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ORIGINAL PAPER

Biotransformation potential of hexavalent chromium by *Bacillus pumilus*-S4, *Pseudomonas doudoroffii*-S5 and *Exiguobacterium*-S8 in association with hydrophytes

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Abstract Three chromium-resistant bacteria *Bacillus pumilus*-S4, *Pseudomonas doudoroffii*-S5 and *Exiguobacterium*-S8 were isolated from chromium-contaminated wastewater/soil and could resist very high concentrations of potassium chromate in Luria agar (up to 25 mg ml^{-1}) and acetate minimal medium (2 mg ml^{-1}). The strains showed growth at diverse pH and temperatures and could resist multiple heavy metals. *Pseudomonas doudoroffii*-S5 reduced (8.27 mg hexavalent chromium 24 h^{-1}) at a lower initial potassium chromate concentration (100 μg ml^{-1}), but overall more chromate (28.4 mg hexavalent chromium 24 h^{-1}) was reduced at a higher initial concentration (1,000 μg ml^{-1}). The addition of various heavy metals (zinc sulphate, copper sulphate, and manganese sulphate at 50 μg ml^{-1}) in the chromium reduction media did not significantly affect the hexavalent chromium reduction potential of these isolates. The chromium removal/detoxification potential of these strains increased when used in conjunction with hydrophytes *Eichornia crassipes* and *Pistia stratiotes*. Interestingly, the whole process runs automatically with less energy input, that is, the bacterial strains support the growth of plant while in turn the plant releases exudates that help bacterial growth.

Keywords Bioremediation · *Eichornia crassipes* · Heavy metals · *Pistia stratiotes*

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Introduction

Chromium compounds have widespread industrial uses in steel production, wood preservation, leather tanning, metal corrosion inhibition, paints and pigments, metal plating, and other applications (Swarnalatha et al. 2009). Chromium can exist in oxidation states ranging from +2 to +6, but is most frequently found in the environment in the trivalent (+3) and hexavalent (+6) states (Parmar et al. 2010). Hexavalent (VI) is about 100-fold more toxic, mutagenic and well-known carcinogen than the trivalent (III) form (Kerger et al. 2009). Conventional methods for removing toxic CrO_4^{2-} from wastewater include chemical reduction followed by precipitation, ion exchange, and adsorption on activated coal, alum, kaolinite, and ash (Camargo et al. 2003; Fathizadeh et al. 2011). When compared with conventional chemical treatment, biological treatment shows some advantages, such as low operational costs and easy recovery of this valuable metal (Mnif et al. 2009). A wide variety of bacteria have been reported to reduce Cr(VI) under either aerobic (Rehman et al. 2011) or anaerobic (Bhowmick et al. 2009) conditions.

Beside bacteria, plants especially hydrophytes can play an important role in metal removal via filtration, adsorption, cation exchange, and through plant-induced chemical changes in the rhizosphere (Hanc et al. 2009). The use of free-floating hydrophytes for the recovery of pollutants from wastewater represents an alternative technology with

a significant potential for application in small and large-scale industrial setups (Danh et al. 2009). Apart from supporting plant growth of the accumulating biomass, rhizosphere bacteria may mobilize heavy metals for enhanced uptake by plant roots (Li et al. 2009). Hence, the present study focuses on the chromium detoxification potential of bacteria and hydrophytes alone and in combination under varying environmental conditions. The study was carried out in the Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore, Pakistan in the year 2010.

Materials and methods

Bacterial isolation and characterizations

Effluent samples from tanneries situated in Din Garh, Kasur, Pakistan were collected in sterilized bottles/bags and were labelled according to the sample. Samples temperature and pH were recorded on site (Table 1). Physicochemical characteristics of soil (Sparks 1996) and wastewater (Clesceri et al. 1998) were also carried out. Serial dilutions (1/10, 1/100, 1/1,000) of effluents and soil suspensions were made and plated on nutrient agar plates supplemented with 1,000 μg ml^{-1} of K_2CrO_4. The plates were incubated at 37 °C for 24 h at pH 7. Colonies obtained were picked and purified by many rounds of streaking. Isolated strains were further characterized morphologically, biochemically, physiologically and genetically following Gerhardt et al. (1994). To confirm the identity of the three isolated strains, 16S rRNA gene sequencing was undertaken. 16S rRNA gene (1,500 bp) was amplified and the amplicon sequenced using fluorescent di-deoxy terminator cycle sequencing chemistry. The extension product was then separated on an ABI PRISM automated DNA sequencer and compared the data to the MicroSeq® databases.

