). Cd can inhibit root and shoot growth, affect nutrient uptake, and is frequently accumulated by agriculturally important crops (Sanita di Toppi and Gabrielli 1999). PGPR, such as *Azospirillum, Azotobacter, Achromobacter, Bacillus, Burkholderia, Gluconacetobacter, Pseudomonas*, and *Serratia*, have been known to improve plant growth through various mechanisms like production of phytohormones, siderophores, and ACC deaminase, and solubilization of mineral nutrients (Ma *et al.* 2009; Rajkumar *et al.* 2008). Plants roots and soil microbes and their interaction improve

Table 3. Identification of elite isolates by 16s rRNA sequencing.

						General properties	
Isolate code	Closest relative in database	Sequence length	GenBank accession no.	Identity (%)	Weed species	Colony color	Gram's reaction
RCB4	*Bacillus* sp.	805	KT384381	98	*Rumex crispus*	Creamy white	+
RCB5	*Bacillus cereus*	903	KT384380	98	*Rumex crispus*	Creamy white	+
TLP2	*Bacillus megaterium*	1497	KT281868	99	*Typha latifolia*	Cream	+

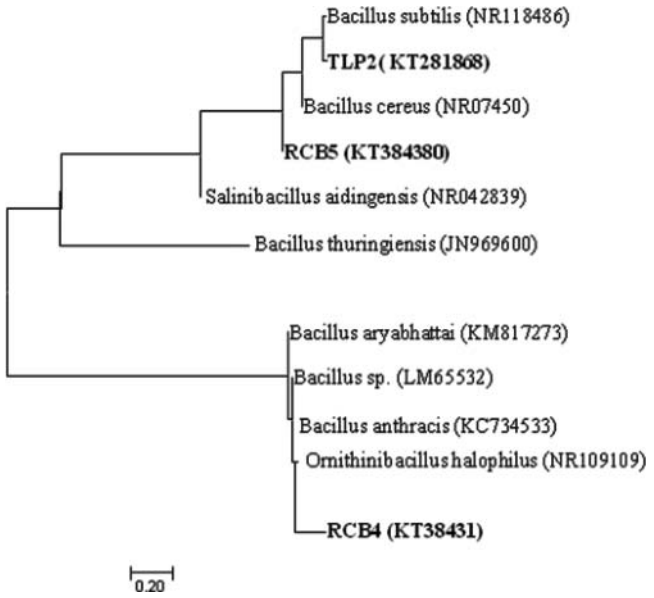

Figure 1. Neighbor-joining phylogenetic tree based on partial 16S rRNA gene of metal-tolerant *Bacillus* sp. isolates showing similarity with other *Bacillus* groups (accession numbers are in parentheses).

metal bioavailability in rhizosphere (Sarathambal and Ilamurugu 2014; Saravanan *et al.* 2007). We studied the effect of heavy metal contamination particularly Cd on plant growth-promoting traits of rhizobacteria associated with rhizosphere of *A. donax*.

Characterization of Cd-tolerant PGPR

Effective microbe-assisted phytoextraction depends on the identification of metal-resistant PGPR capable of improving the plant growth and bioavailability of heavy metals in soils and the selection of suitable plants with potential to tolerate and uptake high concentrations of heavy metals. Hence, this study was carried out to isolate culturable PGPR associated with the selected weed species, which normally grow in various heavy metal-contaminated areas. Plant count analysis revealed that the 58 rhizobacterial isolates were obtained from heavy metal-exposed areas. Among the 58 isolates, 13% isolates were recovered from *A. conyzoides*. The rhizosphere region is a highly favorable habitat for the proliferation, functioning, and metabolic activity of numerous microorganisms. It is characterized by greater microbiological activity near rhizosphere than the soil away from plant roots (Berg *et al.* 2002). However, 20% of the isolated strains of the rhizosphere were tolerant to elevated levels of Cd. Selection of microorganisms both metal-tolerant and efficient in producing plant growth-promoting compounds can be useful to speed up the recolonization of the plant rhizosphere in polluted soils. Microorganisms have developed the mechanisms to cope with a variety of toxic metals for their survival in the environment enriched with such metals (Martin-Laurent *et al.* 2004). Bacterial strains isolated from different habitats may exhibit different degrees of metal resistance, and those from metal-polluted soils are usually more resistant (Ma *et al.* 2011a). As expected, tolerance to heavy metals was found more predominant among rhizobacteria from heavy metal-polluted soil. This study has demonstrated that several bacterial strains (such as *Bacillus* sp., *B. cereus, B. megaterium*) can tolerate Cd. Thus, bacterial strains isolated from contaminated natural soils may be exploited for heavy metal bioremediation. Previous studies have also reported that several bacterial strains are capable of tolerating high concentration of toxic materials (Becerra-Castro *et al.* 2012; Kartik *et al.* 2016; Zarei *et al.* 2010).

The production of various plant growth-promoting attributes may be the reason why microorganisms can survive in the heavy metal-polluted soils. In this study, all the rhizospheric isolates were able to produce siderophore. Siderophore and phosphate solubilizing ability was detected significantly higher among isolates of *Bacillus* sp. Furthermore, bacterial siderophores are able to bind metals other than iron and thus enhance their bioavailability in the rhizosphere of plants. Overall, an increase in plant growth and metal uptake will further enhance the effectiveness of phytoremediation processes (Kartik *et al.* 2016; Rajkumar and Freitas 2008). Under metal-stressed conditions, most metal-resistant PGPB (Plant

Table 4. Effect of plant growth-promoting bacteria on plant biometric characters and Cd uptake in *A. donax*.

