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Original Research

Role of *Acinetobacter* sp. CS9 in Improving Growth and Phytoremediation Potential of *Catharanthus longifolius* under Cadmium Stress

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Abstract

Some rhizobacteria are capable of improving metal tolerance and growth of plants under heavy metal stress. The objective of the current study was isolation and subsequent application of cadmium-resistant rhizobacteria in phytoremediation by *Catharanthus longifolius*. The screened bacterial isolate exhibited growth-promoting attributes, including phosphate solubilization, ACCD activity, auxin, and siderophores production. The inoculation of *Acinetobacter* sp. CS9 under greenhouse trial improved growth and phytoextraction capability of *C. longifolius* plants in soils contaminated with different concentrations (0, 100, and 200 mg kg^{-1}) of Cd. The plants exhibited reduced quantity of total soluble protein, soluble sugars, and chlorophyll contents under Cd stress. On the other hand, improved chlorophyll, soluble protein, and sugar contents were observed in *Acinetobacter* sp. CS9-treated plants. The inoculated plants exhibited improved activity of antioxidant enzymes (SOD and CAT) and reduced malondialdehyde levels. Moreover, higher Cd uptake and translocation ratio was observed in *Acinetobacter* sp. CS9-inoculated plants as compared to un-inoculated ones. The current study showed that *Acinetobacter* sp. CS9 reduced Cd-induced oxidative stress and improved the phytoremediation capability of *C. longifolius*.

Keywords: *Acinetobacter* sp., cadmium, *Catharanthus longifolius*, phytoremediation, stress

Introduction

The agricultural lands of Pakistan adjacent to industrial areas are severely contaminated with heavy metals, including Cd, Cu, Zn, and Ni [1]. Crop productivity is reduced on polluted soils and the presence of these heavy metals in the food chain has caused a number of health issue in humans and animals [2]. Cadmium is one of the injurious heavy metals that may be extremely phytotoxic to some plant species even at very low concentrations [3]. The improper disposal of effluents released during the process of smelting and electroplating in conjunction with burning of urban waste cause Cd contamination [4]. The metal-polluted area may be reclaimed by growing

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hyper-accumulator plants [5]. This process for metals removal, called phytoremediation, is an economically feasible and environmentally safe technique [6]. Our previous research has shown that *Catharanthus* species may be used for the phytoextration of copper and lead [7]. Some other researchers have also demonstrated the potential of these species for phytoremediation of Pb-, Cr-, and Ni-contaminated soils [8-10], which is why *C. longifolius* – a cosmopolitan ornamental plant belonging to *Catharanthus* genus – was evaluated for its potential regarding phytoremediation of Cd-contaminated soil.

The lipid peroxidation caused by increased production of reactive oxygen species (ROS) enhances the membrane permeability in plants under Cd stress [11]. The superoxide dismutase (SOD) helps plants overcome this oxidative stress by dismutation of O_2^- to O_2 and H_2O_2 [12]. Similarly, peroxidase (POD), ascorbate peroxidase (APX), and catalase (CAT) detoxify H_2O_2 [13]. Moreover, the formation of phytochelatins and metal complex help in detoxifying or distributing injurious metals to specific parts of the plants [14]. However, most of the hyper-accumulator plants recognized hitherto exhibit poor growth, biomass production, and phytoremediation potential in soils polluted by higher concentrations of heavy metal [15].

The rhizospheric microbes associated with plant roots may manipulate plant growth and uptake of heavy metals in an environmentally safe manner [16]. These microorganisms are capable of enhancing the mobility and subsequent bioavailability of heavy metals [17]. Certain rhizobacteria that have the ability to produce siderophore and plant hormones improve uptake and translocation of metalloids in assisted plants [18]. The rhizobacteria having growth promoting attributes such as synthesis of phytohormones, nitrogen fixation, solubilization of phosphate, and other nutrients may improve plant growth. These soil-inhabitant bacteria are termed plant growth-promoting rhizobacteria (PGPR). These PGPR assist inoculated plants in alleviating heavy metal stress [19-20]. Consequently, the intentions of current research were: (1) screening and identifying Cd-resistant rhizobacteria, (2) assessing growth-promoting attributes of screened bacterium, and (3) evaluating the Cd phytoextraction and stress-alleviating capability of inoculated and un-inoculated *C. longifolius* plants.

Material and Methods

Procuring Rhizobacteria

For the isolation of Cd-resistant rhizobacteria, 10 g composite rhizospheric soil obtained from healthy *C. longifolius* plants growing in soil polluted by heavy metal-contaminated industrial effluents was mixed thoroughly in 90 ml distilled sterilized water [21]. From soil sample 10^{-6} serial dilution was spread plated on LB media supplemented with $CdCl_2$ (200 mg l^{-1} Cd) and incubated for 3 days at 28°C. The rhizobacteria with distinguished bacterial colony was cultured on Cd-contaminated media according to the spread plate technique [22].

Analyzing Minimum Inhibitory Concentration (MIC)

The obtained bacterial isolates were cultured on different concentrations of Cd-contaminated LB media at 28ºC for 72 h to assess MIC value according to the European food safety authority (EFSA) in Parma, Italy (2012) [23].

Identifying Screened Rhizobacteria

From the isolated Cd-resistant rhizobacteria, the uppermost Cd-resistant CS9 (250 mg l^{-1} Cd; MIC) was identified by observing morphological and biochemical tests [24]. The bacterial identification was further confirmed by 16 s rRNA gene analysis. The genomic DNA of bacterial isolate was extracted with the help of a QIAGEN genomic DNA isolation kit following the manufacturer's guidelines. The universal primers 27F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1492R (5′- GGT TAC CTT GTT ACG ACT T-3′) were employed for the amplification of 16S rRNA gene followed by performing PCR in the thermocycler. After amplifying 16S rRNA, products were purified using a PCR purification kit. The big dye terminator cycle sequencing kit was used to sequence the product. The sequence obtained for *Acinetobacter* sp. CS9 was matched with an existing sequence in the database Gene Bank by means of NCBI BLAST and submitted to the Gene Bank. The screened rhizobacteria was also deposited in the Bacterial Conservatory, Institute of Agricultural Sciences, University of the Punjab Lahore Pakistan under accession number 686.

