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Characterization of multifarious plant growth promoting traits of rhizobacterial strain AR6 under Chromium (VI) stress

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ABSTRACT

Plant growth promoting rhizobacteria (PGPR) can increase the host plant tolerance to cope up with heavy metal induced stress, which can be improve plant growth. Thus, the present study was designed to isolate Cr(VI) tolerant PGPR strain and evaluate its plant growth promoting (PGP) properties under Cr(VI) stress. Rhizobacterial strain AR6 was isolated from the rhizosphere of *Phaseolus vulgaris* L. and showed 99% homology with *Cellulosimicrobium funkei* (KM032184) in BLASTn analysis. Strain AR6 was specifically selected due to its high Cr(VI) tolerance (1200 µg/ml) and substantial production of PGP substances. Strain AR6 produced 36.75 µg/ml of indole acetic acid (IAA), 60.40 µg/ml of ammonia and 14.23 µg/ml of exopolysaccharide (EPS). Moreover, strain AR6 showed positive results for catalase, protease, amylase, lipase production and phosphate solubilization. A trend of Cr(VI) concentration dependent progressive decline for PGP traits of strain AR6 was observed excluding EPS which was regularly increased on increasing concentrations of Cr(VI). Among the four tested Cr(VI) concentrations, 250 µg/ml showed the maximum toxicity to PGP activities of strain AR6. Inoculation of rhizobacterial strain AR6 significantly increased the root length of test crops in the presence of Cr (VI) and produced a considerable number of colonizes on the root of versatile dicot and monocot plants. Moreover, strain AR6 exhibited strong antagonistic activity against phytopathogen *Aspergillus niger*. Thus, the present study suggests that metal tolerant and PGP activities of the rhizobacterial strain AR6 could be exploited for environmental and agricultural issues.

1. Introduction

Globally, environmental pollutant is a major issue of agricultural soil. Heavy metals are the most problematic contaminants during agricultural practices; which contribute around 60% of soil contamination (Panagos et al., 2013). Among the heavy metals, Chromium (Cr) is one of the most predominant heavy metal pollutants, primarily presents in the natural environment and released from various industrial sources. Chromium commonly exists as Cr(VI) and Cr(III), among this Cr(VI) is more toxic, mobile and permeable (Oves et al., 2013). Because of its carcinogenic and mutagenic nature, the United States Environmental Protection Agency (USEPA) has classified Cr as a “Priority pollutant” or Class A” pollutant (USEPA, 1996).

Accumulation of Cr into the agricultural soil leads to changes in microbial activity and soil fertility. Consequently, higher concentrations of Cr have undesirable effects on plant growth. For instance, accumulation of Cr(VI) in plants can reduce the seed germination rate, growth, cell damage, chlorosis, degradation of pigments, alteration in the enzymatic function and nutrient balance (Karthik et al., 2016). Moreover, Cr contamination significantly affects the productivity of agricultural crops. Approximately, 35,000 ha of agriculture land has become unfit for cultivation due to the Cr pollution in Vellore district, Tamil Nadu, India, which consequently reduce the yield of paddy (75%), coconuts (52%) and sugarcane (48%) (Rangasamy et al., 2015; Kennedy, 1999). Therefore, an ecofriendly and affordable remediation strategy is highly desirable.

In this scenario, application of Cr(VI) tolerant PGPR is a useful and convenient method for reacclimation of leather industrial effluent contaminated sites. Naturally occurring rhizospheric microorganisms from leather industrial effluent contaminated soil has the ability to acquire resistance against chromium and other heavy metals due to prolonged exposure of the contaminants. Rhizobacterial detoxification of toxic Cr(VI) can occur directly by enzymatic reduction to less toxic Cr (III) by chromate reductase or indirectly through making edifices with

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metabolites (Thatoi et al., 2014; Karthik et al., 2017). Moreover, these rhizospheric bacterial strains have the ability to enhance the plant growth through the release of phytohormones (auxins, cytokinins, gibberellic acid etc.), phosphate solubilization, nitrogen fixation, synthesis of ammonia and antimicrobial products, thereby improving heavy metal remediation efficiency and growth of plants under adverse metal toxic conditions (Gopalakrishnan et al., 2015). Chromium (VI) tolerant rhizobacterial strain with PGP properties has raised high hope for cost effective and eco-friendly measures for sustainable agriculture in soil tract contaminated with Cr. Therefore, improvement of the interaction between plants and beneficial rhizosphere microbes would be an important component of bioremediation technology in agricultural sites. Considering the facts stated above, the present study was designed to determine the effect of different concentrations of Cr(VI) on PGP traits of the rhizobacterial strain isolated from rhizosphere of *P. vulgaris*.

