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Original Article

Characterization of a Cd^{2+}-resistant plant growth promoting rhizobacterium (*Enterobacter* sp.) and its effects on rice seedling growth promotion under Cd^{2+}-stress *in vitro*

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ABSTRACT

Rhizosphere contamination due to heavy metals in agriculture leads to decreased production of edible crops. High water solubility and permeability of these life-threatening metals lead to increased translocation to the upper part of plants and the accumulation of these metals in food crops including cereal seeds. Apart from conventional methods, plant growth promoting rhizobacteria (PGPR)-influenced bioremediation is now an emerging, eco-friendly and inexpensive tool. The K2 isolate is one such multi-heavy-metal-resistant PGPR isolated from the metal-contaminated rice rhizosphere. The isolate was identified as *Enterobacter* sp. using phenotypic characterization and MALDI-TOF MS-based ribosomal protein data. The strain was able to resist a group of heavy metals/metalloids (Cd^{2+}, Pb^{2+}, As^{3+}, Ni^{2+}, Hg^{2+}) up to certain levels. The strain possessed a collection of PGP traits such as phosphate solubilization, Indole-3-acetic acid production, 1-aminocyclopropane-1-carboxylic acid deaminase activity and nitrogen fixation. *In vitro* growth enhancement of a rice cultivar by the strain was investigated using Cd^{2+} stress, as observed in various growth parameters (seed germination, root-shoot length, root-shoot biomass, seedling vigor index, chlorophyll content). Hence, the K2 strain can be exploited regarding multi-heavy-metal/metalloid contamination for effective growth promotion under stress conditions for sustainable agriculture.

Introduction

The soil is affected daily by a host of contaminants due to industrial wastes containing toxic chemicals including metals (Pandey et al., 2013; Chen et al., 2016). Augmentation of such compounds in the soil has exhibited adverse effects on plant growth, reproduction, and yield (Lin and Aarts, 2012; Tran and Popova, 2013). Moreover, those contaminants even appear sometimes as a life-risk factor by invading the food chain through many food crops, cereals, and vegetables (Meharg et al., 2013).

Heavy metals (such as Cd, Cr, Pb, and Hg) and a few metalloids (such as As and Ni) have no biological role and are becoming a global enigma due to their massive toxicity (Abbas et al., 2014). This metal-induced toxicity appears as one of the important factors regarding the shortage of food crisis especially in some over-populated countries like China and India (FAO, 2003). To meet this high demand for food, several physicochemical techniques are being employed to reduce the effect of such metals but these conventional methods are not always eco-friendly and cost-effective (Quartacci et al., 2006).

However, in this context, heavy metal alleviation by heavy metal-resistant plant growth promoting rhizobacteria (PGPR) is not only eco-friendly and inexpensive but also they promote plant growth by ameliorating the metal-induced stress, producing plant growth promoting substances etc. (Pandey et al., 2013; Pramanik et al., 2016). *Ochrobactrum* sp. (Pandey et al., 2013), *Bacillus* sp. (Pandey et al., 2013; Ahmad et al., 2016), *Raoultella* sp. (Pramanik et al., 2016), *Klebsiella* sp. (Ahmad et al., 2016; Pramanik et al., 2017; Mitra et al., 2018a), *Leifsonia* sp. (Ahmad et al., 2016), *Enterobacter* sp. EG16 (Chen et al., 2016) and *Enterobacter* sp. (Ahmad et al., 2016; Pramanik et al., 2018; Mitra et al., 2018b) are few instances of reported heavy metal-resistant PGPR that exhibited various plant growth promoting (PGP) traits. The possible mechanism of heavy metal resistance by a PGPR strain might be due to bioaccumulation (the accumulation of heavy metals inside the cell; Ahmad et al., 2016; Chen et al., 2016) or biotransformation

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(alteration of the valence states of metals from a toxic form to a non-toxic one; Pramanik et al., 2016). Although some Cd-resistant bacteria have already been reported (Sharma and Archana, 2016) there are some limitations of these strains when used directly as biofertilizer. First, the strains can withstand selective heavy metals but the soil is contaminated with a number of toxic metals and chemicals. Second, not all strains are able to be active in all types of environmental factors including pH and temperature. Third, not all heavy-metal-resistant bacteria are plant growth promoters. Fourth, not all heavy-metal-resistant PGPR remain viable after accumulating metals inside the cells (Pramanik et al., 2018). Hence, the discovery of multi-metal-resistant PGPR is of high global demand today for bioremediation.