Treatment	Plant height (cm)	No. of tillers	Dry weight (g)	Cd uptake (mg/kg)
Control (0 ppm)	45.6 (±3.1)^{e}	11.0 (±2)^{cd}	10.3 (±1.1)^{g}	0.5 (±0.1)^{e}
Cd 3 ppm	56.8 (±2.7)^{cde}	13.0 (±1)^{bcd}	11.6 (±1.2)^{fg}	0.6 (±0.1)^{e}
Cd 5 ppm	53.6 (±1.8)^{de}	10.0 (±2)^{d}	13.5 (±1.7)^{efg}	0.7 (±0.2)^{e}
Cd 3 ppm + *Bacillus* sp.	60.5 (±3.3)^{cde}	15.0 (±3)^{abcd}	15.6 (±2.2)^{cdefg}	0.9 (±0.1)^{de}
Cd 5 ppm + *Bacillus* sp.	62.6 (±4.2)^{bcde}	14.0 (±1)^{abcd}	14.6 (±1.1)^{defg}	1.5 (±0.4)^{bc}
Cd 3 ppm + *B. cereus*	69.8 (±1.4)^{abcd}	16.0 (±2)^{abc}	13.4 (±1.7)^{efg}	1.6 (±0.3)^{bc}
Cd 5 ppm + *B. cereus*	70.3 (±1.8)^{abcd}	14.0 (±2)^{abcd}	18.9 (±3.2)^{bcde}	1.5 (±0.3)^{bc}
Cd 3 ppm + *B. megaterium*	71.9 (±2.4)^{abcd}	13.0 (±1)^{bcd}	17.5 (±1.1)^{bcdef}	1.4 (±0.2)^{c}
Cd 5 ppm + *B. megaterium*	72.9 (±1.9)^{abcd}	17.0 (±2)^{ab}	16.9 (±1.9)^{cdef}	1.3 (±0.3)^{cd}
Cd 3 ppm + *Bacillus* sp.+ AM	73.8 (±2.0)^{abcd}	15.0 (±1)^{abcd}	17.6 (±1.2)^{bcdef}	1.4 (±0.2)^{c}
Cd 5 ppm + *Bacillus* sp.+ AM	75.9 (±2.7)^{abcd}	16.0 (±2)^{abc}	20.6 (±2.6)^{abcd}	1.3 (±0.3)^{cd}
Cd 3 ppm + *B. cereus* + AM	84.9 (±3.1)^{ab}	19.0 (±3)^{a}	25.4±2.5^{a}	2.3±0.5^{a}
Cd 5 ppm + *B. cereus* + AM	89.4 (±2.1)^{a}	18.0 (±1)^{ab}	23.5±3.5^{ab}	1.9±0.9^{ab}
Cd 3 ppm + *B. megaterium* + AM	72.5 (±1.6)^{abcd}	17.0 (±2)^{ab}	20.5±1.0^{abcd}	1.5±0.2^{bc}
Cd 5 ppm + *B. megaterium* + AM	73.5 (±1.1)^{abcd}	16.0 (±2)^{abc}	20.4±1.3^{abcd}	1.4±0.2^{c}
Cd 3 ppm + AM	79.6±(2.7)^{abc}	18.5 (±2)^{a}	21.2±2.8^{abc}	1.2±0.1^{cd}
Cd 5 ppm + AM	80.6±(1.9)^{abc}	18.8 (±1)^{a}	21.1±2.2^{abc}	1.2±0.2^{cd}

Each value is the mean of triplicates (n = 3); values followed by the same letter in each column are not significantly different from each other as detected by Tukey's HSD (p = 0.05).
AM, arbuscular mycorrhizae; Cd, cadmium.

Table 5. Effect of inoculation of plant growth-promoting bacteria on antioxidant enzyme activity in *A. donax*.