2. Materials and methods

2.1. Screening of Cr(VI) tolerant rhizobacterial strain

For rhizobacterial isolation, soil samples were collected from the rhizospheric region of *P. vulgaris,* which has been grown in leather industrial effluent contaminated soil. Collected rhizospheric soil samples were subjected for rhizobacterial bacterial isolation. In primary screening, a total of 36 bacterial isolates were screened with 100 μg/ml concentration of Cr(VI) tolerance. In order to screen potential Cr(VI) tolerant rhizobacterial isolates, all the 36 rhizobacterial isolates were subjected for secondary screening. Among the selected isolates, strain AR6 showed maximum tolerance against Cr(VI) at a concentration of 1200 μg/ml and was selected for further studies. In molecular characterization study, rhizobacterial strain AR6 showed 99% homology with the sequence of *C. funkei* (KM032184) in BLAST analysis (http://www.ncbi.nlm.nih.gov/BLAST).

2.2. Multi-metal tolerance of rhizobacterial strain AR6

Multi-metal tolerance of the strain AR6 was studied by agar plate dilution method (Holt et al., 1994). Rhizobacterial strain AR6 was inoculated in LB agar plate supplemented with different concentrations of $Pb(NO_3)_2$ (100–800 μg/ml), $ZnSO_4$ (100–200 μg/ml), $CuSO_4{\cdot}5H_2O$ (100–350 μg/ml) and $MnCl_2{\cdot}4H_2O$ (50–1550 μg/ml). Inoculated plates were incubated at 35 ± 2 °C for 2–5 days to determine the maximum tolerance of strain AR6. Bacterial growth on heavy metal amended medium was considered as metal tolerance of the bacterial strain.

2.3. Antibiotic resistance of strain AR6

Antibiotic resistance of the strain AR6 was determined by disc diffusion method (Gadd, 1990). Mueller Hinton Agar (MHA) plates were prepared and overnight grown culture of strain AR6 was swabbed evenly. Disks of ampicillin (10 μg/ml), gentamicin (10 μg/ml), methicillin (10 μg/ml), vancomycin (30 μg/ml), neomycin (30 μg/ml), penicillin (2 μg/ml) and streptomycin (10 μg/ml) were placed on MHA medium and incubated for 24 h at 35 ± 2 °C to measure the zone of inhibition.

2.4. PGP traits of AR6

Production of PGP substances like IAA, ammonia, EPS, catalase, amylase, protease and lipase, phosphate solubilization and root elongation ability of the strain AR6 was performed with different concentrations of Cr(VI) (100, 150, 200 and 250 μg/ml).

2.4.1. IAA production

IAA production by the strain AR6 was quantitatively analysed as described by Libbert et al. (2006) with 50 and 100 μg/ml supplementation of L–tryptophan as a precursor. The inoculated tubes were incubated at 35 ± 2 °C for 36 h with shaking at 200 rpm. Two milliliter of cell free supernatant was mixed with 2 ml of Salkowski's reagent (2% 0.5 M $FeCl_3$ in 35% Perchloric acid) and incubated in dark for 30 min at room temperature. The absorbance of the developed pink colour read spectrophotometrically at 530 nm. Further, bacterial IAA was confirmed with C-18 column equipped with reverse phase high performance liquid chromatography (HPLC). Elution was performed with a ratio of H_2O and methanol (60:40). The elution was monitored at 280 nm by Shimadzu UV–vis detector (model LC–20AD, Japan) with a flow rate of 1 ml/min. Commercially purchased pure IAA (HiMedia, India) used as a standard.

2.4.2. Ammonia production

Ammonia production by the strain AR6 was estimated quantitatively as described by Cappuccino and Sherman (1992). Strain AR6 was inoculated in the peptone water broth (g/L: Peptone 10; NaCl 5 and pH 7.0 ± 0.2) and incubated at 35 ± 2 °C with shaking at 200 rpm. After incubation, 1 ml cell free supernatant was mixed with 1 ml of Nessler's reagent and volume of this reaction mixture was made up to 10 ml by addition of sterile ammonia free distilled water. Optical density (OD) was measured spectrophotometrically at 450 nm with ammonium chloride as the standard.