This study investigated the isolation of a potent, cadmium-resistant PGPR which can substantially enhance plant (rice) growth as well as resist a wide range of toxic metals.

Materials and methods

Site characterization, isolation of heavy metal resistant rhizobacteria

A rhizospheric (*Oryza sativa* L.) soil sample was collected from a metal-contaminated area near Durgapur Steel Plant (DSP), Durgapur, Burdwan district, West Bengal (23°33N, 87°15E). The physicochemical characteristics (pH, nutrient contents) of the soil sample were analyzed and atomic absorption spectroscopy was used to quantify the heavy metal contents. The soil sample was serially diluted and plated in Davis Mingioli (DM) medium (K_2HPO_4, 7 g/L; KH_2PO_4, 3 g/L; $(NH_4)_2SO_4$, 1g/L; $MgSO_4$; $7H_2O$, 200 mg/L; $C_6H_5O_7Na_3.2H_2O$, 500 mg/L; $C_6H_{12}O_6$, 10 g/L; pH 7.0) with a cadmium chloride ($CdCl_2$) supplement of 500 μg/mL Cd^{2+}. Among the morphologically distinct colonies after overnight incubation at 32 ± 1 °C, five different isolates were selected for further study and maintained in DM slants with 500 μg/mL Cd^{2+} at the same temperature with a sub-culturing period of 15 d.

Screening of selected Cd-resistant isolates

The five morphological distinct colonies were further screened on the basis of their heavy metal resistance ability and PGPR traits. For determination of Cd-resistance ability, the selected isolates were grown in DM plates supplemented with Cd in the range 500–1000 μg/mL. Qualitative estimation of solubilized phosphorus by the isolates used the Pikovskaya agar plate method (Pikovskaya, 1948). Indole-3-acetic acid (IAA) production was determined according to Bric et al. (1991). Nitrogen fixation ability was tested in nitrogen-free DM medium and siderophore production using chrome azurol S agar plate assay (Schwyn and Neilands, 1987). ACCD (1-aminocyclopropane-1-carboxylic acid deaminase) assay followed the protocol described by Shrivastava and Kumar (2013). Ammonia production and HCN production were qualitatively tested according to Cappuccino and Sherman (2013) and Lorck and Veterinary (1948), respectively. On the basis of the PGPR traits and Cd-resistant ability, the K2 isolate was selected for further study.

Determination of a minimum inhibitory concentration of heavy metals/metalloids and viability test

After primary screening by supplementing Cd^{2+} in the medium, resistance was also checked with a few other toxic heavy metals/metalloids (Pb^{2+}, As^{3+}, Ni^{2+}, Hg^{2+}) in terms of determining the minimum inhibitory concentration (MIC) in the same DM medium (Andrews, 2001). For this, DM plates were prepared to supplement individual heavy metals/metalloids at different concentration grades depending on the metals/metalloids. The inoculation was done by streaking the K2 strain and incubating at 32 ± 1 °C for 48 h. The MIC was further confirmed by inoculating the strain in liquid DM medium with similar chemical constituents and incubating in a rotary incubator shaker at 120 rpm and 32 ± 1 °C for 48 h. The viability of the cells under these metals was tested using the triphenyltetrazolium chloride (TTC) test (Pandey and Bhatt, 2015) for which DM plates were prepared as described for the MIC above and maintained in the same manner. After the appearance of growth, 1% TTC (weight per volume, w/v) was flooded onto plates and the appearance of red colored colonies was considered to indicate viable cells (metabolically active) while colorless colonies were considered as non-viable cells.

Identification of the selected K2 isolate

Phenotype-based identification followed the standard methods of Benson (1990) and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) proteomic analysis-based identification was according to the method described by Pulcrano et al. (2013). The mass spectrum generated by MALDI-TOF MS was calibrated using standard calibrant mixture of *Escherichia coli* cell extracts including the additional proteins RNase A and myoglobin (Bruker Daltonics GmbH; Bremen, Germany). Identification utilized the MALDI Biotyper software 3.0 (Bruker Daltonics GmbH; Bremen, Germany) to visualize mass spectra.

Antibiotic sensitivity test of the selected K2 strain

The antibiotic sensitivity of the K2 strain was tested using 13 antibiotics [erythromycin (ER), norfloxacin (NX), chloramphenicol (CH), doxycycline (DX), tetracycline (T), cephalexin (PR), cloxacillin (CX), nalidixic acid (NA), tobramycin (TB), ciprofloxacin (RC), ampicillin (A), lincomycin (LM) and gentamicin (GM)]. The concentration of each antibiotic disc (HiMedia Laboratories Ltd.; Mumbai, India) was 10 mcg/disc.

