Exploration of heavy metal resistant rhizobacteria *Enterobacter cloacae* PC3 to enhance growth and metal remediation potential of *Zea mays* L. under Cd and Pb stress

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Abstract: Root-associated rhizobacteria and their ability to grow in the toxic metal polluted environment are of great interest; however, their potency to thrive in toxic metal contaminated sites and promote plant growth is still vague. The present study aimed to explore the possible mechanisms used by rhizobacteria to promote plant growth and enhanced metal uptake under Cd and Pb heavy metal stress, for this, *Enterobacter cloacae* strain PC3 was isolated from metal contaminated site and characterized for heavy metal tolerance and plant growth promoting attributes. Results revealed that rhizobacterial strain *Enterobacter cloacae* PC3 was showing resistant for 500 mg L^{-1} of Cd^{2+} ions and 500 mg L^{-1} of Pb^{2+} ions and exhibiting multiple PGP traits. Further, inoculation of strain PC3 with *Zea mays* L. increased total biomass under Cd^{2+} and Pb^{2+} ions stress. Increased biomass might be due to reduced oxidative stress caused by Cd^{2+} and Pb^{2+} ions through multiple PGP characteristics such as ACC, IAA, PO_4^{2+} solubilization and siderophore production by strain PC3. Moreover, rhizobacterial strain *Enterobacter cloacae* PC3 promoted available and exchangeable Cd^{2+} and Pb^{2+} metal ions concentration in rhizosphere demonstrated their potential to induced metal phytoextraction efficiency in *Zea mays* L. plants. Hence the findings of the study clearly indicated that *Enterobacter cloacae* strain PC3 could be a promising and sustainable tool for enhancing the efficiency of metal phytoremediation technology in heavy metal contaminated soil sites.

Keywords: Cadmium; Lead; *Zea mays*; PGPR; Phytoremediation.

1. Introduction

Soil heavy metal contamination has become one of the most severe environmental hazards and considered as major barrier to sustainable development, particularly in developing or underdeveloped nations (Ma et al. 2011). Amongst all heavy metals, cadmium (Cd) and lead (Pb) have emerged as serious toxic environmental pollutants in the past few years because of their excessive use in manufacturing and agricultural industries (Lal et al. 2011). Their elevated concentrations in the soil enormously pollute the natural ecosystem as well as alter or destruct the soil texture by reducing its fertility and nutrient availability (Pramanik et al. 2018). With the growing concern and more industrialization, there is an urgent need to address this severe problem because of their continuous accumulation in agricultural soils and water resources that pose a great threat to contaminate the food chain and cause potential risk to human health and ecosystem (Singh et al. 2017). Different conventional approaches such as physical, chemical, thermal and electrokinetic techniques have been developed by the researchers in recent years to mitigate the contamination of heavy metals from soil but unfortunately they all have failed with several demerits such as cost, non-eco-friendly nature, labour intensive, generate secondary pollutants that are more complex than previous contaminants (Glick, 2012). Taking into consideration all these problems, a plant based biological technique "Phytoremediation" has been identified around five decades as a low cost emerging, eco-friendly and sustainable solution for the remediation of heavy metal contaminated soils (Glick, 2010). Phytoremediation is a widely accepted technique in which plants are used as a remediator or accumulating agents for removal of toxic heavy metals from contaminated soils (Muthusaravanan et al. 2018). Plants used in this technique have an increased rate of heavy metal uptake, rapid translocation from root-to-shoot and excellent ability to detoxify and sequester heavy metals in their aerial parts. Because of sustainable features, phytoremediation has been considered as a best alternate for removal of heavy metals from contaminated soil without affecting the biological activity, structure and fertility (Lal et al. 2018).

Although phytoremediation is easily applicable and cost-effective technique, but it does have some inherent technical constraints like, it is restricted to the site with low pollutant concentration; the higher concentration of contaminant may check the plant growth (Bauddh and Singh 2012). Moreover, hyperaccumulator plants are usually limited to their slow growth and low biomass. Limited bioavailability of tightly bound fraction of metal ions from the soil is another demerit of phytoremediation technology. Slow transfer rate of metal from soil to root and root to shoot makes this technique inefficient (Cameselle and Gouveia 2019). To mitigate these drawbacks use of beneficial soil microbiota colonizing the rhizosphere, often known as Plant Growth Promoting Rhizobacteria (PGPR), endowed with the unique property of heavy metal resistant and plant growth promotion, have been considered as an important tool to promote phytoremediation technology by increasing plant biomass (Glick, 2010). PGPR's increase plants biomass by various mechanisms such as fixation of atmospheric nitrogen, mitigate stress by utilization of 1 aminocyclopropane-1-carboxylic acid (ACC) as a sole N source, production of siderophores and anti-pathogenic substances, production of plant growth regulators (phytohormones, such as auxins), and also through the transformation of nutrient elements like phosphorous and potassium (Lal et al. 2019). The current study was aimed with isolation and characterization of Cd and Pb resistant plant growth promoting rhizobacterial strains and their exploitation in the phytoremediation of Cd and Pb contaminated soil using *Zea mays* L. plant.