Treatment	Catalase (units/μg H_2O_2/g/min)	Peroxidase (units/μmol tetraguaiacol/min/mg/protein)	Superoxide dismutase (units/mg protein/min)
Control (0 ppm)	5.6 ($\pm$1.4)^{g}	2.3 ($\pm$0.7)^{d}	2.1 ($\pm$0.3)^{d}
Cd 3 ppm	8.6 ($\pm$1.1)^{fg}	2.5 ($\pm$1.3)^{cd}	3.8 ($\pm$1.6)^{abc}
Cd 5 ppm	9.3 ($\pm$2.4)^{efg}	2.6 ($\pm$1.1)^{cd}	3.5 ($\pm$0.6)^{bc}
Cd 3 ppm + *Bacillus* sp.	9.6 ($\pm$1.7)^{efg}	3.4 ($\pm$1.3)^{abc}	3.6 ($\pm$0.9)^{bc}
Cd 5 ppm + *Bacillus* sp.	10.3 ($\pm$1.5)^{def}	3.5 ($\pm$0.5)^{abc}	2.8 ($\pm$0.5)^{cd}
Cd 3 ppm + *B. cereus*	10.5 ($\pm$1.8)^{cdef}	2.6 ($\pm$0.9)^{cd}	3.5 ($\pm$0.7)^{bcd}
Cd 5 ppm + *B. cereus*	11.5 ($\pm$0.9)^{bcdef}	2.5 ($\pm$0.5)^{cd}	4.2 ($\pm$1.1)^{abc}
Cd 3 ppm + *B. megaterium*	12.3 ($\pm$2.7)^{bcdef}	2.8 ($\pm$1.1)^{cd}	4.5 ($\pm$1.7)^{ab}
Cd 5 ppm + *B. megaterium*	11.5 ($\pm$1.2)^{bcdef}	2.5 ($\pm$0.4)^{cd}	5.1 ($\pm$1.4)^{a}
Cd 3 ppm + *Bacillus* sp.+ AM	10.7 ($\pm$0.9)^{cdef}	3.5 ($\pm$0.8)^{abc}	4.4 ($\pm$2.1)^{ab}
Cd 5 ppm + *Bacillus* sp.+ AM	13.8 ($\pm$2.2)^{abcd}	3.4 ($\pm$1.2)^{abc}	4.6 ($\pm$0.8)^{ab}
Cd 3 ppm + *B. cereus* + AM	15.6 ($\pm$3.1)^{ab}	4.3 ($\pm$1.0)^{a}	4.7 ($\pm$1.7)^{ab}
Cd 5 ppm + *B. cereus* + AM	16.5 ($\pm$1.8)^{a}	3.9 ($\pm$0.4)^{ab}	4.6 ($\pm$1.1)^{ab}
Cd 3 ppm + *B. megaterium* + AM	14.5 ($\pm$2.6)^{abc}	2.9 ($\pm$1.0)^{bcd}	4.3 ($\pm$0.6)^{ab}
Cd 5 ppm + *B. megaterium* + AM	13.0 ($\pm$1.8)^{abcde}	2.9 ($\pm$0.8)^{bcd}	4.7 ($\pm$1.5)^{ab}
Cd 3 ppm + AM	12.7 ($\pm$2.2)^{abcdef}	3.0 ($\pm$1.1)^{bcd}	4.1 ($\pm$1.0)^{abc}
Cd 5 ppm + AM	12.3 ($\pm$3.1)^{bcdef}	3.1 ($\pm$1.4)^{bcd}	4.1 ($\pm$0.8)^{abc}

Each value is the mean of triplicates (n = 3); values followed by the same letter in each column are not significantly different from each other as detected by Tukey's HSD ($p = 0.05$).
AM, arbuscular mycorrhizae; Cd, cadmium; H_2O_2, hydrogen peroxide.

growth-promoting bacteria) can either convert these insoluble phosphates into available forms through acidification, chelation, exchange reactions, and release of organic acids (Chung *et al.* 2005; Ma *et al.* 2011b; Zhang *et al.* 2011) or mineralize organic phosphates by secreting extracellular phosphatases (Gyaneshwar *et al.* 2002). An increase in P availability to plants through the inoculation of phosphate-solubilizing bacteria has been reported in pot experiments and under field conditions (Sarathambal *et al.* 2010; Zaida *et al.* 2003).

In nature, ACC deaminase has been commonly found in soil bacteria that colonize plant roots (Glick *et al.* 1999). The distribution of ACC deaminase activity is common among plant growth-promoting bacterial groups. In this study, 41% of rhizosphere isolates showed the ACC deaminase activity. Similar to our results, Sarathambal *et al.* (2015) found that *Bacillus* sp. isolated from different weeds possessed the ACC deaminase along with phosphate-solubilizing ability, siderophores, and phytohormones production. Bacteria containing ACC deaminase bind to roots and/or seed coats and stimulate root elongation by lowering the ethylene level in plants. Many of these microorganisms are identified by their ability to grow on minimal medium containing ACC as its sole N source.

Mechanisms by which bacteria can promote plant growth include mobilization of nutrients (Lugtenberg and Kamilova 2004) and production of phytohormones (Frankenberger and Arshad 1995). The microbial synthesis of plant growth regulators like IAA is an important factor in soil fertility. Indeed, under stress conditions, including heavy metal, the plant hormone ethylene endogenously regulates plant homeostasis and results in reduced root and shoot growth. This study also showed that apart from heavy metal tolerance, the strains also showed production of IAA and siderophore, and solubilization of phosphate and potassium. It is important to select metal-resistant bacteria with multiple plant growth-promoting attributes to alleviate metal toxicity.

Identification of new isolates based on phenotypical and physiological criteria is difficult, if the features displayed by a particular isolate are not fully identical with a described species. Based on the sequence analysis of the 16S rRNA gene, the isolates found in the selected grasses belong to family Bacillaceae. In this study, Firmicutes were mainly dominated by different groups of *Bacillus*, which have been isolated from selected grass species in accordance with the findings of Chowdhury *et al.* (2009). In the case of *Bacillus* sp., one of the most common soil bacteria groups, because of their spore-forming ability, strains have high tolerance to adverse ecological conditions, and this fact could determine the high number of isolations obtained from weeds growing in extreme heavy metal stress.

Plant inoculation effects

Plants, as important components of the ecological system, transfer metals from abiotic to biotic environments. The metal fraction in the soil that interacts with a biological target

Table 6. The correlation coefficients among Cd uptake, enzymatic activities, and plant biometric characteristics.