Quantitative plant growth promoting traits of selected K2 strain

Quantitative estimation of solubilized phosphorus by the strain followed the ammonium molybdate method (Fiske and Subbarow, 1925). For estimation of indole acetic acid (IAA), DM liquid medium supplemented with 0.5% tryptophan (Sigma-Aldrich Co.; Darmstadt, USA), inoculated with K2 and kept in a rotary incubator shaker at 120 rpm and 32 ± 1 °C for up to 72 h. IAA production was estimated according to Bric et al. (1991) by adding Salkowski reagent in the culture supernatant obtained by centrifugation at 8000 rpm for 15 min every 12 h interval up to 72 h. The effect of Cd stress (3000 μg/mL) on the growth and IAA production of this strain was also tested. Nitrogen fixation ability by the strain was recorded by the activity of nitrogenase enzyme using gas chromatography (Dilworth, 1966) and calculations used the modified method of Kaushal and Kaushal (2015). ACCD assay followed the protocol described by Shrivastava and Kumar (2013). DM medium without $(NH_4)_2SO_4$ but supplemented with 5 mM ACC was used for this study. ACCD activity was measured by estimating the amount of α-ketobutyrate produced by the ACCD catalyzed the reaction and measured by taking the absorbance at 540 nm. The optical density (OD_{540}) values were compared with a previously prepared calibration curve of α-ketobutyrate (Sigma-Aldrich Co.; Darmstadt, USA). Like the IAA production assay, all other PGP traits were checked in the presence of 3000 μg/mL Cd^{2+} in the medium and analyses were performed in triplicate.

In vitro plant growth promotion by the K2 strain

Viable rice seeds (*Oryza sativa* L. var. Swarnamasuri, accession no.: MTU7029) were collected from Hooghly Krishi Vigyan Kendra, Indian Council of Agricultural Research (ICAR), West Bengal, India and were used for this study. Cd^{2+} was selected as the stress factor of rice seedling and EC_{50} (the stress concentration in which 50% inhibition of germination occurred) for the rice seeds was determined. The germination percentage was determined using Equation (1):

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100 \quad (1)$$

In total, three sets were prepared in 200 mL glass beakers for detection of the influence of the K2 strain on heavy metal stress amelioration. The first set consisted of Hoagland solution (Ahmad et al., 2016) and seeds (Set-I), the second set consisted of seeds as well as the EC_{50} concentration of Cd^{2+} along with Hoagland solution and the final set (Set-III) additionally consisted of the K2 strain ($OD_{540} = 0.02$; 1×10^8 CFU/mL) along with all the constituents of Set-II. The bacterial (K2) culture was grown in DM liquid medium overnight at 32 ± 1 °C, centrifuged to collect the pellet and supernatant discarded. Each pellet was washed three times with phosphate buffer saline and then seed bacterization was performed. The number of seeds in each set was fixed at 80. All the sets were prepared in triplicate and maintained in a seed germinator at 32 ± 1 °C for 3 d in the dark followed by another 7 d in intermittent light and dark conditions. After 10 d of growth, the root-shoot length, root-shoot fresh and dry weights and seedling vigour index (SVI) were measured using Equation (2) according to Bal et al. (2013).

$$\text{SVI} = (\text{mean root length} + \text{mean shoot length}) \times \text{germination percentage} \quad (2)$$

The chlorophyll content in those sets was also estimated spectrophotometrically by the method described by Arnon (1949) and was calculated using Equations (3)–(5) for chlorophyll-a, chlorophyll-b and total chlorophyll, respectively (Arnon, 1949):

$$\text{Chl} - \text{a (ng/g fw)} = 0.0127_{A663} - 0.00269_{A645} \quad (3)$$

$$\text{Chl} - \text{b (ng/g fw)} = 0.0029_{A663} - 0.00468_{A645} \quad (4)$$

$$\text{Total Chl(ng/g fw)} = 0.0202_{A663} + 0.00802_{A645} \quad (5)$$

Statistical analysis

All experiments were performed in triplicate and results were calculated as a mean ± standard deviation (SD). The significance of differences between the control and treatments was tested using Student's t-test. Differences between groups were determined using one-way analysis of variance in the EXCEL 2010 software package (Microsoft Corp.; Redlands, WA, USA). Differences at $p \leq 0.05$ were treated as statistically significant.