2. Materials and Methodology

2.1. Heavy metal resistant (Cd and Pb) rhizobacteria isolation and identification

The rhizospheric soil samples were collected (3-6 cm depth) from multi metal polluted soil sites of Kanpur province Uttar Pradesh, India (Gowd et al. 2010). The heavy metal contents in soil samples were determined by wet acid digestion method with the help of Inductive coupled plasma-optical emission spectroscopy (ICP-OES; Analyst Model Optima 5300V Perkin-Elmer). The average contents of heavy metals Zn, Cu, Cd, Ni, Pb, and Cr in the soil samples were determined 354.04, 10.21, 5.03, 12.44, 78.67, 889.54 mg kg^{-1} respectively. The rhizobacterial strains were isolated by serial dilution method on Luria Bertani (LB) agar medium containing 100 mgL^{-1} Cd^{2+} and Pb^{2+}, added as $Cd(NO_3)_2$ and $Pb(CH_3COO)_2$ individually or in combination, in separate petri dishes and kept for incubation at 32±2 ^oC (Chen et al. 2016). In order to select pure heavy metal (Cd and Pb) resistant bacteria, after 48 hours of incubation, morphologically distinct bacterial colonies were selected and incubated twice in the same medium. Pure culture of selected strain was preserved in 40 % glycerol stock at -80 ^oC for further experiments. Further strain PC3 exhibited high level of Cd^{2+} and Pb^{2+} resistant was selected for present study. In the whole study, $Cd(NO_3)_2$ and $Pb(NO_3)_2$ salts were used for Cd^{+2} and Pb^{+2} respectively, stock solution of 1000 mgL^{-1} respected metals was prepared in Millipore distilled water (Merck). Further, Cd^{2+} and Pb^{2+} resistant rhizobacterial strain was identified by morphologically, biochemically and 16SrRNA gene sequencing analysis. The 16S rRNA gene sequences were submitted to the NCBI Gene Bank Database and homology with the archived 16S rRNA sequences by using BLASTn programme of National Centre for Biotechnology Information database. The evolutionary analysis of the selected heavy metal resistant strain was inferred and bootstrap analysis (1000 replicates) was conducted by MEGA7 software using neighbour-joining method.

2.2. Growth response of rhizobacteria under Cd^{2+} and Pb^{2+} stress

To measure the growth response under Cd^{2+} and Pb^{2+} stress a batch experiment was performed in Erlenmeyer flask. The overnight grown fresh culture of bacterial strain was incubated in liquid LB medium supplemented with 0, 100, 200, 300, 400 and 500 mgL^{-1} Cd^{2+} and 0, 100, 200, 300, 400 and 500mgL^{-1} Pb^{2+}. The optical density was recorded at 600nm (OD_{600}) at definite time intervals using UV-visible spectrophotometer (Evolution 201, Thermo fisher Scientific USA). The nominal and actual metal ions in media was observed before and after incubation period by using Differential pulse anodic stripping voltammetry for Pb^{2+} ions while, square wave anodic stripping voltammetry for Cd^{2+} ions concentration. The minimum inhibitory concentration (MIC) (minimum concentration of toxic metal causing no visible bacteria growth), and effective concentration EC_{50} estimated statistically.

2.3. Plant growth promoting attributes

Plant growth promoting attributes such as IAA (Bric et al., 1991), ACC deaminase (Honma and Shimomura, 1978), Siderophore (Schwyn and Neilands 1987), Phosphate solubilization (Nautiyal 1999) and N_2 fixation of selected strain were performed as per the standard method reported by Mitra et al. 2018. For the utilization of 1-aminocyclopropane-1-carboxylic acid (ACC) as nitrogen source could be predicted as a consequence of the

enzymatic activity of ACC deaminase (E.C. 4.1.99.4). Cd^{2+} and Pb^{2+} resistant rhizobacterial isolate was checked for ACC deaminase activity. In brief, overnight grown bacterial isolate was streaked on NFb medium supplemented with 3.0 mM ACC (Sigma Chemical Co., USA) as the substitute of nitrogen source. After transferring bacterial cultures, NFb plates were incubated at 30ºC for four days. The ability of the isolate to utilize ACC was verified by comparing it with the control having the same isolate grown the absence of nitrogen source (Siddikee et al. 2010).

In order to estimate IAA production, the rhizobacterial isolate was grown in TSB medium containing L-tryptophan (100µg mL^{-1}) at 30±2ºC for 48 hours in shaking condition (110 rpm). The culture suspension was centrifuged at 8,000 g for 10 min. 1 mL supernatant and 2 mL of Salkowsky reagent (1 mL of 0.5 M $FeCl_3$ in 50 mL of 35% $HClO_4$) was allowed to react in dark at 28 ± 2°C for 20 min at room temperature. Development of pink colour confirms the presence of IAA. Further, quantitative estimation of IAA was done by measuring the optical density at 535nm. The optical density values were interpolated with standard calibration curve to estimate the IAA concentration.
To determine the phosphate solubilization activity, the rhizobacterial isolate was grown on NBRIP media (Nautiyal 1999) containing (gL^{-1}):- 10.0 glucose, 5.0 $Ca_3(PO_4)_2$, 5.0 $MgCl_2 .6H_2O$, 0.25 $MgSO_4.7H_2O$, 0.2 KCl, 0.1 $(NH_4)_2SO_4$, 0.5%, TCP (tricalcium phosphate) used as a source of inorganic phosphate and Bromophenol blue dye as an indicator. A brown colour zone surrounding the culture confirmed the positive result of phosphate solubilization. Further, quantification was performed by Molybdate Blue Method (Fiske and Subbarow 1925) after 5th day of incubation. The optical density of culture supernatant was recorded at 660 nm using spectrophotometer for quantitative estimation. The solubilized phosphate in culture supernatant was interpolated with standard calibration curve of KH_2PO_4. Siderophore production was detected by bacterial growth with yellow colour in CAS agar plate assay (Schwyn and Neilands 1987). Each experiment was conducted in triplicate manner to reduce experimental error.