Collections of hydrophytes

Collected the *Pistia stratiotes* and *Eichhornia crassipes* plants in sterilized polyethylene bags from a freshwater pond of the University of the Punjab, Lahore, Pakistan, which had not been previously exposed to a contaminated environment. Plants were washed with tap water, blot dried and the weights of roots and shoots were recorded. Plants were grown in nutrient solution [grams per liter: KNO_3 0.505; Ca $(NO_3)_2$ 0.820; $NaH_2PO_4{\cdot}7H_2O$ 0.208; $MgSO_4{\cdot}7H_2O$ 0.369; $FeC_6H_5O_7$ 0.0245; $MnSO_4$ 0.00223; $CuSO_4{\cdot}5H_2O$ 0.00024; $ZnSO_4{\cdot}7H_2O$ 0.000296; H_3BO_3 0.00186; $(NH_4)_6$ $Mo_7O_{24}{\cdot}4H_2O$ 0.000035; $CoSO_4{\cdot}7H_2O$ 0.000028; $CoSO_4{\cdot}7H_2O$ 0.00585] (Hewitt 1963) and maintained in a growth chamber at a temperature of 25 ± 2 °C with a 12 h photoperiod (10 Klux). Equal-sized plants were selected for each experiment.

Cr(VI) reduction experiments

For the bacterial Cr(VI) reduction experiment, 100 ml of DeLeo and Ehrlich (1994) medium (grams per liter: tryptone 10, yeast extract 5, NaCl 5, citric acid 1 and Na_2HPO_4 6.9) was used supplemented with one of the three initial K_2CrO_4 concentrations (100, 500 and 1,000 μg ml^{-1}). Following incubation by one of the three isolated strains of bacteria, cultures were maintained on incubating shaker with 150 rpm at 37 °C. After 24 h of incubation, samples were taken aseptically and were analyzed for Cr(VI) reduction. The degree of reduction of Cr(VI) by bacterial strains was monitored in the supernatant of the cultures using the standard spectrophotometric method (Clesceri et al. 1998) by reacting with diphenylcarbazide in an acidic solution of phosphoric acid. The absorption was measured at 540 nm. The effects of various temperatures (28, 37 and 45 °C) on Cr(VI) reduction were also examined. Cultures were incubated at various temperatures and after 24 h incubation, Cr(VI) reduction was measured as described

Table 1 Physicochemical characteristics of wastewater\soils samples used for bacterial isolation

Bacterial isolates	Nature of sample	Locality	Sample	Physicochemical characteristics
CrSS4	Contaminated soil	Din Garh (Kasur)	A3	pH 6, EC 58, Cr(VI) 310 μg g^{-1}; Fe 18 μg g^{-1}; Cu 10 μg g^{-1}; Zn 7 μg g^{-1}; Ni 7 μg g^{-1}; Co 2 μg g^{-1}; Mn 14 μg g^{-1}, Organic matter 285
CrSS5	Contaminated soil	Din Garh (Kasur)	B4	pH 6.4, EC 30, Cr(VI) 211 μg g^{-1}; Fe 14 μg g^{-1}; Cu 12 μg g^{-1}; Zn 9 μg g^{-1}; Ni 4 μg g^{-1}; Co 3 μg g^{-1}; Mn 12 μg g^{-1}, Organic matter 347
CrSS8	Wastewater	Din Garh (Kasur)	D2	pH 5.6, EC 5; temperature 27 °C, Cr(VI) 278 μg ml^{-1}, suspended solid 24-40 mgL^{-1}, Sulphide 1-3 mg S^2 L^{-1}, Sulphate 590-876 mg L^{-1}, Chlorides 758-987 mg L^{-1}, Cr(III) 123 mg L^{-1}, Zn 8 μg ml^{-1}; Ni 25 μg ml^{-1}; Pb 4 μg ml^{-1}; Mn 3 μg ml^{-1}

above. The initial K_2CrO_4 concentration used for this experiment was 100 and 1,000 μg ml^{-1}. The reduction potential of these bacterial strains was also examined at various pH levels (5, 7 and 9) using DeLeo and Ehrlich (1994) medium supplemented with an initial K_2CrO_4 concentration of 100 and 1,000 μg ml^{-1}. The pH and temperature of the reduction medium was controlled during the experiment. The Cr(VI) reduced after 24 h was determined as mentioned above.

The effects of different heavy metals on chromium reduction by the bacterial strains were also studied. For this purpose, cultures were also separately amended with salts of $ZnSO_4$ 50 μg ml^{-1}, $CuSO_4$ 50 μg ml^{-1} and $MnSO_4$ 50 μg ml^{-1} at initial Cr(VI) concentrations of 100 μg ml^{-1}. Incubation took place at 37 °C and pH 7.0. After 24 h, cultures were harvested, and were processed as described above to determine the amount of Cr(VI) reduced to Cr(III).

Removal of Cr(VI) by hydrophytes and bacteria

The effects of bacterial strains on Cr(VI) removal/detoxification in conjunction with *Pistia stratiotes* and *Eichhornia crassipes* were examined at three pH levels (5, 7 and 9) in aqueous solutions with no nutritional ingredients added to the test at an initial K_2CrO_4 concentration of 300 μg ml^{-1}. A bacterial inoculum of 100 μl of a freshly prepared overnight culture was added to the solution with incubation at ambient temperature.

After 15 days of incubation, cultures were harvested and Cr(VI) reduction was determined both in plants and bacteria as described by Rand et al. (1979). Weighed plant material (1 g) was taken in two labeled (respective treatment) flasks and digested. 10 ml of HNO_3, 2 ml of $HClO_4$ were added in each flask and flasks were heated on sand bath. In the beginning brown fumes of HNO_3 were emitted from flask, but later on flasks were full with white fumes. When solutions become clear, flasks were removed from sand bath and after cooling volume were made up to 15 ml with distilled water. Samples were now ready for chromium estimation. Bacterial pellet was also digested by the same method, and all samples were analyzed for cell chromium content. Standard errors of the means were calculated following Steel and Torrie (1981).