Quantifying Water-Extractable Cadmium from Soil

For determining water-extractable Cd, 4 g of inoculated and un-inoculated soil sample was mixed in 40 ml water and placed in a shaker at 25ºC. After 48 h, soil suspension was centrifuged and filtered supernatant was used for assessing Cd [25].

Growth-Promoting Attributes of *Acinetobactor* sp. CS9

We observed the indole acetic acid (IAA) production capability of screened bacterial isolate. For this purpose, the 50 µl of bacterial cells suspended in saline solution (0.9%) were inoculated in an agar plate placed in shade for 72 h at 28ºC. Then Salkowsky reagent (200 µl) was also applied over bacterial cells. The formation of pink colour after 0.5 h was an indication of auxin-producing

capability [26]. The screened bacterial isolate was inoculated in plates containing Pikovskyaya's medium containing 6 gl^{-1} NaCl supplemented with insoluble tricalcium to determine phosphate solubilization capability. The inoculated plates were placed at 38ºC for 72 h. The formation of clear halo in plates represented positive testing for phosphate solubilization [27].

The screened bacterial isolate was inoculated on chrome azurol S agar plates placed at 28ºC for 72 h. The formation of an orange halo surrounding the bacterial colony was regarded as a positive test for siderophore production capability [28]. The 1-aminocyclopropane-1-carboxylate (ACC) deaminase (ACCD) activity was analyzed accordingly [29].

Preparing the Bacterial Inoculum

The rhizobacteria was cultured on LB broth placed on a rotary shaker (120 rpm) at 28°C. After 24 h, the bacterial culture was centrifuged at 6,000 × *g* for 5 min. The concentration of bacterial cells was adjusted (10^8 CFU ml^{-1}) in sodium chloride solution (0.8 %) by taking absorbance at 610 nm.

Greenhouse Experiment

The composite soil sample was air-dried and passed through a 6-mm plastic sieve. The physicochemical characteristics of loamy soil obtained from the Conventional and Non-Conventional Agricultural Farm at the University of the Punjab exhibited: P 0.81 g kg^{-1}, K 2.95 g kg^{-1}, N 81.3 mg kg^{-1}, Cd 0.49 mg kg^{-1}, Ni 0.96 mg kg^{-1}, Zn 60.11 g kg^{-1}, and OM 4.03 g kg^{-1}, with 7.3 pH. All the living organisms from soil were killed by sterilizing at 121ºC for 35 min [30]. While using $CdCl_2$, this soil was contaminated with 0, 100, and 200 mg Cd kg^{-1} soil. The contaminated soil was subjected to equilibrium with distilled sterilized water saturating for 30 days in a greenhouse at 25±3ºC. The soil samples (2 kg) were filled in the allotted plastic pots (50 cm^3) devoid of bottom holes. The seeds of *C. longifolius* were soaked in *Acinetobacter* sp. CS9 inoculum for 30 min. The distilled water was used for seed-soaking in case of non-inoculated treatments. The treated seeds were sown equidistantly in allotted pots (5 seed pot^{-1}). Each treatment had 3 replicates (three pots) and all pots were placed under greenhouse conditions at 25±3ºC for 60 days and irrigated with equal amounts of distilled water every 2nd day.

Analyzing Lipid Peroxidation

Lipid peroxidation was estimated by quantifying malondialdehyde (MDA) produced by the thiobarbituric acid reaction [31]. For this purpose, a fresh plant sample was mixed with ice-cold extraction buffer (0.7% of NaH_2PO_4. 2 H_2O and 1.6% Na_2HPO_4. 12 H_2O) using a mortar and pestle. The homogenate was centrifuged at 14,000 × g for 0.5 h. The one ml resulting supernatant was homogenized with 4 ml of 0.5% (w/v) *thiobarbituric acid* solution supplemented with 20% (w/v) *thiobarbituric acid*. This solution was placed at 95ºC for 0.5 h and cooled immediately. The colorimetric value of the solution was observed at 532 nm, while correction for non-specific absorbance was accomplished by deducting the absorbance value obtained at 600 nm. The amount of MDA was evaluated from its molar extinction coefficient (155 $mM^{-1}cm^{-1}$).

Assessing Enzymatic Activity

The frozen fresh plant samples were ground with the help of an ice-cold mortar and pestle. For evaluating SOD activity, a ground plant sample was homogenized with 3 mL of 100 mM K-phosphate extraction buffer (pH 7.8), EDTA (0.1 mM), 2-mercaptoethanol (14 mM), and Triton X-100 (0.1% v/v). Whereas for evaluation of CAT and APX activity, the ground plant sample was homogenized with 50 mM K-phosphate extraction buffer (pH 7.0), EDTA (2 mM), ascorbate (20 mM), and Triton X-100 (0.1% v/v). The homogenates were centrifuged at 15000 ×g at 4ºC for 15 min and supernatant were used for determining total soluble protein [32] and subsequent enzyme activity.

For evaluating SOD cativity, the absorbance of reaction mixture having 50 mM K phosphate buffer (pH 7.8), EDTA (0.1 mM), methionine (13 mM), nitrobluetetrazolium (75 µM), riboflavin (2 µM), and enzyme extract (100 µl) was observed at 560 nm [33]. For assessing CAT activity, enzyme extract (20 µl) was homogenated with reaction mixture (3 mL) having 100 mM phosphate extraction buffer (pH 7.0), EDTA (0.1 mM), and H_2O_2 (0.1%). The reduced quantity of H_2O_2 in homogenate was estimated at 240 nm and calculated with the help of extinction coefficient [34]. For determining POD activity, enzyme extract (0.1 ml) was homogenized with 20 mMl^{-1} guaiacol (50 µl) and 10 mMl^{-1} phosphate buffer (2.8 ml at pH 7.0). The reaction mixture contained 40 mMl^{-1} H_2O_2 (50 µl). The oxidation of guaiacol to tetraguaiacol was observed at 470 nm for 1 min [35].