2.4. Experimental Design

To determine the PGP effects and metal mobilization efficiency of rhizobacterial strain (PC3), a pot experiment was conducted with Cd^{2+} and Pb^{2+} spiked soil for phytoremediation strategy of *Zea mays* L. plant. For this, garden soil was collected; air dried, sieved through 2 mm sieve and spiked with different concentrations of Cd (0, 100, 200, 300, 400 and 500 mg kg^{-1}) and Pb (0, 100, 200, 300, 400 and 500mg kg^{-1}). The physico-chemical characteristics of experimental soils were also done before the metal application. The bacterial inoculum was prepared 48 hours prior to inoculation by transferring single colony in 50 ml Erlenmeyer flask containing LB medium and incubated on rotary shaker (110 rpm) at 30°C. Further, the culture was centrifuged at 7000 rpm and washed in phosphate buffer and then re-suspended in deionized-sterilized water. The isolate suspension was adjusted to 1 optical density at 600 nm which was equivalent to approximate population 10^9 CFU ml^{-1}. Further, pots (height x diameter: 6 x 10 cm) were filled with heavy metals spiked soil, further 10 seeds of *Zea mays* L. were sown in each pot. Before sowing, seeds were surface sterilized using mercuric chloride (Lal et al. 2019) and treated with *Enterobacter cloacae* PC3. The pot experiment was conducted in green house. The sampling was done at different time intervals i.e. 30, 60 and 90 days. The uprooted plants were washed with tap water and separated into root, and shoot for morphological, biochemical and heavy metals estimation.

2.5. Interaction study of rhizobacteria with *Zea mays* L. plant

Plant microbe-interaction study between selected isolate and Zea mays L. plant under heavy metal stress was explored by measuring the different plant growth parameters i.e., shoot height, root length, total dry biomass, chlorophyll and carotenoid contents.

Total Chlorophyll and carotenoid contents of intact leaves were estimated by extracting pigments in 80% (v/v) aqueous acetone solution by continuous shaking. The assay mixture was centrifuged at 6000 rpm for 10 min. The optical density in terms of light absorbance of the supernatant was measured by double beam UV-visible spectrophotometer (Evolution 201, Thermo fisher Scientific USA) against 80 percent aqueous acetone solution as blank. Chlorophyll (Chl-total) and carotenoid content was estimated by following the formula reported by Arnon (1949).

2.6. Estimation of Cd and Pb heavy metal content in treated *Zea mays* L. plant

For the determination of heavy metal accumulation in plant tissues the whole plant was harvested and washed with tap water, followed by 0.1 M HCl washing and with deionized water. Further, plant was separated into root and shoot. The dry biomass of plant was determined after shoot and root oven dried at 70 ^{o}C for 48 hours. The dried biomass (root and shoot separately) were then grinded in sterilized mortars and pestles. The grounded samples were then digested by wet acid digestion method with HNO3 and HClO4 (3:1 v/v ratio) and heated on a hot plate at 180 ^{o}C until solution became transparent. Afterwards the samples were collected and filtered. The heavy metal contents in plant tissues were determined with the help of Inductive coupled plasma-optical emission spectroscopy (ICP-OES; Analyst Model Optima 5300V Perkin-Elmer).

2.7. Data processing and statistical analysis

Total heavy metal (Cd and Pb) contents accumulated by Zea mays L. plant was calculated as:

$$\text{Total metal accumulation by plant} = \frac{\text{Shoot metal content} + \text{Root metal content}}{\text{Total dry biomass of plant}}$$

Translocation factor (TF) was calculated to evaluate the percentage of metal translocated from root to shoot of plant species following the formula by Barman et al. (2000).

$$\text{TF} = \frac{\text{Concentration of metal in plant shoots}}{\text{Concentration of metal in plant roots}}$$

All the experiments in this study were performed in triplicates to reduce the experimental error. The values given are the mean of three replicates and standard error (SE) was calculated using mean. SE represented as error bars in figures and ± in tables. The significance of differences between control and treatments was determined by the application of the post-hoc test-Tukey's t-test using SPSS version 20.0 (SPSS, Chicago, IL) statistical analytical software. Different letters represented significant differences at $p < 0.05$.

3. Results and Discussion

3.1. Isolation and characterization of Cd^{2+} and Pb^{2+} resistant rhizobacteria

Root-associated plant growth promoting rhizobacteria (PGPR) dwelling in extreme environments are likely to more prone to tolerate heavy metal stress (Pramanik et al. 2018; Lal et al 2018). Application of PGPR to promote plant growth and combat plants from heavy metal toxicity is a widely accepted method for the remediation of terrestrial environment. Therefore, the rhizospheric soil samples were taken from the metal contaminated industrial sites of Kanpur province India, where heavy metal resistant rhizobacteria naturally enriched. These sites were already reported for heavy metal contamination by Gowd et al. (2010). Further, soil samples were tested to its physicochemical characteristics and the values are depicted in the table (Table 1). It has been previously reported that bacteria isolated from metal polluted rhizospheric soil showed higher heavy metal tolerance to cope up with such environments (Mitra et al. 2018). Recently, several other Cd and Pb resistant PGPR under the genus *Enterobacter* have been reported by various workers (Chen et al. 2016; Mitra et al. 2018; Pramanik et al. 2018). In the present study, initially two strains PC1 and PC3 were growing under 100 mg L^{-1} of Cd^{2+} and Pb^{2+} stress and showing multiple PGP traits. Further screening was done on the basis of maximum tolerance limit and quantitative PGP characteristics (Table 2). It was observed that isolate PC3 exhibited maximum tolerance limit of Cd^{2+} and Pb^{2+} 500mg L^{-1}. Our findings was in accordance with Mitra et al. (2018) reported that rhizobacterial strain *Enterobacter* sp. showed tolerance upto 350mg L^{-1} Cd^{2+} and 250mg L^{-1} Pb^{2+} whereas; Pramanik et al. (2018) reported *Enterobacter aerogenes* strain K6 showed 300mg L^{-1} Cd^{2+} and 350mg L^{-1} Pb^{2+} tolerance. The selected isolate PC3 was possessing 500mg L^{-1} Cd^{2+} and 500mg L^{-1} Pb^{2+} tolerance which is noteworthy in this regard. Moreover, the selected isolate possesses multiple plant growth promoting traits *viz.* ACC deaminase activity, IAA and siderophore production and phosphate solubilization activity among the selected isolates. Thus the strain PC3 was selected finally as a heavy metal resistant PGPR with the aim to explore its effects on *Zea mays* L. plant growth and accumulation of metals under Cd^{2+} and Pb^{2+} stress. Furthermore, identification of the isolate on the basis of morphological, biochemical (data not shown) and comparative analysis of the 16SrRNA sequences with already available database was done and it was found that isolate (PC3) was close to the bacterial genera *Enterobacter cloacae*. For the evolutionary relationship, a phylogenetic tree was constructed using neighbour joining method (Fig. 1). Further, obtained sequences were submitted to GeneBank (NCBI) under the accession number KX668494 (PC3).