Results and discussion

Site characterization, isolation, and screening of heavy metal resistant plant growth promoting rhizobacterium

The isolation of multi-heavy-metal-resistant PGPR was initiated by supplementing Cd in the medium from the rhizospheric soil of rice. The soil profile analysis revealed that in addition to micronutrients there were significant amounts of heavy metals present in the soil (Table 1). After screening (Table 2), an isolate, named as "K2" growing profusely in the highest Cd concentration (1000 μg/mL) as well as exhibiting the highest number of PGP traits was selected for further studies. Isolation of heavy-metal-resistant PGPR from heavy-metal-contaminated rhizospheric soil was practiced previously by Pandey et al. (2013), Ahmad et al. (2016), Chen et al. (2016), Pramanik et al. (2016, 2017, 2018) and Mitra et al. (2018a, b).

Determination of minimum inhibitory concentration of heavy metals/metalloids and viability testing

The MIC determination experiment clearly revealed that the K2 strain not only resistant to a number of hazardous heavy metals/metalloids but also had a good degree of tolerance to those metals (Fig. 1), the effect of which can also be corroborated by the consistency of viable cells after the TTC test. Hence, the isolate K2 established itself as a multi-heavy-metal-resistant bacterium. MIC determination of heavy metals represents the quantitative efficacy of a strain to tolerate stress and such experiments are practiced by many workers (Pandey et al., 2013; Pramanik et al., 2016, 2017, 2018). The TTC test was also performed by Pandey and Bhatt (2015) while working on arsenic-resistant bacteria isolated from arsenic-contaminated sites.

Identification of the selected K2 isolate

From the primary phenotypic characterization of the strain it was found that the K2 isolate was a Gram-negative rod forming creamy white, raised and round colonies and the physio-biochemical features (positive results in amylase, protease, catalase, citrate utilization, Voges-Proskauer and lysine decarboxylase tests) indicated the isolate to be a part of the Enterobacteriaceae family. The identification was confirmed using MALDI-TOF MS ribosomal protein-based analysis (Table 3, Fig. 2) and it was detected that K2 isolate belongs to the genus *Enterobacter* of the Enterobacteriaceae family. MALDI-TOF MS-based identification was successfully introduced in modern bacterial taxonomy for accurate and rapid identification of bacterial taxa and has been practiced in the last few years (Starostin et al., 2015). The authentication of the MALDI-TOF MS-based analysis of ribosomal protein analysis for the identification of bacterial strain is now considered another standard method compared with 16S rDNA sequence analysis. Seng et al. (2009), while working with 1660 isolates, compared the discrepancies between the MALDI-TOF MS results and 16S rRNA gene sequencing and found that 95.4% of the isolates were correctly identified by MALDI-TOF MS among which 84.1% were identified at the species level. Thus, due to a lack of 16S rDNA sequences and DNA-DNA hybridization results, the K2 strain was identified as *Enterobacter sp.* based on phenotypic characters and MALDI

Table 1
Location, nutrient content and heavy metal contents of the collected soil sample.

Feature	Value
pH	5.6 ± 0.05
Organic carbon (%)	3.55 ± 0.14
Nitrate (μg/g)	2.9 ± 0.15
Phosphate (μg/g)	1.25 ± 0.09
Salinity (psu)	2.1 ± 0.41
Cd (μg/g)	110.23 ± 3.09
Pb (μg/g)	33.30 ± 2.68
As (μg/g)	45.82 ± 3.17

Note. Data are the mean of three replications ± SD.

Table 2
Screening of heavy metal resistant plant growth promoting rhizobacterium.

Strain	Cd^{2+} Resistance (μg/mL)	PO_4 Solubilization	IAA Production	N_2 Fixation	ACC Deaminase activity	Siderophore activity	NH_3 Production	HCN Activity
K1	500	−	++	++	−	−	++	−
K2	1000	++++	++++	+++	+++	+++	+++	+++
K3	600	−	+	+	−	−	++	−
K7	500	−	++++	+	−	+	++++	−
K8	700	+++	++++	++	−	−	++	−

IAA = indole acetic acid.
"+" indicates a positive result for the experiment, "−" indicates a negative result. A greater number of symbols indicates greater production of the substances or more growth of bacterial cells whichever is applicable.