Determining Soluble Sugar Contents

For analysis of soluble sugars, the fine powder of plant samples (100 mg) was thoroughly mixed with 80% (v/v) ethanol. The spectrophotometric value of this solution was observed after 72 h at 585 nm and compared with the standard curve of glucose [36].

Determining Chlorophyll Content

The chlorophyll contents from fresh leaves were analyzed by taking SPAD value with the help of a chlorophyll meter.

Determining Plant Growth, Bioconcentration, and Translocation Factors

After 60 days, the treated *C. longifolius* plants were uprooted carefully, washed with tap water, and air-dried at room temperature. The number of leaves, root length, shoot lengths, and their respective fresh weight were measured. The dry weight of leaves, roots, and shoots were also measured by drying these samples in an oven at 120°C for 12 h. The oven-dried leaves, root, and shoot samples were finely ground and the fine powders of these samples were digested with nitric acid-perchloric acid (4:1, v/v) solution. The quantity of Cd present in plant tissues was analyzed using flame atomic absorption spectrophotometry. The bioconcentration factor (BCF) and translocation factor (TF) for Cd were measured from the following formulas:

$$\mathrm{BCF} = \frac{\text{Cd contents in shoot}}{\text{Cd contents in soil}}$$

$$\mathrm{TF} = \frac{\text{Cd contents in shoot}}{\text{Cd contents in root}}$$

Statistical Analysis

The research trials were conducted using completely randomized design. There were three replicates for each treatment. The data were subjected to analysis of variance and tested at $P \leq 0{\cdot}05$ significance level using DSAASTAT software. Significance differences were estimated by Duncan's test.

Results and Discussion

Cd-tolerance, Bacterial Identification, and Analysis of Growth-Promoting Characteristics

The bacterial isolate that showed maximum MIC value for Cd (250 mg l^{-1}) was screened for downstream experimentation. This rod-shaped bacterium showed pale circular colony on petri plates. This rhizobacterium exhibited positive results for catalase, citrate, and arginine hydrolysis tests. It showed negative results for oxidase, motility, urease, nitrate reduction, hemolysis, gelatin hydrolysios, and Gram tests. According to Bergey's Manual, the screened bacterium was identified as *Acinetobactor* sp. This identification was confirmed by molecular identification. The isolate was assigned accession number *Acinetobactor* sp. CS9: KY026606 from NCBI. The similarity index analysis of said bacterium with already existing bacteria demonstrated that it had 98.56%, 96.53%, 95.87%, and 91.30% alignment similarity with *Acinetobacter* sp. strain SZ051, *Acinetobacter* sp. strain Y15, *Acinetobacter* sp. strain 5L4, and *Acinetobacter* sp. AG-LSL1, respectively.

On the basis of minimum inhibitory concentration, bacterium was found resistant up to 250 mg l^{-1} Cd. Results showed that *Acinetobacter* sp. CS9 exhibited positive results for auxin and siderophores production. The strain also demonstrated phosphate solubilization and ACCD activity. According to our information, this is the first time that a native *Acinetobacter* sp. has exhibited plant growth-promoting attributes and Cd resistance.

Effect of *Acinetobactor* sp. CS9 on Growth of *C. longifolius* Plants

During our current study, reduced growth in terms of root length, shoot length, number of leaves, root, shoot, and leaves dry biomass was observed in *C. longifolius* plants cultivated in Cd-contaminated soils as compared to those growing in non-contaminated soils. However, *Acinetobactor* sp. CS9 inoculation improved the growth of *C. longifolius* plants grown either in the absence or presence of Cd stress (Fig. 1). Our results revealed that the shoot length, root length, number of leaves, and root, shoot, and leaves dry biomass declined up to 13%, 22%, 24%, 19%, 21%, and 11%, respectively, under the influence of 100 mg kg^{-1} Cd as compared to non-contaminated control, whereas *Acinetobactor* sp. CS9 inoculation improved shoot length, root length, number of leaves and roots, and leaves as well as shoot dry weight up to 11%, 13%, 14, 20%, 33%, and 15%

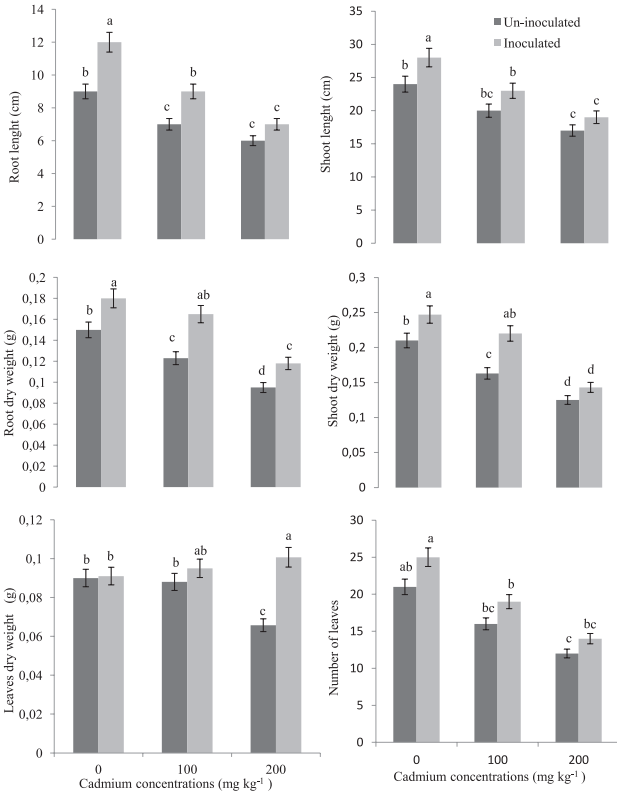

Fig. 1. Effect of *Acinetobacter* sp. CS9 on growth attributes of *C. longifolius* grown in Cd-contaminated soil; data presented are the means ±SD from three replicates of each treatment; values with different letters demonstrate significant differences at $P \leq 0.05$.

under 200 mg kg^{-1} Cd, respectively, as compared to corresponding un-inoculated treatment.