2.4.3. EPS production

EPS production was quantified as described by Mody et al. (1989). Strain AR6 was inoculated in 100 ml LB broth supplemented with 5% sucrose. The inoculated culture flasks were incubated at 35 ± 2 °C for 120 h with shaking at 200 rpm. After incubation, culture broth was centrifuged at 10,000 rpm for 30 min. The EPS was extracted by adding three volumes of ice-cold acetone to one volume of the cell free supernatant. The precipitated EPS was rapidly washed thrice alternately with sterile distilled water and acetone, transferred to a filter paper and weighed after overnight drying at room temperature.

2.4.4. Phosphate solubilization and catalase production

Phosphate solubilization of the strain AR6 was assessed using Pikovskaya medium, that contains 0.5% of insoluble phosphate in the form of tricalcium phosphate (TCP) with bromothymol blue (0.05 g/l) as an indicator (Pikovskaya, 1948). Catalase production of the strain AR6 was studied qualitatively as described by Cappuccino and Sherman (1998). Overnight grown bacterial culture was mixed with an approximate amount of 3% hydrogen peroxide on a glass slide to observe the evolution of oxygen gas.

2.4.5. Hydrolytic enzyme production

For protease production, the bacterial strain was streaked on casein hydrolyzed medium and the plates were incubated at 35 ± 2 °C for 24 h. Formation of clear zone around the bacterial colonies indicated the proteolytic activity of the strain (Smibert and Krieg, 1994). In case of amylase production, the strain AR6 was streaked on starch agar plates and incubated at 35 ± 2 °C for 24 h. After incubation, 1% of iodine solution was flooded on the starch agar plate for the production of a clear zone around the bacterial colonies, which indicated hydrolysis of starch (Mesa et al., 2015). The lipolytic activity of the strain AR6 was confirmed by tributyrin and tween 20 agar plate methods (Kumar et al., 2012).

2.4.6. In vitro root elongation assay

The root elongation and root colonization of the strain AR6 were tested with major agricultural crops such as *Zea mays, Vigna mungo*, *Vigna radiata, P. vulgaris* and *Sesbania aculeata*. The root elongation assay was conducted using modified method of Belimov et al. (2005). The root length of seedlings was measured by a centimetre scale after 6 days of incubation at dark.

Table 1
Heavy metal and antibiotic tolerance of the rhizobacterial strain AR6.

Heavy metals	Concentration (μg/ml)
Cr(VI)	1200
Pb(II)	750
Zn(II)	150
Cu(II)	300
Mn(II)	700
Antibiotics	**Zone of inhibition (mm)**
Ampicillin	–
Gentamicin	22
Methicillin	–
Neomycin	19
Penicillin	12
Streptomycin	17
Vancomycin	17

Note: (−) resistant/no zone formation.

2.5. In vitro root colonization assay

Root colonization efficiency of the strain AR6 was confirmed using Scher et al. (1984) method. Briefly, test tubes (25 × 150 mm) were filled with garden and leather industrial effluent contaminated soil up to 6 cm and 5 ml of sterile distilled water was added into each tube (properties of leather industrial effluent contaminated soil – pH – 7.7, moisture – 49.12%, Nitrogen – 162 kg/ha, Phosphorus – 2.5 kg/ha, Potassium – 80 kg/ha, electro conductivity (dsm-1) – 3.6 and Cr – 42.65 mg/kg). Surface sterilized and AR6 inoculated seeds were planted in the tubes and incubated in a growth chamber. After incubation, root suspensions of the test crops were serially diluted and plated on LB agar medium. Only colonies showing morphological resemblance to AR6 were counted and colony-forming units were calculated. Colonies were isolated, 16S rDNA of the isolates were sequenced, and tentative identification of the isolates were done by sequence similarity studies using BLAST program (NCBI).

2.6. Antagonistic activity

The antagonistic activity of the strain AR6 was evaluated against major plant pathogens such as *Alternaria* sp., *Aspergillus niger, Fusarium* sp., and *Rhizopus* sp. using the disc diffusion method as described by Yoshida et al. (2001) and zone of inhibition was measured after 3 days of incubation.

2.7. Statistical analysis

All the experiments were carried out in triplicates (n = 3) and the data was recorded. Values were analysed by one-way ANOVA and significance of the means were compared with the Tukey's test, using the SPSS software (version 20, SPSS Inc., www.spss.com).