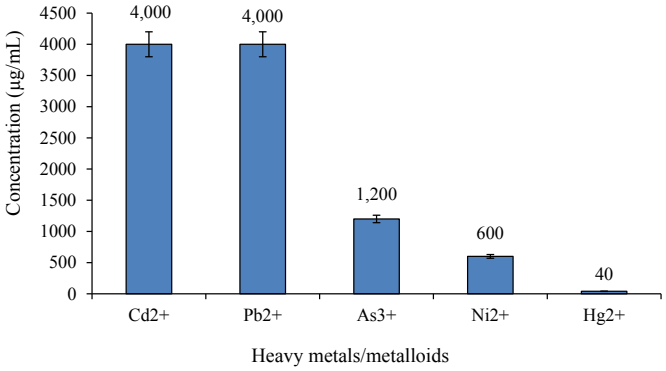

Fig. 1. Determination of minimum inhibitory concentration for different heavy metals/metalloids. Data are the mean of three replicates ± SD.

Biotyper classification as the MALDI score was above 2.0 (Table 3). The strain showing a log value of 1.7 or greater with the strain in the database was confirmed as a member of that genus and a strain showing a log value of 2.0 or greater was confirmed at the species level.

Antibiotic sensitivity of the selected K2 strain

Apart from heavy-metal resistance, the isolates were resistant to some antibiotics (Table 4). Out of the 13 antibiotics, K2 was resistant to ER, PR, CX, A and LM (Table 4). Similarly, a number of heavy-metal-resistant bacteria have also been reported to exhibit resistance to a range of antibiotics (Kimiran-Erdem et al., 2015) although they were not PGPR. The reason behind this coupled resistance pattern might be due to the physiological response, cross-resistance (Chapman, 2003) or genetical response, co-resistance (Chapman, 2003) by the PGPR. Instances of cross-resistance were demonstrated by Hernández-Martínez et al. (2009), where several multi-drug efflux pumps mediate decreased susceptibility toward a number of antibiotics and heavy metals by rapid extrusion of the toxic substances out of the cell. In the case of co-resistance, the genes specifying different resistant phenotypes (such as both heavy-metal and antibiotic resistance) might be located together on a mobile genetic element (plasmid, transposon) and development of resistance of one antimicrobial agent accompanied by another (Chapman, 2003). These might be possible mechanisms of multi-heavy-metal resistance and multi-antibiotic resistance by the K2 strain.

Table 3
MALDI-TOF-based identification of K2 strain.

Strain	MALDI-TOF MS (Best match)		MALDI-TOF MS (Second match)	
	Identification	Score	Identification	Score
K2	*Enterobacter kobei*	2.122[a]	*Enterobacter cloacae*	2.016[a]

[a] The strain showing a log value of 1.7 or greater identified at genus level whereas the value of 2.0 or greater was identified at the species level. (according to MALDI Biotyper classification).

Table 4
Sensitivity test of antibiotics for K2 strain.

Strain	Antibiotic sensitivity	
	Sensitive to	*Resistant to*
K2	NX[a], CH, DX, T, NA, TB, RC, A, GM	ER, PR, CX, A, LM

[a] erythromycin (ER), norfloxacin (NX), chloramphenicol (CH), doxycycline (DX), tetracycline (T), cephalexin (PR), cloxacillin (CX), nalidixic acid (NA), tobramycin (TB), ciprofloxacin (RC), ampicillin (A), lincomycin (LM) and gentamicin (GM).