Biochemical analysis

Many bacterial strains produce phytohormone auxin which benefits plant growth. Auxin production was estimated following Mahadevan (1984). Plant material was crushed and transferred into properly labeled test tubes. 2 ml of ethyl ether was added in each test and covered tightly to avoid evaporation of ethyl ether and shaked thoroughly. They were kept at 4°C for 4 h. After extraction of samples, 2 ml of sodium bicarbonate was added in sample tubes and was thoroughly mixed. 1 ml of 5 % sodium bicarbonate was added in each test tube having supernatant, and was shaked thoroughly and sodium bicarbonate layer was transferred into other set of properly labeled respective test tubes. This process was repeated twice. The bicarbonate extraction was acidified to pH 3 with HCl (6 N). 1 ml of ethyl ether was added in each test tube having bicarbonate extract. The inorganic layer was separated and discarded and in the residue (organic layer) 2 ml of Salkowski reagent was added in each test tube. A blank was also prepared by adding 1 ml of ethyl ether and 2 ml of Salkowski reagent. All test tubes were kept in dark at room temperature for color development for 25–30 min. Auxin content in the extract was estimated with the help of Beckman D-2 Spectrophotometer at 535 nm wavelengths. Optical densities of various concentrations of IAA (standard) were also measured.

Under stress condition, bacteria also produce acid phosphatase. The enzyme was extracted following the method of Iqbal and Rafique (1987). Plant material was crushed in cold 0.1 M Tris HCl buffer (pH 6.5) with 4:1 (v/w) ratio of buffer to plant material. The crushed material was centrifuged at 14,000 rpm for 10 min. Pellet obtained was discarded and supernatant thus obtained was used for the estimation of enzyme acid phosphatase. As a consequence of hydrolysis of acid phosphatase, phenol was released from the substrate phenyl phosphate under specific conditions of time, temperature and pH. To observe the activity of acid phosphatase enzyme, the time duration was 1 h, temperature was 37°C and pH was 4.9. For the quantitative estimation of enzyme, series of reactions i.e., test, control, standard and blank were carried out.

In test reaction, 1 ml of citrate buffer pH 4.9 was mixed with 1 ml of substrate phenyl in properly labeled set of test tube. The set was placed in water bath at 37 °C for 3 min. After incubation, 0.2 ml of enzyme extract was added and incubated again at 37 °C for 1 h. Then, 1 ml of 0.5 N NaOH was added. In control, 1 ml of citrate buffer pH 4.9 was mixed with 1 ml of substrate phenol. The samples were incubated at 37 °C for 1 h in water bath. After that 1 ml of 0.5 N NaOH was added followed by 0.2 ml of enzyme extract in each tube and mixed thoroughly. In standard, 1.2 ml of citrate buffer (pH 4.9), 1 ml of phenol standard and 1 ml of 0.5 N NaOH were mixed together in a test tube, which was used

as standard. In blank, 1.2 ml of citrate buffer (pH 4.9), 1 ml of distilled water and 1 ml of 0.5 N NaOH was added. Test tube containing this mixture is treated as blank. In all the tubes (test, control, standard and blank), 1 ml of 0.5 N sodium bicarbonate was added followed by the addition of 1 ml of 4-aminoantipyrine solution and 1 ml of potassium ferricyanide solution. Test tubes were gently shaked and the optical density was taken immediately against water at 510 nm on spectrophotometer. The total enzyme activity was calculated by using the following formula,

$$\text{Acid phosphatase (K.A units/100 ml)} = T - C/(S - B) \text{ x } W$$

where K.A unit is the liberation of 1 mg of phenol in 1 h. T is absorption of test, C is absorption of control, S is absorption of standard, B is absorption of blank, W is weight of plant material (g).

Root colonization study

To study the bacterial colonization, roots of *Pistia stratiotes* and *Eichhornia crassipes* were surface sterilized and were inoculated with bacterial suspension and control roots were treated with sterilized distilled water. Plants were grown for 15 days at an initial Cr(VI) concentration of 300 μg ml^{-1} and then harvested to test the colonization potential of introduced bacterial strains. To confirm the surface colonization of bacteria, the plant roots were treated with 0.01 % tetrazolium dye. After tetrazolium treatment, the plant roots turned pink on portions where bacterial cells were present. Un-inoculated control plants showed no pink coloration after tetrazolium treatment. The study of root surfaces under microscope revealed that the portions of roots which were stained darker had much dense surface bacterial population than the lighter stained roots. To assess the intensity of bacterial root colonization, the bacterial strains on rhizoplane and in the rhizosphere were also estimated by culturing on L.agar medium.