The plants may tolerate heavy metal contamination up to certain limit above which plants exhibit symptoms of metal phytotoxicity [37]. It was revealed that Cd reduces plant growth and biomass production in a dose-dependent manner. Some other researchers have also reported the negative effect of Cd stress on growth and biomass production of subjected plants [38-39]. The reduced biomass production may be a result of Cd phytotoxicity that reduces nutrient uptake and photosynthetic activity in plants [40]. The stress may reduce turgidity of plant cells [41]. Results of the previous study also revealed that bacteria inoculation increased biomass production in *Solanum nigrum* plants subjected to Cd stress [42]. The bacteria capable of synthesizing auxin and with phosphate solubilization capability boost plant growth by increasing cell division and elongation, root initiation, and stress alleviation [43-44].

Effect of Bacterial Inoculation on Physiological traits of *C. longifolius* Plants

The *C. longifolius* were challenged by being grown in soils amended with different concentrations of Cd. The physiological parameters such as activity of antioxidant enzymes (CAT, POD, SOD) and quantification of MDA, chlorophyll, soluble sugar, and protein contents were employed to determine the extent of Cd stress in *C. longifolius* plants. During our current study, un-inoculated *C. longifolius* plants exhibited enhanced SOD activities in 100 mg kg^{-1} Cd as compared to un-contaminated control. However, relatively decreased SOD activities were observed in 200 mg kg^{-1} Cd in contrast to 100 mg kg^{-1} Cd (Fig. 2). On the other hand, the inoculation of *Acinetobactor* sp. CS9 improved activities of SOD in the leaves and roots of *C. longifolius* under 0, 100, and 200 mg Cd kg^{-1} soils as compared to respective un-inoculated treatments. During our present experiments, CAT activities decreased when un-inoculated plants were subjected to higher concentrations of Cd. The inoculated plants showed relatively higher CAT activity in contrast to respective un-inoculated treatment. The inoculated *C. longifolius* plants showed 32% and 13% reduced POD activity in their leaves under 100 and 200 mg kg^{-1} Cd-treated soils as compared to corresponding un-inoculated treatments, respectively. A similar trend of decreased POD activity was observed in roots of inoculated plants subjected to Cd stress (Fig. 2). The increased activity of antioxidant enzymes may reduce the production of ROS in plants under stress. The current study revealed increased levels of CAT and SOD in plants under Cd stress [45]. The activity of these enzymes was further increased in inoculated plants under Cd stress. However, increased POD activity was observed in plants growing in Cd-contaminated soils. The inoculated plants exhibited reduced POD activity in all treatments as compared to respective un-inoculated treatment. Our results regarding POD activity are in accordance with already reported findings by some researchers [46].

The quantity of soluble sugar contents in plant leaves was dose dependent and reduced by increasing the concentration of Cd in soils. However, the application of *Acinetobactor* sp. CS9 further improved the amount of soluble sugar contents in *C. longifolius* as compared to un-inoculated control (Fig. 3). Reduced SPAD values were observed in plants under Cd stress. This reduction in chlorophyll content was dose-dependent and least chlorophyll contents were recorded in un-inoculated plants grown in 200 mg kg^{-1} Cd. The bacterial inoculation improved chlorophyll contents in all treatments in contrast to respective un-inoculated plants. Significantly low soluble protein contents were observed in 200 mg kg^{-1} Cd-treated soils compared to un-contaminated control. The inoculated *C. longifolius* plants showed relatively higher soluble protein contents in their leaves and roots in contrast with corresponding un-inoculated plants.

It has been observed that metal-resistant growth-promoting rhizobacteria improve photosynthetic contents and subsequent plant growth in plants growing under heavy metal stress [47]. Some researchers have revealed that plants exhibit reduced total sugar contents under stress [48]. However, inoculation of growth-promoting bacteria enhance both total sugar and protein contents

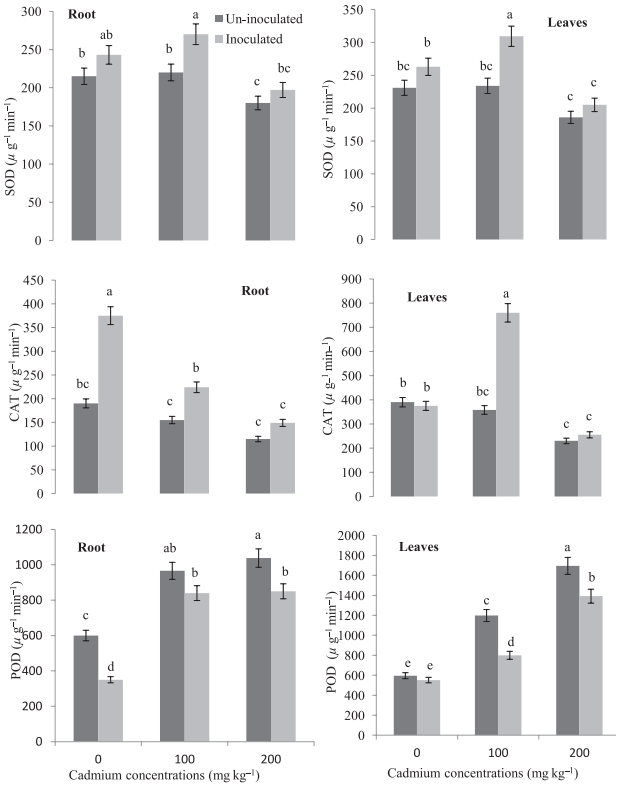

Fig. 2. Effect of *Acinetobacter* sp. CS9 on the antioxidant enzymes of *C. longifolius* grown in Cd-contaminated soil; data presented are the means ±SD from three replicates of each treatment; values with different letters demonstrate significant differences at $P \leq 0.05$.