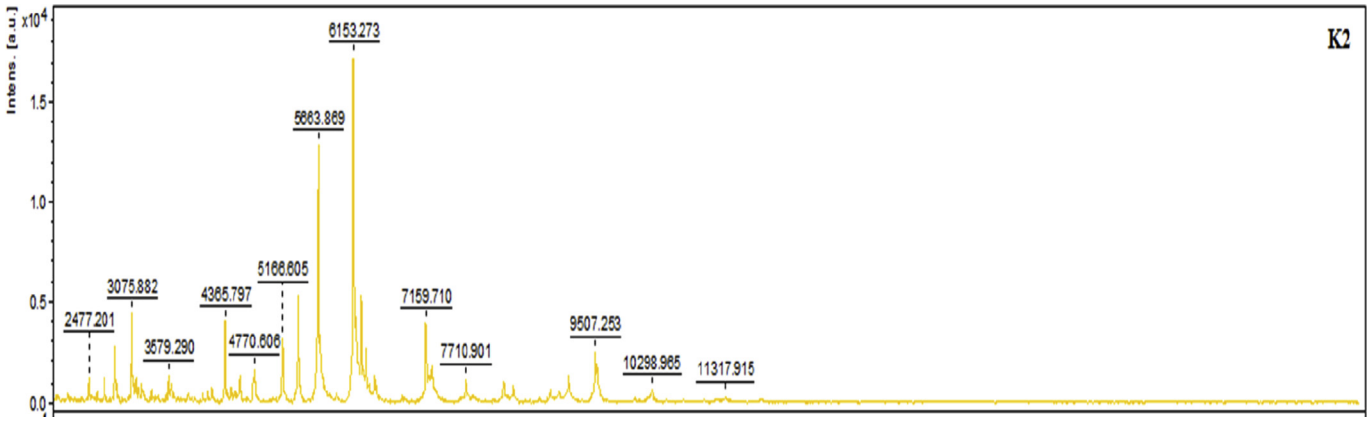

Fig. 2. MALDI-TOF MS spectrum of K2 strain.

Quantitative plant growth promoting traits of selected K2 strain

The presence of PGP traits in this strain indicated its ability to enhance plant growth effectively. The selected strain K2 exhibited a propensity to solubilize inorganic phosphate. A similar result was reported by Pandey and Bhatt (2016) and a phosphate-solubilizing bacterium was reported to promote phosphate availability to the plant under Cd-stress (Park et al., 2010). This strain was also able to fix atmospheric nitrogen and to produce ammonia (Tables 2 and 5). Many PGPR strains have been reported to fix atmospheric nitrogen (Pramanik et al., 2016, 2017, 2018) and it was reported that the Cd-tolerant N_2 fixing *Klebsiella mobilis* increased the grain yield of barley (Pishchik et al., 2002). ACC deaminase promoted plant growth by cleaving the ACC produced by the plant under stress including heavy-metal stress and by reducing the level of "stress ethylene" by ACCD activity (Glick, 2005). The selected K2 strain possessed ACC deaminase activity (Tables 2 and 5) and this might have been one of the reasons it alleviated metal stress by lowering the stress ethylene level under metal stress. Moreover, the growth and IAA production of the K2 strain (Fig. 3A and B) were other important PGP traits essential for plant growth promotion. However, reduced growth and IAA production were observed under Cd stress (Fig. 3B) compared with the control (Fig. 3A) which is likely to have helped plant growth promotion. A similar reduction of IAA under Cd stress has also been reported by Chen et al. (2016) in *Enterobacter* sp. This IAA production might also help the plant with root growth promotion which is essential for nutrient acquisition under Cd stress (Pramanik et al., 2017, 2018; Mitra et al., 2018a; b). The IAA production of PGPR played an important role under heavy metal toxicity of the plant. Under Cd stress, plant cells produce reactive oxygen species (ROS) which are responsible for much damage particularly regarding the disruption of the hormonal pathway and the induction of senescence (Lin and Aarts, 2012; Tran and Popova, 2013). IAA and other phytohormones produced by PGPR reduced this damage and promoted the seedling growth of rice (Bhattacharyya and Jha, 2012). Moreover, the selected K2 strain also produced siderophore and HCN (Table 2). Siderophores are secreted by PGPR which scavenges and chelate Fe^{3+} ions for their high affinity and thus improves iron availability to the plant and reduces the uptake of Cd, thus conferring Cd tolerance to the plant (Yoshihara et al., 2006). Siderophore produced by the PGPR strain *Streptomyces* was shown to increase iron uptake and simultaneously reduced Cd uptake. (Dimkpa et al., 2009). Hence, this strain can be utilized as a biofertilizer, phytostimulator and stress ameliorator in crops to enhance plant growth.

Table 5
Quantification of the plant growth promoting traits of K2 strain under Cd stress.

Strain	PGP traits		
K2	N_2 fixation (μg N_2 fixed/h/mg protein	PO_4 solubilization (ppm)	ACC deaminase activity (ng α-ketobutyrate/mg protein/h)
	1.05 ± 0.11	46.58 ± 2.07	32.00 ± 2.16

PGP = plant growth promoting, ACC = 1-aminocyclopropane-1-carboxylic acid. Data are mean of three replications ± SD.