Results and discussion

Strains characterizations

Industries are discharging hazardous chemicals in their wastewaters which find their way into fresh water rivers and streams (Tarcan et al. 2010). The present research work deal with the reduction of Cr(VI) chromium by chromium-resistant bacterial strains in conjunction with hydrophytes. Strain CrSS8 was isolated from wastewater sample D2 while strains CrSS4 and CrSS5 were isolated from contaminated soil samples A3 and B4, respectively (Table 1). These strains could resist very high concentrations of K_2CrO_4 both in L.agar (up to 25 mg ml^{-1}) and acetate minimal medium (2 mg ml^{-1}). Strain CrSS4 is a gram-positive aerobic spore-forming rod, while strain CrSS5 is gram-negative facultative anaerobic motile rod. Strain CrSS8 is gram-positive, aerobic and motile cocci (Table 2). All the strains are capable of hydrolyzing starch. On the basis of 16S rRNA ribotyping, strains CrSS4, CrSS5 and CrSS8 were identified as *Bacillus pumilus*-S4, *Pseudomonas doudoroffii*-S5 and *Exiguobacterium*-S8, respectively.

To assess the heavy metal resistance profile of the bacteria, six heavy metals ($HgCl_2$, $CdCl_2$, $CuSO_4$, $ZnSO_4$, $MnSO_4$, and Na_2HAsO_4) were used. A variable response towards different metals was observed for each strain at different concentrations of the respective metal salts (Table 3). All the strains showed resistance against Na_2HAsO_4 up to 1,500 μg ml^{-1}, whereas only strain *Exiguobacterium*-S8 was resistant to all the metals at the highest concentrations used in this study (Table 3). For $HgCl_2$ the highest concentration of 150 μg ml^{-1} was tolerated by strains *Pseudomonas doudoroffii*-S5 and *Exiguobacterium*-S8 (Table 3). This high level resistance against Cr(VI) and other metals helps these strains to survive in metal-polluted environment. Chromium-resistant bacteria isolated by Horton et al. (2006) found to be resisting Cr(VI) up to 1,000 μg ml^{-1} while in another study by Camargo et al. (2003), bacterial strains tolerated Cr(VI) up to 2,500 μg ml^{-1} initial concentration. The temperature and pH for optimum growth of these strains is 37 °C and 7, respectively.

Cr(VI) reduction

A variable response in terms of reduction potential was observed by the three strains at different initial Cr(VI) concentrations. Strains *Bacillus pumilus*-S4, *Pseudomonas doudoroffii*-S5 and *Exiguobacterium*-S8 showed 82.4, 71.2 and 52.1 % reduction respectively, from an initial Cr(VI) concentration of 100 μg ml^{-1} (Fig. 1). The percentage Cr(VI) reduction in *Pseudomonas doudoroffii*-S5 was greater [8.27 mg Cr(VI) 24 h^{-1}] at a lower initial K_2CrO_4 concentration (100 μg ml^{-1}) but overall more chromate [28.4 mg Cr(VI) 24 h^{-1}] was reduced at a higher initial Cr(VI) concentration (1,000 μg ml^{-1}) (Fig. 1). Wang and Xiao (1995) showed that the rate of Cr(VI) reduction by *Bacillus* sp. increased with initial Cr(VI) concentrations ranging from 20 to 70 μg ml^{-1} and decreased at higher

Table 2 Morphological and biochemical characteristics of strains

Characteristics	Strains			Characteristics	Strains		
	CrSS4	CrSS5	CrSS8		CrSS4	CrSS5	CrSS8
Colony shape	Concentric	Round	Filamentous	Lactose	−	+	−
Colony elevation	Flat	Convex	Convex	D-mannitol	+	+	−
Colony size (mm)	1.9	3.6	3.9	Inositol	−	−	−
Colony margin	Irregular	Entire	Entire	D-sorbitol	−	−	−
Cell shape	Rod	Rod	Cocci	L-rhamnose	−	−	−
Cell size (μm)	0.5–1.2	0.6–1.0	0.7–0.7	D-sucrose	+	+	+
Gram staining	G+	G−	G+	D-melibiose	−	−	−
Capsules staining	+	+	+	Amygdalin	+	−	+
Spore staining	+	−	−	L-Arabinose	+	−	+
Motility	−	+	+	Maltose	+	+	+
2-Nitrophenyl-D-galactopyranoside	+	−	−	Oxidase	−	+	+
L-lysine	−	−	−	Catalase	+	+	+
L-ornithine	−	−	−	Nitrate reduction	−	+	+
Urea	−	−	−	OF test	A	FA	A
L-tryptophane	−	−	+	Gas production	−	−	−
Indole production	−	−	−	Starch hydrolysis	+	+	+
Gelatin	+	−	+	Arginine hydrolysis	−	+	−

OF Oxidation fermentation, − negative, + positive, *A* aerobic, *FA* facultative anaerobic

Table 3 Heavy metals resistance profile of chromium-resistant bacterial strains

Strains	Heavy metals (μg ml^{-1})					
	Hg^{2+} 200	Cd^{2+} 200	Cu^{2+} 1,500	Zn^{2+} 1,500	Mn^{2+} 1,500	As^{3+} 1,500
Bacillus pumilus-S4	100	200	1,500	1,000	1,500	1,500
Pseudomonas doudoroffii-S5	150	200	500	500	1,000	1,500
Exiguobacterium-S8	150	200	1,500	1,500	1,500	1,500