3. Results and discussion

3.1. Screening of Cr(VI) tolerant rhizobacterial strain AR6

In the present study, a total of 36 Cr(VI) tolerant bacterial isolates have been screened from the rhizosphere of *P. vulgaris* grown in leather industry effluent contaminated soil. Screened bacterial isolates were further subjected to test their maximum Cr(VI) tolerance level. Among 36 isolates, rhizobacterial strains AR6 and AR8 showed maximum tolerance to Cr(VI) up to 1200 and 1100 μg/ml concentrations, respectively. Followed by this, a considerable level of Cr(VI) tolerance have been attained from AR1, AR2, AR3, AR4, AR5 and DI 9 isolates with 550 μg/ml concentration. Rhizobacterial isolates AR7, NB2, NC, DI 2 and DI 7 showed tolerance up to 450 μg/ml of Cr(VI) concentration and isolate DI 10 showed tolerance up to 400 μg/ml concentration of Cr(VI). Furthermore, rhizobacterial isolates DI 6, NA, AR9, AR12, AR15 and DI 5 were showed tolerance up to 350 μg/ml concentration and isolates NB1, AR13, AR18, AR19 and DI 1 showed Cr(VI) tolerance up to 300 μg/ml concentration. Rhizobacterial isolates DI 4, AR16 and AR20 showed tolerance up to 250 μg/ml concentration of Cr(VI). However, few rhizobacterial isolates like AR10, AR11, AR14, DI 8, AR17, DI 3, NA1 and NB were showed Cr(VI) tolerance up to 200 μg/ml concentration. Among the tested isolates, rhizobacterial isolate DI 3 showed least tolerance against Cr(VI) (up to 100 μg/ml concentration). Even though, these bacterial strains isolated from the same leather industrial effluent contaminated sampling sources, they exhibited varied Cr(VI) tolerance level. This may be due to the variation in their genetic makeup, growth medium and biochemical composition of the bacteria. Similarly, Viti et al. (2003) isolated eight bacterial strains from a chromium-polluted vegetated area of leather tannery, with the difference in Cr(VI) tolerance level. Based on maximum Cr(VI) tolerance, rhizobacterial strain AR6 was further exploited for different properties such as PGP traits, antibiotic tolerance and broad spectrum tolerance against other heavy metals.

3.2. Multimetal and antibiotic tolerance

Interestingly, the rhizobacterial strain AR6 demonstrated substantial growth on LB plates supplemented with different concentrations of other heavy metals such as Pb(II), Zn(II), Cu(II) and Mn (II). However, strain AR6 showed varied tolerance level against four tested heavy metal salts. Among them, strain AR6 showed the maximum tolerance to Pb (750 μg/ml) followed Mn (700 μg/ml), Cu (300 μg/ml) and Zn (150 μg/ml). Moreover, strain AR6 showed resistance against ampicillin and methicillin, however, was found to be sensitive to penicillin, gentamicin, neomycin, streptomycin and vancomycin (Table 1). These results concluded that, the heavy metal and antibiotic resistance provide survival and adaptation ability to the bacterial strain under natural selection in harsh environmental conditions. Previously, Hobman and Crossman (2014) reported that, genetic elements are responsible for heavy metal resistance. These genetic elements play a key role in assisting multi-drug resistance and horizontal gene transfer, through co-carriage and/or co-selection of antibiotic resistance along with metal tolerance. Similarly, Wani and Irene (2014) have reported that the importance of combined heavy metal and antibiotic resistance are basis in bacterial survival. In general, soil microorganisms developed various resistance mechanisms such as biosorption,

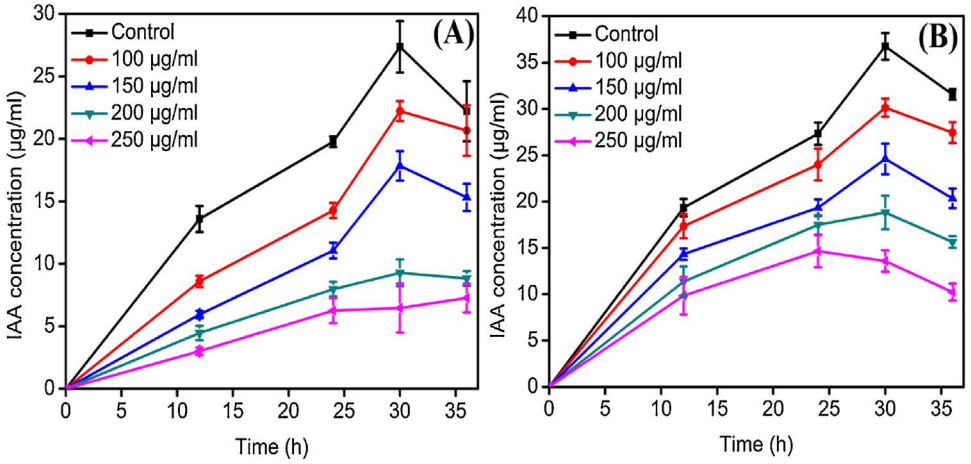

Fig. 1. Effect of different concentrations of Cr(VI) on IAA producing ability of the strain AR6. (A) IAA production with 50 µg/ml of L-tryptophan supplementation and (B) IAA production with 100 µg/ml of L-tryptophan supplementation. Experiments were performed in triplicate and the values are represented as mean ± SE.