In vitro plant growth promotion by the K2 strain

In Asia especially in India, rice is one of the most consumed staple foods (FAO, 2003); thus, it was selected in this study to monitor growth under metal stress and at the same time investigate the effect of the PGPR strain K2 on its growth under stress. The EC_{50} concentration of Cd^{2+} determined for the Swarnamasuri rice cultivar was 150 μg/mL. An almost 40% increase in the germination percentage was observed after inoculation of the K2 strain (Fig. 4A) under Cd^{2+} stress. The enhanced germination percentage after K2 treatment might have been due to the increased activity of the α-amylase activity of the rice seedling due to reduced stress. Moreover, the root length, shoot length and root-shoot biomass were significantly enhanced (Fig. 4B–D), the effect of which was exhibited in the seedling vigor index (Fig. 4E). A similar type of PGP study was also performed by Pandey et al. (2013) working on a heavy-metal-resistant PGPR (*Bacillus* spp., *Ochrobactrum* sp.) and its effect on the promotion of rice seedling growth under Cd-stress. Similar enhancement of the root length, shoot length and root-shoot biomass was also shown by Lin et al. (2016) while experimenting with the effect of *Stenotrophomonas acidaminiphila* and *Pseudomonas aeruginosa* on a rice cultivar. Prapagdee and Khonsue (2015) also reported increased root-shoot length and biomass of *Ocimum gratissimum* L. by applying the Cd-resistant bacteria *Ralstonia sp.* TISTR 2219 and *Arthrobacter* sp. TISTR 2220. Enhancement of the root and shoot lengths might have been due to the production of IAA by the K2 strain which triggers the apical meristems to divide even faster under Cd-stress. This led to increased water uptake by the seedlings. Hence, IAA production by a PGPR strain (here K2) appears to be vital for plant growth promotion. In this context, it is important to note that the EC_{50} of the selected rice cultivar was 150 μg/mL Cd which inhibited germination of 50% of the seeds but this specific concentration was not so high for the K2 strain to ameliorate as it could tolerate up to 4000 μg/mL Cd as

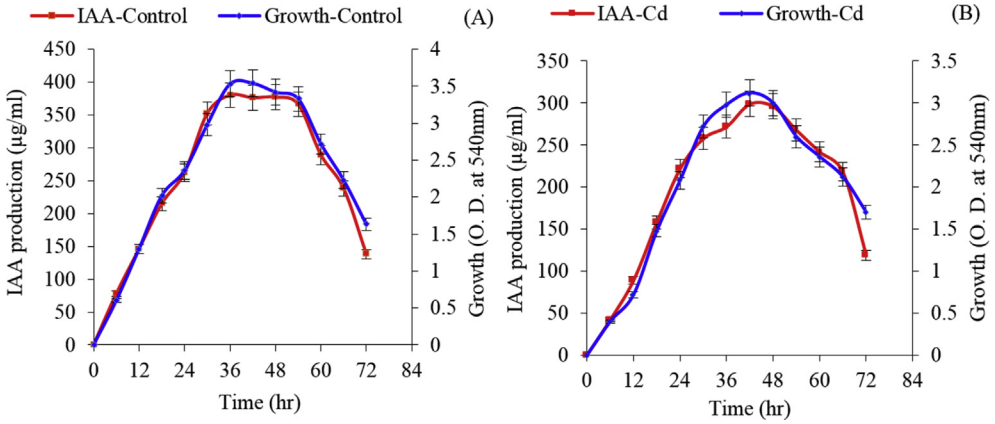

Fig. 3. (A) Growth of K2 strain without Cd stress (B) Effect of Cd on growth and indole acetic acid (IAA) production of K2 strain. OD_{540} = Optical density taken at 540 nm. Data are the mean of three replicates ± SD.