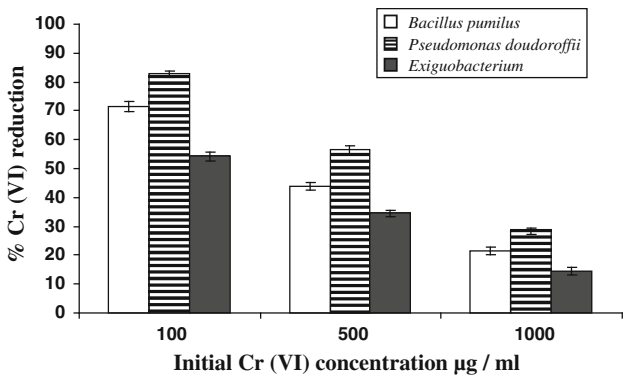

Fig. 1 Hexavalent chromium reduction potential of bacterial strains at different initial K_2CrO_4 concentrations of 100, 500 and 1,000 μg ml^{-1} after 24 h of incubation at 37 °C and pH 7

concentrations. Another study by Camargo et al. (2003), revealed that chromium-resistant bacteria can tolerate 2,500 μg ml^{-1} of Cr(VI), but most of the isolates reduced Cr(VI) at concentrations lower than 1,500 μg ml^{-1}. In contrast to the others studies, the strains used in the present study can resist and reduce much high concentration of chromate.

To assess the effect of various pH on chromium reduction, three pH levels (5, 7 and 9) were selected at initial Cr(VI) concentration of 100 and 1,000 μg ml^{-1}. After 24 h incubation, it was found that strains showed reduction potential at different pH values, but maximum reduction was observed at pH 7 (Fig. 2a, b). *Pseudomonas doudoroffii*-S5 reduced 74.6 and 32.4 % of Cr(VI) at initial Cr(VI) concentration of 100 and 1,000 μg ml^{-1}, respectively, at pH 7 (Fig. 2a, b). Strains *Bacillus pumilus*-S4, and *Exiguobacterium*-S8 reduced maximum Cr(VI) at pH 7 at both 100 and 1,000 μg ml^{-1} initial Cr(VI) concentration (Fig. 2a, b).

The optimum temperature for maximum Cr(VI) reduction for the three strains was 37 °C but also took place at both 28 and 45 °C when initially supplied with 100 or

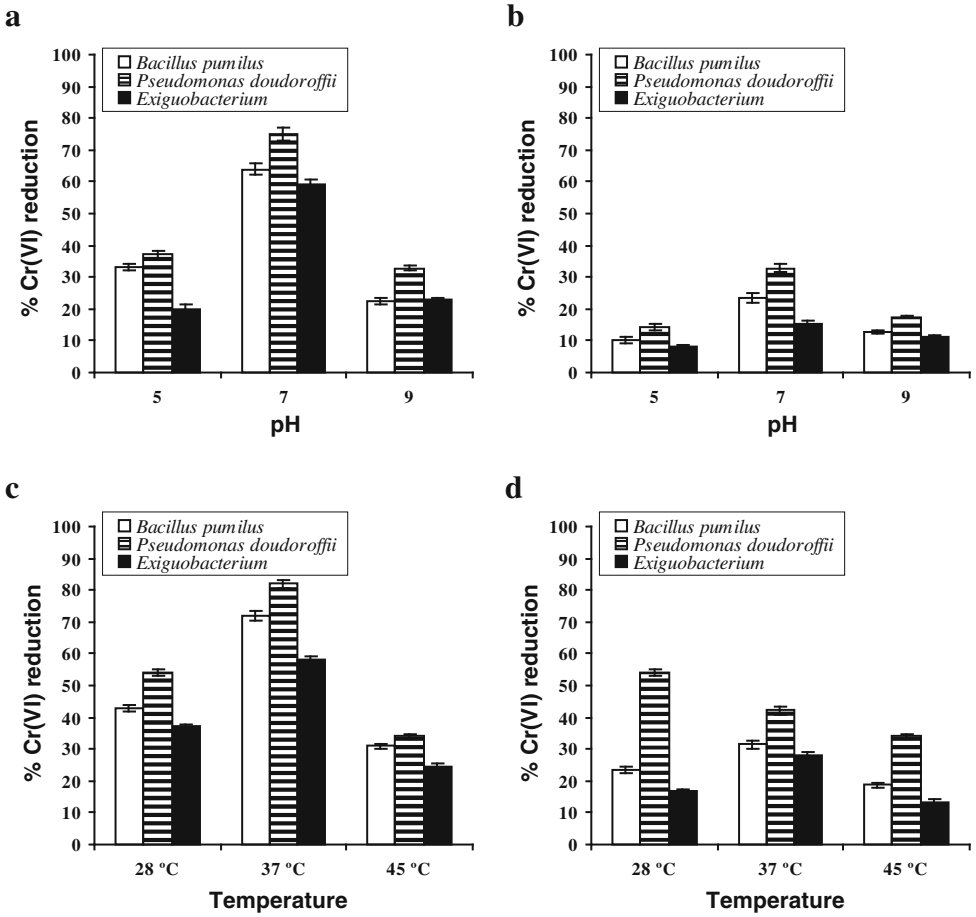

Fig. 2 Hexavalent chromium reduction potential of bacterial strains at different initial pH (5, 7 and 9) and temperature (28, 37 and 45 °C) after 24 h of incubation when initially supplied with 100 (**a**, **c**) and 1,000 (**b**, **d**) μg ml^{-1} of K_2CrO_4