	Number of tillers	Cd uptake	Catalase activity	Peroxidase activity	Superoxide dismutase activity	Dry weight
Cd uptake	0.691**					
Catalase activity	0.776**	0.831**				
Peroxidase activity	0.619**	0.689**	0.694**			
Superoxide dismutase activity	0.673**	0.593**	0.806**	0.447**		
Dry weight	0.830**	0.778**	0.927**	0.708**	0.768**	
Plant height	0.901**	0.810**	0.913**	0.687**	0.818**	0.920**

**Correlation is significant at the 0.01 level (two-tailed).

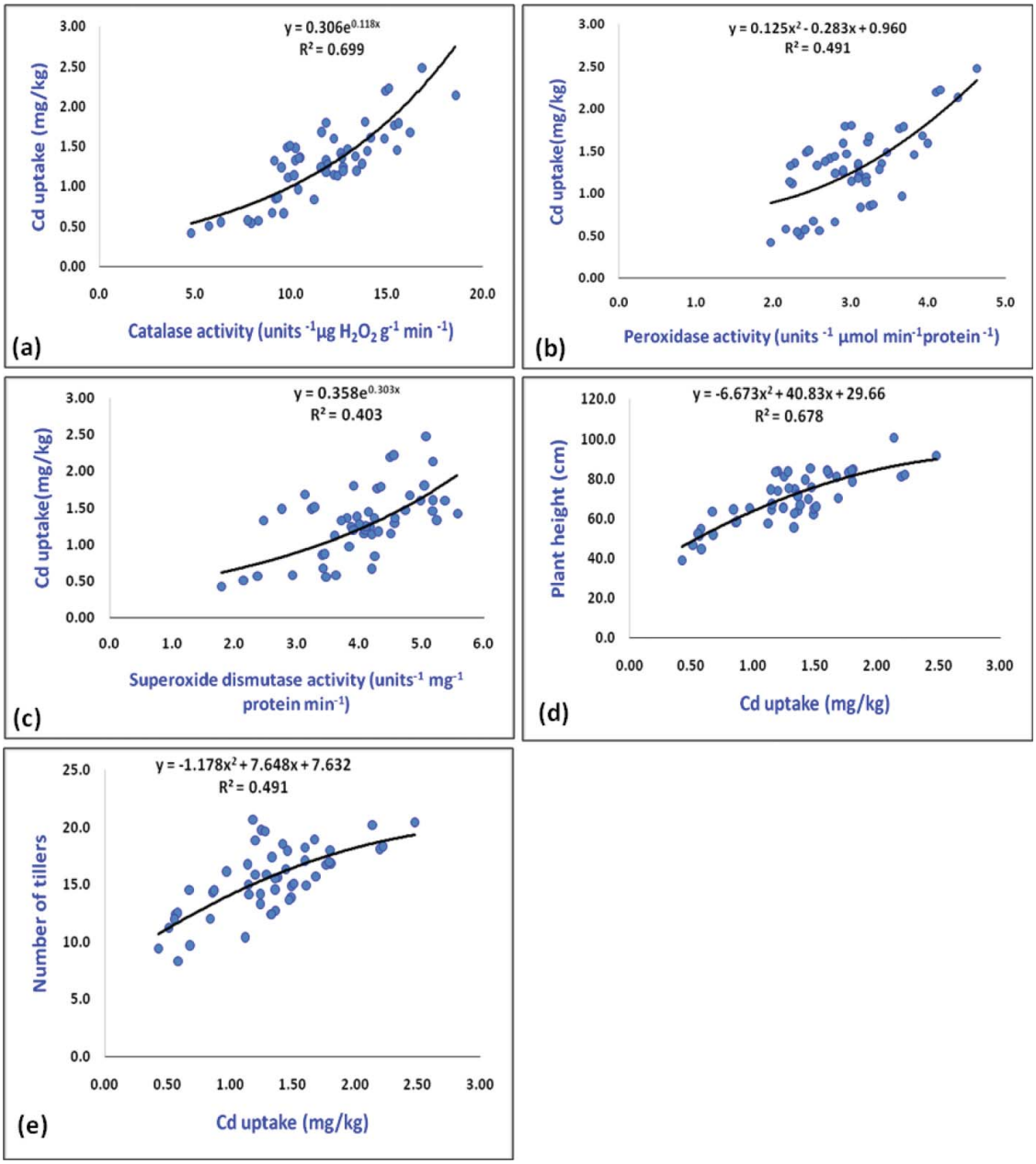

Figure 2. Relationship of Cd uptake with catalase activity (a), peroxidase activity (b), superoxide dismutase activity (c), plant height (d), and number of tillers (e).

determines its bioavailability (Chojnacka *et al.* 2005; Mishra *et al.* 2009). In our experiments, some differences in metal concentration series order in the soil extracts and the upper parts of *A. donax* plants were found.

Plant-associated bacteria can potentially improve phytoextraction by altering the solubility, availability, and transport of heavy metal and nutrients by reducing soil pH, release of chelators, P solubilization, or redox changes. Among the various metabolites produced by PGPB, the siderophores play a significant role in metal mobilization and accumulation (Rajkumar *et al.* 2010; Sessitsch *et al.* 2013), as these compounds solubilize unavailable forms of heavy metal-bearing Fe and also form complexes with bivalent heavy metal ions that can be assimilated by root-mediated processes.