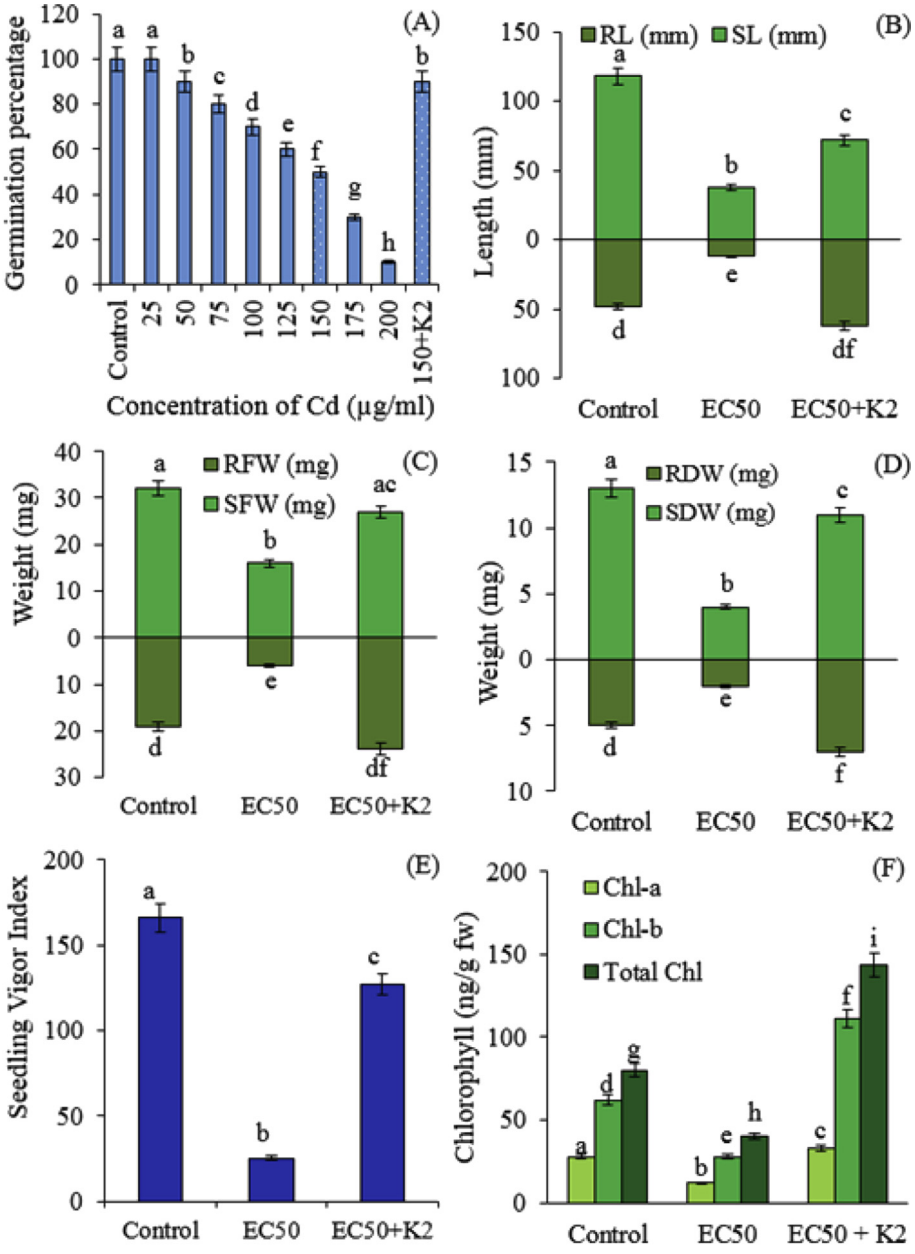

Fig. 4. Effect of K2 strain on (A) seed germination; (B) root length (RL), shoot length (SL); (C) root fresh weight (RFW), shoot fresh weight (SFW); (D) root dry weight (RDW), shoot dry weight (SDW); (E) seedling vigor index; (F) chlorophyll content under Cd stress. Values are the mean of three replicates ± SD represented as error bars. Similar letters above the bars indicate not significantly ($p < 0.05$) different.

determined by the MIC (Fig. 1). The ACC deaminase activity of the K2 strain (Table 5) reduced the "stress ethylene" produced due to Cd stress (Glick, 2005), thereby preventing stress-induced senescence and increasing the root elongation of the plant. A notable increase in the total chlorophyll content after the K2 treatment under Cd^{2+} stress indicated that K2 not only increased plant growth but also recovered Cd-induced impairment of photosynthesis as shown in Fig. 4F (Haneef et al., 2014). This observation was also corroborated by Guo and Chi (2014) while working on the effect of the Cd-tolerant PGPR strain *Bradyrhizobium* on plant growth and on the Cd uptake by *Lolium multiflorum* Lam. and *Glycine max* (L.) Merr.

Thus, the *Enterobacter* sp. strain K2 isolated from the rice rhizosphere adjacent to an industrially polluted area possessed a number of heavy metals/metalloids resistant characters and promoted rice seedling growth under Cd-stress. The PGP traits shown by the strain indicated the strain was a phytostimulator, biofertilizer, and stress ameliorator. Therefore, this strain might be very useful in agricultural applications acting as a potential PGPR in metal-contaminated areas to achieve sustainable agriculture.

Conflict of interest

The authors declare no conflict of interest.

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