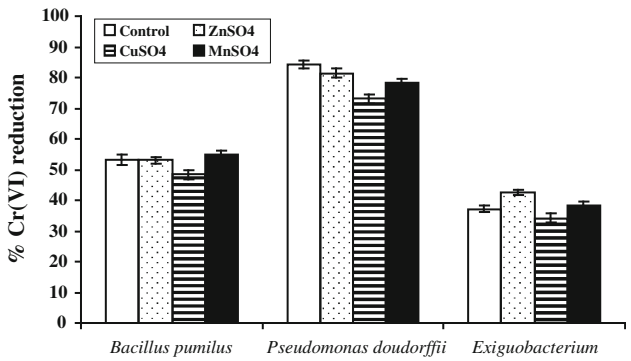

Fig. 3 Hexavalent chromium reduction potential of bacterial strains in the presence of various heavy metals ($ZnSO_4$, $CuSO_4$ and $MnSO_4$) at an initial concentration of 50 μg ml^{-1}. The initial Cr(VI) concentration used was 100 μg ml^{-1} at pH 7 and 37 °C for 24 h incubation period

1,000 μg ml^{-1} of K_2CrO_4. Interestingly, strain *Pseudomonas doudoroffii*-S5 reduced maximum Cr(VI) at 45 °C when initially supplied with 1,000 μg ml^{-1} of K_2CrO_4 (Fig. 2c, d). However, its percentage reduction was more at 28 °C when initial Cr(VI) concentration was 100 μg ml^{-1} of K_2CrO_4. Komori et al. (1990) showed that bacteria can reduce chromium at an optimal temperature of 37 °C, whereas in another study conducted by Dhal et al. (2010), the chromate reduction in *Bacillus* sp. was more than 90 % at initial Cr(VI) concentration of 100 mg L^{-1} after 144 h at a temperature of 35 °C.

Overall, the reduction potential of the strain *Pseudomonas doudoroffii*-S5 slightly decreased 3.4, 8.9 and 6.05 % with the addition of $ZnSO_4$, $CuSO_4$ and $MnSO_4$, respectively, at 50 μg ml^{-1}. In case of strain *Bacillus pumilus*-S4, the addition of heavy metals had no impact on its reduction potential apart from $CuSO_4$ where a slight decrease (8.2 %) was observed (Fig. 3). In case of *Exiguobacterium*-S8, addition of $ZnSO_4$ and $MnSO_4$ resulted 13 and 8.1 % increment, while $CuSO_4$ caused 5 % decreased in the reduction potential (Fig. 3).

Effect of hydrophytes on Cr(VI) reduction

Most of the strains and plant showed increased reduction at pH 7. After 15 days, it was observed that the reduced levels

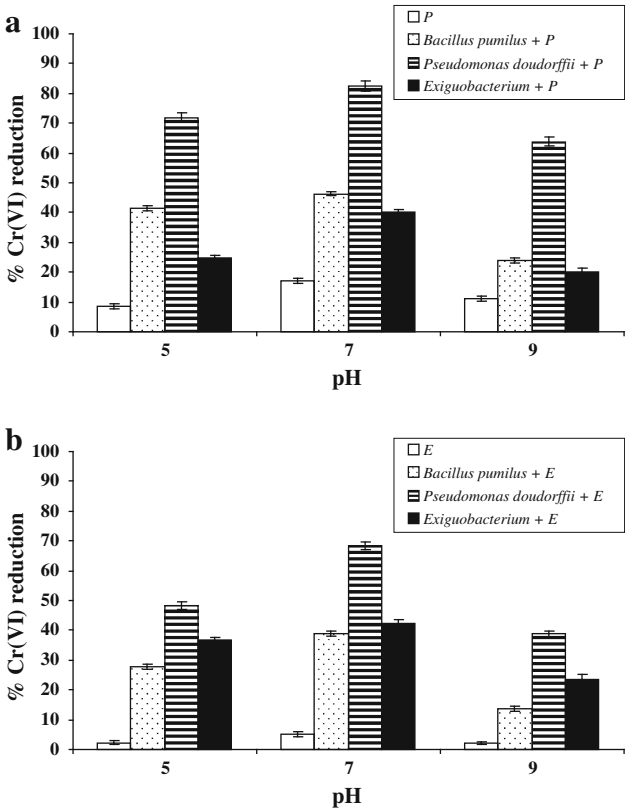

Fig. 4 Hexavalent chromium reduction potential of bacterial strains in the presence of **a** *Pistia stratiotes* and **b** *Eichornia crassipes* at an initial K_2CrO_4 concentration of 300 μg ml^{-1} for 15 days at 37 °C. *P* stands for *Pistia stratiotes* and *E* for *Eichornia crassipes*. Plants were used alone (P, E) as well as in combination (P + *Bacillus pumilus*-S4, P + *Pseudomonas doudoroffii*-S5, P + *Exiguobacterium*-S8 and E + *Bacillus pumilus*-S4, E + *Pseudomonas doudoroffii*-S5, E + *Exiguobacterium*-S8) with each strain