Application of *B. cereus* along with AM enhances the plant biometric character and Cd uptake in *A. donax*. Similar to our results, Sheng *et al.* (2008) showed that the inoculation of soils with biosurfactant producing *Bacillus* sp. J119 significantly enhanced biomass of tomato plants and Cd uptake in plant tissue. The results are in agreement with previous studies in which metal-resistant bacteria like *Brassica napus* (Sheng and Xia 2006), *Ricinus communis* (Rajkumar and Freitas 2008), *Brassica juncea* (Ma *et al.* 2009), *Sedum plumbizincicola* (Ma *et al.* 2015b), and *Sesbania bispinosa* (Kartik *et al.* 2016) possessed various plant growth-promoting traits, such as root elongation, growth, and metal uptake. Mycorrhizae may often lower Cd mobility and toxicity by increasing soil pH, sequestering Cd inside extraradical mycelium, and binding Cd to glomalin. Glomalin is an insoluble glycoprotein synthesized and released by AMF (arbuscular mycorrhizal fungi), and may bind heavy metals in the soil (González-Chávez *et al.* 2004).

The elevation of catalase activity in Cd-treated plants was also noted in our study. Kafel *et al.* (2010) showed a similar phenomenon—an increase of catalase activity in the above-ground parts of *Philadelphus coronarius* grown in conditions of environmental pollution. In a pot experiment, Lin *et al.* (2007) showed that in *Vicia faba* exposed to Cd at 5 μg/mL, the activities of peroxidase, catalase, and SOD were significantly decreased, thus leading to an accumulation of reactive oxygen species. Peroxidase enhancement was registered in the plants treated with *B. cereus* + AM. The experiments of Shamsi *et al.* (2008) and Hassan *et al.* (2008) also showed peroxidase enhancement in conditions of metal contamination in soybean plants exposed to Cd and in rice plants exposed to Cd in a hydroponic experiment. However, elevated SOD activities in *B. megaterium* along with an accumulation of Al, Cd 3 ppm and the similar results were reported by Guo *et al.* (2007). They reported that *B. cereus* and AM interactively influenced Cd uptake, plant growth, antioxidant enzymes in Cd-polluted environments.

In this study, potential bacterial isolates *Bacillus* sp., *B. megaterium*, and *B. cereus* tolerant to elevated level of Cd, efficient siderophore, and ACC deaminase and P solubilizers were subjected to elucidate their role in stimulation of plant growth in the presence of heavy metals. The plant growth promotion by beneficial strains of *Bacillus* has often been related to their active but low level of auxin secretion in the rhizosphere (Patten and Glick 2002). Similar findings were supported by Gholami *et al.* (2009) and Ramteke *et al.* (2012).

Conclusion

The selected three elite strains were performed well with AM, since we can develop a consortia formulation with broad spectrum of action of metal tolerance and growth promotion. *Bacillus* sp. along with mycorrhizae inoculation significantly improves the growth, antioxidants enzymes, and Cd uptake in *A. donax* than *Bacillus* alone. The positive results on specific plant growth-promoting traits of *Bacillus* isolates found in this study suggest that these organisms can promote plant growth by more than one mechanism and that these traits could be better exploited as bioremediation.

It can be concluded that inoculating the rhizosphere soils with selected metal solubilizing bacteria along with AM can be an ecologically viable option to elevate bioavailable metal concentration in soil for plant uptake and thereby improving overall phytoextraction potential in metal-contaminated soils.

Funding

We wish to thank ICAR-NFBSFARA scheme for providing the financial support in the form of chemicals and instruments.

References

Abbas SZ, Rafatullah M, Ismail N, Lalung J. 2014. Isolation, identification, characterization, and evaluation of cadmium removal capacity of *Enterobacter* species. J Basic Microbiol 54:1279–1287.

Abhilash PC, Jami S, Singh N. 2009. Transgenic plants for enhanced biodegradation and phytoremediation of organic xenobiotics. Biotechnol Adv 27:474–488.

Aebi H. 1984. Catalase in vitro. Methods Enzymol 105:121–126.

Ahmad M, Kibret M. 2014. Mechanisms and applications of plant growth promoting rhizobacteria. Curr Perspec J King Saud Univ Sci 26:1–20.

Aneja KR. 2003. Experiments in Microbiology plant pathology and biotechnology. New Delhi: New Age International Limited.

Angelini LG, Ceccarini L, Bonari E. 2005. Biomass yield and energy balance of giant reed (*Arundo donax* L.) cropped in central Italy as related to different management practices. Eur J Agron 22:375–389.

Arnow E. 1937. Colorimetric estimation of the components of 3,4-dihydroxyphenylalanine-tyrosine mixtures. J Biol Chem 118:531–537.

Beau-Champ C, Fridovich I. 1971. Superoxide dismutase: improved assays and assay applicable to acrylamide gels. Anal Biochem 44:276–287.

Becerra-Castro C, Monterrosob C, Prieto-Fernández A, Rodríguez-Lamas L, Loureiro-Viñas M, Acea MJ, Kidd PS. 2012. Pseudometallophytes colonising Pb/Zn mine tailings: a description of the plant–microorganism–rhizosphere soil system and isolation of metal-tolerant bacteria. J Hazard Mater 218:350–359.

Berg G, Roskot N, Steidle A, Eberl L, Zock A, Smalla K. 2002. Plant-dependent genotypic and phenotypic diversity of antagonistic rhizobacteria isolated from different *Verticillium* host plants. Appl Environ Microbiol 683:3328–3338.