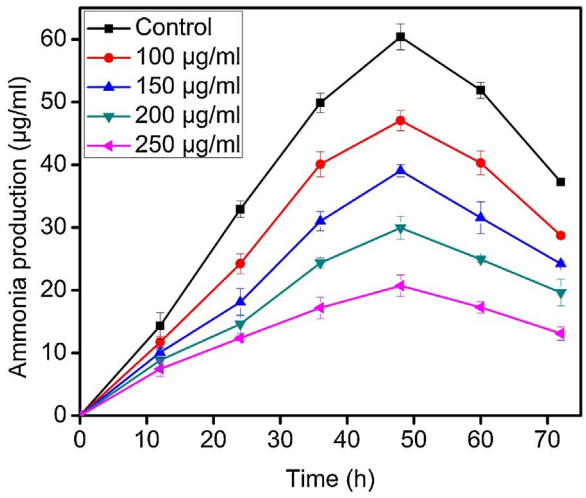

Fig. 2. Effect of different Cr(VI) concentrations on ammonia production of strain AR6. Experiments were performed in triplicate and the values are represented as mean ± SE.

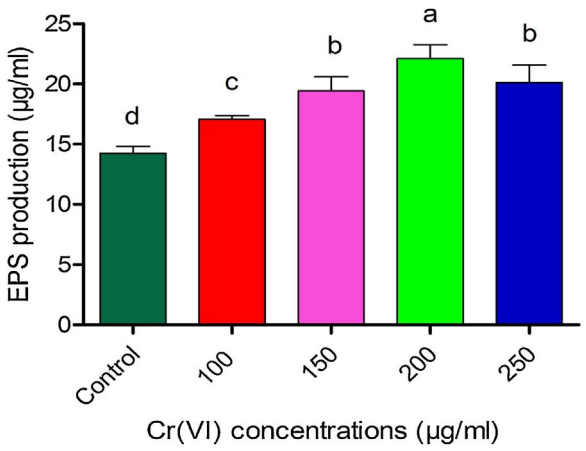

Fig. 3. Effect of different concentrations of Cr(VI) on EPS production by strain AR6. Experiments were performed in triplicate and the values are represented as mean ± SE. Values are significantly different followed by Tukey test at $P < 0.05$.

bioaccumulation (both external and intracellular spaces), biotransformation, immobilization, chelation, exclusion etc., against heavy metal stress (Rajkumar et al., 2010).

3.3. IAA production and HPLC analysis

Production of IAA by the rhizobacterial strain AR6 increased gradually based on concentration of L-tryptophan in time dependent manner. The maximum quantity of IAA production (36.75 µg/ml) was observed at 100 µg tryptophan/ml concentration without Cr(VI) supplementation after 30 h incubation, which was 25.76% higher than 50 µg/ml of L-tryptophan supplementation (Fig. 1). This result confirmed that L-tryptophan is a vital precursor compound for IAA production in strain AR6 and utilizes the indole moiety from tryptophan to produce IAA. On the other hand, quantity of IAA produced by the rhizobacterial strain AR6, reduced progressively with the graded increment of Cr(VI). Among the tested Cr(VI) concentrations, the maximum reduction (63.04%) was observed at 250 µg/ml concentration, compared to control. This progressive decline of IAA production denoted that, under stress condition bacterial cells were actively involved in stress management than other metabolic process. Phytohormones are well known for their plant growth and it's enhance the physiological processes of plants. Similarly, Glick (2012) reported that, bacterially synthesized IAA could enhance the root growth directly by stimulating cell elongation and division. At lower concentration of IAA synthesized by PGPR, stimulates primary root elongation, whereas at higher IAA concentration induce lateral and adventitious root formation, rather inhibiting primary root growth (Ma et al., 2009). Moreover, IAA producing ability of the rhizobacterial strain was confirmed by HPLC analysis (Supplementary Fig. 1). In this, standard IAA and methanol extract of strain AR6 showed similar characteristic peak at 2.9 retention times.