of Cr(VI) was less in *Pistia stratiotes* as compared to *Eichornia crassipes* at various pH. *Pistia stratiotes* reduced more Cr(VI) at pH 7 as compared to pH 5 and 9 where a very low amount of chromium was detected (Fig. 4a). When bacterial strains were used in combination with *Pistia stratiotes*, it was found that strain *Pseudomonas doudoroffii*-S5 reduced 78.6 % of Cr(VI) at pH 7 (Fig. 4a). In this case, the Cr(VI) reducing efficiency of strain *Exiguobacterium*-S8 was much better and it reduced more chromate as compared to strain *Bacillus pumilus*-S4 at all pH levels (5, 7 and 9) in the presence of *Pistia stratiotes*. *Eichhornia crassipes* reduced 8.2, 14.8 and 11.3 % of Cr(VI) from the aqueous solution when initially supplied with 300 μg ml^{-1} of K_2CrO_4 (Fig. 4b) at pH of 5, 7 and 9, respectively. In the presence of *Eichornia crassipes*, strains *Bacillus pumilus*-S4, *Pseudomonas doudoroffii*-S5 and *Exiguobacterium*-S8 reduced 83, 44.7 and 38.4 % of Cr(VI), respectively, at pH 7. Becerra-Castro et al. (2009) investigated bacterial assisted phytoremediation where bacterial strains could solubilise Ni in the soil and potentially improve phytoextraction strategies. The endophytic fungi *Neotyphodium* not only increase the host plant tolerance to cadmium stress, but also caused more accumulation of Cd in root and shoot of *Festuca arundinacea* and *Festuca pratensis* (Soleimani et al. 2010). In another study by Abou-Shanab et al. (2003), chromium-resistant bacteria helps *Eichornia crassipes* plants for the removal/phytoremediation of chromium in wastewater.

Biochemical analysis

In general, there was a pronounced increase in the auxin content of *Eichornia crassipes* plants inoculated with bacterial strains but maximum increase in this parameter was observed in case of *Pseudomonas doudoroffii*-S5 where it was 3.42 μg g^{-1} fresh weight as compared to non-inoculated control *Eichornia crassipes* plants where it was 1.31 μg g^{-1} fresh weight (Fig. 5a). Almost same trend was observed by inoculating *Pseudomonas doudoroffii*-S5 (2.83 μg g^{-1} fresh weight) in case of *Pistia stratiotes* plants when compared with non-inoculated control plants (1.72 μg g^{-1} fresh weight) (Fig. 5a).

Eichornia crassipes plants which were inoculated with bacterial strain *Pseudomonas doudoroffii*-S5 showed significant increment (9.28 μg g^{-1} fresh weight) in the activity of acid phosphate enzyme when compared with non-inoculated control plants (5.8 μg g^{-1} fresh weight) (Fig. 5b). Although in case of *Pistia stratiotes*, strain *Pseudomonas doudoroffii*-S5, resulted an enhancement (10.18 μg g^{-1} fresh weight) of acid phosphatase activity in comparison to that of non-inoculated control plants (5.26 μg g^{-1} fresh weight) (Fig. 5b).

Root colonization study

It was observed that the number of bacterial cells present on the rhizoplane of roots was much higher as compared to the surrounding rhizosphere (1–5 cm) (Fig. 6). In both plants *Eichhornia crassipes* and *Pistia stratiotes*, the bacterial strains occupy the rhizoplane with high intensity (13 to 16-folds) when compared with rhizosphere at 1 cm a distance from root. In addition, this intensity decreased with the increase in distance (Fig. 6). The present study showed that the combined action of hydrophytes and bacterial strains resulted more removal/reduction of Cr(VI) from the aqueous solution. These strains *Bacillus pumilus*-S4, *Pseudomonas doudoroffii*-S5 and *Exiguobacterium*-S8 live in the vicinity of *Pistia stratiotes* and *Eichornia crassipes* roots which were evident in the colonization study (Fig. 6). Plant roots release up to 20 % of the total