Blaylock MJ, Huang JW, Raskin I. 2000. Phytoremediation of toxic metals: using plants to clean up the environment. Vol. 53. New York: John Wiley and Sons Inc.

Carlot M, Giacomini A, Casella S. 2002. Aspects of plant microbe interactions in heavy metal polluted soil. Acta Biotechnol 22:1–13.

Cheng S. 2003. Heavy metal pollution in China: origin, pattern and control. Environ Sci Pollut Res 10:192–198.

Chojnacka K, Chojnacki AH, Górecka H. 2005. Bioavailability of heavy metals from polluted soils to plants. Sci Total Environ 337:175–182.

Chowdhury SP, Schmid M, Hartmann A, Tripathi AK. 2009. Diversity of 16S-rRNA and nifH genes derived from rhizosphere soil and roots of an endemic drought tolerant grass *Lasiurus sindicus*. Eur J Soil Biol 45:114–122.

Chung H, Park M, Madhaiyan M, Seshadri S, Song J, Cho H. 2005. Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea. Soil Biol Biochem 37:1970–1974.

Congeevaram S, Dhanarani S, Park J, Dexilin M, Thamaraiselvi K. 2007. Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates. J Hazard Mater 146:270–277.

Dahmani-Muller H, Van Oort F, Gelie B, Balabane M. 2000. Strategies of heavy metal uptake by three plant species growing near a metal smelter. Environ Pollut 109:231–238.

Dahshan H, Abd-Elall AM, Megahed AM. 2013. Trace metal levels in water, fish, and sediment from River Nile, Egypt: potential health risks assessment. J Toxicol Environ Health A 76:1183–1187.

Fang WC, Kao C. 2000. Enhanced peroxidase activity in rice leaves in response to excess iron, copper and zinc. Plant Sci 15:871–76.

Figueiredo NL, Areias A, Mendes R, Can_ario J, Duarte A, Carvalho C. 2014a. Mercury-resistant bacteria from salt marsh of Tagus Estuary: the influence of plants presence and mercury contamination levels. J Toxicol Environ Health A 77:959–971.

Figueiredo NL, Can_ario J, Duarte A, Serralheiro ML, Carvalho C. 2014b. Isolation and characterization of mercury resistant bacteria from sediments of Tagus Estuary (Portugal): implications for environmental and human health risk assessment. J Toxicol Environ Health A 77:155–168.

Frankenberger JWT, Arshad M. 1995. Microbial synthesis of auxins. In: Frankenberger WT, Arshad M, editors. Phytohormones in soils. New York: Marcel Dekker. p. 35–71.

Gadd GM. 1990. Heavy metal accumulation by bacteria and other microorganisms. Experientia 46:834–840.

Gholami A, Shahsavani S, Nezrat S. 2009. The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize. Proc World Acad Sci Eng Technol 3(7):2070–3740.

Girdhar M, Sharma NR, Rehman H, Kumar A, Mohan A. 2014. Comparative assessment for hyperaccumulatory and phytoremediation capability of three wild weeds. Biotechnol 4(6):579–589.

Glick B. 2010. Using soil bacteria to facilitate phytoremediation. Biotechnol Adv 28:367–374.

Glick B, Patten C, Holguin G, Penrose D. 1999. Biochemical and genetic mechanisms used by plant growth promoting bacteria. London: Imperial College. p. 267.

González-Chávez MC, Carrillo-González R, Wright SF, Nichols KA. 2004. The role of glomalin, a protein produced by arbuscular mycorrhizal fungi, in sequestering potentially toxic elements. Environ Pollut 130:317–323.

Gorden SA, Paleg LG. 1957. Quantitative measurements of indole acetic acid. Physiol Plant 4:24–27.

Guo TR, Zhang GP, Zhang YH. 2007. Physiological changes in barley plants under combined toxicity of aluminium, copper and cadmium. Colloids Surfaces B 57:182–188.

Gyaneshwar P, James EK, Reddy PM, Ladha JK. 2002. *Herbaspirillum* colonization increases growth and nitrogen accumulation in aluminium-tolerant rice varieties. New Phytol 154:131–145.

Harmon ME, Lajtha K. 1999. Analysis of detritus and organic horizons for mineral and organic constitutes. In: Robertson GP, Coleman DC, Bledsoe CS, Sollins P, editors. Standard soil methods for long-term ecological research. New York: Oxford University Press, Inc. p. 143–165.

Hassan R, El-Ramady , Abdalla N, Alshaal T, Elhenawy AS, Shams MS, Salah E, Faizy DA, El-Sayed BB, Shehata SA, Ragab MI, Amer MM, Fári M, Sztrik A, Prokisch J, Selmar D, Schnug E, Pilon-Smits EAH, El-Marsafawy SM, Szabolcsy ED. 2015. Giant reed for selenium phytoremediation under changing climate. Environ Chem Lett 13(4):359–380.

Hassan JM, Shafi M, Zhang G, Zhu Z, Qaisar M. 2008. The growth and some physiological responses of rice to Cd toxicity as affected by nitrogen form. Plant Growth Regul 54:125–132.

Kafel A, Nadgórska-Socha J, Gospodarek A, Babczyńska A, Skowronek M, Kandziora M, Rozpendek K. 2010. The effects of *Aphis fabae* infestation on the antioxidant response and heavy metal content in field grown *Philadelphus coronarius* plants. Sci Total Environ 408:1111–1119.