Table 1. Physico-chemical analysis of soil samples

Parameters	Mean value ± SD
pH	6.89±0.2
E.C (dS m^{-1})	2.62±0.02
OC (%)	0.5±0.02
TC (%)	2.13±0.05
TN (%)	0.15±0.02
TP (%)	0.52±0.06
TK (%)	1.32±0.05
Fe (ppm)	561.64±2.8
Mn (ppm)	38.86±0.70
Zn(ppm)	354.04±4.2
Cu (ppm)	10.21±0.28
Cd (ppm)	5.03±0.08
Ni (ppm)	12.44±0.21
Pb (ppm)	78.67±1.4
Cr (ppm)	1.54±0.03

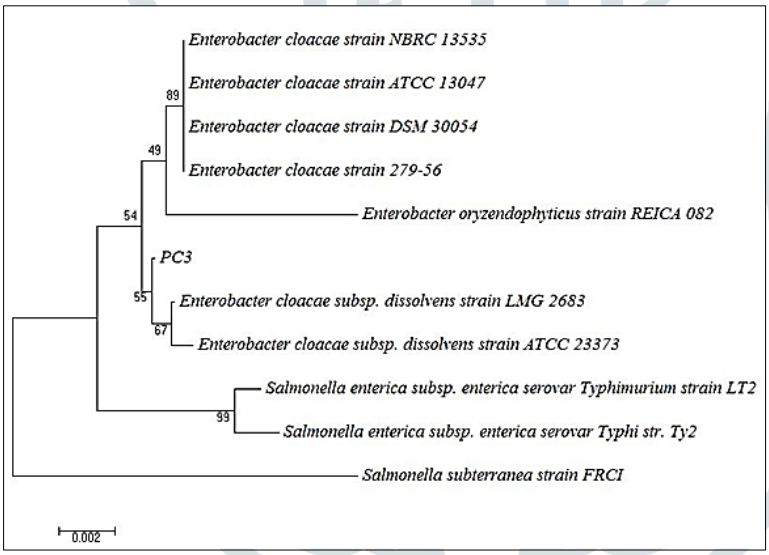


Figure 1. Evolutionary relationships of *Enterobacter cloacae* PC3. The optimal tree with the sum of branch length=0.16657409, and associated taxa clustered together in the bootstrap tests (100 replicates) are shown next to the branches. Bar indicates % similarity.

3.2. *In-vitro* plant growth promoting characteristics of selected rhizobacterial strain

Bacteria reside in rhizosphere having the ability to tolerate heavy metal toxicity and promote plant growth. Different root-associated rhizobacteria were previously reported from various heavy metal contaminated sites which have property of heavy metal resistance and plant growth promotion (Roman-Ponce et al. 2017). The selected isolate *Enterobacter cloacae* PC3 was showing dual property, multiple plant growth promotory characteristics and capability to tolerate Cd^{2+} and Pb^{2+} up to 500mg L^{-1}.

IAA is well known to be involved in cell division, cell enlargement, tissue differentiation, lateral as well as adventitious root initiation, and resistance to stressful condition. In the present study, isolate PC3 was showing ability to produce a good amount of IAA (Table.2) under Cd^{2+} and Pb^{2+} stress. Moreover, iron is essential element required for plant growth and metabolism but the soil contaminated by heavy metals usually showed Fe deficiency (Ojuederie and Babalola, 2017). Rhizobacterial strains producing siderophores solubilize and chelate Fe from soil and supply plants with Fe thereby enhancing plant growth. The selected isolate having the capability to produce siderophore (Table 2) in the presence of Cd^{2+} and Pb^{2+}. Similar findings were also reported by Dutta et al. (2018) with *Klebsiella pneumoniae* (HR1) which was able to produce siderophore and enhanced the *Vigna mungo* growth under Cd^{2+} stress. Ethylene is a gaseous hormone found in all higher