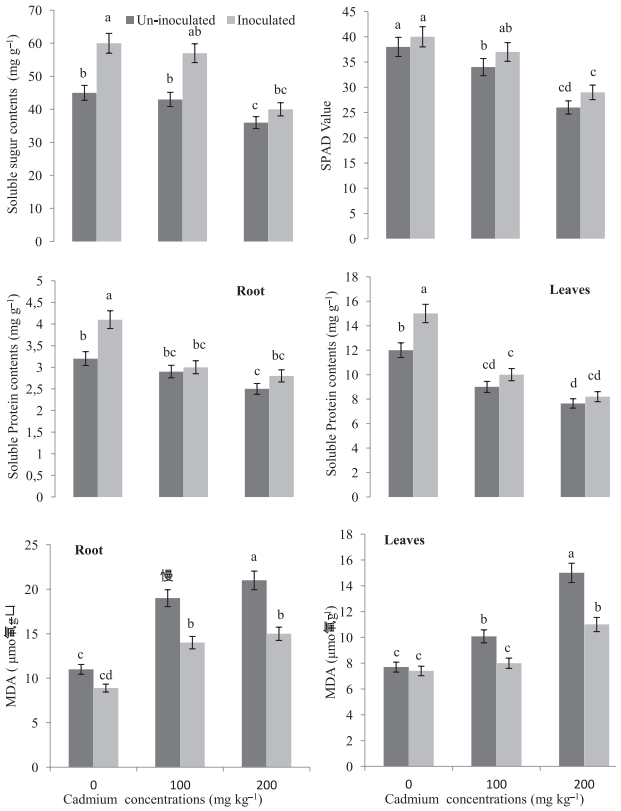

Fig. 3. Effect of *Acinetobacter* sp. CS9 on soluble sugar, SPAD value, soluble protein, and MDA of *C. longifolius* grown in Cd-contaminated soil; data presented are the means ±SD from three replicates of each treatment; values with different letters demonstrate significant differences at $P \leq 0.05$.

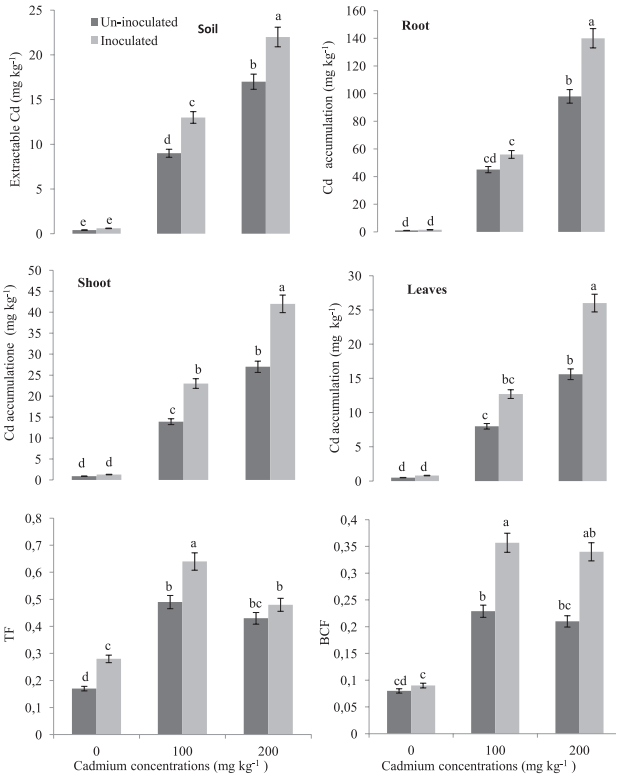

Fig. 4. Effect of *Acinetobacter* sp. CS9 on water-extractable Cd from soil, Cd accumulation (roots, stems, and leaves), TF, and BCF of *C. longifolius* grown in Cd-contaminated soil; data presented are the means ±SD from three replicates of each treatment; values with different letters demonstrate significant differences at $P \leq 0.05$.

in metal-stressed plants and help in alleviating relative stress [49-50].

Higher MDA levels in plants were observed at higher Cd concentrations. However, inoculated plants showed relatively low MDA contents as compared to corresponding un-inoculated plants under Cd stress (Fig. 3). The reduced MDA contents in inoculated plants advocate for *Acinetobacter* sp. CS9 helping mitigate Cd-induced oxidative stress [51].

Effect of *Acinetobactor* sp. CS9 on Bioavailability and Uptake of Cd

The value of water-extractable Cd in un-inoculated soil was 9 and 17 mg kg^{-1} under 100 mg kg^{-1} Cd and 200 mg kg^{-1} Cd-amended soil, respectively. However, bacterial inoculation increased the value of water-extractable Cd up to 41% and 26% in 100 mg kg^{-1} Cd and 200 mg kg^{-1} Cd-treated soils, respectively, as compared to corresponding un-inoculated soils (Fig. 4). During the present study, higher contents of Cd were observed in roots as compared to aerial parts of *C. longifolius* plants under Cd stress. The cadmium may be absorbed in roots through symplastic or apoplastic pathways [52]. Similar to our findings, higher Cd contents were observed in roots of tall fescue and bluegrass plants subjected to Cd stress [53]. The un-inoculated plants exhibited 41, 8, and 15 mg kg^{-1} Cd uptake in their roots, leaves, and shoots, respectively, under 100 mg kg^{-1} Cd. Moreover, the un-inoculated plants showed 98, 17, and 26 mg kg^{-1} Cd in their roots, leaves, and shoots under 200 mg kg^{-1} Cd, respectively. After bacterial inoculation, the Cd uptake enhanced up to 29%, 41%, and 46% in the roots, leaves, and shoots under 100 mg kg^{-1} Cd, respectively. Similarly, the Cd accumulation enhanced up to 25%, 37%, and 39% in roots, leaves, and shoots of inoculated *C. longifolius* plants, respectively, as compared to relevant un-inoculated treatments. During the current study, Cd translocation from root to shoot was significantly improved in *Acinetobactor* sp. CS9-inoculated plants grown in 100 mg kg^{-1} Cd-amended soils as compared to un-inoculated ones. Similarly, the bioconcentration factor of Cd also significantly enhanced in inoculated *C. longifolius* plants treated with 100 mg kg^{-1} Cd in contrast to un-inoculated plants. With the application of *Acinetobactor* sp. CS9 on *C. longifolius* plants, the TF and BCF increased up to 13% and 38% under 200 mg kg^{-1} Cd with respect to corresponding un-inoculated treatments, respectively (Fig. 4).