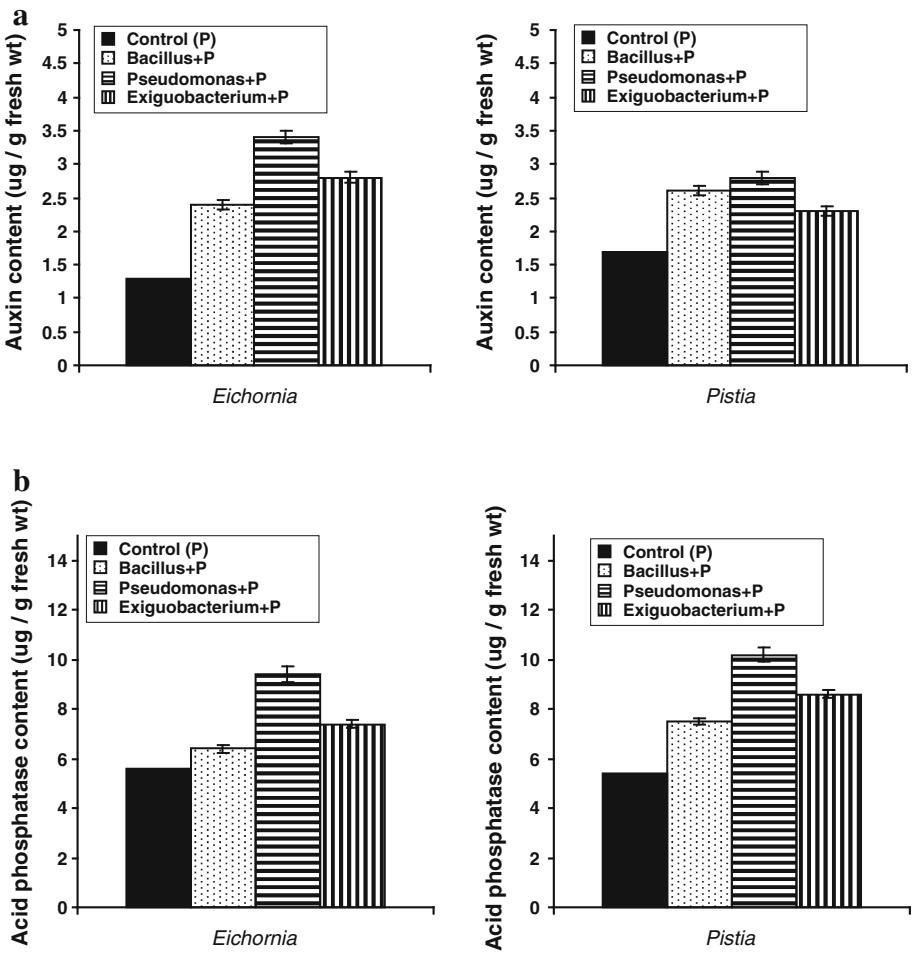

Fig. 5 Impact of bacterial strains on **a** auxin and **b** acid phosphatase production in the *Pistia stratiotes* and *Eichornia crassipes* plants grown at an initial concentration of 300 μg ml^{-1} of K_2CrO_4 at pH 7 and 37 °C for 15 days of incubation period

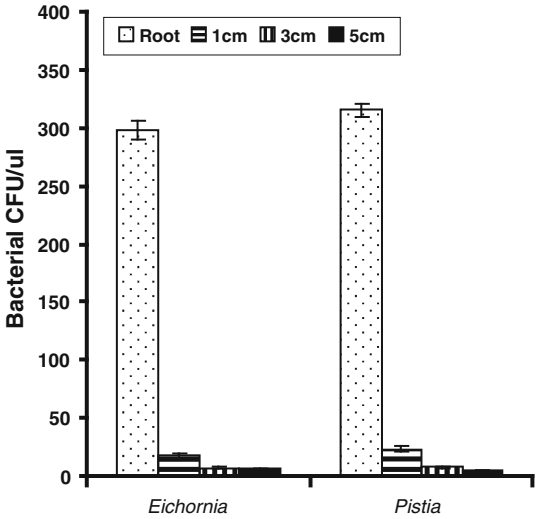

Fig. 6 Root colonization study of *Pistia stratiotes* and *Eichornia crassipes* plants when initially supplied with 300 μg ml^{-1} of K_2CrO_4 at pH 7 and 37 °C for 15 days of incubation period

photosynthesized carbon into the rhizospheric environment. Root exudates primarily contain various organic acids, amino acids, sugars, proteins/enzymes, and other organic macromolecules which can stimulate soil microbial growth and activities in the rhizosphere by providing important substrates for microbial metabolism. Thus, concentrations of heterotrophic microorganisms in the rhizospheric soil can be over 4 to 100-fold greater than the microbial concentrations in the bulk soil (Donnelly et al. 1994; Siciliano et al. 2003). This is a new report of the Cr(VI) reduction/removal using hydrophytes (*Pistia stratiotes* and *Eichornia crassipes*) along with *Exiguobacterium*-S8.

Conclusion

These results suggest that the capacity of *Pistia stratiotes* and *Eichornia crassipes* to remove Cr(VI) from solution

was accelerated in the presence of bacterial strains (*Bacillus pumilus*-S4, *Pseudomonas doudoroffii*-S5 and *Exiguobacterium*-S8). This is because the plants release root exudates which help the bacteria strains. In turn, the bacterial strains release phytohormones which support the growth of hydrophytes growth. Many investigators also used *Bacillus* and *Pseudomonas* for the removal of heavy metals in the presence of different hydrophytes but chromate removal by *Pistia stratiotes* and *Eichornia crassipes* in the presence of *Exiguobacterium* is a new report for wastewater treatment. From these findings, it is easy to construct a wastewater bioreactor to treat chromium-polluted industrial wastewater.

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