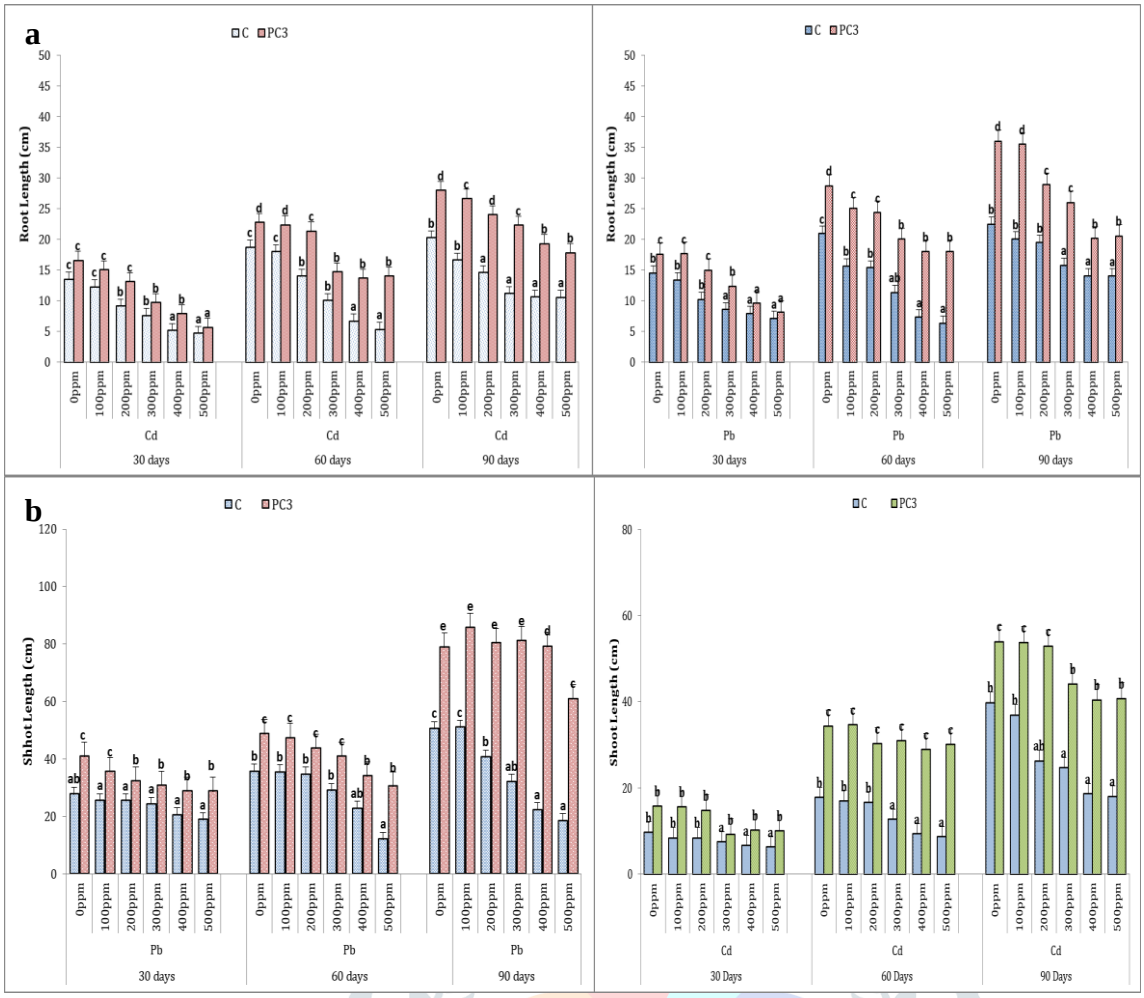
plants, and plays a crucial role in plant growth and development and possesses a key feature in a wide range of stress response. However, in stressed condition higher level of ethylene produced that negatively affects the plant growth through reducing the growth of different plant organs such as roots, shoots, leaves, flowers and fruits (Glick, 2014). On the other hand, PGPR showing property to produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme responsible for the cleavage of the plant ethylene precursor, ACC, into ammonia and α-ketobutyrate (Honma and Shimomura 1978). ACC deaminase producing rhizobacteria decrease the ethylene level in plants by decreasing the ACC in plant tissues (Glick et al., 2007). The selected isolate exhibited a positive ACC deaminase activity in the presence of Cd^{2+} and Pb^{2+} heavy metals. The results are in accordance with previous study reported by Rizvi and Khan (2017). Despite, plenty of phosphorous is found in the soil, however, most of the phosphorous in the soil founds insoluble forms (*viz.* apatite, soil phytate, phosphomonoesters etc.). Plants cannot avail insoluble form of phosphorus directly from soil (Glick, 2012). Bacteria that thrive in the rhizosphere having the characteristics to solubilize insoluble form of phosphorous into soluble form *viz.* the monobasic ($H_2PO_4^-$) and the dibasic ($H_2PO_4^{2-}$) ions by secretion of organic acids, acidification, chelation, protonation or exchange reaction (Bhattacharyya and Jha, 2012). The selected isolate PC3 was exhibiting the characteristic to solubilize phosphorous under Cd^{2+} and Pb^{2+} heavy metal stress (Table 2). This finding corroborates that phosphate solubilization activity of PC3 strain might involve either proton exchange or organic acid production mechanisms that is responsible for inorganic phosphorous solubilization in the medium (Marzban et al. 2016). Moreover, PC3 isolate could perform as potent bio-fertilizer as it was found to able fix N_2 under heavy metal stress (Table 2). Supplementation of nitrogen increases heavy metal tolerance to plants by enhancing photosynthetic activity. Similar heavy metal resistant N_2 fixing rhizobacterial strains have also been reported by several workers (Pramanik et al., 2017, Mitra et al. 2018).