The symbiotic relationships between plant-microbes play a key role in phytoremediation of Cd-polluted soils

[54]. Growth-promoting bacteria may alleviate metal stress by restricting the movement of metalloids in roots or the dilution of metal contents within specific tissues of assisted plants [55]. Heavy metals obstruct the uptake and translocation of phosphorus and other nutrients in plants. Nevertheless, bacteria capable of synthesizing IAA with phosphate-solubilizing capability boost nutrient uptake, stress mitigation, and phytoremediation in assisted plants [56]. Similarly, *Acinetobacter* sp. CS9 is capable of producing siderophore, which may have increased Cd mobility toward plants, resulting in enhanced phytoextraction by inoculated plants [57]. The present results show that *Acinetobacter* sp. CS9 is capable of reducing Cd phytotoxicity in inoculated *C. longifolius* plants by increasing the quantity of photosynthetic pigments, shoots, and root growth, along with subsequent biomass production. The improved biomass enhances Cd phytoextraction in inoculated plants. In general, the current study shows that *Acinetobacter* sp. CS9 may mitigate Cd stress and improve the phytoextraction potential of *C. longifolius* plants.

Conclusions

The current study demonstrates that *Acinetobacter* sp. CS9 improves stress alleviation, growth, and phytoremediation potential of *C. longifolius* under Cd stress. The growth-promoting attributes of this bacterial isolate such as capability for siderophore production, phosphate solubilization, ACCD, and auxin production makes it an ideal candidate for exploitation in trials regarding metal stress alleviation and phytoremediation. The further genetic and molecular research will help to elucidate plant-microbe synergistic relationships to develop effective phytoremediation strategies.

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Conflict of Interest

The authors declare no conflict of interest.

References

1. HAMID A., RIAZ H., AKHTAR S., AHMAD S.R. Heavy Metal Contamination in Vegetables, Soil and Water and Potential Health Risk Assessment. American-Eurasian Journal of Agricultural and Environmental Sciences, **16** (4), 786, **2016**.
2. BIAN B., LIN C., LV L. Health risk assessment of heavy metals in soil-plant system amended with biogas slurry in Taihu basin, China. Environmental Science and Pollution Research, **23**, 16955, doi: 10.1007/s11356-016-6712-3. **2016**.
3. DATTA J.K., GHOSH D., BANERJEE A., KUMAR, MONDAL N. Studies on the Impact of cadmium on Growth, Yield Attributes, Yield and Biochemistry of Mung Bean (*Vigna radiata* L Wilczek) Under Natural Field Condition, Burdwan, West Bengal.. Scientia Agriculturae, **14** (1), 202, (DOI: 10.15192/PSCP.SA.2016.14.1.202209), **2016**.
4. GALLEGO S.M., PENA L.B., BARCIA R.A., AZPILICUETA C.E., IANNONE M.F., ROSALES E.P., ZAWOZNIK M.S., GROPPA M.D., BENAVIDES M.P. Unravelling cadmium toxicity and tolerance in plants: insight into regulatory mechanisms. Environmental and Experimental Botany, **83**, 33, **2012**.
5. LIU K., YU F., CHEN M., ZHOU Z., CHEN C., LI M.S., ZHU J. A newly found manganese hyperaccumulator *Polygonum lapathifolium* Linn. International Journal of Phytoremediation, **18**(4), 348, doi: 10.1080/15226514.2015.1109589, **2016**.
6. HAMZAH A., HAPSARI R.I., WISNUBROTO E.I. Phytoremediation of Cadmium-contaminated agricultural land using indigenous plants. International Journal of Environmental and Agriculture Research, ISSN 2454-1850, **2**(1), **2016**.
7. KHAN W.U., AHMAD S.R., YASIN N.A., ALI A., AHMAD A. Effect of *Pseudomonas fluorescens* RB4 and *Bacillus subtilis*189 on the phytoremediation potential of *Catharanthus roseus* (L.) in Cu and Pb contaminated soils. International Journal of Phytoremediation, **19** (6), 514, DO:10.1080/15226514.2016.1254154, **2017**.
8. KUMAR S.P., VARMAN P.A.M., KUMARI R. Stress response in *Catharanthus roseus* leaves through proteomic approach. 10th International Conference on Biology, Environment and Chemistry, **1**, IACSIT press Singapore, **2011**.
9. SUBHASHINI V., SWAMY A.V.V.S. Phytoremediation of Pb and Ni Contaminated Soils Using *Catharanthus roseus* (L.). Universal Journal of Environmental Research and Technology, **3** (4), 465, **2013**.
10. EHSAN N., NAWAZ R., AHMAD S., KHAN M.M., HAYAT J. Phytoremediation of chromium contaminated soil by an ornamental plant Vinca (*Vinca rosea* L.). Journal of Environmental and Agricultural Sciences, **7**, 29, **2016**.
11. NOCTOR G., FOYER, C.H. Intracellular redox compartmentation and ROS-related communication in regulation and signaling. Plant Physiology, **171**, 1581, **2016**.
12. BOWLER C., MONTAGU M.V., INZE D. *Superoxide dismutase and stress tolerance.* Annual Reviews in Plant Physiology and Plant Molecular Biology, **43**, 83, **1992**.
13. GILL S.S., TUTEJA N. *Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants.* Plant Physiology and Biochemistry, **48**, 909, **2010**.
14. ANDRADE S.A.L., GRA P.L., AZEVEDOB R.A., SILVEIRA A.P.D., SCHIAVINATO M.A. *Biochemical and physiological changes in jack bean under mycorrhizal symbiosis growing in soil with increasing Cu concentrations.* Environmental and Experimental Botany, **68**, 198, **2010**.