Kamaludeen SP, Ramasamy K. 2008. Rhizoremediation of metals: harnessing microbial communities. Ind J Microbiol 48:80–88.

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 (11):1061–1066. DOI:10.1080/15226514.2016.1183576

Katznelson H, Bose B. 1959. Metabolic activity and phosphate-dissolving capability of bacterial isolates from wheat roots, rhizosphere and non-rhizosphere soil. Can J Microbiol 5:79–85.

Khankhane PJ, Varsheny JG. 2008. Accumulation of heavy metals by weeds grown in drains of Jabalpur, Madhya Pradesh. Ind J Weed Sci 55–59.

Kirkham MB. 2006. Cadmium in plants on polluted soils: effects of soil factors, hyper accumulation and amendments. Geoderma 13:719–732.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol Biol Evol 33 (7):1870–1874.

Li Z, Ma Z, Vander Kuijp TJ, Yuana Z, Huang L. 2014. Review of soil heavy metal pollution from mines in China: pollution and health risk assessment. Sci Total Environ 843–853.

Lin A, Zhang X, Chen M, Cao Q. 2007. Oxidative stress and DNA damages induced by cadmium accumulation. J Environ Sci 19:596–602.

Lugtenberg BJJ, Kamilova FD. 2004. Rhizosphere management: microbial manipulation for biocontrol. In: Goodman RM, editor. Encyclopedia of plant and crop science. New York: Marcel Dekker. p. 1098–1101.

Lum AF, Ngwa ES, Chikoye D, Suh CE. 2014. Phytoremediation potential of weeds in heavy metal contaminated soils of the Bassa Industrial Zone of Douala, Cameroon. Int J Phytorem 16(3):302–319.

Ma Y, Dickinson NM, Wong MH. 2006. Beneficial effects of earthworms and arbuscular mycorrhizal fungi on establishment of leguminous trees on Pb/Zn mine tailings. Soil Biol Biochem 38:1403–1412.

Ma Y, Prasad MNV, Rajkumar M, Freitas H. 2011a. Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotechnol Adv 29:248–258.

Ma Y, Rajkumar M, Luo YM, Freitas H. 2011b. Inoculation of endophytic bacteria on host and non-host plants—effects on plant growth and Ni uptake. J Hazard Mater 196:230–237.

Ma Y, Rajkumar M, Freitas H. 2009. Improvement of plant growth and nickel uptake by nickel resistant-plant growth promoting bacteria. J Hazard Mater 166:1154–1161.

Ma Y, Rajkumar M, Vicente J, Freitas H. 2010. Inoculation of Ni-resistant plant growth promoting bacterium *Psychrobacter* sp. strain SRS8 for the improvement of nickel phytoextraction by energy crops. Int J Phytorem 13:126–139.

Ma Y, Oliveira RS, Nai FJ, Rajkumar M, Luo YM, Rocha I, Freitas H. 2015a. The hyperaccumulator *Sedum plumbizincicola* harbors metal-resistant endophytic bacteria that improve its phytoextraction capacity in multi-metal contaminated soil. J Environ Manage 156:62–69.

Ma Y, Rajkumar M, Rocha I, Oliveira RS, Freitas H. 2015b. Serpentine bacteria influence metal translocation and bioconcentration of *Brassica juncea* and *Ricinus communis* grown in multi-metal polluted soils. Front Plant Sci 5:757.

Martin-Laurent F, Cornet L, Ranjard L, Juan-Carlos López-Gutiérrez, Philippot L, Schwartz C, Chaussod R, Catroux G, Soulas G. 2004. Estimation of atrazine degrading genetic potential and activity in three French agricultural soil. FEMS Microbiol Ecol 48:425–435.

Mass F, De Kok L, Peters J, Kuiper PA. 1987. Comparative study on the effects of H_2S and SO_2 fumigation on the growth and accumulation of sulfate and sulfhydryl compounds in *Trifolium pratense* L., *Glycine max* Merr., *Phaseolus vulgaris* L. J Exp Bot 38:1459–1469.

Mavrogianopoulos G, Vogli V, Kyritsis S. 2002. Use of wastewater as a nutrient solution in a closed gravel hydroponic culture of giant reed (*Arundo donax*). Bioresour Technol 82:103–107.

Mirza N, Pervez A, Mahmoud Q, Shah MM, Shafqat MN. 2011. Ecological restoration of arsenic contaminated soil by *Arundo donax* L. Ecol Eng 37(12):1949–1956.

Mishra V, Tiwari A, Shukla B, Seth CS. 2009. Effects of soil amendments on the bioavailability of heavy metals from zinc mine tailings. Environ Monit Assess 155:467–475.

Olsen SR, Sommers LE. 1982. Phosphorus. Methods of soil analysis, Part 2, Chemical and microbial properties. In: Miller AL, Keeney RH, editors. Madison (WI): American Society of Agronomy. p. 403–430.

Onofri A, Carbonell EA, Piepho HP, Mortimer AM, Cousens RD. 2010. Current statistical issues in weed research. Weed Res 50:5–24.

Papazoglou EG, Karantounias GA, Vemmos SN, Bouranis DL. 2005. Photosynthesis and growth responses of giant reed (*Arundo donax* L.) to the heavy metals Cd and Ni. Environ Int 31:243–249.