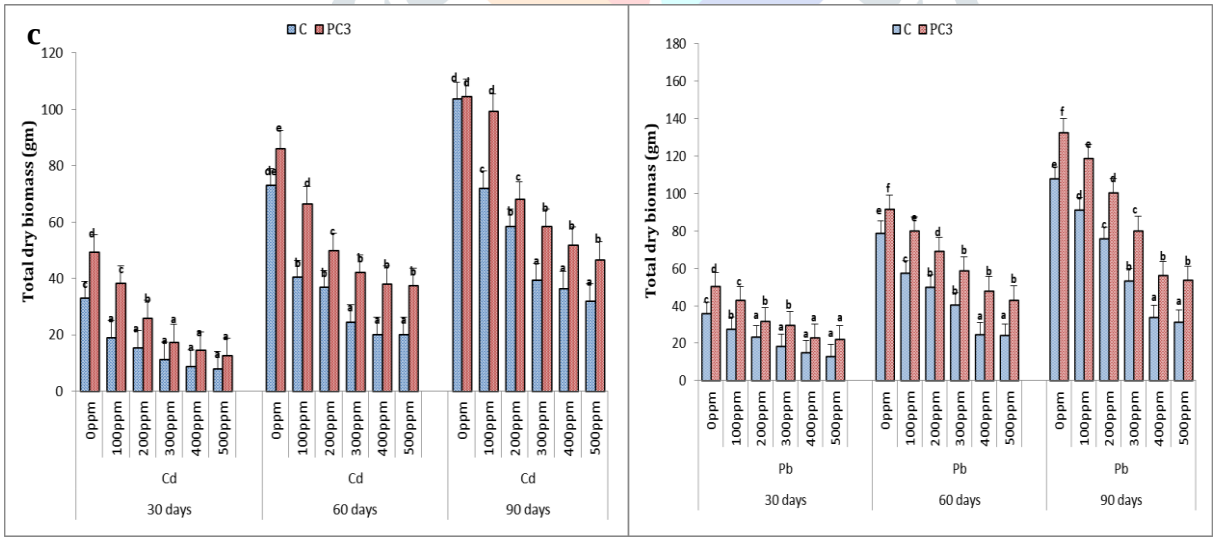
Table 2 Different PGP traits by rhizobacterial strain *Enterobacter cloacae* PC3

	PGP Traits				
Treatment	IAA production (µg/ml)	ACC deaminase production (nMAKB/µg protein)	Phosphate solubilisation (µg/ml)	Siderophore production	N_2 Fixation
-Cd, -Pb	40.73	47.58	121.23	++	++
+Cd	26.42	22.61	82.43	++	--
+Pb	36.34	33.23	110.09	++	++

3.3. Interactive effects of strain PC3 on *Zea mays* L. growth under Cd^{2+} and Pb^{2+} stress

Generally, different plant growth parameters *viz.* root length, shoot length and dry biomass are considered as indicator of plant growth and substantially supported by different nutrients, water availability, climatic factors and soil physico-chemical characteristics. However, root and shoot length provided very important information to gauge the potential of any specific plant in stress condition (Kamran et al. 2015). The present experiment investigated the interactive effects of heavy metals (Cd^{2+} & Pb^{2+}) and PC3 isolate on different growth parameters of *Zea mays* L. plant. Results revealed that strain PC3 significantly enhanced root length (> 6 fold) (Fig. 2a), shoot length (> 1.5 fold) (Fig. 2b), and total dry biomass (> 8 fold) (Fig. 2c) of *Zea mays* L. compared to their respective uninnoculated control. It was noticed that the toxic effects of Cd^{2+} and Pb^{2+} on physical growth parameters were diminished by inoculation of PC3 strain and alleviated the heavy metal toxicity of *Zea may* L. plant. Moreover, root-shoot and total biomass were directly associated to plant growth which was reported to enhance after the inoculation of PC3 under heavy metal stress. Similar findings were also reported by Kamran et al. (2015) and Mitra et al. (2018). Photosynthesis is a vital process for plant growth and development and chlorophyll is the basis of this process. Frequently, in heavy metal stress photosynthetic activity and chlorophyll biosynthesis inhibition occurs under heavy metal stress. The results of this study showed the reduction of chlorophyll and carotenoid content in the presence of Cd^{2+} and Pb^{2+} (Fig. 2d & e). It might be due to either by degrading or inhibiting the enzymes involved in chlorophyll synthesis or by blockage of photosynthetic electron transport chain (Pietrini et al. 2009). However, PC3 strain inoculated plants showed increased chlorophyll (total chlorophyll and Carotenoid) content than Cd-treated non-innoculated plants. Similar findings were also corroborated by Tirry et al. (2018).


a
C PC3
Root Length (cm)
0ppm 100ppm 200ppm 300ppm 400ppm 500ppm
Cd
30 days
60 days
90 days
Pb
b
Shoot Length (cm)
Shoot Length (cm)
30 Days
60 Days
90 Days