15. SOBARIU D.L., FERTU D.I., DIACONU M., PAVEL L.V., HLIHOR R.M., DRĂGOI E.N., CURTEANU S., LENZ M., CORVINI P.F., GAVRILESCU M. Rhizobacteria and plant symbiosis in heavy metal uptake and its implications for soil bioremediation. N Biotechnology, 1871-6784 (16), 32403. doi: 10.1016/j.nbt.2016.09.002, **2016**.
16. WU F.Y., HU J.L., WU S.C., WONG M.H. Grain yield and arsenic uptake of upland rice inoculated with arbuscular mycorrhizal fungi in As-spiked soils. Environmental Science and Pollution Research, **22**, 8919, **2015**.
17. KHAN W.U., YASIN N.A., AHMAD S.R., ALI A., AHMED S., AHMAD A. Role of Ni-tolerant *Bacillus* spp. and *Althea rosea* L. in the phytoremediation of Ni-contaminated soils. International Journal of Phytoremediation, **19** (5), 470, DOI: 10.1080/15226514.2016.1244167, **2017**.
18. ULLAH A., HENG S., MUNIS M.F.H., FAHAD S., YANG X. Phytoremediation of heavy metals assisted by plant growth promoting (PGP) bacteria: A review. Environmental and Experimental Botany, **117**, 28, **2015**.
19. AHMAD I., AKHTAR M.J., ASGHAR H.N., GHAFOOR U., SHAHID M. Differential effects of plant growth-promoting rhizobacteria on maize growth and cadmium uptake. Journal of Plant Growth Regulation, **35** (2), 303, **2016**.
20. KHAN W.U., AHMAD S.R., YASIN N.A., ALI A., AHMAD A., AKRAM W. Application of *Bacillus megaterium* MCR-8 improved phytoextraction and stress alleviation of nickel in *Vinca rosea*. International Journal of Phytoremediation, **19** (9), 813, DOI: 10.1080/15226514.2017.1290580, **2017**.
21. GHAZIFARD K., TAVAKOL. Identification of bacteria resistant to heavy metals in the soils of Isahan provice. Iranian Journal Science and Technology, **31**, 122, **2007**.
22. SHRIVASTAVA A., SINGH V., JADON S., BHADAURIA S, Heavy metal tolerance of three different bacteria isolated from industrial effluents. International journal of pharmaceutical research and bio-science, **2**, 137, **2013**.
23. EUROPEAN FOOD SAFETY AUTHORITY, PARMA, ITALY (EFSA). Guidance on the assessment of bacterial susceptibility to antimicrobial of human and veterinary importance. EFSA Journal, **10** (6), 2740, **2012**.
24. BERGEY D.H., HOLT J.G., KRIEG N.R., SNEATH P.H.A. Bergey' s manual of determinative bacteriology, 9th ed., (Breed RS, Murray EGD and Smith NR, eds.) WILLIAMS and WILKIMS, Baltimore, **1994**.
25. YASIN N.A., ZAHEER M.M., KHAN W.U., AHMAD S.R., AHMAD A., ALI A., AKRAM W. The beneficial role of potassium in Cd-induced stress alleviation and growth improvement in *Gladiolus grandiflora* L. International Journal of Phytoremediation, (accepted), **2017**.
26. SARWAR M., KREMER R.J. Enhanced suppression of plant growth through production of L-tryptophan-derived compounds by deleterious rhizobacteria. Plant and Soil, **172**, 261, **1995**.
27. PIKOVSKYAYA R.I. Mobilization of phosphorus in soil in connection with vital capacity of source microbial species. Microbiologia, **17**, 326, **1948.**
28. SCHWYN B., NEILANDS J.B. Universal chemical assay for detection and determination of siderophores. Analytical Biochemistry, **160**, 47, **1987**.
29. HONMA M., SHIMOMURA T. Metabolism of 1-aminocyclopropane-1carboxylicacid. Agricultural Biology and Chemistry, **42**, 1825, DOI:10.1271/bbb1961.42.1825, **1978**.
30. ZHOU W, QIN S., LYU D., ZHANG P. Soil sterilisation and plant growth-promoting rhizobacteria promote root respiration and growth of sweet cherry rootstocks. Archives of Agronomy and Soil Science, **61**(3), 361, **2015**.
31. HEATH R.L., PACKER L. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics, **125**, 189, **1968**.
32. BRADFORD M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, **72** (1-2), 248, DOI: 10.1016/0003-2697 (76) 90527, **1976**.
33. GIANNOPOLITIS C.N., RIES S.K. Superoxide dismutases: I. Occurrence in higher plants. Plant Physiology, **59** (2), 309, DOI: 10.1104/pp.59.2.309, **1977**.
34. HAVIR E.A., MCHALE N.A. Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. Plant Physiology, **84** (2), 450, DOI: 10.1104/pp.84.2.450, **1987**.
35. FIELDING J.L., HALL J.L. A biochemical and cytological study of peroxidase activity in roots of *Pisum sativum*. Journal of Experimental Botany, **29**, 969, **1978**.
36. SHIELDS R., BURNETT W. Determination of protein-bound carbohydrate in serum by a modified anthrone method. Analytical Chemistry, **32**, 885, **1960**.
37. LIN Y.F., AARTS M.G. The molecular mechanism of zinc and cadmi-um stress response in plants. Cell and Molecular Life Sciences, **69**, 3187, **2012**.
38. HOSSAIN M.A., PIYATIDA P., DA-SILVA J.A.T., FUJITA M. Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. Journal of Botany, **2012**, 37, DOI:10.1155/2012/872875, **2012**.
39. NIE J., LIU Y., ZENG G., ZHENG B., TAN X., LIU H., XIE J., GAN C., LIU W. Cadmium accumulation and tolerance of *Macleaya cordata*: a newly potential plant for sustainable phytoremediation in Cd-contaminated soil. Environmental Science and Pollution Research, DOI 10.1007/s11356-016-6263-7, **2016**.
40. KHAN A.R., ULLAH I., KHAN A.L., PARK G.S., WAQAS M., HONG S.J., JUNG B.K., KWAK Y., LEE I., SHIN J.H. Improvement in phytoremediation potential of *Solanum nigrum* under cadmium contamination through endophytic assisted *Serratia* sp. RSC-14 inoculation. Environmental Science and Pollution Research, **22**, 14032, doi:10.1007/s11356-015-4647-8, **2015**.
41. KANG S.M., KHAN A.L., WAQAS M., YOU Y.H., KIM J.H., KIM J.G., HAMAYUN M., LEE I.J. Plant growth-promoting rhi-zobacteria reduce adverse effects of salinity and osmotic stress by regulating phytohormones and antioxidants in *Cucumis sativus*. Journal of Plant International, **9**, 673, **2014**.
42. MOHAMMADZADEH A., TAVAKOLI M., MOTESHAREZADEH M., CHAICHI M.R. Effects of plant growth-promoting bacteria on the phytoremediation of cadmium-contaminated soil by sunflower. Archives of Agronomy and Soil Sciences, DOI:10.1080/03650340.2016 .1235781, **2016**.
43. GHOSH P., RATHINASABAPATHI B., MA L.Q. Arsenic-resistant bacteria solubilized arsenic in the growth media and increased growth of arsenic hyperaccumulator *Pteris vittata* L. Bioresource Technology, 102, 8756, **2011**.