3.4. Ammonia production

Similar to IAA production, AR6 strain produced a considerable quantity of ammonia in all the tested Cr(VI) concentrations. Among them, the maximum quantity of ammonia production (60.40 µg/ml) was observed at control condition after 48 h of incubation. On the other hand, ammonia production by the strain AR6 decreased significantly by 22.06, 35.33, 50.41 and 65.69% at 100, 150, 200 and 250 µg/ml concentrations of Cr(VI), respectively (Fig. 2). Ozugul and Ozugul (2007) have highlighted that, degradation and decarboxylation of various amino acids were utilized from the complex growth medium, which leads to the production of biogeneic amines as well as ammonia. Plants utilize nitrogen and nitrogen based compounds for growth and metabolic function, which are one of the essential and primary plant nutrients. Previously, Marques et al. (2010) reported that inoculation of ammonia producing PGPR increases the root and shoot length and biomass of *Z. mays*.

Table 2
PGP activities of rhizobacterial strain AR6 under different concentrations Cr(VI).

Cr(VI) conc. (μg/ml)	P[a] solubilization	Catalase	Protease	Amylase	Lipase	
					TBA[b]	T20[c]
Control	+	+	+	+	+	+
100	+	+	+	+	+	+
150	+	+	+	+	+	+
200	+	+	+	+	+	+
250	+	+	+	+	+	+

[a] P-phosphate.
[b] TBA-tributyrin agar.
[c] T20-tween 20.

Table 3
Effects of different concentrations of Cr(VI) and rhizobacterial strain AR6 inoculation on root elongation of test crops.

Treatment	Cr(VI) cons. (μg/ml)	Root length (cm)				
		Z. mays	*V. mungo*	*V. radiata*	*P. vulgaris*	*S. aculeata*
Uninoculated seedling	Control	13.75 ± 1.71[a]	4.85 ± 1.29[a]	5.7 ± 1.16[b]	7.13 ± 0.83[b]	3.8 ± 1.11[b]
	100	7.7 ± 2.41[c]	3.2 ± 0.87[c]	3.75 ± 2.31[d]	4.5 ± 2.26[d]	2.3 ± 1.58[c]
	150	5.7 ± 0.61[d]	2.48 ± 0.54[d]	2.63 ± 1.65[e]	3.41 ± 1.06[e]	1.63 ± 0.61[d]
	200	3.9 ± 0.85[e]	1.90 ± 2.16[e]	1.95 ± 0.78[f]	2.04 ± 1.62[f]	0.93 ± 1.94[e]
	250	2.40 ± 2.01[f]	0.6 ± 1.40[f]	1.03 ± 1.54[f]	1.16 ± 1.83[g]	0.35 ± 1.63[f]
Inoculated seedling	Control	14.47 ± 0.94[a]	5.33 ± 2.85[a]	6.66 ± 1.21[a]	9.6 ± 2.21[a]	4.5 ± 2.07[a]
	100	9.87 ± 1.19[b]	4.16 ± 0.82[b]	4.60 ± 2.01[c]	6.15 ± 1.86[c]	3.6 ± 2.17[b]
	150	6.11 ± 2.23[d]	3.7 ± 2.23[c]	3.9 ± 0.91[d]	4.51 ± 1.49[d]	2.81 ± 1.04[c]
	200	4.3 ± 1.68[e]	2.8 ± 1.14[d]	2.95 ± 2.11[e]	2.93 ± 0.81[f]	1.85 ± 1.49[d]
	250	2.7 ± 1.41[f]	0.93 ± 2.70[f]	1.23 ± 1.32[f]	1.86 ± 1.74[g]	0.6 ± 1.19[e]

Note: Results are expressed as the mean three replicates ± SE. Mean values followed by different letters are significantly different according to the Tukey test at $P < 0.05$.

3.5. EPS production

Apart from other PGP activities, EPS production by the AR6 strain gradually increased with increase in Cr(VI) concentration. Among the tested concentrations of Cr(VI), the maximum stimulation of EPS secretion (22.11 μg/ml) was observed at 200 μg/ml concentration of Cr (VI). However, EPS production of the strain AR6 decreased at 250 μg/ml concentration of Cr(VI) (Fig. 3). Similar pattern of EPS production was reported in *Pseudomonas aeruginosa* strain by Oves et al. (2013) under different concentrations of Cr(VI). Bacterially produced EPS has greatly accepted to play a key role in the interaction of the rhizobacterial cells with varied surfaces; osmoregulation and ion transport. Bacterial EPS can enhance plant growth by stimulating the microbial activity in the rhizosphere by enhancing the soil organic content and thereby providing stability to soil aggregate with plant roots. Moreover, excessive production of EPS through PGPR denotes the innate protection mechanism by masking the effect of metal toxicity while growing in stress environments (Karthik et al., 2016).