c
C PC3
Total dry biomass (gm)
0ppm 100ppm 200ppm 300ppm 400ppm 500ppm
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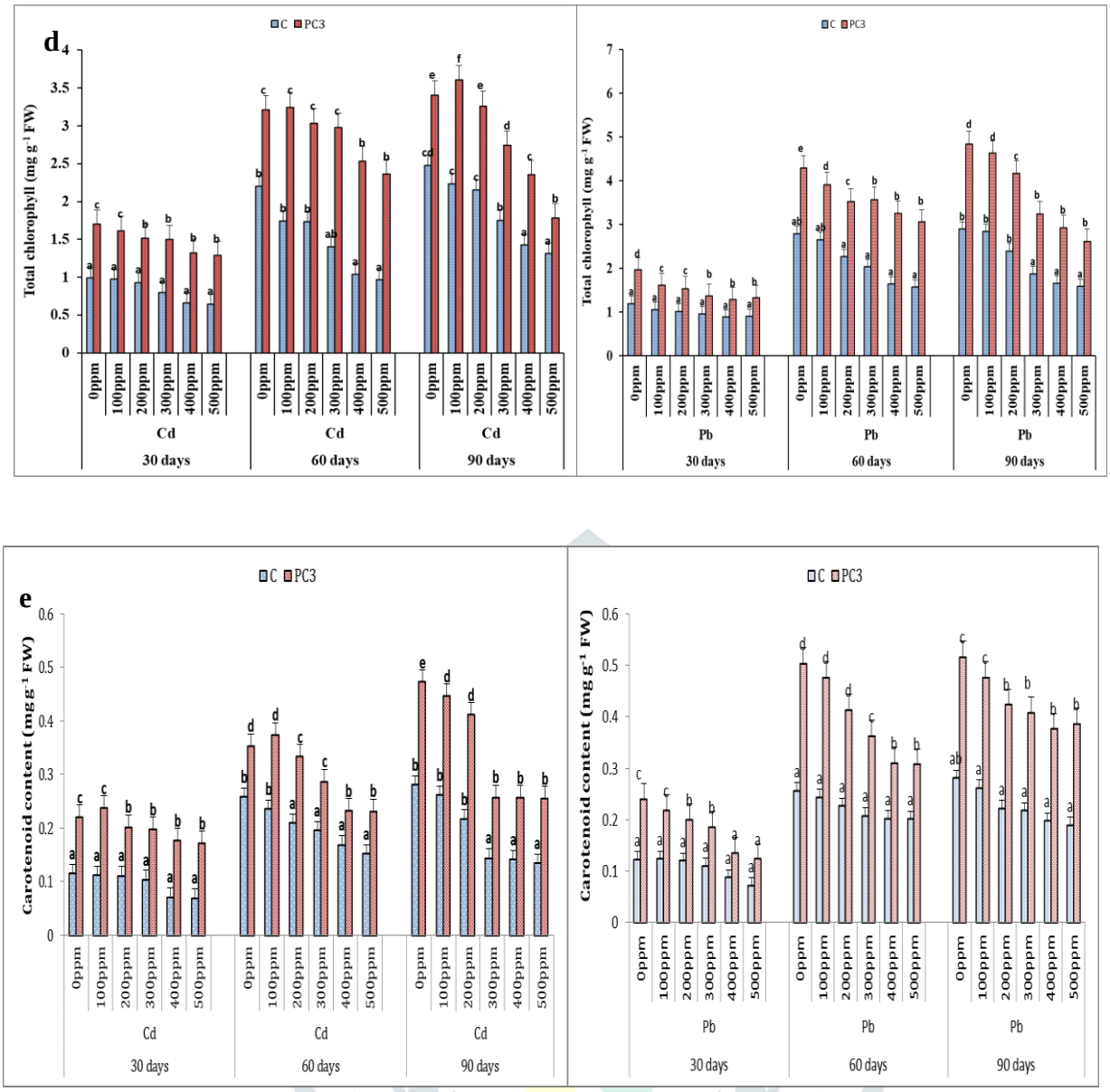


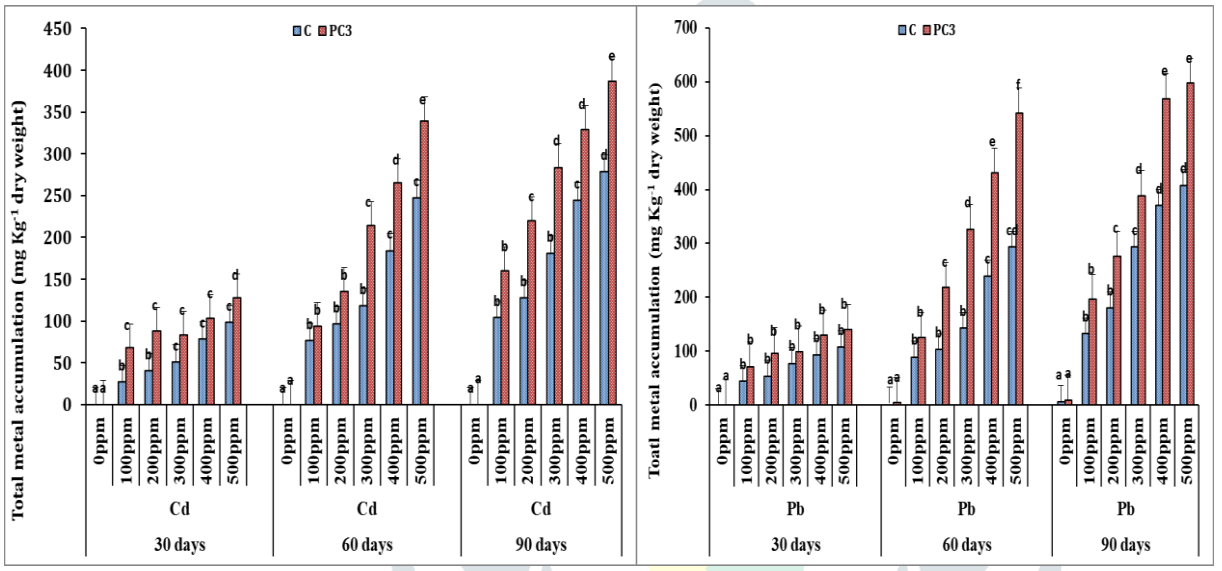
Figure 2. Showing root length (a), shoot length (b), total dry biomass (c), total chlorophyll and carotenoid content (d) in *Zea mays* plant treated with rhizobacteria *Enterobacter cloacae strain* PC3 under Cd and Pb stress.