Patten C, Glick B. 2002. Role of *Pseudomonas putida* indole acetic acid in development of the host plant root system. Appl Environ Microbiol 683:3795–3801.

Penrose DM, Glick BR. 2003. Methods for isolating and characterizing ACC deaminase-containing plant growth promoting rhizobacteria. Physiol Plant 118:10–15.

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

Rajkumar M, Freitas H. 2008. Influence of metal resistant-plant growth promoting bacteria on the growth of *Ricinus communis* in soil contaminated with heavy metals. Chemosphere 71:834–842.

Rajkumar M, Ma Y, Freitas H. 2008. Characterization of metal-resistant plant growth promoting *Bacillus weihenstephanensis* isolated from serpentine soil in Portugal. J Basic Microbiol 48:1–9.

Ramteke PW, Joseph B, Mani A, Chacko S. 2012. *Pisum sativum* and associated plant growth promoting rhizobacteria: effect of normal and sewage irrigation. Int J Soil Sci 18:4970–4978.

Reeves RD, Baker AJM. 2000. Metal-accumulating plants. In: Raskin I, Ensley BD, editors. Phytoremediation of toxic metals: using plants to clean up the environment. New York: John Wiley & Sons, Inc. p. 193–229.

Saitou N, Nei M. 1987. The neighbour-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425.

Sanita di Toppi L, Gabrielli R. 1999. Response to cadmium in higher plants. Environ Exp Bot 41:105–130.

Sarathambal C, Ilamurugu K. 2014. Phosphate solubilising diazotrophic bacteria associated with rhizosphere of weedy grasses in India. Ind J Weed Sci 46:80–85.

Sarathambal C, Ilamurugu K, Balachandar D, Chinnadurai C, Yogita G. 2015. Characterization and crop production efficiency of diazotrophic isolates from the rhizosphere of semi-arid tropical grasses of India. Appl Soil Ecol 87:1–10.

Sarathambal C, Thangaraju M, Paulraj C, Gomathy M. 2010. Assessing the Zinc solubilization ability of *Gluconacetobacter diazotrophicus* in maize rhizosphere using labelled ^{65}Zn compounds. Ind J Microbiol 50:S103–S109.

Saravanan VS, Madhaiyan M, Thangaraju M. 2007. Solubilisation of zinc compounds by the plant growth promoting bacterium *Gluconoacetobacter diazotrophicus*. Chemosphere 66:1794–1798.

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.

Shamsi IK, Wie K, Zhang GP, Jilani GH, Hassan MJ. 2008. Interactive effects of cadmium and aluminium on growth and antioxidative enzymes in soybean. Biol Plant 52:165–169.

Shatalov AA, Pereira H. 2005. *Arundo donax* L. reed: new perspectives for pulping and bleaching. Part 4. Peroxide bleaching of organosolv pulps. Bioresour Technol 96:865–872.

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

Sheng XF, He LY, Wang QY, Ye HS, Jiang C. 2008. Effects of inoculation of biosurfactant producing *Bacillus* sp. J119 on plant growth and cadmium uptake in a cadmium amended soil. J Hazard Mater 155:17–22.

Smith SR. 2009. A critical review of the bioavailability and impacts of heavy metals in municipal solid waste composts compared to sewage sludge. Environ Int 35:142–156.

Varghese R. 2012. Bioaccumulation of cadmium by *Pseudomonas* sp. isolated from metal polluted industrial region. Environ Res Eng Manage 61:58–64.

Varun M, Jaggi D, D'Souza R, Paul M, Kumar B. 2015. *Abutilon indicum* L.: a prospective weed for phytoremediation. Environ Monit *Assess* 187(8):527.

Vernersson T, Bonelli EG, Cerrella AL, Cukierman PR. 2002. *Arundo donax* cane as a precursor for activated carbons preparation by phosphoric acid activation. Bioresour Technol 83:95–104.

Wan Y, Luo SL, Chen JL, Xiao X, Chen L, Zeng GM, Liu CB, He YJ. 2012. Effect of endophyte-infection on growth parameters and Cd-induced phytotoxicity of Cd-hyperaccumulator Solanum nigrum L. Chemosphere 89:743–750.

Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 1991. 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 173:697–703.

Wenzel WW. 2009. Rhizosphere processes and management in plant-assisted bioremediation (phytoremediation) of soils. Plant Soil 321:385–408.

Zaida A, Khan MS, Amil MD. 2003. Interactive effect of rhizotrophic microorganisms on yield and nutrient uptake of chickpea (*Cicer arietinum* L.). Eur J Agron 19:15–21.

Zarei M, Hempel S, Wubet T, Sch€afer SH, Savaghebi GR, Jouzani GS, Nekouei MK, Buscot F. 2010. Molecular diversity of arbuscular mycorrhizal fungi in relation to soil chemical properties and heavy metal contamination. Environ Pollut 158:2757–2765.

Zhang YF, He LY, Chen ZJ, Wang QY, Qian M, Sheng XF. 2011. Characterization of ACC deaminase producing endophytic bacteria isolated from copper-tolerant plants and their potential in promoting the growth and copper accumulation of *Brassica napus*. Chemosphere 83:57–62.

Zhu LJ, Guan DX, Luo J, Rathinasabapathi B, Ma LQ. 2014. Characterization of arsenic-resistant endophytic bacteria from hyperaccumulators *Pteris vittata* and *Pteris multifida*. Chemosphere 113:9–16.