3.6. Phosphate solubilization, catalase and hydrolytic enzyme production

The release of insoluble and fixed forms of phosphate is an important facet of increasing the availability of soil phosphate. Thus, the uses of phosphate solubilizing bacteria inoculation concurrently increase the availability of phosphate there by increase the agricultural productivity (Ji et al., 2014). Strain AR6 shows a positive result for phosphate solubilization, in the presence and absence of Cr(VI) (Table 2). Phosphate is the second major nutrient source for plant, after nitrogen. However, phosphorus compounds are enormously available in agricultural soils in the form of insoluble state. Phosphate solubilizing

Table 4
Root colonization efficiency of the rhizobacterial strain AR6.

Test crops	cfu/g roots	
	Garden soil	Tannery effluent contaminated soil
Z. mays	1.6×10^5	1.3×10^3
V. mungo	3.7×10^5	3.1×10^4
V. radiata	3.2×10^4	3.0×10^4
P. vulgaris	4.3×10^5	3.9×10^5
S. aculeate	2.3×10^4	1.8×10^5

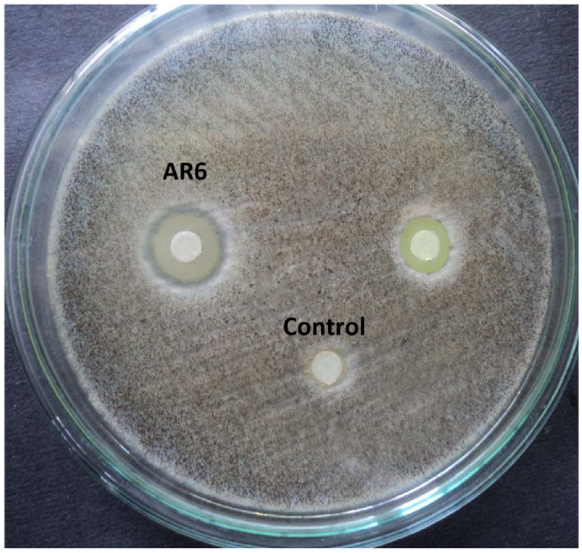

Fig. 4. Antagonistic activity of the rhizobacterial strain AR6 against *A. niger*.

bacteria being able to adapt insoluble phosphate into available forms for plants during the processes of chelation, acidification, exchange reactions and the production of gluconic acid (Gulati et al., 2010), thereby playing a key role in plant growth promotion, yield and nutrient uptake. Apart from this, biological nitrogen fixation needs a considerable quantity of the absorbed phosphate from soil to produce ATP, which is required by the plant for atmospheric nitrogen fixation (Dey et al., 2004). Similar to phosphate solubilization, catalase and hydrolytic enzyme production by strain AR6 was remain unaffected with different concentrations of Cr(VI) (Table 2).

3.7. In vitro root elongation assay

The production of various PGP substances by strain AR6 in control and Cr(VI) stress environment motivate us to evaluate its impact on the root elongation of major agriculture crops. Based on our results, Cr(VI) toxicity significantly reduces the root length of test crops. For instance, when seeds treated with 250 µg/ml of Cr(VI) showed the maximum phytotoxicity effect and decreased root length by 82.54, 87.62, 81.92, 83.73 and 90.78% in *Z. mays, V. mungo, V. radiata, P. vulgaris* and *S. aculeata* plants respectively, when compared to uninoculated control plant. At initial seed germination and seedling developmental stage, heavy metals affect the root initiation and growth by their innate toxicity nature, also inhibiting water and nutrient intake (Kranner and Colville, 2011). When rhizobacterial strain AR6 was used as a bioinoculant, root length of *Z. mays*, *V. mungo*, *V. radiata*, *P. vulgaris* and *S. aculeata* increased significantly (Table 3). This increased root length might be due to the production of IAA by the rhizobacterial strain AR6 under Cr(VI) stressed environment. IAA known to be a major plant growth regulator, which increases the plant growth by modulating various physiological behaviour of the host plant (Wani and Khan, 2010). Another possible reason is strain AR6 has the ability to reduce toxic Cr(VI) into less toxic Cr(III) (Karthik et al., 2017). Hence, rhizobacterial strain AR6 with PGP activities can promote the plant growth without major effects on plant physiology when grown in Cr(VI) contaminated soil.