1.1. Accumulation and translocation of Cd and Pb by *Zea mays* L. plant

Heavy metal accumulation and translocation of Cd and Pb in *Zea mays* L. plant was directly correlated with the exposure time and concentration of heavy metals (Cd and Pb) in the soil. Results revealed that there was a linear increase of Cd and Pb concentration (100-500 mg kg-1) with increased exposure time i.e. more the exposure time, more will be the accumulation of Cd and Pb in *Zea mays* L. plant. Interestingly, inoculation of rhizobacterial strain PC3 further increased the total metal accumulation in *Zea mays* L. plant as compared to their respective control (uninoculated plant) at 90 days after sowing (DAS) (Fig. 3). Our findings were in the accordance with Kamran et al., (2015) for Cd uptake by *Eruca sativa* inoculated with PGPR. The enhancement of metal uptake of plants by PGPR inoculation has been reported in several studies (Ghosh et al., 2011; Ma et al., 2013; Prapagdee et al., 2013; Liu et al., 2015).

Translocation factor (TF) of the plant describes the ratio of heavy metal concentration in shoot tissue over heavy metal concentration in root tissues. Results from this test revealed that the TF value of *Zea mays* L. plant under Cd and Pb stress were < 1 at all concentration and exposure time (30, 60, and 90 DAS). The maximum TF for *Zea mays* L. plants were found to be 0.16.±0.01 and 0.43±0.05 at 500 ppm concentration of Cd and Pb at 90 DAS, respectively. Further, it was observed when the rhizobacterial strain PC3 was inoculated, increase in TF values were recorded (27.47% and 58.44%) at 90 DAS under 500 ppm concentration of Cd and Pb respectively in *Zea mays* L (Fig. 4). Translocation factor lower than 1 in most cases were obtained with both metals (cadmium and lead) for *Zea mays* L. plant. This leads to conclusion that Zea mays L. plant has adopted a tolerance/immobilization strategy, as suggested earlier by Marques et al., (2013) for *Helianthus annuus* exposed with Cd and Zn. The similar pattern of TF for Pb translocation from root to shoot in *Canna indica* were reported by Bose et al., (2008) under the soil amended with industrial waste. Moreover, in present study, inoculation of rhizobacterial strain PC3 consequently increased the TF value for both metals (Cd and Pb). The result was in accordance with the study of Moreira et al., (2014) in *Zea mays* L. plant inoculated with PGPR exposed to Cd.

Figure 3. Total metal accumulation by *Zea mays* L. plant grown under Cd and Pb stress treated with *Enterobacter cloacae strain* PC3.



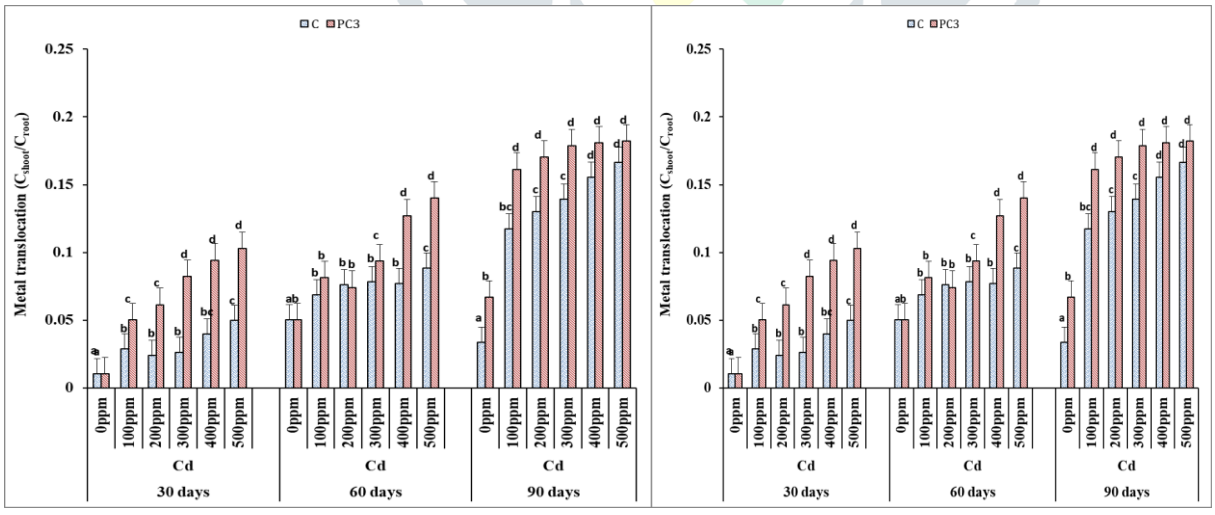


Figure 4. Showing Cd and Pb translocation in *Zea mays* L. plant grown under Cd and Pb stress treated with *Enterobacter cloacae strain* PC3.

2. Conclusion

Based on the findings obtained from experimental results, study concluded that rhizobacterial strain PC3 (Accession No. KX668494) isolated from metal contaminated site was showing multiple PGP traits as well as Cd and Pb tolerance ability. Root/shoot length, plant dry biomass chlorophyll, carotenoid contents and metals accumulation in *Zea mays* L. plants were significantly improved by the inoculation of strain *Enterobacter*

cloacae PC3 over control in a pot experiment. With these findings, study concluded that the heavy metal resistant rhizobacterial bioinoculant and its multifarious plant growth promoting properties such as ACC deaminase, IAA, siderophore, phosphate solubilisation alleviated the toxic effects of Cd and Pb. Consequently, rhizobacterial strain PC3, could be exploited for remediation of metal contaminated sites by functioning as a phyto-stimulant for plants under heavy metal contaminated soils.

3. References

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