44. MESA V., NAVAZAS A., GONZÁLEZ-GIL R., GONZÁLEZ A., WEYENS N., LAUGA B., JOSE-LUIS R., SÁNCHEZ G.J., PELÁEZ A.I. Use of endophytic and rhizosphere bacteria to improve phytoremediation of arsenic-contaminated industrial soils by autochthonous Betula celtiberica. Applied & Environmental Microbiology, **10**, doi:10.1128/AEM.03411-16, **2017**.
45. GRATÃO P.L., MONTEIRO C.C., TEZOTTO T., CARVALHO R.F., ALVES L.R, PETERS L.P., AZEVEDO R.A. Cadmium stress antioxidant responses and root-to-shoot communication in grafted tomato plants. Biometals, **28**(5), 803, DOI: 10.1007/s10534-015-9867-3. Epub 2015 Jun 16. **2015**.
46. WANG Q., XIONG D., ZHAO P., YU X., TU B., WANG G. Effect of applying an arsenic-resistant and plant-growth promoting rhizobacterium to enhance soil arsenic phytoremediation by *Populus deltoides* LH05-17. Journal of Applied Microbiology, **111**, 1065, **2011**.
47. ISLAM F., YASMEEN T., ALI Q., MUBIN M., ALI S., ARIF M.S., HUSSAIN S., RIAZ M., ABBAS F. Copper-resistant bacteria reduces oxidative stress and uptake of copper in lentil plants: potential for bacterial bioremediation. Environmental Science and Pollution Research, **23**, 220, DOI 10.1007/s11356-015-5354-1, **2016**.
48. SANDHYA V., ALI Z.S.K., GROVER M., REDDY G., Venkateswarlu B. Effect of plant growth promoting *Pseudomonas* spp. on compatible solutes, antioxidant status and plant growth of maize under drought stres. Plant Growth Regulation, **62** (1), 21, **2010**.
49. NASEEM H., BANO A. Role of plant growth-promoting rhizobacteria and their exopolysaccharide in drought tolerance of maize. Journal of Plant Interactions, **9**(1), 689, DOI: 10.1080/17429145.2014.902125, **2014**.
50. FANG Q., FAN Z., XIE Y., WANG X., LI K., LIU Y. Screening and evaluation of the bioremediation potential of Cu/Zn-resistant, autochthonous *Acinetobacter* sp. FQ-44 from *Sonchus oleraceus* L. Frontiers in Plant Sciences, **7**, 1487. DOI: 10.3389/fpls.2016.01487, **2016**.
51. MOHAMED A.A., CASTAGNA A., RANIERI A., SANITA D.I. TOPPI L. Cadmium tolerance in *Brassica juncea* roots and shoots is affected by antioxidant status and phytochelatin biosynthesis. Plant Physiology and Biochemistry, **57**, 15, **2012**.
52. KUSHWAHA A., RANI R., KUMAR, S., GAUTAM A. Heavy metal detoxification and tolerance mechanisms in plants: implications for phytoremediation. Environmental Reviews, **24**, 39, **2016**. DOI: 10.1139/er-2015-0010.
53. DONG Q., XU P.X., WANG Z.L. Differential cadmium distribution and translocation in roots and shoots related to hyper-tolerance between tall Fescue and Kentucky blue grass. Frontiers in Plant Sciences. **8**, 113, **2017**. DOI: 10.3389/fpls.2017.00113.
54. CHEN L., LUO S., LI X., WAN Y., CHEN J., LIU C. Interaction of Cd-hyperaccumulator *Solanum nigrum* L. and functional endophyte *Pseudomonas* sp. Lk9 on soil heavy metals uptake. Soil Biology and Biochemistry, **68**, 300, **2014**.
55. ASHRAF M.A., HUSSAIN I., RASHEED R., IQBAL M., RIAZ M., ARIF M.S. Advances in microbe-assisted reclamation of heavy metal contaminated soils over the last decade: A review. Journal of Environmental Management, **1** (198), 132, doi: 10.1016/j.jenvman.2017.04.060. Epub 2017 Apr 26, **2017**.
56. PRAPAGDEEA B., CHANPRASERTA M., MONGKOLSUKB S. Bioaugmentation with cadmium-resistant plant growth-promoting rhizobacteria to assist cadmium phytoextraction by *Helianthus annuus*. Chemosphere, **92**, 659, **2013**.
57. DREWNIAK L., STYCZEK A., MAJDER-LOPATKA M., SKLODOWSKA A. Bacteria, hypertolerant to arsenic in the rocks of an ancient gold mine, and their potential role in dissemination of arsenic pollution. Environmental Pollution, **156**, 1069, **2008**.