3.8. In vitro root colonization assay

Root colonization efficiency of the strain AR6 has been confirmed by root colonization assay. The maximum bacterial population was recorded on the root of *P. vulgaris* (4.3×10^5 cfu/g root) which was followed by *V. mungo* (3.7×10^5 cfu/g root), *V. radiata* (3.2×10^4 cfu/g root), *S. aculeate* (2.3×10^4 cfu/g root) and *Z. mays* (1.6×10^5 cfu/g root), respectively, while grown in garden soil (Table 4). However, root colonization ability of the strain AR6 was reduced when plants grown in leather industrial effluent contaminated soil. Similar type of versatile root colonization ability of *Pseudomonas putida* 06909 strain was observed by Wu et al. (2006) in cowpea, corn, wheat and sunflower under heavy metal stress. In order to confirm the similarity between re-isolated bacterial strains and strain AR6, molecular 16S rDNA gene sequencing was performed. Results showed that re-isolated bacterial isolates belonged to *C. funkei*. Root colonizing and its associated microbes are able to enhance host plant growth by production of phytohormones, stabilization of soil structure and detoxification of phytotoxins (Gaiero et al., 2013). Hoflisc et al. (1995) observed increased root length, lateral root numbers and root nodules in *Rhizobia* and *Pseudomonas* strains colonized agriculture crops. The versatility of the strain is to adapt to a wide array of host plants under heavy metal polluted soil will be invaluable for future rhizo-remediation under various site and plant growth conditions.

3.9. Antagonistic activity

Interestingly, strain AR6 showed strong antagonistic activity against plant pathogenic fungi *A. niger* (Fig. 4). In general, cell wall of *A. niger* majorly consists of carbohydrate (73–83%) hexosamine (9–13%), lipid (2–7%), protein (0.5–2.5%) and phosphorus (less than 0.1%) (Johnston, 1965). Thus, rhizobacterial strain AR6 with protease, lipase and amylase producing ability can easily hydrolyze the cell wall of *A. niger*. This result suggests that the production of hydrolytic enzymes from rhizobacterial strain would be the most prominent traits of antagonism. In the presence of phytopathogens, the bacterial strains may induce the production of lytic enzymes by utilize the carbon source from the pathogenic cell wall. Previously, Dunne et al. (1997) have observed the suppression of phytopathogenic fungus in the rhizosphere of sugarcane was due to the production of extracellular protease by *Stenotrophomonas maltophilia* W81.

3.10. Schematic representation of PGP ability of the bacterial strain AR6

Based on our results, plant growth promoting mechanisms of strain AR6 can be mainly divided into two different mechanisms: (i) direct plant growth promoting mechanism and (ii) indirect defense mechanism. In direct plant growth promoting mechanism, strain AR6 produces plant growth regulators (IAA), provide N source and facilitate soil nutrients uptake to the host plant. These PGP substances directly stimulate the growth of the host plant by inducing cell division, root and shoot elongation, subsequently increase plant biomass and metabolic activities. Whereas, indirect defense mechanism occurs by three unique PGP activities: (i) EPS production: bacterial EPS has diverse PGP properties. Specifically, anionic charged bacterial EPS molecules can easily entrap the cationic charged Cr(VI), which leads to the reduction of Cr(VI) availability and their mobility in rhizosphere, thereby providing stress free environment to the host plant. (ii) Catalase production: In general, catalase production by rhizospheric microorganisms involved in the degradation of H_2O_2 into water and oxygen. Thus, bacterial strain AR6 with catalase activity protects their host plant from oxidative stress and (iii) Hydrolytic enzyme production: rhizobacterial hydrolytic enzymes are involved in bio-control activity (by lysing the cell wall in close vicinity of rhizospheric phytopathogens) and nutrient recycling (organic matter decomposition), thereby strain AR6 increase the nutrient source and reduce pathogen population around the host plant.

4. Conclusion

The PGP traits of the strain AR6 were not completely lost even at higher concentrations of Cr(VI). Strain AR6 inoculation either alone or

in combination with Cr(VI) improved root length of test crops by reducing toxicity of Cr(VI). Strain AR6 able to interact easily with a versatile plant species which may provide Cr(VI) free environment and rapidly promote the growth of host plant. In addition, our results clearly highlighted that strain AR6 is a potential biocontrol agent against plant pathogen. Thus, the rhizobacterial inoculation in contaminated sites might be a powerful applicant for soil restoration and as alternative bio–fertilizer instead of hazardous chemical fertilizer and fungicides.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.micres.2017.07.008.

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